nanoDDS

12TH INTERNATIONAL NANOMEDICINE & DRUG DELIVERY SYMPOSIUM

Carolina Club, University of North Carolina
550 Stadium Dr, Chapel Hill, NC

It is our pleasure to welcome you to the 12th International Nanomedicine and Drug Delivery Symposium (nanoDDS'14) in Chapel Hill, NC. Revolutionary advances in the area of nanomedicine and drug delivery require collaboration amongst researchers working in a diverse array of fields including nanotechnology, materials science, imaging, cell biology, tissue engineering, drug and gene delivery as well as clinical research. The symposium aims to shorten the gap between research communities in academia, government and industry. NanoDDS'14 has attracted over 250 registered participants from elleven countries all over the world. This year nanoDDS for the first time features an exhibition of local and national companies and academic institutions (nanoEXPO).

Enjoy the symposium!

Monday, October 6th

8:00-9:00 **Breakfast, check-in, poster setup**

9:00-9:10 **Opening remarks by Chancellor Carol L. Folt and Dean Robert Blouin**

9:10-10:10 **Keynote Lecture**

Session Chair: Robert Blouin, UNC Chapel Hill

Leaf Huang, UNC Chapel Hill
In memory of Prof. Feng Liu

Nanoparticle Remodeling of Tumor Microenvironment to Improve Therapy

10:10-10:40 **Coffee break, posters, expo**

Cancer Nanotechnology I

Session Chairs: **Tatiana Bronich,** University of Nebraska Medical Center
Andrew Wang, UNC Chapel Hill

10:40-11:10 **Hsing-Wen Sung**
National Tsing Hua University, Hsin-chu, Taiwan

Bubble-Generating Carrier Systems for Localized Controlled Release

11:10-11:30 **Biana Godin Vilentchouk**
Houston Methodist Research Institute

Microenvironment affects delivery of nanovectors in primary tumors and tumor metastasis

11:30-12:00 **Tatiana Bronich**
University of Nebraska Medical Center

Targeted nanogels for chemotherapy of cancer

12:00-1:30 **Lunch, posters, expo**

Special Lecture

Moderator: Alexander Tropsha, UNC Chapel Hill

1:30-1:50 **Anthony Hickey**,
RTI International

The NIH Nanomaterial Registry

Targeted Drug Delivery

Session Chairs: Arash Hatefi, Rutgers University
Matthew Parrott, UNC Chapel Hill

1:50-2:20 **Suzie Pun**
University of Washington

Biomaterials Science Lecture:
Phage panning, peptides and polymers: from ligand identification to in vivo *medical applications*

2:20-2:40 **Simona Mura,**
University Paris-Sud XI

Peptide-functionalized nanoparticles for selective targeting of pancreatic tumor

2:40-3:00 **Horacio Cabral**
University of Tokyo

Targeting intractable tumors and metastasis by using supramolecular nanodevices

3:00-3:30 **Coffee break, posters, expo**

Abstract Winners Presentations

Session Moderators: Sam Lai, UNC Chapel Hill
Lei Miao, UNC Chapel Hill
Hemant Vishwasrao, University of Nebraska Medical Center & UNC Chapel Hill

Gene & siRNA Delivery

Session Chairs: Adah Almutairi, University of California, San Diego
Devika S. Manickam, UNC Chapel Hill

4:20-4:40 **Gaurav Sahay**
Massatchusetts Institute of Technology

Endo/lysosomal transport of lipid nanoparticles in-vitro *and* in-vivo

4:40-5:00 **Kathryn Whitehead,**
Carnegie Mellon University

Lipid nanoparticles for the delivery of siRNA to immune cells

5:00-6:00 **Welcome Reception**
Carolina Club

Tuesday, October 7th

8:00-8:30 **Breakfast**

Applied Nanomedicine

Session Chairs: Kay Wagoner, Wagoner Discovery and Development Consulting, LLC
Xiang Yi, UNC Chapel Hill

8:30-9:00 **Steve Zale**
Chief Technology Officer, Bind Therapeutics

BIND Accurins: Targeted nanomedicines through medicinal nanoengineering

8:30-9:00 **Benjamin Yerxa**
Chief Scientific Officer, Envisia Therapeutics and Liquidia Technologies.

Commercialization of PRINT® Technology

9:30-10:30 **Panel Discussion:**

How to advance nanomedicine from lab to marketplace

Panel Moderator: Ralph Lipp, President and CEO, Lipp Life Sciences

Neal Fowler, Chief Executive Officer, Envisia Therapeutics and Liquidia Technologies

Andrew Geall, RNA vaccine platform leader, Novartis Vaccines and Diagnostics, Inc.

