nanomedicine and drug delivery symposium - indianapolis

October 5-6, 2009
Indianapolis, Indiana

NanoDDS'09

Keynote Speaker

Scott McNeil, Director, Nanotechnology Characterization Laboratory, National Cancer Institute at Frederick

Confirmed Speakers

Saghir Akhtar, Kuwait University
Susan Clare, Indiana University School of Medicine
Omid Farokhzad, Harvard Medical School
M. Laird Forrest, University of Kansas
Rogério Gaspar, University of Lisbon
Gershon Golomb, The Hebrew University of Jerusalem
Ick Chan Kwon, Korea Institute of Science and Technology
Vinod Labhasetwar, Lerner Research Institute
Claus-Michael Lehr, Saarland University
Elaine Merisko-Liversidge, Elan Drug Technologies
Samir Mitragotri, University of California, Santa Barbara
S. Moein Moghimi, Copenhagen University
Kinam Park, Purdue University
Soon Hong Yuk, Hamnam University

Organizing Committee

Ralph Lipp, (Co-Chair) Eli Lilly & Co.
Kinam Park, (Co-Chair) Purdue University
Randall Mrsny, (Co-Chair) University of Bath
Christine Allen, University of Toronto
Henry Havel, Eli Lilly & Co.

Alexander Kabanov, University of Nebraska Medical Center
Marsha Fau, University of Nebraska Medical Center
Keith Sutton, University of Nebraska Medical Center

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NanoDDS'09

October 5 - 6, 2009
Hilton Indianapolis
Indianapolis, Indiana

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NanoDDS'09

Seventh International Nanomedicine and Drug Delivery Symposium

October 5 – 6, 2009

Venue: Hilton Indianapolis

Indianapolis, Indiana, USA

Symposium Chairs

Ralph Lipp, Eli Lilly and Co., Indianapolis, IN, USA
Kinam Park, Purdue University, West Lafayette, IN, USA
Randall Mrsny, University of Bath, Bath, England

Immediate past and future chairs

Christine Allen, University of Toronto (Toronto 2008)
Alexander Kabanov, University of Nebraska Medical Center (Omaha 2010)

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Preface

The 7th International Nanomedicine and Drug Delivery Symposium (NanoDDS'09) will be held on October 5th and 6th, 2009 at the Hilton in downtown in Indianapolis, Indiana (USA). Drs. Ralph Lipp (Lilly Research Labs), Kinam Park (Purdue University), and Randy Mrsny (University of Bath) will be the co-chairs for this year's event.

Consistent with the goals of previous NanoDDS meetings (initiated by Sasha Kabanov, Kalle Levon, and Hamid Ghandehari), the 2009 edition of this meeting focuses on a program that highlights the latest groundbreaking discoveries and developments in the field of nanomedicine focused on efficiently and effectively delivering a wide range of therapeutic drug entities (proteins, peptides, genes, siRNA, etc). As the applications of nanomedicines in drug delivery are focused at moving these novel approaches to the clinic, the NanoDDS'09 program will focus on efforts to make this a reality: the realities of preparation and testing of nanomedicines that are required for moving potentially effective agents safely into clinical studies. Thus, the NanoDDS'09 program will not only have talks from the most noted scientists in the fields of nanotechnology, materials science, imaging, cell biology, and tissue engineering, it will also have presentations by those individuals who consider the essential aspects of preparation and testing of materials to qualify them for clinical studies.

In order to highlight various elements required to translate exciting new possibilities using nanomedicines to clinical testing, the NanoDDS'09 program includes talks from individuals who have successfully taken nanomedicines to the clinical as well as presentations on essential requirements to qualify nanomedicines for clinical testing. The preliminary program includes presentations by new promising investigators and world-renowned scientists on the following selected topics:

- Emerging Regulatory Frame Works for Nanomedicines
- Translation of Nanomedicines to Clinical Applications
- Nanomaterials with Biofunctions
- Manufacturing (Making little things in big scale)
- In vitro / in vivo comparisons of nanomaterials

This 2-day symposium will feature a series of presentations by experts in the field, poster sessions (with oral presentations of selected posters) and a round-table discussion. We hope that you like this symposium!

Sincerest regards,

Ralph Lipp, Ph.D.
Vice President Pharmaceutical Sciences R&D
Eli Lilly and Co.

Kinam Park, Ph.D.
Showalter Distinguished Professor of Biomedical Engineering & Professor of Pharmaceutics
Purdue University

Randall Mrsny, Ph.D.
Professor of Epithelial Cell Biology
University of Bath

Biographies of Symposium Chairs

Kinam Park is currently the Showalter Distinguished Professor of Biomedical Engineering and Professor of Pharmaceutics at Purdue University in West Lafayette, Indiana. He received his Ph.D. degree in pharmaceutics from the University of Wisconsin in 1983. After a postdoctoral training at the Department of Chemical Engineering of the same university for 2 years, he joined the faculty of the Purdue University in 1986 and was promoted to Full Professor of Pharmaceutics in 1994. Since 1998, he has had the joint appointment in the Department of Biomedical Engineering. His research has been focused on the use of various polymers and hydrogels for controlled drug delivery. His current research includes homogeneous microparticles using nano/micro fabrication, hydrotropic polymeric micelles, superporous hydrogels, fast melting tablet formulations, and drug-eluting stents. He has published more than 260 papers and chapters, and presented 180 abstracts at national and international meetings. He is currently the Editor-in-Chief of the Journal of Controlled Release.

Randall Mrsny obtained a B.S. in Biochemistry and Biophysics at the University of California at Davis, a Ph.D. in Anatomy and Cell Biology at the U.C. Davis School of Medicine and spent four years as an NIH Postdoctoral Fellow in Membrane Biophysics in the Institute of Molecular Biology at the University of Oregon. After running Peptide Biology Group at ALZA Corp and then heading the drug delivery/biology group at Genentech, Inc., he left Genentech to found Trinity Biosystems, a company focusing on the mucosal delivery of macromolecules and antigens for vaccination. He recently left Trinity and started Unity Pharmaceuticals, a company exploring novel methods to treat inflammatory conditions and epithelial-derived cancers. Randy holds a part-time Professor's chair of Epithelial Cell Biology at the University of Bath in the Department of Pharmacy and Pharmacology where he studies biological principles associated with normal epithelia cell function and how these are affected in disease states. He has been elected president of the Controlled Release Society and to co-organize a Gordon Conference on Drug Delivery.

Ralph Lipp is Vice President - Pharmaceutical Sciences Research and Development at Eli Lilly and Company in Indianapolis. His main areas of focus are patient-centered and Quality-by-Design-based formulation and packaging research and development, as well as the GMP-manufacturing and global supply of investigational drugs. Before coming to Lilly in 2005, he headed various R&D functions, including Drug Delivery Systems and Pharmaceutical Development, at Schering AG in Berlin, Germany. In parallel, he taught the subject Pharmaceutical Technology and supervised Ph.D. candidates at the Freie Universität in Berlin. Ralph holds a degree in Pharmaceutics from Johannes Gutenberg Universität in Mainz, Germany, and received a Ph.D. in the area of Medicinal Chemistry as well as a Habilitation for Pharmaceutical Technology from Freie Universität Berlin. He is author of more than 100 publications in the form of original articles, talks, abstracts, book chapters, patent applications and patents.

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NanoDDS 2009: From Laboratory to Clinical Reality
Indianapolis, Indiana. October 5-6, 2009

Symposium Chairs: Ralph Lipp, Kinam Park & Randall Mrsny

October 5 (Monday)

08:00 - 09:00 am Registration / Continental Breakfast

Welcome
Session Chair: Ralph Lipp

09:00 - 09:10 am Introductory Remarks (Symposium Chairs)

09:10 - 10:00 am Keynote Presentation: Nanotechnology for Drug Delivery: Lessons Learned from NCI's Nanotechnology Characterization Lab (NCL) (Scott McNeil)

10:00 - 10:30 am Coffee Break, Poster Set-Up

SESSION 1: Nanomaterials with Biofunctions
Session Chair: Randy Mrsny

10:30 - 11:00 am Complement: Alive and Kicking Nanomedicines (S. Moghimi)

11:00 - 11:30 am Engineering Physical Properties of Nanoparticles for Drug Delivery (Samir Mitragotri)

11:30 - 12:00 pm Nanomedicine for Drug Delivery across Epithelial Barriers (Claus-Michael Lehr)

12:00 - 12:30 pm Nanoparticle-Mediated CNS Delivery of Therapeutics (Vinod Labhasetwar)

12:30 - 2:00 pm Lunch

2:00 - 2:30 pm Immunological Properties of Engineered Nanomaterials (Scott McNeil)

SESSION 2: Manufacturing (Making Little Things in Big Scale)
Session Chair: Hamid Ghandehari

2:30 - 3:00 pm Multifunctional Polymeric Nanoparticles for Medical Applications (Omid C. Farokhzad)

3:00 - 3:30 pm NanoCrystal® Technology: Medical and Diagnostic Applications (Elaine Merisko-Liversidge)

3:30 - 4:00 pm A New Nanofabrication Method Designed for Scale-Up Production (Kinam Park)

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Session Chair: Henry Havel

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10:00 - 10:30 am	Modulation of the Innate Immunity by Nanoparticles (Gershon Golomb)
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SESSION 4: *In vitro / in vivo* Comparisons of Nanomaterials
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11:30 - 12:00 pm	Nanoparticle Fomulation of Drugs for Localized Chemotherapy (M. Laird Forrest)
12:00 - 1:30 pm	Lunch

SESSION 5: Toxicological and Immunological Aspects of Nanomaterials
Session Chair: Gershon Golomb

1:30 - 2:00 pm	Toxicogenomics of Delivery Systems: From Gene Expression Changes to Impact on siRNA Activity (Saghir Akhtar)
2:00 - 2:30 pm	Scientific Issues and Regulation Aspects in the Translation of Nanopharmaceuticals to Clinic (Rogério Gaspar)
2:30 - 3:00 pm	Coffee Break, Poster Take-Down

SESSION 6: Oral Presentations by Poster Awardees
Session Chair: Kinam Park

3:00 - 3:30 pm	Poster Awards and Presentations
3:30 - 3:45 pm	Closing Remarks by Symposium Chairs

Speaker Abstracts and Biosketches

Dr. Scott E. McNeil

Dr. McNeil serves as Director, Nanotechnology Characterization Laboratory for the National Cancer Institute at Frederick. Dr. McNeil coordinates pre-clinical characterization of nanomaterials intended for cancer therapeutics and diagnostics. Prior to joining NCI-Frederick (i.e. SAIC-Frederick), Dr. McNeil served for three years as Senior Scientist in the Nanotech Initiatives Division at SAIC where he transitioned basic nanotechnology research to government and commercial markets. He consults with 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 in the Gulf War. He is an invited speaker to numerous nanotechnology-related conferences and has six patents pending related to nanotechnology and biotechnology. He received his bachelor's degree in chemistry from Portland State University and his doctorate in cell biology from Oregon Health Sciences University.

Keynote: Nanotechnology for Drug Delivery: Lessons Learned from NCI's Nanotechnology Characterization Lab (NCL)

Dr. Scott McNeil

NCI's Nanotechnology Characterization Laboratory (NCL) conducts preclinical efficacy and toxicity testing of nanoparticles intended for cancer therapeutics and diagnostics. The NCL is a collaborating partnership between NCI, the U.S. Food and Drug Administration and the National Institute of Standards and Technology. The NCL characterizes nanoparticles' physical attributes, their *in vitro* biological properties, and their *in vivo* compatibility in animal models. The Laboratory accelerates the transition of basic nanoscale particles and devices into clinical applications by providing critical infrastructure and characterization services to nanomaterial providers, and is a national resource available to investigators from academia, industry and government. NCL's many collaborations with nanotech investigators and expertise with a variety of nanoparticle drug delivery platforms have allowed us to elucidate trends relating physicochemical properties such as size and surface chemistry to nanoparticle behavior in biological systems, biodistribution, safety, and efficacy. This presentation will include some of the NCL's recent findings regarding nanoparticle biocompatibility and toxicity.

Session 1 Presentation: Immunological Properties of Engineered Nanomaterials

The National Cancer Institute's Nanotechnology Characterization Laboratory (NCL) conducts preclinical efficacy and toxicity testing of nanomaterials intended as cancer therapeutics and diagnostics. The NCL's toxicity studies include evaluation of nanomaterial immunological characteristics, such as blood contact properties, complement activation, reticuloendothelial system (RES) uptake, and effects on immune cell function. This presentation will include some of NCL's recent findings regarding trends in nanoparticle immunological properties and highlight *in vitro* models that have proven helpful for understanding nanomaterial *in vivo* interactions with the immune system.

S.M. Moghimi

Moein Moghimi is Professor of Nanomedicine and Director of the Centre for Pharmaceutical Nanotechnology and Nanotoxiocology at the Faculty of Pharmaceutical Sciences and NanoScience Center, University of Copenhagen, Denmark. He is also Guest Professor of Nanomedicine at Multidisciplinary Research Center, Shantou University, China. His research activities are focused on pharmaceutical nanotechnology and experimental nanomedicines. He has pioneered research in design and surface engineering of nanoparticulate delivery vehicles and functional nanosystems for parenteral site-specific targeting and imaging modalities (splenotropic entities, lymphotropic agents and 'phagocyte-resistant' nanoparticles) as well as molecular toxicology of polymeric and particulate complexes. Prof. Moghimi is also the Associate Editor of the *Journal of Biomedical Nanotechnology* and a member of the editorial/advisory board of 7 international journals to include the *Journal of Liposome Research, Drug Delivery* and *Nanomedicine-UK.* He is also a consultant to numerous pharmaceutical, biotechnology, health, and food industries as well as investment banks, management consultancy firms and other entrepreneurial enterprises world-wide. In 1985, he graduated in biochemistry from The University of Manchester (UK) and in 1989 completed a PhD in biochemistry (liposomes immunobiology) at Charing Cross Hospital Medical School (Imperial College, University of London, UK).

Complement: Alive and Kicking Nanomedicines

S.M. Moghimi

Nanomedicine Laboratory, Centre for Pharmaceutical Nanotechnology and Nanotoxicology, department of Pharmaceutics and Analytical Chemistry, University of Copenhagen, Denmark

E-mail: momo@farma.ku.dk

Acute reactions to intravenously injected PEGylated liposomes are well documented. These pseudoallergic responses, which are associated with cardiac anaphylaxis and rapid haemodynamic collapse, are strongly correlated with complement activation. Many consider complement activation by PEGylated liposomes an unexpected phenomenon since surface PEG coverage is generally believed to dramatically suppress particle-protein interaction. Contrary to such belief, we have demonstrated a critical role for the anionic phosphate oxygen moiety of liposomal-incorporated mPEG-phospholipid conjugate in triggering alternative and C1q-dependent pathways of the human complement system. Our studies have further shown that other PEGylated particulate materials such as carbon nanotubes and even PEG in soluble form (but at high concentrations) activate complement and notably through the action of the mannose-activating serine protease-2. This lecture will discuss the molecular basis of complement activation by PEGylated nanomedicines and polymeric constructs as well as strategies for circumventing these events.

Samir Mitragotri

Samir Mitragotri is a professor of Chemical Engineering at the University of California, Santa Barbara. He received his Ph.D. in Chemical Engineering from the Massachusetts Institute of Technology. His research interests include the development of novel methods of drug delivery including transdermal, oral and particle-based methods. His honors include Ebert Prize by American Pharmaceutical Association (1996), Technology Review Young Innovator award (1999), Young Scientist award by International Research Promotion Council (2000), CRS-Dow Corning award for outstanding research (2000), 3M Young Faculty award (2001), Global Indus Technovator Award (2003), Pfizer-Capsugel award for innovative work in oral drug delivery (2004), Hendrick C. Van Ness Lecturer at RPI (2004), Allan P. Colburn award from American Institute of Chemical Engineering (2005), and Young Investigator award from CRS (2008). His teaching honors include outstanding faculty award (2001) and Chancellor's award for excellence in undergraduate research (2003). He is a member of the Editorial Boards of *European Journal of Pharmaceutical Sciences, Journal of Controlled Release, and Journal of Pharmaceutical Sciences*. He is the author of over 100 publications and is an inventor of over 40 issued or pending patents.

Engineering physical properties of nanoparticles for drug delivery

Samir Mitragotri

Polymeric nanoparticles have wide application in varied fields including drug delivery. Particle properties have significant impact on their performance including circulation half-life, drug release rates and toxicity. Our research focuses on engineering particle shape, a design parameter that has received little attention in the past.

We have devised methods to generate particles of several distinct shapes and studied their impact on key processes in drug delivery including phagocytosis, circulation, adhesion of vascular walls, and targeting. Based on this understanding, we have designed novel polymeric particles that demonstrate reduced phagocytosis and enhanced targeting. Our studies demonstrate that particle shape provides a new dimension in engineering of polymeric carriers and opens up new opportunities in drug delivery.

In addition to shape, we demonstrate that controlling mechanical properties of carriers also offers unique opportunities. Specifically, we have synthesized flexible particles made from proteins that mimic the physical and functional properties of body's own circulating cells such as red blood cells and platelets. Particles that mimic the size, shape and flexibility of natural circulating cells offer advantages that are typically lacking in standard spherical polymeric particles.

The motivation to use physical properties of nanoparticles to control biological function is provided by the biology itself. In nature, numerous examples can be found where physical aspects, such as shape, mechanical properties and compartmentalization are crucial to biological function. We demonstrate that physical attributes such as size, shape and mechanical properties form essential building blocks of biology. This recognition forms the basis of new strategies for nanoparticle design.

Prof. Dr. Claus-Michael Lehr

Professor Lehr is head of the Department of Biopharmaceutics and Pharmaceutical Technology at Saarland University, Germany. He studied pharmacy in Germany, obtained his PhD (1991) from Leiden University (The Netherlands), was postdoc at USC (Los Angeles, USA, 92), and had other appointments at Leiden University (93) and Marburg University (Germany, 94). The central theme of his research is drug delivery across biological barriers, in particular those of the lungs, the GI tract and the skin. One line of his research is occupied with cell culture based in-vitro models, a second line with advanced drug carrier systems, in particular nanomedicines, to overcome these barriers. He is (co)author of ~180 papers, which have been cited ~2.300 times (h-index 25). He was the recipient of the CRS Young Investigator Award (2001), the APV Research Award 2006 for outstanding achievements in the Pharmaceutical Sciences and recently was elected for the biannual International Price 2008 of the Belgian Society for Pharmaceutical Sciences.

Professor Lehr is European Editor of the European Journal of Pharmaceutics and Biopharmaceutics, and member of the editorial boards of other peer-reviewed international journals in the Pharmaceutical Sciences, such as Pharmaceutical Research, Advanced Drug Delivery Reviews, and the Journal of Controlled Release. Besides collaborations with the pharmaceutical industry, he is involved in several national and EU research projects, mainly related to nanotechnology, nanomedicine and nanotoxicology. His office coordinates the EU-funded GALENOS network (www.galenos.net) with approx.70 academic and industrial member institutions. The program "GALENOS Euro-PhD® in Advanced Drug Delivery" aims to promote and to recognize in-depth scientific training and international mobility of young pharmaceutical scientists across Europe and worldwide.

Nanomedicine for Drug Delivery across Epithelial Barriers

Claus-Michael Lehr
Saarland University, Saarbrücken, Germany, lehr@mx.uni-saarland.de

The focus of our research over the past ten years has been on the biological barriers of the gastro-intestinal tract, the skin and the lungs. This presentation will highlight some of our recent results or data of work in progress in these three areas, either concerning the development of new in-vitro models or new drug carriers systems, for which the nano-size often has turned out to be advantageous.

Inflammatory bowel diseases, such as Morbus Crohn or Colitis Ulcerosa, are painful for the patient and moreover difficult to treat due to the increased mucus production and the occurrence of diarrhea. We could demonstrate that the anti-inflammatory drug rolipram, when delivered by nanoparticles made of biodegradable PLGA, led to a prolonged alleviation of colitis syndromes in rats and a reduction of central nervous side effects, compared to the same dose of the drug administered as an aqueous solution [1, 2].

With respect to skin drug delivery, there is an interesting new hypothesis that nanoparticles may penetrate along hair shafts and to thus accumulate in hair follicles [3]. However, applying PLGA nanoparticles loaded with flufenamic acid, were mostly seen in the intercellular clefts between the keratinocytes [4]. The observed enhancement of epidermal penetration may instead be explained by an acidic microclimate around the hydrolyzing polymer particles, leading to a reduced dissociation and higher lipophilicity/better penetration of flufenamic acid [5]. This data points out that, besides of their small size, the chemical composition of such nanomaterials remains evenly important.

Due to their large surface area and excellent blood supply, the lungs are an attractive alternative route for drug delivery, both for local as well as for systemic action. By escaping mucociliary or macrophage clearance, inhaled nanopharmaceuticals could perhaps be used as platform for pulmonary sustained release delivery systems. Finally, nanoplexes formed between biodegradable polymeric carriers and DNA/RNA-based drugs can be used to facilitate cellular transfection [6]. We are currently using this approach for the delivery of telomerase inhibiting antisense oligonucleotides to lung cancer cells ,[7,8].

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Dr. Vinod Labhasetwar

Dr. Labhasetwar is Professor and Head, Division of BioMEMS and NanoMedicine at the Department of Biomedical Engineering, Learner Research Institutive, Cleveland Clinic, Cleveland, OH. His laboratory's research focus is on translational NanoMedicine, which involves biomaterial synthesis, formulation design and development, and evaluation of different biocompatible nanoparticle-based platform technologies for targeted drug/gene delivery and imaging agents. Dr. Labhasetwar's primary research interests are in cancer treatment and detection, delivery of anti-oxidant enzymes in stroke, and developing a non-stent approach to inhibition of restenosis. His laboratory also investigates biophysical and molecular mechanisms of nanoparticle-cell membrane interactions and its effect on intracellular trafficking of nanoparticles. Dr. Labhasetwar leads Cleveland Clinic's Cancer NanoMedicine Program, a joint undertaking of the Department of Biomedical Engineering and the Taussig Cancer Center. The program's primary aim is to develop better treatment options for cancer patients through applications of nanotechnology. Dr. Labhasetwar is a fellow of the American Association of Pharmaceutical Scientists.

Nanoparticle-mediated CNS Delivery of Therapeutics

Vinod Labhasetwar, Ph.D.
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Cleveland Clinic, Cleveland, OH 44195, USA
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The blood brain-barrier (BBB) presents a major hurdle to delivering potentially useful therapeutic agents to the central nervous system (CNS) in the treatment of several neurological disorders. Therefore, an effective delivery system that can cross the BBB without disrupting its integrity and deliver therapeutics to the CNS is needed. We are investigating biodegradable *trans*-activating transcription (TAT)-peptide conjugated nanoparticles (TAT-NPs) for this purpose. Our approach to developing an efficient CNS delivery system is based on biophysical interactions of these TAT-NPs with our model endothelial cell membrane (EMM). Our results have shown that both the TAT peptide sequence and the amount of TAT conjugated to NPs significantly affect the biophysical interactions of NPs with the EMM, and these interactions correlate with the effectiveness of cellular delivery of the encapsulated drug. With new knowledge gathered from these NP-EMM interactions, this study will not only help us to design/develop an efficient CNS drug delivery system but also to predict how well it will work.

We have also shown that TAT-NPs bypass the efflux action of P-glycoprotein and increase the transport of encapsulated ritonavir, a protease inhibitor, across the BBB to the CNS without disrupting BBB integrity. A steady increase in the ratio of drug seen in brain parenchyma vs. capillaries over time suggests that TAT-NPs are first immobilized in the brain's vasculature prior to their transport into parenchyma. Localization of NPs in parenchyma was further confirmed with histologic analysis of brain sections. The brain level of ritonavir delivered by conjugated NPs was 800-fold higher than that of drug in solution at 2 wks. TAT-NPs maintained a therapeutic drug level in brain for a sustained period; such a sustained presence of an antiviral drug could be effective in reducing viral load within the CNS, which acts as a reservoir for replicating HIV-1 virus. In another study, we have demonstrated the ability of NPs to deliver the antioxidant enzyme superoxide dismutase (SOD) to brain and thus reduce reperfusion injury in a stroke model. Of the animals treated with SOD-NPs, not only did more survive than controls (75% vs. 0% at 28 d), but also more regained most of their vital neurological functions. As these promising results indicate, NPs could prove to be an effective drug delivery mechanism that also enhances the bioavailability of different therapeutics in the CNS.

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3. **Reddy MK, Labhasetwar V. Nanoparticle-mediated delivery of superoxide dismutase to the brain: an effective strategy to reduce ischemia-reperfusion injury. *FASEB J.* 23:1384-95, 2009.**

Dr Omid Farokhzad

Dr Omid Farokhzad is Associate Professor at Harvard Medical School (HMS) and a physician-scientist in the Department of Anesthesiology at Brigham and Women's Hospital (BWH). He is a co-founder of BIND Biosciences, a biopharmaceutical company developing targeted nanoparticle therapeutics; and Selecta Biosciences, a bipharmaceutical company developing immunomodulatory therapeutics and prophylactic vaccines. He serves as Director on the board of BIND and as Director and Vice Chairman on the board of Selecta. Dr. Farokhzad's research group is focused on developing novel nanotechnologies for medical applications. He has pioneered the application of micro- and nanotechnology for high throughput development and screening of targeted drug delivery systems for a myriad of clinical applications. Dr. Farokhzad was named among the Nano50 winners of 2007 by Nanotech Briefs which awards the most innovative people and design ideas that will revolutionize nanotechnology. He completed his post-doctoral clinical and research trainings, respectively, at the BWH/HMS and MIT in the laboratory of Dr. Robert Langer. Dr. Farokhzad has authored more than 50 papers and is an inventor of more than 40 pending patents. He received his M.D. and M.A. from Boston University School of Medicine.

Multifunctional nanoparticles for medical application

Omid C. Farokhzad

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A variety of organic and inorganic materials have been utilized to generate nanoparticles for drug delivery applications, including polymeric nanoparticles, dendrimers, nanoshells, liposomes, nucleic acid based nanoparticles, magnetic nanoparticles, and virus nanoparticles. The two most commonly used systems are polymeric nanoparticles and liposomes [1, 2]. Controlled release polymer technology has impacted virtually every branch of medicine, including ophthalmology, pulmonary, pain medicine, endocrinology, cardiology, orthopedics, immunology, neurology and dentistry, with several of these systems in clinical practice today such as Atridox, Lupron Depot, Gliadel, Zoladex, Trelstart Depot, Risperidol Consta and Sandostatin LAR. The annual worldwide market of controlled release polymer systems which extends beyond drug delivery is now estimated at $100 billion and these systems are used by over 100 million people each year. Polymeric nanoparticles can deliver drugs in the optimum dosage over time, thus increasing the efficacy of the drug, maximizing patient compliance and enhancing the ability to use highly toxic, poorly soluble, or relatively unstable drugs. These systems can also be used to co-deliver two or more drugs for combination therapy [3]. The surface engineering of these nanoparticles may yield them "stealth" to prolong their residence in blood [4] and the functionalization of these particles with targeting ligands can differentially target their delivery or update 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 summarize the key components required for creating effective targeted nanoparticle conjugates. The structure and properties of various targeting ligands, as well as the development and evaluation of therapeutic and imaging conjugates that take advantage of the unique properties of these of these ligands will be discussed.

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Elaine Merisko-Liversidge

Biography

Elaine Merisko-Liversidge, Ph.D. is Senior Director of Elan's Exploratory Early Stage Development Program for NanoCrystal® Product Opportunities. Dr. Liversidge has worked in the area of drug delivery for over twenty years and holds numerous patents and publications focusing on topics ranging from drug nanoparticles to intracellular trafficking. She received her doctorate degree in Cell Biology and Anatomy from the University of Pittsburgh Medical School. After a research fellowship position in laboratory of the late Nobel Laureate, Dr. George Palade, at Yale University, she pursued an academic career at the University of Kansas Medical Center, focusing her research in the area of intracellular trafficking of macromolecules. In the late 80's, she re-directed her career from academia to the pharmaceutical industry where her interests have focused on drug delivery issues associated with poorly water-soluble molecules. She was a member of the original Eastman Pharmaceuticals/Sterling Winthrop team credited with identifying the benefits of formulating problematic compounds as nanoparticles. Dr. Liversidge continues to play an active role in the discovery, development and evolution of Elan's NanoCrystal® Technology. Her current team is responsible for rapid formulation identification of poorly water-soluble discovery compounds. In addition, she leads a research effort identifying "smart stabilizer" systems for improving the biological performance of nanoparticulate drug delivery systems.

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Abstract

NanoCrystal® Technology: Medical and Diagnostic Applications

This year marks the tenth anniversary for the NDA filing of the first licensed NanoCrystal® product, Rapamune® (Wyeth Corp.). Since then the technology has successfully been utilized to formulate poorly water-soluble therapeutic and diagnostic compounds. Currently, five licensed products incorporating Elan's proprietary NanoCrystal® technology have been approved in the US and many international markets. Typically the approach uses a media milling process that is water-based, wherein, drug is shear-fractured into nanometer-sized particles. The resulting NanoCrystal Colloidal Dispersion® formulations are stable as liquids or are readily processed into the most commonly used dosage forms for oral and parenteral delivery. NanoCrystal® technology has been applied to poorly water-soluble compounds from discovery through development to enhance performance and/or provide more patient compliant dosage forms. Case studies will be presented to demonstrate the various medical and diagnostic applications that have benefited from this formulation approach and opportunities for the future will be discussed.

A new nanofabrication method designed for scale-up production

Ghanashyam S. Acharya and Kinam Park

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Nano/micro particles have been used extensively in controlled drug delivery. There are several techniques of making nano/micro particles, but the most widely used technique is the double emulsion approach. The solvent extraction and solvent evaporation methods have been the methods of choice. While they have been used most frequently, they suffer from a few major limitations. They are low drug loading capacity and high initial burst release.

Recent revolution in nanotechnology has brought many new approaches in making drug formulations in nano/micro scales, known as microfabrication. Several microfabrication techniques for making nano/micro scale drug delivery vehicles have been developed, but most of them in the literature are difficult to use for production of large quantities of nano/micro particles, especially for clinical applications, for several reasons. While the microfabrication techniques described in the literature are highly elegant, the processes are such that they may result in impurities or use of excessive amounts of organic solvents. In addition, the processing steps of making such vehicles are sometimes complicated, making the scale-up production difficult.

In our study, a number of factors required for producing clinically useful drug delivery vehicles were identified first, and then the solution to each problem was tried. The first six factors considered are: easy scale-up, ability to load low molecular weight drugs, ability to load high molecular weight drugs, high drug loading capacity, high drug loading efficiency, and acceptable drug release profiles. Of these, the scale-up issue has not been dealt with in depth in the literature, and this is most likely due to the fact that scale-up process is probably the last thing that a research may consider when a new technique is developed, The drug loading capacity found in the literature is in the range of 2-10% with the average of 5%. This is clearly too low to make efficient drug delivery particles. It is also common to observe that most of the nano/micro particles release a drug with the initial burst release of 50% or so. Such a high burst release is clearly not desirable.

Recently, we developed a new microfabrication method for producing nano/micro particles using a process that is specifically designed with the factors described above in mind. The microfabrication method was designed for easy scale-up manufacturing, high drug loading capacity, and minimized initial burst release. The method we developed utilizes hydrogel materials as a template, and thus, it is named "AquaTemplate" method. The AquaTemplate method met our initial goal of satisfying the six factors for fabrication of homogeneous nano/micro structures.

Dr. Susan Clare

Biography

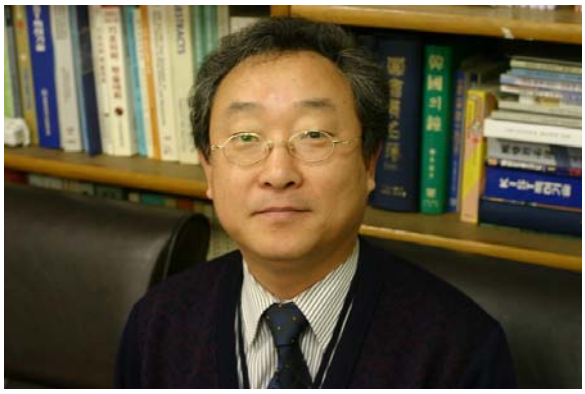
Dr. Clare is an Assistant Professor in the Department of Surgery, Indiana University School of Medicine. She is also a Guest Scientist/Visiting Professor at the Universitäts-Frauenklinik, Tübingen, Germany. She received a BA (Biology), MS (Chemistry) and PhD (Chemistry) from Northwestern University, Evanston, Illinois. Dr. Clare is an Alpha Omega Alpha graduate of Northwestern University Medical School, Chicago, IL. Following completion of an internship and residency in general surgery at Northwestern, Dr. Clare was a postdoctoral fellow in the laboratory of Patricia S. Steeg, PhD, Women's Cancers Section, Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland. She is a member of the Clinical Advisory Board of the Cincinnati/Purdue/University of Illinois at Urbana-Champaign Nanomedicine Development Center.

Abstract

If nanotechnology is to reach its promise for the treatment of solid malignancies, it will need to be delivered efficiently. The physical properties of solid tumors, such as malformed blood vessels, elevated interstitial pressure; and, in addition, large transport distances in the tumor and interstitium, individually or in concert limit or thwart the delivery of oxygen, glucose and therapeutics. In addition, compressive mechanical forces generated by proliferating malignant cells can cause vessel lumen collapse and thus restrict blood inflow and lymph outflow. Frequently, the center of a tumor, the area furthest from the vascular supply, becomes hypoxic, hypoglycemic and cell death is extensive. This microenvironment also exerts a selective pressure on tumor cells in that only those with an aggressive phenotype (e.g., mutated p53) are able to survive hypoxia.

Tumor neovascular leakiness and lymphatic flow compromise are hypothesized to be responsible for what is referred to as the *Enhanced Permeability and Retention (EPR) Effect*. Theoretically EPR should result in the delivery and retention of nanoparticles in tumors. However, does the EPR effect occur in a human malignancy *in situ*? While endothelial fenestrations may be much larger than nanoparticles, the leakiness of the tumor neovasculature has the consequence of diminishing or eliminating the net driving force, i.e., the pressure differential that in normal tissue produces flow from the capillary to the tissue interstitium.

We have developed an alternative delivery strategy, which uses the body's response to hypoxia and necrosis to deliver nanovectors to hypoxic regions of tumors. The body's response is to mobilize peripheral blood monocytes, which once they cross the endothelial basement membrane differentiate into tumor associated macrophages. Monocytes can be loaded *ex vivo* with large numbers of nanovectors. The monocytes can then be reintroduced into the systemic circulation where they home into the necrotic regions of tumors, delivering their nanocargo. In addition to providing active transport of the nanovectors, this approach is one of the few which targets the cells residing in hypoxic, inadequately vascularized regions of tumors inaccessible to conventionally delivered therapies and likely the origin of recurrences. We have also utilized this strategy to deliver nanovectors across the blood-brain barrier.



Ick Chan KWON, Ph.D.

Ick Chan Kwon is currently Head of Biomedical Research Center at Korea Institute of Science and Technology (KIST). He received his Ph. D. in pharmaceutics and pharmaceutical chemistry from University of Utah under the guidance of Professor Sung Wan Kim in 1993. He is currently president of the Korean Society of Molecular Imaging (2008-2010). He also serves as an Asian Editor of the *Journal of Controlled Release* (Elsevier), Asian Editor of the *Journal of Biomedical Nanotechnology* (American Scientific Publisher). His main research interest is targeted drug delivery with polymeric nanoparticles and is now expanding to the development of smart nano-probes for theragnostic imaging. He has published 136 peer-reviewed articles, 11 book chapters, and 8 review articles.

Ick Chan KWON

Abstract unavailable at time of printing.

Dr. Gershon Golomb

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CURRICULUM VITAE

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

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

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

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

Professional Activities (selected)
Organizer, chairman/co-chairman of international conferences. Member of 3 editorial boards, and referee. Member & chairman of several HUJI and national committees; chairman of the National Committee for Licensing Exams in Pharmacy. Over 140 worldwide invited lectures in academia and industry (not including contributed presentations in meetings/conferences).

Teaching (2008/9)
Undergraduate courses in Pharmacy (Introduction to Pharmaceutics, Advanced Drug Delivery Systems); Medicine and Basic Medical Sciences (part of Pharmacology); Graduate courses (Implantable and Injectable Drug Delivery systems). Supervisor of 1 MSc, 6 PhD, and 1 postdoc.

Research (Key words)
Controlled / Sustained release drug delivery systems; Dosage forms; Nanoparticles; Liposomes; Gene Delivery and Therapy, Immunomodulation, bisphosphonates; Pathophysiology & therapy of cardiovascular (restenosis, MI) and bone disorders, mammary carcinoma, endometriosis. Basic-research grants, and industrial projects.

Publications
120 full publications (105 papers & 15 chapters in books), >28 patents, and >150 abstracts & proceedings.

Modulation of the Innate Immunity by Nanoparticles

Gershon Golomb
School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel (gershong@ekmd.huji.ac.il)

Intimal hyperplasia is a universal response of the arterial wall to mechanical injury and it is a major cause of restenosis following angioplasty. Experimental and clinical data indicate that the innate immunity and inflammation are of major importance in the pathophysiology of restenosis. Macrophage recruitment is associated in other clinically important disorders such as endometriosis; characterized by the deposition of endometrial cells in areas outside the uterine cavity.

We validated the hypothesis that systemic and transient depletion of monocytes inhibits the inflammatory cascade. Monocytes/macrophage depletion was achieved with a systemic injection of nanoparticulated dosage forms (PLGA-based NP and liposomes) containing bisphosphonates (BP) formulated for effective phagocytosis. Following phagocytosis the vesicles discharge their encapsulated drug like a Trojan Horses, inactivating the cell with no effect on non-phagocytic cells.

We investigated the effect of different BP, NP type (polymeric or liposomal), and size on the formulation properties and biodistribution. Bioactivity and mechanism was examined in tissue cultures, and in animal models of restenosis and endometriosis. Partial and transient depletion of blood monocytes following NP systemic injection correlated with the therapeutic effect. Phase I clinical studies confirmed the safety and potential efficacy of the nanoparticulate delivery system leading to ongoing Phase II clinical studies in stented patients.

Soon Hong Yuk

Birthday: August 26, 1959

Education

1978-1982 B. S., Chemistry, Department of Chemistry, College of Natural Science, Seoul National University
1982-1987 Ph. D., Chemistry, Department of Chemistry, Korea Advanced Institute of Science and Technology

Career

1987-1989 Post-Doctoral Fellow, Department of Pharmaceutics, University of Utah
1989-1999 Senior/Principal Research Scientist, Korea Research Institute of Chemical Technology
1999-present Professor, Department of Advanced Materials, Hannam University

Interest

Biopolymers and Drug Delivery System

Dr. Soon Hong Yuk is a Professor of Hannam University in Korea. After finishing his research experiences in the department of pharmaceutics, University of Utah, he has continued his research in the field of drug delivery system and biopolymers. Recently, he has focused on the design and characterization of novel drug delivery systems for protein and anticancer drugs based on nanotechnology.

Nanocarriers with core/shell structure for drug delivery

Keun Sang Oh, Hee Hoon Lee, Ji Young Song and Soon Hong Yuk
Department of Advanced Materials, Hannam University, 461-6 Jeonmin Dong, Yusung Gu, Daejeon, 305-811, Republic of Korea

Core/shell nanoparticles with a lipid core were prepared and characterized as a delivery system for VEGF (vascular endothelial growth factor). The lipid core is composed of lecithin and the polymeric shell is composed of Pluronics (poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) triblock copolymer, F-127). With the formation of core/shell nanoparticles, almost zero-order release pattern was observed during a 42-day period. VEGF is a potent mitogen in embryonic and somatic angiogenesis with a unique specificity for vascular endothelial cells. We investigated the functionality and efficiency of core/shell nanoparticles as a sustained delivery system of VEGF delivery *in vivo* with myocardial ischemia-induced rat (MI) models. The significant increase in the capillary density was observed with core/shell nanoparticles. The ejection fraction, which represents heart contractility, was significantly recovered with the application of VEGF-loaded core/shell nanparticles (39±7) comparing with the MI group (22±7) ($p<0.001$) and the heart elasticity (arterial elastance) was also recovered.

Doxorubicin-loaded Pluronic micelles were stabilized with glycol chitosan/heparin complexes. Firstly, the intermolecular interaction between doxorubicin-loaded Pluronic micelles and glycol chitosan was induced via hydrogen bonding. Subsequently, low molecular heparin was introduced for the formation of cationic glycol chitosan/anionic heparin complexes via ionic interaction. With the formation of stabilized nanoparticles, the release rate of doxorubicin was significantly reduced. Because of pH-sensitive association/dissociation of glycol chitosan/heparin complexes, the release rate was changed in response to environmental pH change. We also evaluated, *in vivo* biodistribution for tumor targeting profile using a non-invasive live animal imaging technology and tumor therapeutic effects in tumor-bearing mice.

M. Laird Forrest

Biography

Laird Forrest is an assistant professor of Pharmaceutical Chemistry at the University of Kansas. His lab develops nanoparticles and conjugates for drug and gene delivery and imaging.

Abstract

Nanoparticle formulation of drugs for localized chemotherapy

The lymphatics play an essential role in the metastatic spread of cancer. They drain cellular waste and excess fluids from nearly all tissues of our bodies. The lymphatics play an important role in early cancer metastasis because early tumors hijack the lymphatic drainage system in order to spread to distant organs and tissues; following lymphatic flow and establishing in lymph nodes until eventually entering the systemic circulation. We believe the lymphatics could be used to turn the tables on aggressive cancers; if chemotherapeutics could be localized to the diseased lymphatics before distant metastasis occurs, many patients could forgo destructive surgery, radiation, and whole-body chemotherapy.

Our group builds new materials that can enter the lymphatics, penetrate tumors, and deliver highly localized doses of chemotherapy to lymphatic tumors. Current available materials fail due to their lack of biodegradability and relatively low drug capacity. Hence, we develop new biodegradable, highly water soluble, and transport-optimized lymphatic nanocarriers with large capacities for hydrophobic anticancer drugs that can be used in the targeted treatment of cancer.

Saghir Akhtar

Biography

Saghir Akhtar is currently Professor in the Department of Pharmacology and Toxicology, Faculty of Medicine, Kuwait University and Editor-in-Chief of the Journal of Drug Targeting. He was previously Professor of Drug Delivery in the Welsh School of Pharmacy and Director for the Centre for Genome-based Therapeutics, Cardiff University, UK (2002-2006).

Professor Akhtar gained a First Class honours degree in Pharmacy from the Leicester School of Pharmacy in the UK and his PhD from the University of Bath. From 1990-1991, he held a post-doctoral fellowship at UNC Medical School at Chapel Hill, North Carolina and began his academic career at Aston University, firstly as lecturer from 1991-1997 and then as Reader in Pharmaceutical Sciences (1997-1999). In 1997, Prof. Akhtar was a visiting fellow with Prof. Ed Southern in the Department of Biochemistry, Oxford University. He is the winner of the Lilly Prize (1996), the Pfizer Academic Award (1997), the British Pharmaceutical Conference Science Medal (1998), the Controlled Release Society (USA) Young Investigator Research Achievement Award (2001) and the Kappa Society Science Award (2005).