Scott Minick, President and CEO, Bind Therapeutics

Don Rose, Director, Carolina KickStart, UNC Chapel Hill

Clay Thorp, General Partner, Hatteras Venture Partners

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11:00-11:30 **Ashutosh Chilkoti**
Duke University

Solving drug delivery problems by genetically engineered nanoparticles

11:30-12:00 **Alexander Tropsha**
UNC Chapel Hill

Computer Modeling of Nanomaterials and Liposome-based Drug Delivery Systems

12:00-12:30 **Yi Yan Yang**
National University of Singapore

Targeted drug delivery towards cancer stem cells using polymeric nanostructures stabilized via non-covalent interactions

12:30-1:50 **Lunch, posters, expo**

Nanomedicine & Education

1:50-2:20 **Russell Mumper**
University of Georgia

Flipping the way we train the next generation of scientists

2:20-3:20 **Panel Discussion:**

Nanomedicine & Education

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Piotr Grodzinski, National Cancer Institute

J. Zach Hilt, University of Kentucky

Michael Jay, UNC Chapel Hill

Alexander Kabanov, UNC Chapel Hill

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Carolina Club

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Vivek Mahajan, University of Nebraska Medical Center, UNC Chapel Hill
Zhijian He, UNC Chapel Hill

Sponsor Presentations

Session Moderator: Marina Sokolsky, UNC Chapel Hill

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Perkin Elmer

The use of single particle ICP-MS in nanomedicine and drug delivery systems

9:40-9:50 **Clinton Hupple**
iThera Medical

Dynamic Evaluation of Nanoparticle Pharmacokinetics and Biodistribution using Multispectral Optoacoustic Tomography (MSOT)

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Session Chairs: Rahima Benhabbour, UNC Chapel Hill
Kristy Ainslie, UNC Chapel Hill

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Nanyang Technological University

Nanofiber-mediated gene-silencing for neural tissue regeneration

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University of Michigan

Elicitation of robust cellular and humoral immunity with vaccine nanoparticles

11:00-11:20 **J. Andrew MacKay**
University of Southern California

Protein polymer nanomedicines for ocular drug delivery

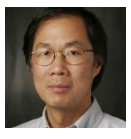
11:20-11:40 **Christian Kastrup**
University of British Columbia

Self-propelling particles that deliver coagulants and other cargos deep into wounds

Closing remarks

nanoDDS 2014 Chairs

Alexander Kabanov, PhD, DrSci
UNC Chapel Hill



Kam Leong, PhD
Duke University

Speakers and Panelists:

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UNC Chapel Hill

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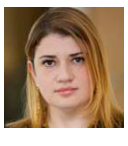

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Houston Methodist Research Institute

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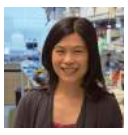

Suzie Pun, PhD
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Neal Fowler, BS, MBA
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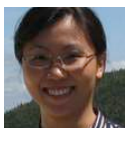

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J. Zach Hilt, PhD
University of Kentucky

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University of Southern California

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UNC Chapel Hill

Christian Kastrup, PhD
University of British Columbia

nanoEXPO

North Carolina Biotechnology Center

UNC Center for Nanotechnology in Drug Delivery

Malvern

QUIDEL

PerkinElmer For the Better

UNC Lineberger

Carolina Center of Cancer Nanotechnology Excellence

CytoViva

ParticleMetrix

CDDN center for drug delivery and nanomedicine

HemaCare BioResearch Products & Services Your Research • Our Cells • Your Way

iTheraMedical Listening to Molecules

Hutchison PLLC

LIQUIDIA TECHNOLOGIES

BIND THERAPEUTICS

WORLDWIDE Life Sciences Division

REMI

Bibby Scientific

SUGroup The Maintenance Solution Innovator

BUCHI SWITZERLAND

nanoDDS

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NC 27514

October 6-8 2014

Symposium Chairs:

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Mescal S. Ferguson Distinguished Professor
UNC Eshelman School of Pharmacy

Kam Leong
James B. Duke Professor
Biomedical Engineering, Duke University

nanoEXPO

SHOW AND EXPOSITION

PREFACE

Dear Colleagues,

It is our pleasure to welcome you to the 12th International Nanomedicine and Drug Delivery Symposium (nanoDDS'14) in Chapel Hill, NC. The history of nanoDDS goes back to January 2003, when Dr. Alexander Kabanov and Dr. Kazunori Kataoka have organized the first nanomedicine meeting in the United States – "The Nanomedicine and Drug Delivery Symposium". Since then nanoDDS has been held annually in different locations across North America. Over years it has attracted over 1,500 participants from 30 different countries and became one of the most authoritative forums in the field of nanomedicine. Each year the meeting comes to a different University campus, thereby promoting knowledge and becoming a world-class scientific event for its students and scholars.