Prof. Akhtar's current research interests include a) studying molecular pharmacology and signal transduction pathways involved in diabetes and/or hypertension-induced cardiovascular dysfunction; b) understanding the biological and pharmaceutical challenges associated with the development of gene silencing nucleic acids (RNA interference/ siRNA/ antisense oligonucleotides) as potential therapeutic agents; and c) studying the toxicogenomics of novel drugs and non-viral drug delivery systems.

Abstract

Effective drug delivery systems must be biocompatible and non-toxic at the organ, tissue and cellular levels in the body. However, delivery vectors should also be geno-compatible especially when used for the delivery of gene-based therapeutics where only specified gene changes are desired. This presentation will review how microarray based toxicogenomics studies can aid in identifying the genomic fingerprint of drug delivery systems commonly used for nucleic acid delivery. Further, it will discuss how specific gene changes induced by these delivery systems could potentially interfere with small interfering RNA mediated gene silencing activity thereby highlighting how toxicogenomic studies can be used to optimize delivery system-nucleic acid drug combinations for maximal activity.

Rogério Gaspar

Rogério Gaspar is currently Associate Professor in the Pharmaceutics Department, Faculty of Pharmacy at the University of Lisbon, and a consultant to the Portuguese Tecnimede Pharmaceutical Group. Early in his career, both at the University of Coimbra and whilst undertaking his PhD studies at the Université Catholique de Louvain in Brussels, he developed an interest in advanced drug delivery systems. He has continued to work in this area, and has more than 20 years experience in the design and evaluation of nanoparticles and liposomes for drug (e.g. Leishmaniasis and cancer) and gene (cytosolic) delivery. More recently, these interests have broadened to other aspects of the nanomedicine field including the design of vectors for MRI imaging and also understanding the cellular mechanisms that govern the action of nanomedicines. Throughout his career Rogerio Gaspar has been called upon to support the development of Portuguese Regulatory strategy and his participation on numerous national committees, and his role as the Vice-chairman of the Medicines National Committee and Management Board (INFARMED) (1996-1999 and 2000-2002 respectively) led to invitations to join EMEA as an advisory expert. He also has been a participant on several EMEA Working Groups developing both European Regulatory Strategy and overseeing International Harmonization. He was a member of the Working Group on Human Medicines of the European Council (European Union) during the Portuguese Presidency, being the Chairman of this group when political agreement was reached on the new European Clinical Trials Directive (2000). These aspects of his career give Rogerio a unique perspective of both nanomedicines research and development and the Regulatory process.

In addition, Rogerio Gaspar has been Chairman of the Spanish-Portuguese Local Chapter of the Controlled Release Society (2002-2005), and member of the expert panel that developed the European Science Foundation's Forward Look on Nanomedicine. Co-chairman of the 2006 ESF Conference on Nanomedicine and for the Nanomedicine 2008, he also chaired the ESF Summer School for advanced Training in Nanomedicine held in Lisbon in 2009.

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Poster Information and Abstracts

Poster Information

1. **Mechanisms of sensitization of multidrug resistance by Pluronic**
Daria Y Alakhova, Natalia Y Rapoport, Elena V Batrakova, Alexander A Timoshin, Shu Li, David Nicholls, Valery Y Alakhov, Alexander V Kabanov

2. **Development of leukocyte-mimetic nanoparticles for tumor targeted drug delivery**
Zohreh Amoozgar, Joonyoung Park, Yoon Yeo

3. **WITHDRAWN**

4. **Complement Activating Properties of Liposome Encapsulated Hemoglobin**
Peter Bedocs, Rolf Bunger, Janos Szebeni

5. **Hemodynamic and Hematologic reactions in Pigs after Repeated Intravenous Administrations of Carbon Nanotubes**
Peter Bedocs, Lara Lacerda, A. Herrero, Miklós Tóth, Alberto Bianco, Maurizio Prato, Zoltan Rozsnyay, Laszlo Rosivall, Janos Szebeni, Kostas Kostarelos

6. **Development of Antioxidant Nanozymes For Parkinson's Disease Therapy**
Anna M. Brynskikh, Elena V. Batrakova, Howard E. Gendelman, Alexander V. Kabanov

7. **Effect of Probucol, AICAR, and TGF-β1 on Smooth Muscle Cell and Endothelial Cell Phenotype and Proliferation**
Somali Chaterji, Namho Kim, Michael S. Sturek and Kinam Park

8. **"Synthesis of Magnetite Nanoclusters Using a Multi-Inlet Vortex Mixer"**
R. Mejia-Ariza, O. Celebi, W. C. Miles, J. S. Riffle, R. M. Davis

9. **Construction of a Novel Bioresponsive Gene Delivery System Based on β-6-Amino-Cyclodextrins and Polyvinylalcohol-Adamantane-PEG Host:Guest Interactions**
Wei Deng, Trisha Eustaquio and David H. Thompson

10. **Blood Borne Macrophage Delivery of Nanoformulated Paclitaxel for Glioma**
C. B. Grotepas, Z. Ma, J.M. McMillan, B. Boyd, H. Chen, H. E. Gendelman, and H. Dou

11. **Engineered PLGA Nanoparticles as Therapeutic Delivery Vehicles**
Elizabeth M. Enlow, Patricia A. Ropp, Shaomin Tian, J Christopher Luft, and Joseph M. DeSimone

12. **Single nanoparticle detection by DNA barcoding for biodistribution studies in nanomedicine**
Trisha Eustaquio, Christy L. Cooper, Lisa M. Reece, James F. Leary

13. **Polymer-Based Delivery of siRNA for Cancer Treatment**
Dana Gary and You-Yeon Won

14. **Self-Assembling System for Delivery of Organophosphate Protectants**
V. Gilman, A. Gaydess, E. Duysen, O. Lockridge, A. Kabanov, T. Bronich

15. **Interfacial Activity Assisted Surface Functionalization – A Novel Approach to Introduce Functional Groups on Polymeric Nanoparticles**
Alex Grill, Udaya S. Toti and Jayanth Panyam

16. **Peritumorally activatable nanoparticles for drug delivery to multidrug resistant tumors**
Emily Gullotti and Yoon Yeo

17. **Levofloxacin encapsulated PLGA nanosuspension for sustained ocular drug delivery**
Himanshu Gupta, M.Aqil, R. K. Khar, Asgar Ali, A. Bhatnagar, Gaurav Mittal

18. **Swelling in Glucose-Sensitive HEMA-DMAEMA Hydrogels**
Hillary Kaylin Ashley Holback, Sungwon Kim, Kinam Park

19. **Efficacy of gentamicin encapsulated pluronic based polymeric nanoplexes against chronic *Brucella melitensis* infection in mice**
Jain N., Seleem M. N., Pothayee N., Restis E., A. Ranjan, Riffle J. S., Sriranganathan N.

20. **Dual-Modality Nanoparticles for cancer diagnosis using Magnetic Resonance and Near Infrared Fluorescence Imaging**
Jaehong Key, Ah Young Kim, Seung Young Lee, Christy Cooper, Kwangmeyung Kim, James F. Leary, Ick Chan Kwon, Kuiwon Choi

21. **Surface Functionalized Nanogels with Cross-Linked Ionic Core for Specific Tumor Targeting**
Jong Oh Kim, Nataliya V. Nukolova, Zigang Yang, Alexander V. Kabanov, and Tatiana K. Bronich

22. **Polyelectrolyte-Biosurfactant based Nanocarriers for Drug Delivery**
Jong Oh Kim, Alexander V. Kabanov, Tatiana K. Bronich

23. **Thermo- and pH-sensitive Hydrotropic Polymer Micelles for Oral Paclitaxel Delivery**
Sungwon Kim, Kinam Park

24. **Development of DPCPX-eluting stents**
Jutarat Kitsongsermthon, Sungwon Kim, Yuanzu (Henry) He, Xin Long, Michael Sturek, and Kinam Park

25. **Surface-Modified Gold Nanorods for Applications in Nanomedicine**.
Alexei P. Leonov, Jiwen Zheng, Jeffrey D. Clogston, Stephan T. Stern, Anil K. Patri, and Alexander Wei

26. **Polyethylene-b-Polycaprolactone (PEO-PCL) Vesicles for Therapeutic Applications**
D.H. Levine, J.S. Katz, Michael J. Therien, J.A. Burdick, J.A. Hadfield, D.A. Hammer

27. **SERS Quantification of Kinase Activity Using a Nanoparticle Based Peptide Biosensor**
Andrew M. Lipchik, G.V. Pavan Kumar, Joseph Irudayaraj, Laurie Parker

28. **Characterization of Nanobioconjugates to Treat Brain and Breast Tumors**
Hui Ding, Satoshi Inoue, Rameshwar Patil, Jose Portilla-Arias, Keith L. Black, Eggehard Holler, Marina A. Dobrovolskaia, Jeffrey D. Clogston, Scott E. McNeil, Julia Y. Ljubimova

29. **Novel Drug-Eluting Stents to Reduce Coronary In-stent Stenosisin a Porcine Model of Metabolic Syndrome**
Xin Long, Yuanzu (Henry) He, Jutarat (Pete) Kitsongsermthon, Kinam Park, Michael Sturek

30. **Therapeutic Immunosuppression with Targeted Nanoparticulate Systems**
Michael Look, Anushree C. Shirali, Eric Stern, Qin Wang, Leah D. Diplacido, Daniel R. Goldstein, Joe Craft, and Tarek M. Fahmy

31. **Surfactant composition affects nanoART uptake and drug release in human monocytes and macrophages**
Zhiya Ma, Ari S. Nowacek, Katie Brown, JoEllyn McMillan, Alec Anderson, Brandon Boyd, Cassi Grotepas, Huanyu Dou and Howard E. Gendelman

32. **Little, BIT, of WONder -they deliver**
Mahesh R.V. and Charulatha G.

33. Polyelectrolyte complexes of antioxidant enzymes and block copolymers – physicochemical characterization and cellular uptake mechanisms
Devika S. Manickam, Natalia L. Klyachko, Elena V. Batrakova, Tatiana K. Bronich and Alexander V. Kabanov

34. **Fabrication of Homogeneous Drug Delivery Systems *via* Hydrogel Template Strategy**
Matthew McDermott, Crystal Shin, Ghanashyam Acharya, Kinam Park

35. Pharmacokinetic properties of indinavir, ritonavir, atazanavir, and efavirenz nanoparticles for human monocyte and macrophage drug delivery
Ari Nowacek, Reagan Miller, JoEllyn McMillan, Alec Anderson, Katie Brown, Huanyu Dou, Sabine Graham, Mahesh Chaubal, Jane Werling, Barrett E. Rabinow, Howard E. Gendelman

36. **Folate-Conjugated Cross-Linked Polymer Micelles as a Nanocarrier for Targeted Delivery**
Nataliya V. Nukolova, Hardeep S. Oberoi, Alexander V. Kabanov and Tatiana K. Bronich

37. **Preparation of Antibody-Bound Cross-Linked Micelles for Targeted Drug Delivery**
Nataliya V. Nukolova, Surinder Batra, Alexander V. Kabanov, Tatiana K. Bronich

38. Biodistribution and comparative pharmacokinetics of cisplatin loaded core cross-linked micelles in mice
Hardeep S. Oberoi, Frederic C. Laquer, Nataliya V. Nukolova, Jiangeng Huang, Yazen Alnouti and Tatiana K. Bronich

39. Collagen-Binding Synthetic Peptidoglycans: A New Therapeutic to Prevent Dermal Scarring
John Paderi, Kate Stuart, Alyssa Panitch, Lynetta Freeman

40. Imaging of Carriers for Therapeutics and Diagnostics on Nano-resolution Scale by Freeze-fracture TEM
Brigitte Papahadjopoulos-Sternberg

41. Enhanced Delivery of Antimicrobial Agents to Intracellular Pathogens by Core-Shell Polyion Complexes
N. Pothayee, T. Vadala, A. Ranjan, N. Jain, M. Seleem, N. Sriranganathan, R. M. Davis and J. S. Riffle

42. Facile Production of Composite Nanoparticles for Drug Delivery with Hydrophobic Actives, Polypeptides, and Proteins, Targeting, and Imaging
Suzanne D'Addio, Varun Kumar, and Prof. Robert K. Prud'homme

43. **Composite Nanoparticles for Photodynamic Cancer Therapy**
Stephanie Budijono, Robert Prud'homme , Jingning Shan and Yiguang Ju

44. **Nanomedicine and Drug Delivery: Efficacy and Toxicity of Rifampicin Loaded Nanoparticles Against *Mycobacterium avium* Infected Murine Macrophages and BALB/c Mice**
Eva Restis, Mohamed Seleem, Judy Riffle, Nikorn Pothayee, Joseph Falkinham III, and Nammalwar Sriranganathan

45. Core-Shell Magnetite-Polyether Nanostructures for MR Imaging
P. P. Huffstetler, M. R. J. Carroll, W. C. Miles, R. C. Woodward, T. G. St. Pierre, R. M. Davis and J. S. Riffle

46. Hydrogel Template Strategy for the Fabrication of Microstructures of Complex Geometries
Thomas Rish, Matthew McDermott, Crystal S. Shin, Keith Hansen, Kyle Amick, Ghanashyam Acharya, and Kinam Park

47. Nonionic block copolymer Pluronic P85 is a promising adjuvant for MUC4 plasmid DNA immunotherapy
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Mechanisms of sensitization of multidrug resistance by Pluronic

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One of the mechanisms of multidrug resistance (MDR) in cancer is overexpression of drug efflux pumps, such as P-glycoprotein, which drastically reduce intracellular concentration of drugs. However, MDR is also associated with significant changes in cellular metabolism. Thus, MDR cells predominantly utilize fatty acids as energy source, when non-MDR cells mainly oxidize glucose. Additionally, changes in cellular metabolism while developing MDR lead to impairment of mitochondria function in those cells: they have lower mitochondria membrane potential and higher proton leakage across inner mitochondria membrane, and overall perform less effective respiration. Therefore compromised mitochondria function in MDR cells can serve as a target for chemosensitization.

Pluronic block copolymer, consisting of hydrophobic polypropylene oxide block flanked on both ends with hydrophilic polyethylene oxide blocks, has shown a promise in clinical trials as a potent chemosensitizer of MDR. However, the mechanism of MDR sensitization by Pluronic remained unknown. Due to its structure Pluronic can incorporate into plasma membrane, alter membrane microviscosity and, probably, interacts with Pgp, what results in inhibition of Pgp ATPase activity. Pluronic also translocates into the cells and causes ATP depletion. Using resistant and sensitive breast carcinoma cells (MCF7/ADR and MCF7) we investigated the effects of Pluronic on their metabolism. We demonstrate that Pluronic inhibits total cellular metabolism, and, on the other hand, stimulates anaerobic metabolism in comparison to non-treated control. It was also shown that Pluronic compromises mitochondria function in MDR cells, what leads to ATP depletion, increase of reactive oxygen species production and release of cytochrome c. Notably, the Pluronic effects mentioned above were selective to MDR phenotype and were much more pronounced in drug resistant cells. Altogether these effects can eventually enhance cell death via apoptosis.

Development of leukocyte-mimetic nanoparticles for tumor targeted drug delivery

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We aim to develop nanocarriers that can extravasate efficiently at the tumor sites and achieve tumor-selective biodistribution of anti-cancer drugs. In order to obtain efficient and safe chemotherapy, drug delivery systems should selectively deliver toxic antineoplastic agents to tumor cells without harming normal cells. One of the popular approaches to achieve this goal is to conjugate a ligand recognized by tumor-cell specific receptors on the delivery systems ("active targeting"). However, the promise of this method has not yet translated to clinically viable products. One of the possible reasons may be that the endothelial barriers limit the access of the delivery systems to the cancer cells. Thus, it is hypothesized that selective accumulation of the anticancer medicine carried by nanoparticles can be enhanced by increasing the interaction of the nanocarrier with the endothelium surrounding tumors ('peri-tumoral endothelium'). To test this, we develop a new nanocarrier system that will establish an interaction with the tumor vasculature mimicking that of leukocytes and the inflamed endothelium (i.e., leukocyte-mimetic nanoparticles, LMNPs). It is anticipated that this interaction will guide circulating nanocarriers to the peri-tumoral endothelium and facilitate their extravasation and access to the underlying tumors, achieving tumor-selective drug delivery.

E-selectin was chosen as an endothelial marker of the peri-tumoral endothelium, because the endothelium harboring various metastatic tumors (e.g., breast, liver, lung, prostate, colon, pancreas, and gastric cancers) expresses a high level of E-selectins. A quinic acid derivative was chosen as a ligand to modify the surface of nanoparticles and guide the particles to the peri-tumoral endothelium. The quinic acid derivative is known to mimic the ability of tetrasaccharide sialyl Lewis-x (sLe^x) to interact with E-selectin but simpler to synthesize and more stable in the blood than sLe^x. The quinic acid derivative (QA-NH_2) was synthesized by esterification of quinic acid hydroxyl groups followed by conjugation to methyl-3,5-diaminobenzoate and deprotection with lithium hydroxide. The structure of QA-NH_2 was confirmed by NMR spectroscopy. LMNPs were created by conjugating the QA-NH_2 to the poly(lactic-co-glycolic acid) nanoparticles with polyethylene glycol termini via peptide bond. The LMNPs had an average size of 189 nm. The affinity of LMNPs to E-selectin will be tested by measuring its ability to interfere with interactions between HL-60 cells (expressing sLe^x) and E-selectin bound on a microwell surface. Once the LMNPs are found to have significant affinity to the E-selectin, their interactions with the endothelium will be evaluated with the human umbilical vein endothelial cell model activated with interleukin-1β under dynamic flow conditions.

WITHDRAWN

Complement Activating Properties of Liposome Encapsulated Hemoglobin

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Background: **Creation of a blood group-free universal, long-lasting and safe oxygen carrier blood substitute, i.e., an artificial blood, has been a generic research topic for a long time and will remain to be one in the future for many reasons, including real medicinal need as well as unmet intellectual challenge. Our project focuses on the development of a long-circulating, biocompatible blood substitute based on liposome-encapsulated hemoglobin (LEH). Long circulation is achieved by attaching polyethylene glycol (PEG).**

Goals: **In this part of the study we examined the immunogenic properties of LEH, particularly its complement activating potentials that could prevent its application in trauma, hemorrhagic shock or other conditions of severe blood loss.**

Methods: **In our swine model of complement activation related pseudoanaphylaxis (CARPA) we injected various doses of PEGylated and non-PEGylated forms of LEH while monitoring and recording physiological parameters (systemic arterial and pulmonary arterial blood pressure, ECG, oxygen saturation, expired CO2) and collecting blood samples for further analysis.**

Results: **The non-PEGylated LEH vesicles did not cause any immediate hypersensitivity reactions. The first injections of PEGylates LEH provoked severe anaphylactic reactions, but the hypersensitivity substantially diminished or completely disappeared upon subsequent administration of PEGylated LEH.**

Conclusions: **Non-PEGylated liposomes and LEH did not induce hypersensitivity reactions. LEH is nonreactogenic even in relatively high bolus dose. Upon first exposure to PEGylated liposomes potentially lethal physiologic reactions occur. PEGylation lends reactivity to these vesicles that can be prevented by tolarization with empty pegylated liposomes. If PEG is used in LEH, tolerization with placebo might be necessary for safe application as the PEGylated liposomes seems to have a tachyphilactic properties. This hypothesis needs to be investigated in details before proceeding to application of LEH as a blood substitute.**

Hemodynamic and Hematologic reactions in Pigs after Repeated Intravenous Administrations of Carbon Nanotubes

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Intravenous administration of nanomaterials can lead to a previously reported complement activation-related pseudoallergy. The manifestation of this reaction is evidenced by changes in pulmonary and systemic arterial blood pressure, changes in white blood cell and platelet counts, and elevation in circulating thromboxane B2 levels. In this work, we tested whether the repeated administration of carbon nanotubes (CNTs) would provoke such changes and examined how various types of functionalized carbon nanotubes influenced the occurrence of these symptoms. Pigs were injected intravenously by repeated administrations with pristine, oxidized, amino-functionalized, and both oxidized and amino-functionalized carbon nanotubes up to 3.41 mg/kg. Electrocardiogram and hemodynamic parameters were recorded continuously and blood samples were collected.

Results: Injection of pristine CNTs induced an immediate, transient, and dose-dependent increase in the pulmonary arterial pressure, while all types of functionalized carbon nanotubes did not lead to any such increases. Pristine CNTs also caused a decrease in the total white blood cell (WBC) count. Low doses of oxidized amino-functionalized nanotubes caused a decrease in WBC count, while dose escalation led to increased WBC counts. Between the types of functionalized CNTs injected, the oxidized CNTs (CNT-COOH) induced a prominent and statistically significant rise in WBC count, while the amino-functionalized CNTs (CNT-NH_3) did not. Reduction of the number of platelets was obtained for all CNTs, except in case of CNT-NH_3, which increased the thrombocyte levels. Histological examination indicated endothelium-associated depositions of pristine CNT clusters in the lung, liver and spleen.

Conclusion: Functionalization of CNT can markedly improve the adverse hemodynamic reactions that can be generated by injections of pristine CNT. The type of CNT functionalization also has been shown to affect the induction of such hematological changes, with oxidized CNTs leading to more severe changes. The mechanism of such observed changes in reaction to CNT administrations needs to be further explored, particularly with represent to the occurrence of complement activation related pseudoallergies.

DEVELOPMENT OF ANTIOXIDANT NANOZYMES FOR PARKINSON'S DISEASE THERAPY

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Introduction and Purpose: Oxidative stress is a process that accompanies a number of neurological disorders, such as neurodegeneration, brain trauma, cerebral ischemia and infectious brain diseases. As a potential therapeutics, antioxidants are used in a broad variety of studies and were proven to be neuroprotective in models of Parkinson's disease. Superoxide dismutase and catalase are the examples of enzymes that quench reactive oxygen species and completely degrade them thus preventing biochemical cascades leading to oxidation of cellular compounds and cell death. We propose the application of antioxidative formulations, developed in our laboratory, for the therapy of neuroinflammation-related pathologies. Proposed therapeutic properties are based on anti-inflammatory role of catalase and superoxide dismutase, the active compounds of the formulations.

Experimental Methods: To protect enzymatic activity, enzyme molecules were coupled with PEI-PEG, a synthetic polyelectrolyte of opposite charge, and cross-linked to obtain stable polyion complexes, the nanozymes. Stability of nanozymes and preservation of enzymatic activity was shown in physiological conditions. We employed a neuroimmunological approach for the nanozyme delivery in MPTP mouse model of Parkinson's Disease (PD). Considering leukocytes infiltration into the brain parenchyma during the course of PD, we developed a cell-based delivery system that involved bone marrow-derived macrophages (BMM) as carriers for the formulation particles. We propose that the cell-based delivery allows transport of formulations across the blood-brain barrier. Anti-inflammatory efficacy was assessed by means of immunochemical evaluation of glial activation and neuron-specific markers.

Results and Discussion: We demonstrated that BMM can rapidly accumulate nanoparticles and then spontaneously release them into the external media over the period of 4-5 days. Intravenous injection of BMM, loaded with nanozyme in vitro, significantly decreased expression of neuroinflammation markers and promoted neuronal survival in substantia nigra pars compacta in MPTP mouse model of PD. Profound leukocytes unfiltration into brain parenchyma was not observed.

Conclusions: We suggest that BMM facilitate effect of nanozyme in the model of Parkinson's diease. We hypothesize that BMM play the role of reservoirs and thus help maintaining therapeutic concentrations of the drug in the blood and provide prolonged antioxidative effect.

Effect of Probucol, AICAR, and TGF-*β*1 on Smooth Muscle Cell and Endothelial Cell Phenotype and Proliferation

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Cardiovascular drug-eluting stents (DESs) have been used to prevent restenosis occurring after the implantation of bare metal stents (BMSs). Recent studies, however, have indicated that while DESs reduced the restenosis problem, they can result in late in-stent thrombosis (LST) with a probability comparable to that of BMSs. LST can be life-threatening. LST can be best prevented by promoting endothelial cell (EC) growth in the stented region. One way of achieving this is to deliver a drug that can both facilitate EC growth and prevent smooth muscle cell (SMC) hyperplasia, preferably by differentiating SMCs.

With this need in mind, we recently developed an *in vitro* EC-SMC direct co-culture platform. In this platform, the beneficial effect of combined TGF-*β*1 and heparin treatment and of direct co-culture of ECs and SMCs on SMC differentiation was revealed. Further, it was shown that co-culturing ECs with SMCs pre-treated with TGF-*β*1 and heparin resulted in greater differentiation of SMCs when compared to SMCs treated with TGF-*β*1 alone. This evidence was provided through immunocytochemistry followed by confocal imaging. This was then further quantified using an In-Cell Western technique via the use of infrared probes tagged to the secondary antibodies and scanned using an Odyssey Infrared Imaging System (Licor).

In our current study, probucol in the order of 10^{-5} M and AICAR (5-aminoimidazole-4-carboxyamide ribonucleoside) in the order of 10^{-3} M were tested on separate monocultures of ECs and SMCs. The objective was to observe the effect of these drugs on three factors—first, EC and SMC proliferation; second, the expression of SMC differentiation markers, namely, smooth muscle actin and calponin; and third, the expression of EC adhesion molecules, namely intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). The objective was also to determine the optimal concentration of these drugs.

In our future work, we plan on testing the optimal concentrations of the above drugs (probucol and AICAR) in our developed EC-SMC co-culture model to determine the more physiologically relevant effects on EC and SMC phenotype and proliferation. Further, we plan to challenge our model by the use of inflammatory cytokines such as tumor necrosis factor-α and then study the effects of the above drugs.

"Synthesis of Magnetite Nanoclusters Using a Multi-Inlet Vortex Mixer"

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Functional nanoparticles that have well-defined size distributions and are colloidally stable in aqueous media are important for biomedical applications. Clusters of superparamagnetic particles are particularly interesting for applications as magnetic resonance imaging (MRI) contrast agents and in hyperthermia. Thus, development of a method to control the size of sterically stabilized nanoparticles is needed. We have used a multi-inlet vortex mixer (MIVM)[1] to create clusters of superparamagnetic particles of magnetite. In the MIVM, particle formation occurs by the rapid mixing of an organic active and a stabilizing amphiphilic polymer, dissolved in an organic solvent, with an antisolvent to create solutions with high supersaturation values. To produce nanoparticles of homogeneous particle size, the mixing time has to be lower than the nucleation and growth time scale.

The MIVM process was used to make clusters comprised of hydrophobically modified magnetite nanoparticles (diameter ~10 nm) dispersed in Tetrahydrofuran (THF) stabilized with an amphiphilic polymer, Pluronics F-127 (a triblock of Poly (ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide)). Clusters of these magnetite nanoparticles were formed with hydrodynamic diameters ranging from 60-230 nm in aqueous media. The control of the composition of these clusters and the response of the cluster colloidal stability to an external magnetic field will be discussed.

References:

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Construction of a Novel Bioresponsive Gene Delivery System Based on β-6-Amino-Cyclodextrins and Polyvinylalcohol-Adamantane-PEG Host:Guest Interactions

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＜Introduction＞

Several novel cationic gene carriers have been investigated based on self-assembly of cationic cyclodextrin (CD) derivatives with adamantine-modified (Ad) polyvinylalcohol (PVA). PVA, linked to Ad via a pH-sensitive acetal linkage, provided a scaffold for binding of cationic CD amines. DLS and AFM experiments indicate that small particles <200 nm were formed. Cell culture data suggests that these materials have substantially lower cytotoxicities than 25 kD PEI.

＜Results and Discussion＞

Initially, the PVA scaffold was studied for pH-dependent response. 1H NMR spectra showed that the PVA-Ad acetal linkage signal was lost after incubation at pH 3.6 for 1 hr, indicating that the acetal linkage was hydrolyzed under these conditions, leading to separation of the Ad groups from the PVA backbone. ^{1}H NMR data also confirmed that only the β-CD derivatives, but not α-CD and γ-CD, formed supramolecular complexes with modified PVA through host:guest interactions between β-CD and Ad.

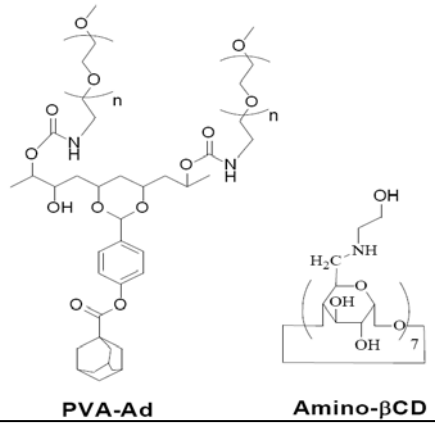

Figure 1. Structures of PVA-Ad and amino-β-CD.

Two different methods were used to evaluate the complexation efficiency of PVA-Ad:amino β-CD and pDNA. In Method A, PVA-Ad was pre-complexed with amino β-CD before addition of the pDNA. In Method B, the pDNA was first complexed with amino β-CD, followed by addition of PVA-Ad. QLS data showed that both methods produced excellent plasmid condensation abilities, however, Method B consistently produced the lowest diameters, reaching ~ 160 nm at N/P ratios ≥ N/P = 4. These complexes were directly observed by AFM as shown in the inset of Figure 2.

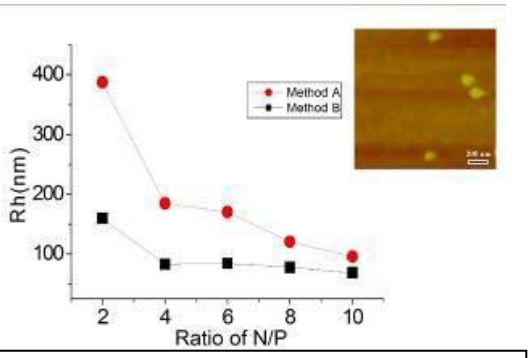

Figure 2. Particle sizes of complexes of PVA-Ad:amino-β-CD:pDNA at various N/P ratios using two different complexation methods. The inset shows an AFM image on mica of particles prepared by Method B at N/P=2.

The cytotoxicity of these materials were studied in HeLa cell culture. All of these compounds, including the free amino-β-CD, PVA-Ad and PVA-Ad: amino-β-CD complexes, displayed cytotoxicities below 20% dead cells (measured by LIVE/DEAD assay) at concentrations as high as 1 mM (e.g., LD_{50} values >1000 μg/mL), indicating that they are substantially less toxic than PEI (25K Da). In vitro gene transfection efficiency experiments with these materials are now in progress.

Blood Borne Macrophage Delivery of Nanoformulated Paclitaxel for Glioma

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Despite combined and clinically aggressive therapies that include radiation and chemotherapy, high-grade malignant gliomas presents a significant therapeutic challenge. The reasons are multifaceted and include the tumors diffuse infiltrative and growth behaviors and marked cytologic heterogeneity together with the known poor penetrance of therapeutic agents across the blood–brain barrier. As cells of mononuclear phagocyte (MP) lineage, principally histiocytes, are prominent around vessels, within the tumor and contained in tumor giant cells we reasoned that the same mechanisms that attract MP into the cancer can be harnessed to bring MP loaded with anti-tumor nanoparticles (NP) to disease sites. MP can readily cross the BBB and home to tumor sites following the establishment of a chemokine gradient as part of a glioma-induced neuroinflammatory response. To this end, we manufactured paclitaxel NP (PTX-NP) and loaded them into blood borne monocytes and monocyte-derived macrophages (MDM). Such PTX-NP MDM demonstrated robust chemosensitivity (p<0.01) after 3 days of co-cultivation with U87 cells compared to PTX-NP alone. Increased chemosensitivity was observed for PTX-NP than free-PTX when administered for 12 hours to U87 cells. Differences amongst the preparations were seen for inhibition of tumor growth after days 3 (p<0.05), and 5 (p<0.01), and 7 (p<0.01). PTX-NP was loaded into bone marrow derived macrophages (BMM-PTX-NP) and adoptive transferred or given intravenously as PTX-NP to severe combined immunodeficient mice. Tissue concentrations of PTX were determined by reverse phase high performance liquid chromatography. PTX-NP, BMM-BMM-NP significantly limited PTX levels in the liver at post treatment day 1, 3, 7, 10 and 15 (P<0.05). In contrast, PTX-NP showed lower concentration at post-treatment day 3, 7, 10 and 14 except a narrow peak present at day 1. Specific BMM-PTX-NP trafficking into the brain was readily demonstrated to the tumor site by immunocytochemical examination. The data support the potential utility of MP-based NP drug delivery systems for glioma.

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Engineered PLGA Nanoparticles as Therapeutic Delivery Vehicles

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Advances in nanotechnology have brought about innovative solutions to disease treatment and new methods for the fabrication of drug delivery vehicles. Traditional nanocarriers, such as liposomes and polymer conjugates, have provided the foundations for the field of advanced drug delivery; however, new carriers, based on polymeric nanoparticles that combine the best features of each of these traditional delivery vectors, yet offer new flexibility to overcome some of the current delivery barriers in the field are gaining momentum. Of particular interest is poly(lactic acid-co-glycolic acid) (PLGA) because it is biocompatible, bioabsorbable, has already shown promise in medical applications and can easily be tailored to vary release and degradation profiles. Various methods for fabricating PLGA particles, including emulsions, spray drying, and flow focusing, are currently under investigation by a number of research groups. A substantial volume of literature has been dedicated to investigating the effects of process parameters on particle properties and the attempts to develop trends to guide particle design. The PRINT® (Particle Replication In Non-wetting Templates) process, a soft lithography platform, simplifies fabrication and particle design with the unique ability to control size and shape independent of process variables. In addition, the PRINT process creates truly monodisperse particles, is not limited to spheres, and allows for easy encapsulation of a wide range of cargos including hydrophilic or hydrophobic therapeutics, biologicals, proteins, oligonucleotides, siRNA, and imaging agents. Soft lithography in general offers a great alternative for the fabrication of highly engineered particles, but has not been used in the fabrication of PLGA nanoparticles. To demonstrate the efficiency of PLGA PRINT nanoparticles as drug delivery agents, we have applied them to the delivery of a sensitive biological cargo, siRNA, and a chemotherapeutic agent, Docetaxel. siRNA exploits an inherent pathway of gene knockdown and is recognized as a therapeutic treatment options for a variety of diseases including cancer, however siRNA is easily degraded *in vivo* and does not readily cross the cell membrane, The development of effective therapies will require the development of efficient and protective delivery systems. Docetaxel, on the other hand, has dose limiting toxic side effects when administered systemically and would benefit from a carrier system in order to reduce systematic exposure and maintain therapeutic drug concentrations at the site of disease.

We have shown delivery of biologically active siRNA and Docetaxel *in vitro* with 200nm x 200nm cylinders and 80nm x 360nm high aspect ratio cylinders. Encapsulation of cargo is straightforward and applies to all cargos regardless of solubility or sensitivity; the cargo is simply mixed into a one phase precursor solution which will be used to fabricate particles. High cell uptake is achieved through the incorporation of PEI, giving the particles a positive charge. siRNA containing particles exhibit no toxicity *in vitro* and dose dependent knockdown in an *in vitro* luciferase model has been shown. Docetaxel containing particles exhibit similar IC_{50} values to free docetaxel while the base particle alone shows no toxicity. Current objectives are the investigation of *in vivo* biodistribution and delivery in both these systems.

Single nanoparticle detection by DNA barcoding for biodistribution studies in Nanomedicine

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In nanomedicine, biodistribution studies are absolutely critical to evaluate the safety and efficacy of any given nanoparticle formulation. Detection of small numbers of nanoparticles in whole tissues using standard imaging techniques, however, is feasible only when they are agglomerated and subsequently able to generate a sufficient detection signal. Moreover, standard imaging techniques do not allow the direct association of nanoparticle uptake with a specific cell type. Single-cell imaging techniques, such as electron microscopy (EM) and atomic force microscopy (AFM), are able to detect small numbers of (or even single) nanoparticles in individual cells, but these techniques only scan over very small areas and are not practical for extensive biodistribution studies. We have developed a novel method for single nanoparticle detection that incorporates a non-endogenous oligonucleotide on the nanoparticle surface for use as a unique "barcode." After these functionalized nanoparticles are internalized by cells, the barcode can then be amplified by *in situ PCR* inside the cells of a fixed tissue section and the amplicons detected by fluorescence or colorimetric systems at the optical level. The DNA barcoding method utilizes the limitations of current imaging methods to its advantage by amplifying the detection signal from single nanoparticles and permitting evaluation of nanoparticle uptake by cell types and their distribution over larger areas of tissue. Preliminary data demonstrate proof-of-principle of our DNA barcoding method using cell-free systems. Work in progress will include detection of single nanoparticles in *in vitro*, *ex vivo*, and *in vivo* cell systems via *in situ* PCR. The DNA barcoding method has the potential to enable the precise, single cell analysis of long-term nanoparticle biodistribution, an important problem in nanomedicine, and to accelerate translation to the clinic.

Polymer-Based Delivery of siRNA for Cancer Treatment

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The need for robust and effective synthetic delivery vehicles for siRNA continues to present a major hurdle to the clinical realization of RNAi therapies. Polycations have become popular subjects of study to researchers who aim to incorporate the powerful delivery, uptake, and release properties of viruses to their synthetic counterparts, minus the harmful immunogenic effects. We have performed extensive in-vitro characterization and testing of three (poly(2-(dimethylamino)ethyl methacrylate)-based delivery systems to determine the extent, if any, to which the size/architecture and/or degree of "PEGylation" of a polycation carrier has on its ability to deliver siRNA and facilitate gene silencing. In particular, our work introduces a novel chemistry for triblock copolymer "micelleplexes", which differ from the conventional single polymer chain-based vehicles commonly used in siRNA delivery. Results suggest that our systems are reasonably non-cytotoxic, stable against enzymatic degradation and mediate up to 85% gene knockdown at the in vitro level. Encouraging preliminary in-vivo biodistribution studies with fluorescently-labeled PEG-PDMAEMA polyplexes and PEG-PnBA-PDMAEMA micelleplexes in athymic mice possessing solid tumors indicate that both complexes do accumulate and get retained in tumor tissue. This is a promising result which suggests that our carriers are good candidates for exploiting the Enhanced Permeability and Retention (EPR) effect, responsible for the nonspecific uptake of favorably-sized nanoparticles in tumors. With some further testing and optimization, we believe our systems will prove to be viable options for in vivo tumor-targeted therapies.

SELF-ASSEMBLING SYSTEM FOR DELIVERY OF ORGANOPHOSPHATE PROTECTANTS

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Providing improved countermeasures to protect military personnel and the civilian population from biological warfare agents is an extremely high priority need. For example, there is currently no effective treatment beyond the difficult to control and implement administration of antidotes to protect central nervous system (CNS) against organophosphate (OP) poisoning or warfare agents. Although new prophylactic and therapeutic enzymatic organophosphate protectants are under accelerated development, none currently provide significant protection of central nervous system due to their rejection by the blood brain barrier. Clearly there is an urgent need for parallel development of new drug delivery technologies with a possibility to deliver desired protectants to the brain. Polyionic complexation was used as a platform for the development of polymer carriers for the delivery of detoxifying enzyme - butyrylcholinesterase (BChE). The nanofabrication of such carriers was achieved by interaction of BChE with ionic copolymer, poly(ethylene oxide)-*graft*-polylysine, and spontaneous formation of stable aqueous dispersion of polymer complexes of nanoscale size. The multimolecular structure of BChE/copolymer complexes was further reinforced by formation of cross-links between the polymer chains. The resulting cross-linked complexes were stable against dilution without significant loss of enzymatic activity. *In vivo* "proof of concept" studies using an animal model evaluated an effectiveness of the delivery of nano-encapsulated protein into the brain. The preliminary findings are promising and will guide further design and optimization of drug delivery systems targeting chemical and biological warfare agents.

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Interfacial Activity Assisted Surface Functionalization – A Novel Approach to Introduce Functional Groups on Polymeric Nanoparticles

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Introduction: Targeted drug delivery using nanocarriers is achieved by functionalizing the nanocarrier surface with a tissue-recognition ligand. Conventional surface modification methods require specific functional groups on nanocarrier surface, which may not always be available. For example, a number of newly reported tumor-targeting peptides require a maleimide group, which is not commonly available on polymeric nanoparticles. Lack of such groups would require sequential synthetic steps, which are labor-intensive and inefficient, and can result in significant loss of the payload. In this report, we describe a novel interfacial activity assisted surface functionalization (IAASF) technique for introducing functional groups previously not-available on polymeric nanoparticles.

Methods: We evaluated the use of IAASF technique to conjugate tumor targeting peptides, cRGD, LyP-1, and CREKA (the last two provided by Prof. Erkki Ruoslahti, UCSB) on the surface of poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles. Polylactide-polyethylene glycol block copolymer with terminal maleimide group (PLA-PEG-maleimide) was synthesized as described previously. PLGA nanoparticles containing 6-coumarin (green fluorescent label) and/or paclitaxel were prepared by emulsion solvent evaporation technique, with modifications. An oil-in-water emulsion was formed by emulsifying a solution of PLGA and drug in chloroform in aqueous polyvinyl alcohol solution by using a probe sonicator. PLA-PEG-maleimide copolymer dissolved in chloroform was added into the polymer emulsion. This resulted in the partitioning of polylactide block into the polymer containing chloroform phase and PEG block into the aqueous phase. Removal of chloroform resulted in the formation of nanoparticles with PEG-maleimide on the surface. Peptide conjugated nanoparticles were prepared by reacting maleimide functionalized nanoparticles with the respective peptide. The effectiveness of the peptide conjugation was determined by comparing the cellular accumulation of peptide-conjugated and unconjugated nanoparticles in different tumor cell lines. Cytotoxicity studies were carried out to establish the anticancer efficacy of targeted nanoparticles loaded with paclitaxel. Finally, *in vivo* tumor accumulation studies were conducted in BALB/c mice to confirm the tumor-targeting potential of peptide-functionalized nanoparticles.

Results: The cRGD and CREKA peptides target endothelial cells and some tumor cells, while LyP-1 targets tumor cells. Nanoparticles conjugated to the cRGD peptide showed up to three-fold higher uptake in NCI-ADR, 4T1, and HUVEC cells compared to non-functionalized nanoparticles. Similarly, nanoparticles conjugated to LyP-1 showed higher uptake in MCF-7, MDA-MB-231 and JC cells compared to CREKA-functionalized and non-functionalized nanoparticles. As expected, CREKA conjugated nanoparticles showed higher uptake in the HUVEC cell line compared to LyP-1 and non-functionalized nanoparticles. Cytotoxicity studies showed that paclitaxel-loaded cRGD functionalized nanoparticles were more effective than non-functionalized nanoparticles in inducing cell death. *In vivo* tumor accumulation studies demonstrated that cRGD functionalized nanoparticles accumulated to a significantly greater extent in tumor than non-functionalized nanoparticles (two-fold higher after 6 hrs and 5-fold higher after 24 hrs; $p<0.05$).

Conclusions: cRGD, LyP-1 and CREKA peptides were successfully conjugated to PLGA nanoparticles using the IAASF technique. Nanoparticles conjugated with such tumor-targeting peptides appear to have significant potential in improving the therapeutic efficacy of anticancer drugs.