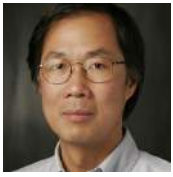
This year the program includes: a) a keynote, plenary and invited lectures by 26 experts in the field on the topics of Cancer nanotechnology, Targeted Drug delivery, Gene & siRNA delivery, Theranostics & Imaging, and Novel Nanomaterials in Biology and Medicine; b) two special sessions on Education and Applied Nanomedicine, followed by panel discussions, each featuring distinguished academic and industrial speakers; c) poster sessions, as well as oral presentations by best abstract and poster winners; and d) an exhibition by industrial partners and academic institutions (**nanoEXPO**).

Enjoy the symposium!

Sincerest regards,
nanoDDS'14 Chairs

Alexander Kabanov

Mescal S. Ferguson Distinguished Professor
UNC Eshelman School of Pharmacy



Kam Leong

James B. Duke Professor
Biomedical Engineering, Duke University

WE THANK OUR SPONSORS!

THANK YOU!

The organizing committee would like to thank the sponsors of the 12th International Nanomedicine and Drug Delivery Symposium whose generosity and support made this symposium possible.

North Carolina Biotechnology Center provided a grant to support the symposium. Our academic sponsors Center for Drug Delivery in Nanomedicine, Carolina Center for Nanotechnology Excellence, UNC Lineberger Comprehensive Cancer Center and Center for Nanotechnology in Drug Delivery also provided generous financial support. We are very grateful to Bind Therapeutics and Liquidia Technologies for their financial support and contribution to the program in Applied Nanomedicine Session and Panel Discussion.

For the first time this year we are hosting a nanoEXPO as part of the symposium and would like to thank our industrial sponsors and exhibitors:Perkin Elmer, Malvern, Quidel, Particle Metrix, Cytoviva, iTheraMedical, HemaCare. Hutchison PLLC, World Wide Medical Products, Remi, Bibby Scientific, Buchi and SU Group. We sincerely hope to continue and our collaborations.

We would also like to thank poster and abstract judges for their valuable time and contribution.

Last but not least we would like to thank graduate students, postdocs, faculty and staff at UNC Eshelman School of Pharmacy who helped with organization of the meeting.

We greatly appreciate and value your support. Thank you!

nanoDDS'14
Organizing Committee

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Microenvironment affects delivery of nanovectors in primary tumors and tumor metastasis

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Tumor Microenvironment-Targeted Cancer Therapy with Nanoparticular Drug Delivery System

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Session Chairs: **Piotr Grodzinsky**, National Cancer Institute
Shawn Hingtgen, UNC Chapel Hill

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Solving Drug Delivery Problems by Genetically Engineered Nanoparticles

11:30-12:00 **Andre Nel,** University of California, Los Angeles
Development of multifunctional nano carrier platforms for cancer treatment

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Russell Mumper, University of Georgia
Flipping the Way We Train the Next Generation of Scientists

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Self-propelling particles that deliver coagulants and other cargos deep into wounds

11:30 **Closing remarks**

Feng Liu

1955–2014

Feng Liu, PhD, was a father to a daughter of whom he was enormously proud, a husband of thirty-one years, a lover of dogs and a lover of ribs, a fan of the Tar Heels and the Steelers, and a brilliant scientist with an NIH-funded research program and a paper that has been cited approximately 1,300 times.

Liu came to the University of North Carolina at Chapel Hill in the summer of 2005 as a research associate professor. He was a colleague and collaborator of Leaf Huang, PhD, and was part of a team that came south from the University of Pittsburgh to join the faculty of the UNC Eshelman School of Pharmacy's Division of Molecular Pharmaceutics.

The small family settled in Durham's Hope Valley where Liu and his wife enjoyed regular evening walks around the neighborhood. Liu indulged his love of food—growing it, grilling it, eating it—but he always stayed thin. His wife accepted a job at "that other school," which inevitably led to good-natured bickering every basketball season.

Liu's research focused on delivering gene therapy and drugs to cells with an emphasis on the treatment of cancer. He was the author of forty-five peer-reviewed papers and ten book chapters and reviews, and he held four patents. Liu's work exploring the use of nanocrystals to treat multidrug-resistant cancer was funded by the National Institutes of Health through 2016. He was promoted to research professor in 2012.