Peritumorally activatable nanoparticles for drug delivery to multidrug resistant tumors

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Nanoparticles have been explored as potential drug delivery vehicles for the targeted therapy of tumors. However, killing multidrug resistant (MDR) tumors remains a challenge. The purpose of this research is to create nanoparticles that are protected by a stealth coating during systemic circulation but activated in the tumor environment to lose the coating. Thus, the peritumorally activatable nanoparticles are expected to have minimal interaction with normal tissues during circulation, and the 'activated' nanoparticles can enter the tumor cells and eradicate them. The uptake of nanoparticles into the tumor cells is especially important in dealing with MDR tumors, which can expel free drug from the cell before it can reach its target. The tumor-specific activation of the nanoparticles will be induced by matrix metalloprotinases (MMPs) overexpressed in the tumors. The internalized nanoparticles are expected to create a drug reservoir in the cell that can overwhelm the MDR pumps on the cellular membrane.

A PLGA-TAT polymer was created according to a method in the literature (Nam et al., *Biotechnology Letters*, 24, 2093). Nanoparticles were made using the single emulsion method, and their size and zeta potential were measured by a Zetasizer. The nanoparticles were incubated in two ovarian cancer cell lines: SKOV-3 (MMP+) and OVCAR-3 (MMP-); their uptake was observed on a confocal microscope. Cleavage of MMP-sensitive peptides in the presence of MMP-9 and MMP-2 was tested via RP-HPLC.
The average particle size for PLGA-TAT nanoparticles was 197.4 ± 1.71 nm. Their positive zeta potential (1.59 ± 0.15 mV at pH 7.4), contrasted with that of plain PLGA nanoparticles (-7.85 ± 0.27 mV at pH 7.4), indicates the presence of TAT peptide on the PLGA-TAT nanoparticles. After incubation with the cancer cells for 3 hours, the PLGA-TAT nanoparticles were clearly visible inside the cells, while the plain nanoparticles were not visible even after 4 hours of incubation. It is thought that the positive charge of the TAT helped the uptake of nanoparticles by the cells. MMP-sensitive peptides were identified and have a wide range of sensitivity.

In summary, PLGA-TAT nanoparticles were shown to increase cellular uptake, and MMP-sensitive peptides were identified. When further modified with an MMP-sensitive peptide and poly(ethylene glycol) (PEG), the PLGA-TAT nanoparticles are expected to remain inert in normal tissues and be activated at the tumor site in the presence of MMPs revealing the TAT peptide, delivering anticancer drugs specifically and effectively to MDR tumors.

Levofloxacin encapsulated PLGA nanosuspension for sustained ocular drug delivery

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Abstract

Poor ocular bioavailability of drugs (< 1%) from conventional eye drops (i.e. solution, suspension and ointments) is due mainly to the precorneal loss factor that include rapid tear turnover, non productive absorption, transient residence time in cul-de-sac and the relative impermeability of the drugs to corneal epithelial membrane. In general, ocular efficacy is closely related to ocular drug bioavailability, which may be enhanced by increasing corneal drug penetration and prolonging precorneal drug residence time. A variety of ocular drug delivery system, such as hydrogels, microparticles, nanoparticles, microemulsion, liposomes and collagen shields have been designed and investigated for improved ocular bioavailability. Amongst them, polymeric nanoparticle is one of the most interesting approach to achieve local sustained delivery system. Levofloxacin is a newer generation hydrophobic floroquinolone. Poor water soluble drugs are difficult to develop as conventional ocular drug delivery system. Nanotechnology can be used to formulate poorly water soluble drugs as nanosuspension and offers the opportunity to address many of the deficiencies associated with such class of drug.

In our present work we develop and evaluate new colloidal system i.e. PLGA nanoparticle for levofloxacin ophthalmic delivery to improve precorneal residence time and ocular penetration. Nanoparticles were prepared by nanoprecipitation technique and characterized for various properties like particle size, zeta potential, in vitro drug release, stability etc. Microbiological assay was carried out against *Pseudomonas aeruginosa* using cup-plate method. Precorneal residence time was studied on albino rabbits by gamma scintigraphy after radiolabelling of levofloxacin by Tc-99m. Ocular tolerance of developed nanosuspension was also studied by HET-CAM method.

The developed nanosuspension showed a mean particle size in the range of 190-230 nm, suitable for ophthalmic application with zeta potential of -22mV. In vitro release from developed nanosuspension showed an extended release profile of levofloxacin. The observation of acquired gamma camera images showed good retention over the entire precorneal area for developed nanosuspension as compared to marketed formulation. Marketed drug formulation cleared very rapidly from the corneal region and reached to systemic circulation through nasolachrymal drainage system, as significant radioactivity was recorded in kidney and bladder after 2h of ocular administration, whereas developed nanosuspension cleared at a very slow rate ($p<0.05$) and remained at corneal surface for longer duration as no radioactivity was observed in systemic circulation. HET-CAM assay further proves the non irritant property of developed nanosuspension. The developed lyophilized nanosuspension found to be stable for longer duration of time imparting good shelf life to the product. In conclusion, the developed PLGA-levofloxacin nanosuspension formulation gives appropriate particle size, extended release with better tolerability and prolonged retention at corneal site. It is suitable for sustained ocular drug delivery and can go up to the clinical evaluation and application.

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Swelling in Glucose-Sensitive HEMA-DMAEMA Hydrogels

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Over the last several decades attempts have been made to employ the swelling behavior of hydrogels in the detection and control of elevated blood glucose levels. Although a multitude of 2-hydroxyethyl methacrylate (HEMA)-based glucose-sensitive hydrogels have been synthesized in the academic arena, such devices have yet to be introduced for clinical use [1, 2]. Until swelling behavior can be accurately predicted from hydrogel properties, implementation of hydrogel sensory devices will require optimization of swelling behavior at every level of hydrogel design. The aims of this research sought to optimize the equilibrium swelling ratio for hydrogels composed of HEMA and 2-(dimethylamino)ethyl methacrylate (DMAEMA) crosslinked by ethylene glycol dimethacrylate (EGDMA) via manipulation of hydrogel properties such as monomer molar ratio and crosslinking density, as well as experimental properties such as the acidity of the hydrogel swelling agent. As the equilibrium swelling ratio limits the extent to which a hydrogel can respond to stimuli, optimizing this property assumed highest priority in developing HEMA-based glucose-sensitive hydrogels for clinical application.

HEMA and DMAEMA monomers as well as EGDMA crosslinking agent were purified by vacuum distillation. Hydrogels were synthesized at the following HEMA:DMAEMA monomer molar ratios: 70:30, 50:50, 30:70. An approximate total monomer concentration of 0.98 M was obtained for each of the monomer molar ratios. Additionally, hydrogel crosslinking density varied as 0, 1, or 2 mole % EGDMA (with respect to the hydrogel total monomer concentration) was incorporated into the hydrogels. Hydrogels were prepared by in situ free radical polymerization in 35×10 mm polystyrene petri dish. Ammonium persulfate (APS) and sodium metabisulfite (NaMBS) were used as a redox initiator system. Hydrogel swelling was examined by varying pH and gluconic acid concentration. Physically crosslinked hydrogels (0 mole % EGDMA) did not possess a good mechanical property, but still showed significant swelling property depending on pH. Dimension and swelling ratio of chemically crosslinked hydrogels depend on monomer feed ratio, crosslinking density, and pH as well. Future work aims to reduce the time for selected hydrogels to swell to equilibrium, as well as characterizing swelling behavior in the presence of varying concentrations of gluconic acid, a byproduct of the oxidation of glucose.

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Efficacy of gentamicin encapsulated pluronic based polymeric nanoplexes against chronic *Brucella melitensis* infection in mice

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Brucellosis caused by a bioterrorism agent *Brucella* species remains one of the most common zoonotic diseases worldwide with more than 500,000 cases annually and billions of dollars of economic burden associated with treatment, hospitalization costs, and loss of productivity. Antibiotics with polar nature, like gentamicin, although are very effective against *Brucella* in vitro, they failed to cross the cell membrane barriers and reach the replicative niche of the pathogen. We investigated the capability of delivering gentamicin by conjugating with the pluronic based polymeric nano sized particles (P85PAA). Pluronics are FDA approved polaxamer family of polyether. Pluronic based core- shell nanostructures are considered as foreign by the body and rapidly taken up by phagocytic cells which are sheltering the bacteria. Efficiency of these polymeric-drug conjugates to deliver drugs has been tested in *Brucella melitensis* infected J774A.1 macrophage cells and chronically infected BALB/c mice. The P85PAA-gentamicin nanoparticles achieved a statistically significant 1.1-log and 0.64-log reduction in the spleens and the livers of infected mice, respectively; while for the free drug there was no reduction. This new approach should improve the capability for targeting intracellular pathogens.

Dual-Modality Nanoparticles for cancer diagnosis using Magnetic Resonance and Near Infrared Fluorescence Imaging

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In the past three decades, there has been an extreme growth in imaging technologies. In modern medicine, *in vivo* imaging technologies have become a necessary method for cancer diagnosis. Various image modalities such as position emission tomography (PET), computed tomography (CT), fluorescence tomography (FMT) and magnetic resonance imaging (MRI) are currently being used and tried for cancer diagnosis. In current cancer research, a common purpose is to get accurate diagnostic images of cancer. However, each imaging technology has advantages and limitations in terms of spatial resolution, penetration depth and sensitivity. Therefore, multi-modal approaches can be a promising way to overcome the limitations by using advantages of each technology at the same time. In this study, we report dual-modality nanoparticles for both magnetic resonance and near infrared fluorescent imaging. The dual-modality nanoparticles consist of hydrophobically modified glycol chitosan (HGC) nanoparticles and superparamagnetic iron oxide (SPIO) nanoparticles. HGC nanoparticles were labeled with near infrared fluorescent dye, Cy5.5. HGC nanoparticles were prepared by a partial modification of glycol chitosan (GC) with 5β-cholanic acid, which represented micelle shapes in distilled water. SPIO nanoparticles capped by oleic acid were made by using the thermal decomposition method on 320°C. The size of SPIO nanoparticles showed a mean diameter of 4.20 nm, with a size distribution of ±1.04 nm. SPIO nanoparticles were loaded into HGC nanoparticles using a probe-type sonicator, which were physically connected each other by hydrophobic interactions between oleic acid and 5β-cholanic acid. The mean hydrodynamic diameter of SPIO-HGC-Cy5.5 nanoparticles was 350 nm. SPIO-HGC-Cy5.5 nanoparticles were evaluated by 3 Tesla MRI and eXplore Optix System respectively. *In vivo* and *ex vivo* results from optical imaging systems showed the primary accumulation of the nanoparticles at tumor sites. Moreover, results from MRI suggested their use as a MRI contrast agent. Overall, SPIO-HGC-Cy5.5 nanoparticles may be a promising dual-modality probe for cancer diagnosis.

Surface Functionalized Nanogels with Cross-Linked Ionic Core for Specific Tumor Targeting

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The novel surface functionalized nanogels with cross-linked ionic cores were developed as a platform for the targeting and detection of tumors. First, poly(ethylene oxide)-*b*-polymetacrylic acid (PEO-*b*-PMA) block copolymers with terminal aldehyde groups were synthesized by atom transfer radical polymerization (ATRP) and characterized using a combination of physicochemical methods such as GPC and ^{1}H NMR. Second, polymeric nanogels was prepared by template-assisted method involving condensation of PEO-*b*-PMA by Ca^{2+} ions, followed by chemical cross-linking of the polyion chains in the cores. The resulting nanogels containing aldehyde functional groups on the surface had the spherical morphology and exhibited pH-dependent swelling behavior. Finally, the tumor-associated glycoprotein 72 (TAG-72) monoclonal antibody, CC49, was successfully conjugated to nanogels. Surface plasmon resonance (SPR) analysis showed high specific binding affinity of CC49-conjugated nanogels to their epitopes. The functionalized nanogels can be a useful nanocarrier for targeted cancer diagnostics and treatment.

Polyelectrolyte-Biosurfactant based Nanocarriers for Drug Delivery

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Polymer-surfactant complexes have found numerous applications in engineering, cosmetics, detergent formulation, bioseparation, and other areas. Unfortunately, the tendency of these systems to phase separate from aqueous milieu has hindered the use of these complexes in nanomedicine. This limitation can be overcome by using double hydrophilic copolymers containing a polyelectrolyte chains and a water nonionic chain (block ionomers). We report a novel family of block ionomer complexes (BIC) formed by cationic copolymer poly(ethylene oxide)-*graft*-poly(ethyleneimine) and biosurfactants such as bile salts and oleic acid. These complexes spontaneously self-assemble in aqueous solutions into particles with average size of 50 - 200 nm and remained soluble over the entire range of the compositions of the mixtures including stochiometric electroneutral complexes. Solution behavior of the resulting BIC was characterized using fluorescence spectroscopy and dynamic light scattering. The physico-chemical properties of these systems such as critical association concentration, particle size and ζ-potential strongly affected by the structure of biosurfactants. A weakly basic drug, doxorubicin, was successfully immobilized into the cores of the biosurfactant-based BIC. Such tertiary complexes can be further used for additional solubilization of hydrophobic drug such as paclitaxel. The utility of biosurfactant-based BIC as carriers for combination drug delivery will be discussed.

Thermo- and pH-sensitive Hydrotropic Polymer Micelles for Oral Paclitaxel Delivery

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Polymer micelles have been intensively studied to solubilize and delivery poorly soluble drugs. Amphiphilic block copolymers are spontaneously assembled into nano-structured polymer micelles in water that have capability to incorporate hydrophobic drugs, biocompatibility, and prolonged circulation time when intravenously injected. Meanwhile, effort has been made to oral dosage formulation of poorly soluble drugs using polymer micelles. To enhance the drug bioavailability, polymer micelles have couples of problems, which include low capacity to load drug and instability of micelle structure under biological fluids. In this study, we introduced thermo-sensitive moiety to improve the micelle stability after oral administration and pH-sensitive moiety to prevent gastric digestion of paclitaxel. To improve the drug loading capacity of polymer micelle, a hydrotropic polymer was employed [1].

The hydrotropic monomer derived from nicotinamide structure, 4-(2-vinylbenzyloxy)-*N,N*-diethylnicotinamide (VBODENA) was prepared by the method described elsewhere [1]. *N*-isopropylacrylamide (NiPAAm) and acrylic acid (AAc) were used as thermo- and pH-sensitive units, respectively. Amphiphilic block copolymers were obtained by atom transfer radical polymerization (ATRP) by varying NiPAAm-to-AAc ratio. Structure and molecular weight of polymers were analyzed by ^{1}H-NMR and GPC. Thermo-sensitivity was observed by turbidimetry using UV spectrometer. The pH-sensitivity was determined by the change of particle size in buffers with various pHs. Paclitaxel was loaded in the polymer micelles by nanoprecipitation method. Drug loading efficiency and content was analyzed by reverse phase HPLC employing binary gradient method (co-solvent of water and methanol). Drug release study was conducted under simulated gastric fluid and simulated intestine fluid. As a result, hydrotropic moieties enhanced the paclitaxel loading efficiency and content compared with a conventional polymer micelle. Also, thermo- and pH-sensitive units were effect to prevent paclitaxel hydrolysis under simulated gastric or intestine fluids. The results suggests that simply formulation of hydrotropic polymer micelle can be a good candidate for oral paclitaxel delivery.

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Development of DPCPX-eluting stents

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DPCPX (1,3 dipropyl-8-cyclopentyl xanthine) is a potent and selective A1 adenosine receptor antagonist. *In vivo* studies showed that coronary artery smooth muscle cell (CASMC) expresses A1 adenosine receptor that playing an important role in cell proliferation.[(1,2)] DPCPX eluted from DES should effectively inhibit mitogenesis and avoid the thrombosis. PMBU and PLGA are biocompatible polymers selected for this study.

To study the release kinetic of DPCPX in polymer coating, two different concentrations of DPCPX were formulated with polymers and appropriate solvents, and coated on the bare metal stents by electro-spray techniques. The *in vitro* release profile in 0.01M phosphate buffer solution with 0.05%Tween20, at 37 °C, was monitored for 28 days and the concentration of DPCPX was determined by HPLC.

DPCPX showed zero-order release kinetic in both polymers. Less than 5% DPCPX was released within first 5 days. 100-200 ug of DPCPX, or 30-40% in a stent, was released slowly within 4 weeks and DPCPX still continued releasing from a stent after 28 days. The release rate from 50%DPCPX matrix was lower than that from 30%DPCPX formulation. When the thickness of coating layer was doubled, delayed release was observed.

In conclusion, DPCPX-eluting stents, with 30% and 50% drug in PMBU and PLGA, can sustain the drug release for more than 4 weeks with zero-order kinetic.

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Surface-Modified Gold Nanorods for Applications in Nanomedicine

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Abstract: Gold nanorods (GNRs) are useful as optical contrast agents for *in vitro* and *in vivo* biomedical imaging, and also have exciting potential as photothermal therapeutic adjuvants. These are derived from their high extinction coefficients and narrow absorption peakwidths in the near-infrared (NIR) region between 800-1100 nm, where biological tissues exhibit highest permittivity of light. GNRs provide excellent contrast for NIR imaging modalities, such as two-photon excited luminescence (TPL) and optical coherence tomography (OCT). In addition, GNRs efficiently convert absorbed optical energy into heat, generating localized photothermal gradients with application to hyperthermic treatments. GNRs can be conjugated with targeting ligands for the efficient delivery and photothermal treatment of cancer cells, and are thus promising agents for antitumor therapies.

With respect to clinical applications, surface-modified GNRs must first undergo preclinical evaluation for absorption, distribution, metabolism, excretion, and toxicity (ADMET). We address the following issues: (1) colloid stability in fluids of high ionic strength, (2) resistance to nonspecific interactions and uptake, (3) targeted delivery to the diseased tissues, and (4) cytotoxicity. To address the issues of toxicity and nonspecific uptake associated with cetyltrimethylammonium bromide (CTAB), a cationic surfactant used in the GNR synthesis, we developed a procedure using polystyrenesulfonate (PSS) as a detergent. Evidence suggests that CTAB forms a stable complex with the anionic polyelectrolyte PSS, which can be replaced by other nontoxic surfactants. The IC_{50} value for GNRs was increased from 12 μg/ml for CTAB-laden GNRs to above 85 μg/ml (the highest concentration tested) for "CTAB-free" GNRs.

Polyethylene-b-Polycaprolactone (PEO-PCL) Vesicles for Therapeutic Applications

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Polymersomes (polymer vesicles) have been shown to possess a number of attractive biomaterial properties compared to liposomes (phospholipid vesicles), including prolonged circulation times, increased mechanical stability, as well as the unique ability to incorporate numerous large hydrophobic molecules within their thick lamellar membranes and hydrophilic molecules within their lumen. We have shown the ability to generate two types of self-assembled nanovesicles ranging in size from hundreds of nanometers to tens of microns. One type is comprised of a biocompatible diblock copolymer polyethyleneoxide (PEO)/polybutadine (PBD) and a second fully-bioresorbable vesicle consisting of two FDA-approved building blocks, polyethyleneoxide (PEO) and polycaprolactone (PCL). In addition, we have successfully loaded imaging agents, such as porphyrin-based near infrared (NIR) fluorophores (for vesicle tracking, biodistribution studies, and potentially diagnostic studies), and therapeutics such as doxorubicin and combretastatin A-4 into these polymersomes and tracked their release.

Tumors require a network of blood vessels to survive and grow; these blood vessels are required to provide oxygen and nutrients to the tumor cells and remove carbon dioxide and waste. However, these tumor blood vessels are immature and poorly developed. As a result, the combination of chemotherapeutics with anti-angiogenesis drugs/vascular disrupting agents (VDA) has emerged as a promising therapy for eradicating tumors. These agents target genetically stable endothelial cells that constitute the blood vessels around tumors, rather than the transformed tumor cells themselves. Combretastatin A-4, a hydrophobic cytotoxic agent, inhibits the polymerization of tubulin and is highly toxic to tumor vasculature, but is known to not affect healthy vasculature. Hence, in addition to delivering drug to tumorogenic cells, the ability to deliver therapeutics to the endothelial cells lining the newly formed vasculature would be advantageous in cancer therapy.

Here, we determined the cytotoxic potential of combretastatin A-4 loaded polymeric vesicles on human umbilical vein endothelial cells (HUVECs) and SKBR3 tumorgenic cells. For both cell lines, toxicity was both concentration and time dependent. For HUVECs, a 50% reduction in viability is seen within 12 hours at high concentrations of combretastatin A-4; at longer times, cellular viability is decreased to approximately 25% viable even at low concentrations of combretastatin A-4. For SKBR3 cells, cell growth for drug treated cells was arrested, and at extended times cells appeared to be dying. Similar results were observed for HUVECs and SKBR-3 cells treated with doxorubicin loaded vesicles.

Furthermore, we utilized NIR-emissive polymersomes formulated from PEO-PCL diblock copolymer and loaded with porphryin (a NIR emissive fluorophore) to determine uptake of polymersomes in HUVECs. The vesicle uptake by HUVECs was dependent on both concentration and incubation time. As vesicle concentration in the media increased, cellular uptake also increased. Furthermore, increased incubation time generally resulted in increased uptake by the HUVECs. At higher cell densities, uptake was seen as early as 45 minutes and increased with extended incubation times. At lower vesicle concentrations, extended time was necessary for significant vesicle uptake. In addition to determining vesicle uptake, toxicity studies were carried out. A viability assay using Cell Titer Blue demonstrated biocompatibility of the nanoparticles without drug or imaging agent with the HUVECS. Thus, this study highlights the feasibility of using polymersomes to deliver vascular disrupting agents to endothelial cells simultaneously with treating tumors directly.

SERS Quantification of Kinase Activity Using a Nanoparticle Based Peptide Biosensor

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Phosphorylation by protein kinases is one of the major mechanisms of communication within a cell. Phosphorylation controls many critical signals within a cell, such as proliferation, cell cycle progression, and apoptosis. Dysfunctional phosphorylation signaling can result in the imbalance between cell proliferation and death, resulting in over proliferative diseases such as cancer or degenerative diseases such as Alzheimer's. Kinases are attractive drug targets for cancer therapeutics because of their critical role in cell fate determination. The paradigm for kinase inhibitor drug discovery has been the drug Gleevec (imatinib mesylate or STI571), an inhibitor of the oncogenic fusion kinase Bcr-Abl, a constitutively active tyrosine kinase expressed in chronic myeloid leukaemia (CML). New therapeutics are always in need due to the emergence of drug resistance and toxic side effects; therefore, new screening assays and technologies that offer high sensitivity and high-throughput analysis of kinase activity are essential. Although nanomedicine is most often associated with drug delivery there are significant opportunities for the development of high sensitivity and high-throughput nanomedical diagnostics to monitor these processes.

Kinase activity is currently detected through the use of antibodies, mass spectroscopy, radiolabeling, and labeled proteins. These methods are limited by dynamic range, throughput, and difficulties in monitoring activity in vivo. Using the Bcr-Abl model we have developed a nanoparticle based Abl specific peptide biosensor to detect relative amounts of phosphorylation using Surface Enhanced Raman Spectroscopy (SERS). SERS is a single molecule sensitive, high-throughput vibrational spectroscopy method that is able to detect structural changes in biomolecules. By functionalizing silver nanoparticles with an Abl specific peptide substrate we are able to detected tyrosine phosphorylation of the substrate in a label free method. The addition of a phosphate to the tyrosine in the substrate results in a change of the tyrosine doublet. These spectral changes allow for relative quantification of phosphorylation to be calculated. Performing MALDI-TOF mass spectrometry analysis of the functionalized nanoparticles allows for validation of this technique. This assay could be adapted to any kinase/substrate pairing and could be used in a high-throughput drug screening assay.

Characterization of Nanobioconjugates to Treat Brain and Breast Tumors

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Introduction. Polymer-conjugate drug (Polycefin) based on polymalic acid (PMLA) nanoplatform has been used for I.V. delivery of anti-tumor agents to treat brain and breast tumors *in vivo*. The variants of Polycefin were engineered for a combined attack on cancer cells inhibiting tumor angiogenesis and inducing apoptosis. These drugs are based on biodegradable, non-immunogenic and non-toxic PMLA with covalently conjugated anti-transferrin receptor (TfR) monoclonal antibody (mAb) for transcytosis across the endothelial system, and with antisense oligonucleotides (AON) to tumor markers to inhibit angiogenesis and/or HER2/*neu*. The preclinical experiments for brain and breast cancer treatment showed significant animal survival and reduction of tumor size. **Experimental Methods.** Polymer-conjugate activity and stability in plasma and cells were evaluated by HPLC, ELISA, and $-SH_2$ and $-NH_2$ group specific chemical testing. Nanosizer techniques were applied to measure sizes, zeta potential and absolute molecular weights. Endotoxin was removed by extraction with Triton X-114. PMLA-nanoconjugates were tested for endotoxin by LAL-tests and rabbit pyrogenic assay. All Polycefin variants were administered by I.V. Xenogen IVIS 200 Imager was used to detect and quantitate *in vivo* Polycefin accumulation and drug distribution in organs and plasma. Confocal microscopy was used to study Polycefin cell delivery and distribution.

Results. (1) Characterization Polycefin conjugates with covalently attached modules were synthesized for vascular escape (anti-host transferrin mAb), endosomal uptake (anti-human TfR mAb or anti-tumor marker mAb), endosomal escape into cytoplasm, drug AON, PEG for protection against enzymatic degradation and RES scavenging, and fluorescent dye Alexa Fluor 680 for imaging. Absolute molecular weights were close to calculated values based on design. Sizes (< 30 nm) of PMLA and Polycefin conjugates varied in accordance with amount and size of conjugated functional groups. Zeta potential was slightly negative and approached neutrality in response to conjugation of AON, mAbs and especially PEG_{5000}. Tandem conjugation of two mAbs on one nanoplatform was tested and confirmed by HPLC and ELISA. Half-life of Polycefin variants was about 24 h in PBS at 37^0C. **(2)** Immunological study At a concentration of 1 mg/mL, nanoconjugates were not hemolytic, and did not induce but inhibited collagen-stimulated platelet aggregation. PMLA (1 mg/mL) delayed plasma coagulation time in APTT and thrombin time tests, nanoconjugates delayed time in the APTT tests. No significant NO^- secretion was observed in culture supernatants from macrophages treated with 1 mg/mL PMLA or nanoconjugates. Only clinically insignificant chemotaxis was observed at 1 mg/mL. No significant phagocytosis was seen by luminol assay. PMLA did not induce significant expression of CD80, CD83, CD86 and did not cause reduction in CD14 expression in monocyte-derived immature dendritic cells (from 3 human plasma donors). No induction of leukocyte proliferation *in vitro* in PBMC of plasma from 3 donors was observed, and PHA-M induced leukocyte proliferation in one of three donors was inhibited (1 mg/mL). **(3)** Pharmaceutical purity Endotoxin contents were at the border of detection by LAL and no animal demonstrated the temperature rise by pyrogenic assay. **(4)** By confocal microscopy with labeled moieties delivery of AON into the tumor cells and mechanism of drug delivery were confirmed. **Conclusion.** The nanobioconjugate Polycefins represents a new generation of cancer therapeutics, with pharmaceutical and preclinical evaluation showing systemic treatment efficacy against brain and breast cancers.

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Novel Drug-Eluting Stents to Reduce Coronary In-stent Stenosis in a Porcine Model of Metabolic Syndrome

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Drug-eluting stents (DES) are the predominant treatment for blood flow-limiting coronary stenosis and are more effective in reducing in-stent stenosis (ISS) compared to bare metal stents. However, due to the non-selective cytotoxicity of the drugs in DES, late stent thrombosis is a life-threatening problem. Proliferation of coronary smooth muscle cells (CSMC) is a pivotal event in the development of ISS. We found that adenosine A1 receptors (A1R) via activation of ERK1/2 mediate mitogenesis in CSMC and intact coronary rings. Ossabaw miniature swine are a large animal model of the metabolic syndrome because of the insulin resistance of lean Ossabaw and predisposition to obesity compared to other breeds (e.g. Yucatan). Our goal is to find a CSMC-specific drug to prevent ISS.

Hypothesis: A1R-selective antagonist DPCPX-eluting stents are effective in reducing coronary ISS compared to bare metal stents in Ossabaw swine.

Methods: Stainless steel stents were coated with polymer and DPCPX in concentrations that optimized in vitro elution kinetics of DPCPX. Polymer- and DPCPX-eluting stents were placed in the coronary arteries of Ossabaw pigs. Coronary ISS was assessed in vivo by intravascular ultrasound imaging after 4 weeks of recovery from stenting and then pigs were sacrificed for tissue analysis. Results: A1R protein was upregulated in both types of stented arteries after 4 weeks of recovery. CCPA, an A1R-selective agonist, induced robust ERK1/2 activation in polymer- vs. DPCPX-eluting stented and non-stented artery segments. In-stent stenosis was reduced by DPCPX-coated stents compared to polymer-coated stents. Conclusion: In vivo data indicate that A1R upregulation and activation of ERK1/2 contributes to coronary in-stent stenosis. **Support: NIH RR013223 and HL062552 to M.S., HL078715 to K.P., and AHA Predoctoral Fellowship to X.L.**

Therapeutic Immunosuppression with Targeted Nanoparticulate Systems

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Abstract

Therapeutic immunosuppression can alleviate the severity of autoimmune diseases and prevent or delay organ transplant rejection. In both autoimmune diseases and organ transplant rejection, CD4 T cells contribute to pathogenesis. Nanoparticulate drug delivery systems targeted to CD4 T cells offer a new strategy for achieving therapeutic immunosuppression. Here, we demonstrate that these drug delivery systems can enhance the efficacy of currently used immunosuppressant drugs by improving therapeutic potency and lowering the dosage required to achieve therapeutic immunosuppression. The mechanism of action of these systems may involve a synergistic immunosuppression of both CD4 T cells and antigen presenting cells. We present work demonstrating the feasibility of using these nanoparticulate drug delivery systems for achieving therapeutic immunosuppression in two different mouse models of CD4 T cell mediated disease: systemic lupus erythematosus (SLE) and allogeneic skin graft rejection.

Surfactant composition affects nanoART uptake and drug release in human monocytes and macrophages

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Despite the development of a broad range of potent antiretroviral therapies (ART), eradication of human immunodeficiency viral infection remains elusive. This is caused, in part, to limited drug penetrance into viral sanctuaries and drug pharmacodynamics, half-life, and biodistribution properties. To address these therapeutic challenges our laboratories developed a mononuclear phagocytes (MP; monocytes and blood borne macrophages)-based antiretroviral drug nanoparticle (nanoART) platform. The aim of this research is towards maintaining continuous drug levels in plasma, improving drug bioavailability and limiting ART toxicities. A series of experiments were performed aimed towards optimizing nanoART size, shape, charge, and composition in order to maximize cell-based delivery. We manufactured then tested commonly used antiretroviral drugs for clinical practice (for example, indinavir, atazanavir, ritonavir and efavirenz). NanoART were prepared by wet milling in an aqueous solution with a broad range of surfactants (Poloxamer 188, PVA, mPEG2000-DSPE, DOTAP and SDS). Nanoparticles (NP) were characterized by dynamic light scattering (DLS), zeta potential and scanning electron microscopy (SEM) for assessments of particle size, surface charge and morphology. MP uptake and release of drugs were dependent on NP size, concentration, surface charge, incubation time, and surfactant composition. These showed significant effects on the drug uptake and pharmacokinetics. NanoART uptake was measured up to four hours and found to be restricted for monocytes but robust, > 1 log increase, in monocyte-derived macrophages (MDM). Surfactant combinations containing Poloxamer 188 demonstrated robust MDM uptake and continuous drug release for up to 14 days for all nanoART manufactured. Diverse release profiles were seen amongst the tested nanoARTs and different formulations of the same drug and strongly dependent on the chosen surfactants. Poloxamer 188 proved most efficient for drug uptake and slow release in the MDM tested system. Both intracellular and extracellular drug levels were readily detected indicating sustained release profiles using an initial treated drug concentration of 100 μM and a treatment time of 2 hours. The study supports the idea that optimizing surfactant composition could improve nanoART pharmacokinetics and eliminate a major obstacle required for human development.

Little, BIT, of WONder
-they deliver

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Nanotech based drug delivery systems have the tremendous potential to **revolutionize** medical treatments and medical practice. With hundreds of nanodrugs in development globally, the opportunities for clinical and market successes are apparent. Most of these nanoparticles have potential as a nontoxic, bioresorbable vehicles, which are highly **specific, accurate and precise** in their action of drug delivery to **specific sites** on cells, tumors and organs in cell culture assays and diseased patients. Nanodrugs are at the forefront of bioengineering for diseases such as cancer, cardiovascular diseases, diabetes, Parkinson's, age-related and other degenerative diseases and represent the next generation of medical therapies that will impact worldwide markets, healthcare, and pharmaceutical industry with new and **multipurpose** nano drugs coming into market, which can solve the existing side-effects and problems in current system of medicine to a greater extent.

Few examples for NanoDDS are, quantum dots, liposomes, dendrimers, micelles, carbon nanotubes, Fullerenes, antibody attached particles, fluorescent labelers etc.

Various features like, Increased drug localization in the target tissue through, Active and Passive targeting, Decreased drug localization in sensitive non-target tissues, Ensuring minimal drug leakage during transit to target, Protecting the drug from degradation and from premature clearance, Retaining the drug at the target site for the desired period of time, Facilitating cellular uptake and intracellular trafficking, Biocompatible and biodegradable nature makes these nanodrugs **the best possible** Drug delivery systems which easily and efficiently help to overcome all problems and undesired effects.

Regular cancer treatments (i.e. chemotherapy) have countless side effects, and they don't really cure the patient. The drugs that patients consume get lost in the bloodstream before they reach their target, thus indirectly "helping" (mutatations) the cancer to grow. However, **nanomedicine** can change it all. **Targeted drug delivery** does wonders.

Certain supramolecular, core-shell nanoparticles offer considerable advantages for cancer diagnosis and therapy. Their relatively small size (10–100 nm), ability to solubilize hydrophobic drugs as well as imaging agents, and improved pharmacokinetics provide a useful bioengineering platform for cancer applications. Several polymeric micelle formulations are currently undergoing phase I/II clinical trials, which have shown improved antitumor efficacy and reduced systemic toxicity. Fictionalization strategies result in enhanced micellar accumulation at tumor sites, higher drug bioavailability, as well as improved tumor diagnosis and visualization of therapy. Ultimately, integrated nanotherapeutic systems (e.g., theranostic nanomedicine) may prove essential to address the challenges of tumor heterogeneity and adaptive resistance to achieve efficacious treatment of cancer.

So, by all these unique features and inherent abilities, nano drug delivery system is a promising and better solution for future.

Key Words: Nanodrugs, Nanoparticles, Drug delivery, Medical therapy, Targeted drug delivery, Cancer treatment

Polyelectrolyte complexes of antioxidant enzymes and block copolymers – physicochemical characterization and cellular uptake mechanisms

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Polyelectrolyte complexes of block copolymers represent a promising class of delivery systems for a variety of macromolecular therapeutics. In particular, they have been widely investigated to utilize their potential to deliver therapeutic proteins [1, 2]. Our ***goal*** is to develop a safe and efficient delivery system to effectively treat neurological disorders. Excessive production of reactive oxygen species (ROS) results from inflammation and subsequently leads to neurodegeneration – a common pathological feature observed in neurological disorders. Published studies have indicated that antioxidant enzymes like superoxide dismutase (SOD) and catalase can scavenge ROS, attenuate inflammation and induce neuroprotection in relevant disease models. We hypothesize that optimized formulations of block ionomer complexes (BICs) of antioxidant enzymes and block copolymers will serve to protect enzymatic activity prior to delivery at the target site of action, improve pharmacokinetics, biodistribution and will thus improve overall efficacy of the delivery process. Optimization of biophysical properties such as hydrodynamic size, surface charge, morphology and stability form a crucial component in the development of BICs as therapeutic entities. We have developed different classes of BICs of catalase and SOD using poly(ethylene glycol)-block-poly(L-lysine hydrochloride). BICs are of narrow polydispersity and demonstrate good retention of enzyme activity compared to naked enzyme. Biophysical properties and possible modes of cellular internalization/transport across an *in vitro* model of blood brain barrier cells will be presented.

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Fabrication of Homogeneous Drug Delivery Systems *via* Hydrogel Template Strategy

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Development of clinically compatible polymeric drug delivery systems with multifunctional attributes requires an in depth understanding of the influence of the physical parameters such as geometry and dimensions of homogeneous microstructures on blood circulation times and cellular uptake. Due to the non availability of efficient fabrication strategies and biocompatibility issues associated with the preparation methods and materials properties, the development of multifunctional drug delivery systems has been slow.

In this direction, we have developed a broadly applicable hydrogel template strategy using sol-gel phase reversible hydrogel forming materials. This strategy enabled the fabrication of homogeneous drug-PLGA microstructures in the size ranging from 200 nm to 50 μm. The hydrogel template strategy is simple, inexpensive, and readily scalable for the fabrication of multifunctional drug delivery systems.

Pharmacokinetic properties of indinavir, ritonavir, atazanavir, and efavirenz nanoparticles for human monocyte and macrophage drug delivery

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INTRODUCTION: Limitations to conventional oral anti-retroviral medications include treatment adherence, high toxicity, poor pharmacokinetics, and a failure to eradicate viral reservoirs. These issues increase the chances of viral mutation and treatment failure. In attempts to bypass these limitations we developed nanoformulations of commonly used anti-retroviral drugs that include ritonavir (RTV), indinavir (IDV), atazanavir (ATZ), and efavirenz (EFV) for cell-based drug delivery. This combination was chosen in order to boost effective sera concentrations of protease inhibitors and nucleoside analogues by reducing drug liver metabolism and degradation. Antiretroviral nanoparticles (nanoART) were manufactured to optimize drug entry and release kinetics into and out of monocytes and monocyte-derived macrophages (MDM). This would allow for cell-mediated delivery of nanoART into sites of active viral replication. Slow release of drug from nanoART laden cells would maintain a high level of drug concentration in infected tissues and plasma for extended periods.

METHODS: In an initial step to achieve these goals we measured RTV, IDV, ATZ, and EFV concentrations by reverse phase high performance liquid chromatography (RP-HPLC) in monocytes and MDM treated with nanoART in concentrations ranging from10^{-4} to 10^{-6}M. Comparisons amongst formulations ensured optimal drug uptake and dissociation of drug. Cytotoxicity was recorded by the 3-(4,5-dimethyl thiazol -2-yl)-2,5-diphenyl tetrazolium bromide assay. Functionality was measured by cell migration across an *in vitro* blood-brain-barrier and cytokine secretion. Antiretroviral efficacy was measured by pre-treating MDM with nanoART and challenging cells with HIV-1 infection up to 15 days post nanoART treatment.

RESULTS: Monocytes and MDM were reproducibly able to "rapidly" uptake (within 30 minutes) nanoART in amounts exceeding the ED_{50} of one log or greater without cytotoxicity or loss of function. The drugs were released into culture media for >14 days. Size, shape, charge, and surfactant coating greatly affected the uptake/release properties, cell handling, and cell viability of nanoART.

CONCLUSION: These results support the contention that nanoART could be used for cell-mediated drug delivery of antiretroviral drugs for treatment of HIV-1 infection in humans. Future experiments will explore drug distribution and anti-retroviral properties *in vivo* upon a single intravenous administration.

Folate-Conjugated Cross-Linked Polymer Micelles as a Nanocarrier for Targeted Delivery

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INTRODUCTION: The folate receptor (FR) is a tumor marker overexpressed in more than 90% of ovarian carcinomas and a large percentage of other tumors. Meanwhile, the normal tissue expression of FRs is highly restricted, making it a useful marker for tumor targeting. The purpose of this study was to design cross-linked polymer micelles coupled to folic acid (FA), a high affinity substrate for the FR (K_d 10^{-9}–10^{-10} M). These FA-conjugated micelles can potentially enhance the delivery of the system to FR-overexpressed cancers.

METHODS: Diblock copolymer poly(ethylene oxide)-b-poly(methacrylic acid) (PEG-b-PMA) was used to form cross-linked polymer micelles with the desired degree of cross-linking. The cross-linked micelles were further conjugated to FA via divinyl sulfone linker. The binding of FA-conjugated micelles to the surface-bound FR was studied by the surface plasmon resonance (SPR) technique. FR over-expressing human ovarian cancer A2780 cell line and non FR expressing A549 lung alveolar epithelial cell lines were used to evaluate cellular uptake at 37°C and 4°C. Competitive inhibition of cellular uptake was studied using cellular uptake in the presence of free FA. Confocal microscopy was used to visualize the cellular uptake.

RESULTS: FA-conjugated micelles represented spherical particles, about 100 nm in size and a negative zeta-potential. SPR measurements indicated the selective and strong interaction of FA-conjugated micelles with the surface immobilized folate binding protein. The FA-conjugated micelles showed much higher uptake than non conjugated micelles by A2780 cells. The uptake is FR specific as seen by the reduced uptake by non FR expressing A549 cell line. Reduced uptake at 4°C by FR expressing A2780 cells indicates the role of active receptor mediated processes. Competition inhibition of cellular uptake for FA-conjugated micelles in the presence of free folic acid confirmed the FR specific uptake of such micelles.

CONCLUSION: The folate-conjugated cross-linked polymer micelles exhibit selective uptake by ovarian cancer cells and can be utilized as nanocarriers for targeted delivery of chemotherapeutic agents to the folate receptor expressing cancers.

Preparation of Antibody-Bound Cross-Linked Micelles for Targeted Drug Delivery

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INTRODUCTION: Targeted drug delivery for cancer treatment is one of the important objectives in nanomedicine. There are two different approaches for delivery of drugs to tumors: (a) utilizing passive targeting of macromolecules achieved by EPR effect; and (b) active targeting through functionalization of the surface of nanoparticles with vector ligands that promote tumor cell-specific recognition and binding. Self-assembled block copolymer nanocarriers have attracted significant attention for delivery of therapeutic agents. In this study, antibody modification of block-ionomer micelles and the ability of the resulting antibody modified nanocarrier to recognize its targeted ligand have been investigated.

METHODS: Block ionomer complexes of poly(ethylene oxide)-b-poly(methacrylic acid) (PEO170-b-PMA180) copolymer and divalent metals cations (Ca^{2+}) were utilized as micellar templates for the synthesis of the polymer micelles with cross-linked ionic core. Specific targeting ligand, monoclonal antibody CC49, was coupled to the surface of the micelles via flexible PEO linker. Unbound antibodies and by-products were removed by gel filtration chromatography (Sepharose CL-6B). These micelles were characterized by dynamic light scattering, SDS-PAGE gel electrophoresis and atomic force microscopy. Specific interaction between antibody modified micelles and its targeted antigen (bovine submaxillary mucin or BSM) were determined by surface plasmon resonance measurements (SPR).

RESULTS: The MAb modified cross-linked polymer micelles have average diameter of 115-130 nm with low polydispersity indices (PDI <0.1) and form stable aqueous dispersion. The antibody-bound nanoparticles were able to effectively recognize the immobilized BSM as shown by the specific binding of the targeted micelles with its antigen coated on the sensor chip. In contrast, polymer micelles lacking the targeting ligand or the micelles modified with unspecific antibodies (IgG) did not show binding to the BSM antigen.

CONCLUSION: Successful antibody modification of polymer micelles was achieved. SPR analysis confirmed a specific binding of the CC49 antibody conjugated cross-linked micelles to its antigen, BSM. This approach can allow specific tumor targeting of nanocarriers towards improved cancer therapy.

Biodistribution and comparative pharmacokinetics of cisplatin loaded core cross-linked micelles in mice

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INTRODUCTION: Benefits of the frequently prescribed platinum (II) chemotherapy drug (cisplatin) are compromised by undesirable side effects, poor pharmacokinetics and development of drug resistance. Polymer micelles derived from amphiphillic block copolymers, offer a novel macromolecular platform for carrier based drug delivery, potentially overcoming such limitations. In this study, the altered biodistribution and pharmacokinetics of cisplatin upon entrapment into core cross-linked block ionomer complex micelles were evaluated in tumor-bearing mice.

METHODS: Poly(ethylene oxide)-poly(methacrylic acid) block copolymer (PEO-b-PMA) based core cross-linked micelles were synthesized via 1) condensation of PEO-b-PMA copolymers by Ca^{2+} into spherical micelles, 2) core cross-linking by using either 1,2-ethylenediamine or cystamine as cross-linkers and 3) removal of Ca^{2+} by extensive dialysis. Cisplatin was incorporated into the ionic core by reversible polymer-metal complex formation. Female nude athymic 4 week old mice bearing ectopic A2780 human ovarian cancer xenograft tumor (300-400 mm^3), were given a single intra venous bolus dose (n=5) of free cisplatin or cisplatin loaded micelles at 7.5 μg cisplatin/g of animal. At various time intervals up-to 72h, the animals were euthanized, perfused with saline, the tissues blood, lungs, liver, kidney, spleen and tumors excised and analyzed for platinum (Pt) content using ICP-MS. The percentage of injected dose per gram of tissue (%ID/g) was determined for each organ. The pharmacokinetic parameters were evaluated with noncompartmental analysis using the WinNonlin software. RESULTS: The cisplatin loaded micelles demonstrated prolonged blood circulation and increased tumor accumulation with significantly higher maximum observed plasma platinum concentration (Cmax), plasma area under the curve (AUC), tumor Cmax and tumor AUC compared to the free cisplatin treatment. Kidney, the primary target organ of cisplatin toxicity, had about threefold lower Pt Cmax than cisplatin-treated mice. Organs responsible for removing micelles from the bloodstream, namely the liver and spleen, had elevated Pt levels.

CONCLUSION: Cisplatin-loaded cross-linked micelles display prolonged circulation and increased tumor accumulation while exhibiting a lower renal accumulation. Hence, polymer micelles utilized as carriers for cisplatin could be promising for targeted therapy of solid tumors.

Collagen-Binding Synthetic Peptidoglycans: A New Therapeutic to Prevent Dermal Scarring

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Hypertrophic scars commonly form during healing of deep dermal wounds and are characterized as bulky, inelastic scars, which can restrict mobility, constrict orifices, and severely compromise physical appearance. According to Aetna, 10-15% of dermal wounds result in hypertrophic or keloid scars. The abnormal healing mechanism giving rise to hypertrophic scars is not fully understood, however advances in the understanding of biochemical differences between healthy and hypertrophic tissue, such as extracellular matrix components and how they affect fibroblast behavior, provide insights for development of therapeutic approaches for improving healing and reducing or eliminating scarring. The proteoglycan decorin is one of the key components in dermal healing due to its many roles, including its influence on collagen organization, the biochemical signaling of the attached glycosaminglycan chain, and its effect on fibroblast behavior. However, decorin only exists in tissues in small quantities, making its wide-spread use in wound healing impractical.

We have developed feasible alternatives in the form of collagen-binding peptidoglycans, inspired by decorin, which can be synthesized in large quantities using unique processing and design controls. Our novel therapeutic mimics many of the functions of decorin, including regulation of collagen fibril formation and increase of collagen gel stiffness. Using adult primary smooth muscle cells, higher elastin production was found in type I collagen gels in the presence of the peptidoglycan relative to collagen gels alone, thus demonstrating a positive impact of peptidoglycans on adult cell healing. Recently, we have shown that one time introduction of the peptidoglycan into a full thickness dermal wound in a rat results in improved healing as determined by a 45% reduction in visible scar and 60% improved wound tensile strength. These results also correlated to more mature tissue located in the wound bed as scored with Masson's Trichrome stained histology. Future studies will examine the efficacy of the peptidoglycan in hypertrophic scar formation using a red Duroc pig model.

Imaging of Carriers for Therapeutics and Diagnostics on Nano-resolution Scale by Freeze-fracture TEM

***Brigitte Papahadjopoulos-Sternberg**, NanoAnalytical Laboratory, San Francisco*

The potency of nano- and micro-particles, loaded with therapeutic and/or diagnostics is frequently depending upon their morphology adopted in a biological relevant environment. Freeze-fracture transmission electron microscopy (ff-TEM) as a cryo-fixation, replica TEM method is a powerful technique to monitor self-assembling of lipid-, polymer-, as well as protein/peptide-based carriers encapsulating drug-, gene-, vaccine, antimicrobial- and imaging molecules[1-3]. At a resolution limit of 2 nm we are able to study the fate of such carriers related to their pay load, application milieu [4], and during their interaction with cells.

Using ff-TEM we studied the morphology of a wide variety of nano- and micro particles suitable as carriers for diagnostics as well as therapeutics including quantum dots (free and coupled to drug-loaded immunoliposomes), gold nano-particles, superparamagnetic iron oxide nano-particles loaded in polymeric immunomicelles [5], micelles (spherical-, disc-, and worm-type micelles) [6], small unilamellar liposome [7], multilamellar liposome, niosomes [8], cationic liposome/DNA complexes [9,10], polymer- or lipid-stabilized gas bubbles [11], cochleate cylinder, depofoam particles, and drug crystals. Recently we explored liposome-, virosome-, and virus-based vaccines, including measles vaccine powders, by ff-TEM.

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Enhanced Delivery of Antimicrobial Agents to Intracellular Pathogens by Core-Shell Polyion Complexes

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A methodology for encapsulating aminoglycoside antibiotics, particularly gentamicin, into colloidal drug carriers with core-shell nanostructures has been developed. The assemblies comprised a polyacrylate-aminoglycoside core surrounded by hydrophilic poly(ethylene oxide) (PEO) or amphiphilic poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (PEO-*b*-PPO-*b*-PEO) shells. High concentrations of the drugs (~25 wt%) were incorporated into the cores of these complexes through cooperative electrostatic attractions among carboxylates on the polyacrylate and ammonium ions on the drugs. Intracellular uptake of the nanostructures with amphiphilic PEO-*b*-PPO-*b*-PEO shells into mouse macrophages was greatly enhanced relative to analogous complexes with only hydrophilic shells. Enhanced *in vitro* and *in vivo* efficacies of these amphiphilic nanostructures in reducing intracellular *Salmonella*, *Listeria* and *Brucella* have been demonstrated.

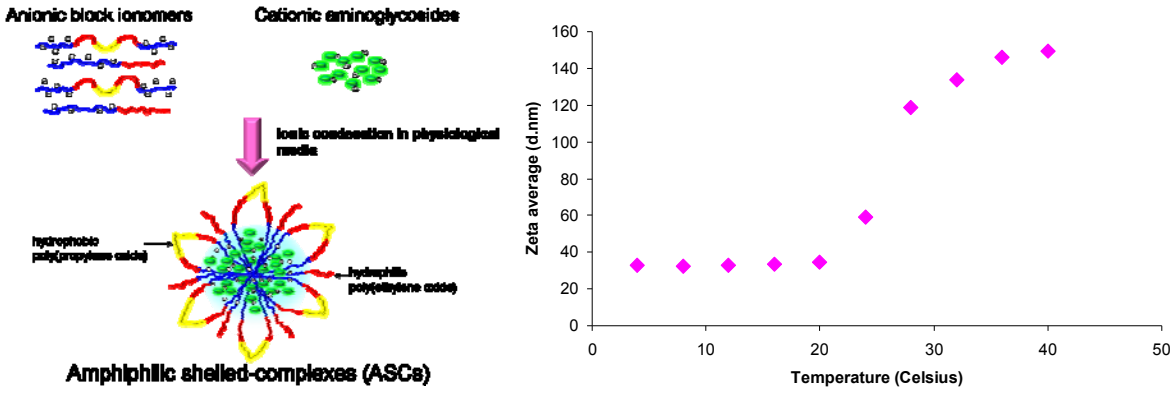

Figure 1. Schematic of block ionomer-aminoglycoside assembly into amphiphilic polyion complexes (left), and thermosensitive sizes of the complexes in PBS (right)

Facile Production of Composite Nanoparticles for Drug Delivery with Hydrophobic Actives, Polypeptides, and Proteins, Targeting, and Imaging

Suzanne D'Addio, Varun Kumar, and Prof. Robert K. Prud'homme, Princeton University

Abstract: Nanoparticle formulations of hydrophobic drugs present unique opportunities for treatment of solid tumor cancers, TB and for the delivery of drugs by aerosol administration. There is also increased interest in the delivery of polypeptide and protein materials which are not hydrophobic. Common requirements of these applications are control of particle size and surface functionality. For cancer therapy particles in the size range of 100-200 nm passively pass through defects in the vasculature in tumors and deposit by "enhanced permeation retention". Particle sizes smaller than 400nm are generally deemed necessary for IV administration. In addition to delivery, the ability to monitor the fate of the nanoparticles is also of important since anti-cancer agents are invariably toxic to healthy tissue. Our process --Flash NanoPrecipitation – is a controlled precipitation process that produces stable nanoparticles at high concentrations using amphiphilic diblock copolymers to direct self-assembly. It produces composite nanoparticles that enable simultaneous imaging and delivery. Uniform particles with tunable sizes from 50-500 nm can be prepared in an economical and scalable manner. The key to the process is the control of time scales for micromixing, polymer self-assembly, and particle nucleation and growth. The PEG protective layer creates long-circulating particles and the inclusion of PEG chains with terminal ligands allows drug targeting. The incorporation of gold nanoparticles, magnetic nanoparticles, or fluorophores into the composite particle enables imaging by x-ray, MRI, or confocal microscopy, respectively. The incorporation of up converting phosphor crystals into the composite nanoparticles enables a highly efficient form of photodynamic therapy.

The poster will emphasize our recent results that focus on the creation of polypeptide nanoparticles with protected PEG surfaces that enable peptide delivery with protection from protease clearance in circulation. We also focus on a new class of biologically activated compounds where the protection in the nanoparticle core enable extended release and protection in circulation.

Composite Nanoparticles for Photodynamic Cancer Therapy

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Robert H. Austin Physics Department, Princeton University

Photodynamic therapy is an approved treatment for pulmonary and pleural mesothelial cancers. The therapy relies on cytotoxic singlet oxygen production due to excitation of a photosensitizer by a visible light. The current method has significant limitations firstly because many photosensitizers are hydrophobic and thus non-deliverable, the visible light used to excite these photosensitizers has a limited penetration distance in tissue and the mode of singlet oxygen delivery is not targeted specifically to cancer cells.

We present a design of composite nanoparticles (CNPs) in which upconverting phosphors, a material capable of emitting visible light, are placed in close proximity to photosensitizer. The biocompatible, 140nm-phosphors, $NaYF_4$:Yb^{3+},Er^{3+}, produces green light upon excitation by infra red (980nm). The utilization of infra-red light in place of green laser light addresses penetration distance problem encountered in current PDT approach. The phosphors and the photosensitizer (*meso*-tetraphenyl porphine (mTPP)) are packaged in a single compartment using biocompatible, FDA-approved polymeric materials. Candidate block-copolymers include PEG-PCL (poly(ethylene glycol)-*block*-poly(caprolactone) and PEG-PLGA (poly(ethylene glycol)-*block*-poly(lactide-co-glycolide) acid). The CNPs are manufactured using Flash NanoPrecipitation technology which provides homogenous mixing, resulting in good control over nanoparticle size with high reproducibility.

Our study suggests that PEG-PLGA provides better nanoparticle size control compared to PEG-PCL block-copolymers. The spherical composite nanoparticles of PEG-PLGA has a diameter of 200 nm while PEG-PCL 400 nm, both of which are stable in water. With a high ratio of photosensitizer to drug (1:3, by weight) in the composite, these CNPs are capable of producing cytotoxic singlet oxygen, as determined by ADPA tests.

Nanomedicine and Drug Delivery: Efficacy and Toxicity of Rifampicin Loaded Nanoparticles Against *Mycobacterium avium* Infected Murine Macrophages and BALB/c Mice

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Currently, about one third of the world's population is latently infected with *Mycobacterium tuberculosis* and about 3 million people die from the disease annually worldwide. In the US, a resurgence of tuberculosis cases has occurred in recent years. It is attributable to emigration from countries with high TB prevalence, the HIV epidemic, outdated control strategies and poor patient compliance. Failure to complete the full treatment course can increase the risk of disease re-emergence, spread of infection and development of drug resistant mycobacteria. An improved treatment approach in humans is urgently needed. Developing a safe and effective colloidal drug delivery system that targets the macrophage directly and releases anti-tuberculosis drugs in a controlled manner, may translate to an overall reduction in the amount and frequency of therapy. Improving the bioavailability of anti-tuberculosis drugs through nanomedicine can ultimately lead to better patient compliance and treatment of tuberculosis.

The goal of this project is to explore, if different nanoparticles loaded with anti-tuberculosis drugs will effectively enter and eliminate *M. avium* in the macrophage as well as treat *M. avium* infection in a mouse model without toxic side effects. *In-vitro* cell culture studies using J774A.1 murine macrophage cell line were done with rifampicin loaded polyacrylate and rifampicin loaded biosilica nanoparticles with equivocal clearance results. Results of MTS and Trypan blue cytotoxicity assays revealed rifampicin polyacrylate nanoparticles were non-toxic in J774A.1 cells. However, several constructs of rifampicin biosilica nanoparticles were toxic. Studies of characterization of zeta potential, drug loading/release profiles, and intracellular localization of the nanoparticles will be reported. Future efforts will include development of nanoparticles with improved efficacy and minimal toxicity in *in vitro* and *in vivo* BALB/c mouse models.

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Core-Shell Magnetite-Polyether Nanostructures for MR Imaging

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Magnetic resonance imaging has been one of the most non-invasive imaging tools used in the detection of various diseased tissues over the past three decades. Enhancement of contrast comes from the use of contrast agents. The most common contrast agents are Gd-based chelates, but there is significant interest in iron oxide nanoparticles due to their inherent high magnetic susceptibilities. Superparamagnetic magnetite-based contrast agents are already used for image enhancement of the liver, spleen and lymphatic system. Our interest is in developing contrast agents as tools that can tag single cells to enable cell tracking or early detection of disease.

In this study, a series of hydrophilic poly(ethylene oxide) and amphiphilic poly(propylene oxide-*b*-ethylene oxide) (PPO-*b*-PEO) copolymers with terminal carboxylate, ammonium and ammonium phosphonate zwitterions were adsorbed onto magnetite nanoparticles via the charged endgroups.[1-3] The hydrophilic nanostructures have a magnetite core surrounded by a PEO brush and the amphiphilic complexes have a magnetite core surrounded by a hydrophobic PPO segment and an outer hydrophilic PEO brush. While all of the complexes remained stably bound in water, only those with the ammonium phosphonate zwitterions survived in PBS. **This is due to the strong adsorption of phosphate anions to the iron oxide surface, as evidenced by highly negative zeta potentials of magnetite in PBS over a wide pH range. The carboxylate and ammonium functional groups on the polymers become partially displaced by phosphate anions in PBS.** The size and aggregation characteristics of the complexes in water and PBS were measured via dynamic light scattering and compared to colloidal predictions. **An adaptation of a density distribution model introduced by Vagberg et al.[4] that is based on a model for star polymers was used—in conjunction with extended DLVO theory—to calculate the colloidal stability of these polyether-magnetite complexes. The diameters of the magnetite-polyether complexes were predicted within 8% of the experimental values obtained by dynamic light scattering.**

Transverse relaxivities (R_2) of these nanostructures as well as commercially available materials were probed in light of their chemical and colloidal properties. **Localized field gradients generated by the particles in applied magnetic fields result in rapid local dephasing of the proton spins, thus increasing R_2.** The magnetic nanoparticles have much higher R_2 values than today's commercial iron oxide contrast agents.

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Hydrogel Template Strategy for the Fabrication of Microstructures of Complex Geometries

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Recent studies have revealed the influence of physical parmeters such as shape and size of the microstructures in triggering cellular responses.[1] Understanding the influence of shape and size of polymeric microparticles in triggering cellular responses will facilitate the development of smart drug delivery systems with increased blood retention times and cellular uptake. Mitragotri *et al* have elegantly demonstrated the effect of shape and size of the polymeric microparticles on phagocytosis.[2] Harnessing this phenomenon for clinical applications requires the development of efficient, inexpensive, and readily scalable fabrication methods that can produce large quantities of homogeneous micro/nanoparticles of simple to complex geometries.

We herein present a hydrogel template strategy for the fabrication of homogeneous polymeric nano/microparticles of complex geometries. Because of its mild processing conditions, the hydrogel template strategy is readily applicable for the large scale production of microparticles incorporated with sensitive drugs without compromising on their biological activity and pharmaceutical efficacy.

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Nonionic block copolymer Pluronic P85 is a promising adjuvant for MUC4 plasmid DNA immunotherapy

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Introduction: Gene therapy based vaccination is a promising approach to facilitate successful immunization against various cancers. Pancreatic cancer, a devastating and aggressive disease affecting 37,000 people each year in US alone, is an attractive target for vaccination because of clearly marked MUC4 overexpression. DNA vaccination approach through delivery and expression of the gene coding for MUC4 might be an interesting prophylactic and therapeutic option, in aim to alert host immune system against MUC4 overexpressing cells. In this study, we report, that amphiphilic block copolymer Pluronic P85 co-administered intramuscularly with plasmid DNA encoding MUC4, significantly enhances plasmid expression in the muscle, spleen and lymph nodes and stimulates expansion of T cells in mice.

Methods: The expression of the transgene coding for a mini-MUC4 was assessed in muscle tissue as well as draining lymph node and spleen at day 4 after direct intramuscular injection of either DNA alone or DNA/Pluronic P85 formulation. Co-localization studies were performed on cryo-sections of each tissue to determine the cell-types expressing mini-MUC4. In addition, activation of the immune response was monitored on spleen and lymph nodes by direct immunostaining of lymphatic cells and further FACS analysis of various subsets of immune cells.

Results: In the muscle tissue, MUC4 expression was observed in either myocytes or keratinocytes, with an increase in transfection efficiency when associated with P85. MUC4 expression also co-localized with antigen presenting cells (dendritic cells and macrophages) as well as T cells. The same pattern of expression was found in cells participating to the immune response in the draining lymph node (inguinal lymph node) and spleen. Moreover, FACS analysis showed a significant expansion of the CD8a+ and CD4+ T cells in the lymph node, but no dendritic cell expansion for the P85/DNA formulation. Dendritic cells maturation was also monitored.

Conclusion: MUC4/P85 formulations might be a promising strategy towards vaccination through gene delivery due to the improved expression of the transgene when associated to the Pluronic copolymer P85, in addition to the expansion of both cytotoxic and helper T cells.

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Single Chain Block Copolymer Employs Pathogen-Like Cellular Trafficking

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Amphiphilic triblock copolymer, poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide), Pluronic® P85, is unexpectedly shown to utilize sophisticated cellular trafficking mechanisms and enter brain microvessel endothelial cells and primary neurons that are poorly penetrable. Though caveolae serve as a primary entry site for the copolymer single chains, in cells devoid of caveolae, the copolymer can still exploit caveolae- and clathrin-independent routes. This parallels the copolymer's trafficking itinerary with that of biological pathogens. The similarity is reinforced since both bypass early endosomes/lysosomes and transport to the endoplasmic reticulum. The copolymer finally reaches the mitochondrion that serves as its final destination. Notably, it also succeeds to gain entry in brain microvessel endothelial cells through caveolae and in primary neurons through caveolae- and clathrin-independent pathway. In neurons the copolymer accumulates in the cell body followed by anterograde trafficking towards the axons/dendrites. Overall, dissecting the trafficking of a synthetic polymer in multiple cell types triggers development of novel delivery systems that can selectively target intracellular compartments and provide entry in cells currently considered impenetrable.

Palmitoyl ascorbate-modified liposomes: Novel carrier for ascorbate-mediated antitumoral therapy

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Purpose: To develop and evaluate palmitoyl ascorbate-modified liposomes as novel carrier for ascorbate-mediated antitumoral effect.

Methods: Palmitoyl ascorbate was incorporated in egg phosphatidylcholine/ cholesterol (70:30) liposomes by the lipid film hydration method. When needed, 0.4 mM of paclitaxel was added to the lipid mixture. The formulations were evaluated for palmitoyl ascorbate content by RP-HPLC. Size distribution of the formulations was studied using the dynamic light scattering. *In vitro* cytotoxicity of the liposomal formulations towards various cancer cell lines was investigated using the cell viability assay. Cancer cell targeting of fluorescently labeled palmitoyl ascorbate liposomes was investigated by fluorescence microscopy and flow cytometry with various cancer and non-cancer cell line. To elucidate the mechanism of action of cell death, the effect of various H_2O_2 scavengers, metal chelators on palmitoyl ascorbate-mediated cytotoxicity was studied *in vitro*. *In vivo* anticancer activity by palmitoyl ascorbate-liposomes with and without paclitaxel, and ascorbate-free paclitaxel-loaded liposomes in murine mammary carcinoma (4T1 cells) was studied in the female Balb/c mice. Fourteen days after the tumor inoculation, different liposomal formulations equivalent to 10 mg/Kg of palmitoyl ascorbate and 2 mg/Kg of paclitaxel were intravenously administered via the tail vein three times at days 0, 2, 4. Normal saline was used for the control group. The tumor volumes were monitored on alternate days.

Results: The palmitoyl ascorbate was stably incorporated at 30 mol% into liposomes with the uniform size (146.6±29.0 nm), and no evidence of precipitation upon standing at room temperature for up to 1 week. These liposomes showed dose-dependent toxicity towards variety of cancer cell lines. Further, it is interesting to note that the incorporation of a non-toxic dose of paclitaxel into palmitoyl ascorbate-liposomes significantly increased their toxicity over the empty palmitoyl ascorbate-liposomes, indicating that this low dose of paclitaxel can contribute to toxicity when incorporated in palmitoyl ascorbate liposomes. The H_2O_2 scavengers were completely protective suggesting role of peroxide in palmitoyl ascorbate-mediated cell death. The results from fluorescence microscopy and flow cytometry confirmed selective targeting of cancer cell lines with palmitoyl ascorbate liposomes. The results of the *in vitro* studies were further confirmed *in vivo* in 4T1 tumor bearing mice. The palmitoyl ascorbate-liposomes suppressed tumor growth quite efficiently. There was no significant difference in tumor volume in mice treated with low dose (2mg/Kg) of paclitaxel compared to control. The most remarkable tumor suppression was observed in the group treated with paclitaxel loaded palmitoyl ascorbate-liposomes. Thus, palmitoyl ascorbate-modified liposomes significantly improved the effect of a non-toxic dose of paclitaxel, which is in agreement with our *in vitro* data.

Conclusion: The incorporation of palmitoyl ascorbate into liposomes effectively suppressed the tumor growth in mice and also enhanced the anticancer effect of paclitaxel.

Effective Repair of Traumatically Injured Spinal Cord by Block Copolymer Micelles

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Spinal cord injury (SCI) results in immediate disruption of cell membranes followed by extensive secondary neurodegenerative processes. A key approach for repair of SCI is sealing the damaged membranes at the early stage. Herein we demonstrate effective repair of axonal membranes using self-assembled monomethoxy poly(ethylene glycol)-poly(D,L-lactic acid) di-block copolymer micelles of 60 nm diameter. Rapid restoration of compound action potential following compression injury and reduction of calcium influx into the axons were achieved by incubating the injured site of spinal tissue with micelles at a concentration of 6.7 μg/mL. Intravenously injected micelles effectively improved the locomotor function recovery, and also reduced lesion volume and inflammatory response in SCI rats. The micelles showed no adverse effects after systemic administration to live rats. These results demonstrate the applicability of copolymer micelles as a membrane sealing agent to interrupt the spread of SCI from primary to secondary damages with minimal toxicity.

Fabrication of Homogeneous Drug Delivery Systems With Controlled Release Kinetics

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Development of homogeneous drug delivery systems with high drug content, minimal burst release, and controlled release kinetics has been the focus of current drug delivery research. Drug delivery platforms that have been developed over the past few years include liposomes, polymeric micelles, polymeric nanoparticles, DNA-polymer conjugates, and dendrimers. These platforms yield polydisperse particles having sizes that usually range between hundreds of nanometers to tens of microns. Clinical applicability of these systems has been limited due to low drug loading efficiency and burst release pharmacokinetics. Furthermore, liposomes and polymeric micelles are self assembled dynamic systems and have limited life time in the medium.

We have fabricated homogeneous felodipine-PLGA microstructures of a series of dimensions *via* hydrogel template methodology and studied their drug release kinetics. This method enabled the loading of as high as 48% of felodipine in PLGA microstructures. *In vitro* drug release studies have revealed a minimal initial release followed by slow release for up to a month.

Collagen-Binding Synthetic Peptidoglycans: A New Therapeutic to Prevent Early Stage Thrombosis

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Early stage thrombosis following balloon angioplasty has a mortality rate of up to 25%, resulting in an estimated 9300 deaths per year in the US alone. Most therapies to prevent thrombosis target platelets systemically by interfering with platelet activity; however, bleeding problems are a significant consequence of systemic inhibition of platelet function, and early stage thrombosis remains a clinical problem despite these therapies. We have developed a class of novel molecules called collagen-binding synthetic peptidoglycans which target prevention of early stage thrombosis by preventing platelet binding and activation specifically at the site of exposed collagen of denuded vessels following balloon angioplasty. These molecules are composed of collagen binding peptides conjugated to polysaccharide, and specifically bind to collagen, preventing platelet interaction with the exposed collagen surface and subsequent platelet activation. As these synthetic peptidoglycans can be synthesized with relative ease, at low cost, and with unique design control, multiple variations have been developed for this application.

Preliminary studies indicate that the peptidoglycans developed remain bound to a collagen surface after extensive washing. In static studies, peptidoglycan treatment significantly inhibits platelet binding and activation on collagen surfaces as measured by a reduction in number of bound platelets and release of platelet activation factors PF4 and β-Thromboglobulin. Studies to optimize the design of the synthetic peptidoglycan for anti-thrombotic activity are ongoing, and future studies will examine delivery of the molecule during balloon angioplasty in pigs. The preliminary studies indicate that the collagen binding synthetic peptidoglycans have great potential as a novel therapeutic to prevent early stage thrombosis.

PAMAM-Camptothecin Conjugate: Synthesis, Characterization and In Vitro Activity

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Poly (amido amine) (PAMAM) dendrimers are polymeric macromolecules with a defined structure and multiple surface groups that enable attachment of drugs and biorecognizable moieties for targeted drug delivery. The objective of this study was to evaluate the in vitro activity of a novel conjugate of camptothecin (CPT) and amine terminated PAMAM generation 4 dendrimer. The PAMAM-CPT conjugate was synthesized using a glycine-succinic acid (S-G) spacer and characterized by SEC & HPLC profiles for purity, UV for drug loading and DLS for size measurements. The stability of the conjugate in PBS (pH 7.4) and growth media (with 10% FBS) was studied. Results indicate that the conjugate was stable under these conditions with approximately 4% & 6% release of camptothecin at 48 hrs respectively. A conventional cytotoxicity assay (modified WST-8) performed on HCT-116 cells demonstrates activity of the conjugate (Figure 1). To monitor cell cycle progression cells were stained with propidium iodide and analyzed using flow cytometry. Results indicate cell cycle arrest in the G2 phase for PAMAM-CPT treatment (Figure 2). To visualize apoptosis caused as a consequence of the cell cycle block the cells were stained with DAPI and images were captured on a confocal microscope. The images (Figure 3) suggest apparent nuclear fragmentation leading to formation of apoptotic bodies. This study suggests the potential of the PAMAM-CPT system as an anticancer therapeutic. Future research will focus on evaluating the in vivo efficacy of this system for effective treatment of colorectal cancer.

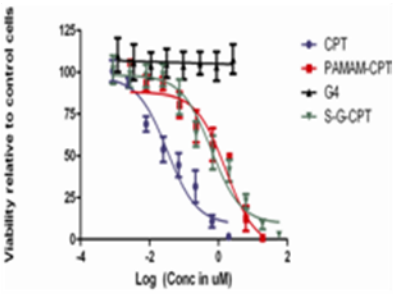

Figure 1. Cytotoxicity of various treatment groups on HCT-116 cells.

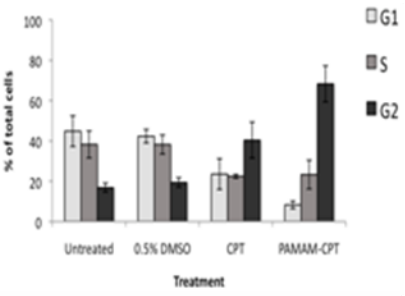

Figure 2. Percentage of cells in different phases of the cell cycle for various treatment groups.

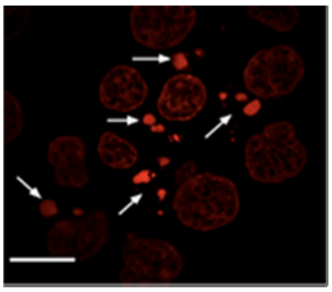

Figure 3. Nuclear fragmentation and apoptotic body formation in HCT-116 cells due to treatment with PAMAM-CPT as indicated by arrows. Bar in picture is 20um.

Protein-Poly(2-oxazoline) Conjugation: Synthesis, Characterization and Enhanced Cellular Uptake

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Protein-Pluronic® (amphiphilic block copolymer poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide)) conjugation has been shown to significantly enhance the cellular uptake and the transcellular transport of protein across the blood-brain barrier *in vivo* and the model *in vitro*. To investigate if the same enhancement can be found in other block copolymers, we synthesized and characterized the protein-poly(2-oxazoline) conjugates. Horseradish peroxidase (HRP) was selected as the model protein and four poly(2-oxazoline)s, including two block copolymers (PMOx-b-PBOx and PEOx-b-PBOx), one random copolymer (P (EOx-ran-BOx) and one homopolymer (PMOx) were chosen for the conjugation. Disuccinimidyl propionate (DSS) and dithiobis(succinimidyl propionate) (DSP) were used as non-biodegradable and biodegradable linkers separately. The HRP-poly(2-oxazoline) conjugates were characterized by amino group titration, SDS-PAGE, isoelectric focusing, enzymatic activity assay and conformation analysis. The conjugates contained average from one to two polymer moieties and 70%-90% of enzymatic activity was preserved in most cases. Circular dichroic analysis revealed that polymer conjugation had effects on the secondary structure of apo-protein but the tertiary structure and heme environment were well maintained. Enhanced cellular uptake was found in the conjugates of two block copolymers using both MDCK cells and Caco-2 cells, but not in the conjugates of random copolymer and homopolymer. HRP-PMOx-b-PBOx had the highest cellular uptake than other conjugates. These data indicate that poly(2-oxazoline) modification has the potential to enhance cellular delivery of proteins.

KEYWORDS
Protein-polymer conjugation, poly(2-oxazoline)s, horseradish peroxidase, cellular uptake, Caco-2 cells

Gold Nanorod-Mediated Photothermolysis Induces Apoptosis to Macrophages via Damage of Mitochondria

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Alterations in macrophage functions contribute to a variety of autoimmune and inflammatory diseases. Diagnosis and eradication of activated macrophages is becoming a valid pharmaceutical target. In this work, gold nanorods conjugated with arginine-rich peptides were applied as theragnosis agents to detect activated macrophages via two-photon luminescence (TPL) and subsequent eradicate through optical hyperthermia. Functionalized gold nanorods could be selectively internalized by activated macrophages *in vitro* and in live animals. Apoptosis of macrophage was induced by controlling laser irradiation to reduce secondary inflammation. Cell death mechanism was further investigated for necrosis and apoptosis pathways. Necrosis was related with the compromise of plasma membrane integrity and apoptosis was through intracellular damage and mitochondria-dependent pathway.

Efficient suppression of human immunodeficiency virus in macrophages by Nano-NRTIs

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Macrophages serve as a natural depot for HIV-1 in the peripheral and central nervous system. To develop drug delivery vehicles that can efficiently target macrophages, we evaluated several cationic nanogel networks for the encapsulation of the 5'-triphosphates zidovudine and didanosine, which are nucleoside inhibitors of the viral reverse transcriptase (NRTIs). The hypothesis underlying the choice of these Nano-NRTIs was that nanoencapsulated phosphorylated drugs would be more potent, less neurotoxic, and able to cross the blood-brain barrier (BBB). Four types of nanogels, consisting of PEG-cl-PEI (NG1) or Pluronic F68-cl-PEI (NG2) biodegradable networks, and star PEG-g-PEI (NG3) or PAMAM-X-PEI-g-PEG (NG4) networks, have been synthesized and fractionated in order to isolate particles with a hydrodynamic radius of 30-40 nm. The triphosphates immediately associated with protonated amino groups inside the nanogel networks during the mixing of aqueous solutions of the drug and the nanocarrier, and the Nano-NTRI formulations were freeze-dried for storage before application. Drug content in the Nano-NRTIs was from 8 to 21%. Nano-NRTIs formed compact stable nanoparticles with a 6- to 8-fold smaller volume than unloaded nanogels. Human monocyte-derived macrophages efficiently consumed the fluorescently-tagged nanogels in the following order: NG3>NG2>NG1>NG4. All nanogels demonstrated relatively low cytotoxicity, and did not affect HIV-1 multiplication as determined by an *in vitro* RT activity test. We determined the Nano-NRTIs which had the highest degree of HIV-1 inhibition, and optimized preincubation time based on the cellular uptake kinetics. Among the best results, Nano-NRTIs showed a 5-50-fold increase in antiviral efficacy, as compared to parental drugs, while the onset of cytotoxicity was not observed until up to 200 times higher concentrations. Brain-targeted nanocarriers were obtained by the decoration of nanogels (via PEG linker) with multiple short peptide molecules binding an apolipoprotein E (ApoE) receptor, which is overexpressed in the BBB. Although this modification slightly decreased the observed antiviral efficacy of Nano-NRTIs, the binding of modified nanogels with brain capillary endothelial cells (bEnd3) was enhanced by 70 to 150%. The selected efficient Nano-NRTIs will be further tested with regards to neurotoxicity, mitochondrial toxicity, *in vivo* brain accumulation, and anti-HIV efficacy in a humanized mouse model.

Drug Free Nanomedicine using PRINT® Nanotechnology: Converting Human Serum Transferrin and Transferrin Receptor Antibodies into Potent Anticancer Drug for B-cell Lymphoma Treatment

Jin Wang, Shaomin Tian, Robby A. Petros, Mary Napier, Joseph M. DeSimone

Transferrin receptor is an essential protein involved in iron uptake and regulation of cell growth. Many kinds of tumor cells overexpress transferrin receptors due to the necessity of large amount of iron to maintain rapid proliferation. The surface of PRINT nanoparticles (200 nm × 200 nm, cylindrical) was conjugated with mouse anti-human transferrin receptor monoclonal antibody (TfR mAb, clone number: OKT-9, mouse IgG1) or human serum transferrin. PRINT particles coated with OKT9 show promising targeting efficiency (63-98% of cell uptake) on seven human cancer cell lines employed in this study (HeLa, cervical carcinoma; Sup-B8, Burkitt's B-cell lymphoma; Ramos, Burkitt's B-cell lymphoma; H125, lung adenocarcinoma; SK-OV-3, ovarian adenocarcinoma; MGR3, human glioblastoma; LNCaP, prostate adenocarcinoma) compared with PRINT particles coated with OKT9 isotype control IgG1. Particles coated with OKT-9 or isotype control IgG1 show little uptake on mouse embryonic fibroblast (MEF), which was used as negative control cell line and does not bind OKT-9 or isotype control mouse IgG1. The fact that OKT9 coated nanoparticles show specific uptake on human cancer cell lines and little uptake on a non-human cell line MEF suggests TfR-mediated cell uptake. Based on 72 hr MTS assays, the nanoparticles coated with OKT9 or IgG1 show little cytotoxicity on all the seven human cell lines employed in this study except Ramos cells.

More than 80% of the Ramos cells were killed by OKT9 coated nanoparticles at a concentration of 100 μg/mL. In contrast, free OKT9 does not induce Ramos cell death. Ramos cell viability was also studied as a function of OKT9 density on particle surface. There was a strong correlation between TfR ligand density on particle surface and cell viability. Using confocal microscopy, OKT9 coated particles were both found in early endosomes and lysosomes of Ramos cells, suggesting that the transferrin receptors (TfRs) may enter lysosomes along with the nanoparticles, which may cause degradation of TfRs and depletion of surface TfRs and subsequent cell death. Apoptosis was also observed for Ramos cells treated with OKT9 coated particles based on a caspase activity assay.

Similar results were also observed for transferrin conjugated PRINT particles on Ramos cells. More than 80% of the Ramos cells were killed by transferrin coated nanoparticles at a concentration of 100 μg/mL, as effective as OKT9 coated particles. In summary, we converted non-toxic human serum transferrin and transferrin receptor antibodies into an effective therapy for a B-cell lymphoma through multiplexing with PRINT nanoparticles. We propose that multiplexing cancer cell targeting ligands with nanoparticles may induce unique cell biology and can be used as a special cancer therapy with minimal side effects.

Zwitterionic chitosan derivatives with tunable isoelectric points

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pH-responsive polymers are widely used in biomedical applications, such as drug delivery, microfluid control, and biosensor devices. One of the potential applications is to create nanoparticulate drug carriers that can be activated by the weakly acidic tumoral pH. However, most of the existing polymers respond to specific pH's; thus, designing a material that may respond to delicate changes in the body requires laborious efforts to synthesize a unique polymer for each application. In order to address this limitation, we developed zwitterionic chitosan derivatives with tunable isoelectric points (IEP). Succinic anhydride-modified low molecular weight chitosan (SALM-CS) were synthesized by amidization of chitosan primary amines with succinic anhydride. By changing the feed molar ratio of succinic anhydride to chitosan primary amine, we obtained a series of zwitterionic chitosan derivatives with IEPs ranging from 4.9 to 7.1. Above IEP, the zwitterionic chitosans were negatively charged, whereas below IEP they displayed positive charges. To evaluate its potential as a material to modify nanocarriers for systemic use, the interaction between the SALM-CS and serum albumin was investigated. Albumin adsorption to the cationic polymer (E-100) film coated with SALM-CS was 50% less than that to the uncoated film. The reduction of albumin adsorption to the E-100 film is likely due to the negative charge of the SALM-CS on the film surface, which reduced the electrostatic interaction between albumin and the E-100 film. To evaluate the hematocompatibility of the polymer, rat red blood cells were incubated with SALM-CS's, and the degree of hemolysis (absorbance of hemoglobin at 540 nm in the supernatant) was monitored. SALM-CS was non-hemolytic at physiological pH.

In summary, a series of zwitterionic chitosan derivatives with tunable IEPs, ranging from 4.9 to 7.1, were created by amidization of chitosan. The protein adsorption assay and the hemolysis assay suggest that the zwitterionic chitosan is a promising material for surface modification of nanocarriers for systemic application.

Optimization of Leptin and Pluronic Conjugation: Potential to Improve Leptin Brain Delivery for The Treatment of Obesity

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INTRODUCTION

Leptin is a major body fat regulator, which acts within the brain and controls appetite and thermogenesis. However in obesity leptin transporters are often impaired at the level of the blood-brain barrier (BBB), which prevents the efficient entry of leptin to the brain to exert its normal function. We hypothesize that by conjugating leptin with poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) block copolymer (also named Pluronics), might improve leptin delivery to the brain and provide therapeutic efficacy in the treatment of obesity.

RESULTS

Two leptin-Pluronic conjugates were produced: (A) leptin lysine modification by mono-amine Pluronic P85 through a degradable linker; and (B) leptin N terminal amine modification by mono-propionaldehyde Pluronic P85 in the presence of cyanoborohydride. Mass spectra and electrophoresis SDS-PAGE showed that leptin was attached by various numbers of Pluronic chains in product A; while in product B it contained leptin attached by one Pluronic and non-modified protein. Interestedly, the dissociation constant (K_D) of leptin-Pluronic (A) was 300 times less than leptin alone (313.67e-10 vs 1.18e-10), whereas leptin-Pluronic (B) displayed 30 times less binding affinity than leptin alone (31.5e-10 vs 1.18e-10), as measured by surface plasma resonance (SPR) in the Biacore 3000. These data suggest that site specific modification, such as Pluronic modification at leptin N terminal produce less heterogeneity, and as such preserves better binding capacity. Our previous studies have shown that leptin modified by various Pluronic chains (product A) enhanced leptin metabolic stability and preserved BBB penetration in mice. In comparison to leptin, this conjugate (A) also showed less food intake at higher doses but produced a more reliable dose-response curve after i.c.v administration. All together these data warrants studies to investigate the BBB permeability of N terminal modified leptin (B) and to test its effect in food intake in mice.

CONCLUSIONS In summary, optimization of the leptin-Pluronic conjugation reduced leptin conjugates heterogeneity, improved leptin binding capacity, and it might provide therapeutic potential for the treatment of obesity.

Approaches to Determining the Apparent pK_a of Cationic Lipids Used to Assemble siRNA Lipid Nanoparticles

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Lipid nanoparticles consisting of cationic lipids with ionizable amine head groups are being pursued as one of the most promising approaches to delivering small interfering RNA (siRNA), and research is being conducted to elucidate the structure-activity relationships (SAR) of these cationic lipids. The pK_a of cationic lipid head groups is one of the critical physiochemical properties of interest due to the strong impact of lipid ionization on the assembly and performance of lipid nanoparticles. This research focuses on developing approaches to determining the relevant pK_a of the ionizable amines in the head groups of cationic lipids. Three distinct approaches have been investigated: 1) potentiometric titration of cationic lipids dissolved in neutral surfactant micelles; 2) pH-dependent partitioning of fluorescence dye to cationic lipid nanoparticles or cationic liposomes; and 3) potentiometric titration of dialyzed cationic lipid nanoparticles or cationic liposomes. Using the approaches developed here, pK_a's of cationic lipids with distinct head groups are measured and compared to calculated values. To determine the potential impact of pK_a's on the interaction between cationic lipids and cell membranes, pHdependent lysis of model membranes caused by cationic lipids is evaluated. The pK_a methods reported here can be used to support the SAR screen of cationic lipids for siRNA delivery, and the information revealed through studying the impact of pK_a on the interaction between cationic lipids and cell membranes will contribute significantly to the design of more efficient siRNA delivery vehicles.

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