He was well known for his work in developing a hydrodynamic technique for introducing nucleic acids into animal cells—a process known as transfection—during his doctoral studies. This methodology is used worldwide, and the *Gene Therapy* article describing the breakthrough has been cited approximately 1,300 times since it was published in 1999.

Born March 28, 1955, Liu lived much of his life in Shenyang, the capital city of Liaoning Province and the largest city in northeastern China. He was a middle child with an older and a younger sister. He attended Shenyang Pharmaceutical University from 1978 to 1982. After earning a bachelor's degree in pharmacy, he worked as a research associate at the university before returning to the classroom at Shenyang to pursue a master's degree in pharmaceutics, which he received in 1988.

Liu married and started a family in Shenyang but almost didn't. He was so nervous before a blind date that he suffered a panic attack and backed out of the first meeting with the woman who would become his wife. He regrouped. The second attempt was much more successful and led to marriage in 1983 and the birth of a daughter in 1985 who would go on to graduate from the UNC School of Medicine.

In 1993, Liu came to the United States as a visiting scientist at the University of Pittsburgh. He began work on a PhD in pharmaceutics at Pitt in 1996 and earned his doctorate three years later. He then completed a postdoctoral fellowship at the Pitt School of Pharmacy's Center for Pharmacogenetics and joined the center as an instructor in 2001 before being promoted to research assistant professor in 2002. In 2004 he was the recipient of an NIH Career Development Award.

Although he had been in the U.S. for more than twenty years, Liu retained strong ties to China. He had served as a guest professor at Xi'an Jiaotong University in Xi'an since 2004 and at his alma mater, Shenyang, since 2008.

If you asked him what his greatest accomplishment was, he probably wouldn't even think to mention that *Gene Therapy* paper that everyone else talks about. Instead he might have told you that over the past five years on his nightly walks with his wife he had found four lost dogs and successfully returned them to their owners.

Liu is survived by his wife, who is a research analyst at Duke University, and his daughter, who is a physician in Asheville, North Carolina.

Speaker Bios and Abstracts

Leaf Huang graduated from the physics department of National Taiwan University and received a PhD from the Michigan State University. He taught at the University of Tennessee and the University of Pittsburgh. He is now the Fred Eshelman Distinguished Professor, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill. His research focuses on non-viral gene therapy and targeted chemotherapy for cancer. A vector developed in his lab was used in the first non-viral clinical trial in1992, which pioneered the clinical studies in non-viral gene therapy. Professor Huang has published more than 500 scientific articles (H-index 101), 21 patents, and has received the Bangham Achievement Award. He was awarded the 2013 Distinguished Pharmaceutical Scientist Award of the American Association of Pharmaceutical Scientists. This is the highest scientific recognition of AAPS. Professor Huang has co- founded 6 biotech start-up companies.

Hsing-Wen Sung is a Tsing Hua Chair Professor, Department of Chemical Engineering and the Director of Institute of Biomedical Engineering, National Tisng Hua University. He received his PhD degree from Department of Chemical Engineering and Biomedical Engineering Center, Georgia Institute of Technology in May 1988. His research interests are nanobiomaterials, nanomedicine, drug/gene delivery, and tissue engineering. Professor Sung has received numerous awards such as Fellow of American Institute for Medical and Biological Engineering, Fellow of International Union of Societies for Biomaterials Science and Engineering, Academician of Asia Pacific Academy of Materials, Ho Chin Tui Outstanding Research Award, National Science Council Outstanding Research Award, and Professor Tsai-The Lai Award. He has published 240 scientific papers and received 70 international patents.

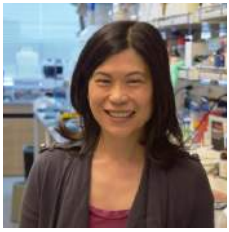
Biana Godin Vilentchouk earned her Ph.D. in Pharmaceutical Sciences from the Hebrew University of Jerusalem in 2006. During her Ph.D. studies, Dr. Godin Vilentchouk focused on designing a non-invasive treatment for the hard-to-treat skin deep infections and on nasal delivery of proteins. She was recruited as a Postdoctoral trainee in the field of Cancer Nanotechnology to the research team of Dr. Mauro Ferrari at the University of Texas Health Sciences Center. Her postdoctoral research focused on injectable multistage nanovectors (MSVs) for cancer treatment and imaging. Her work provided insightful understanding of the interactions of MSVs with immune cells in vivo and in vitro, biocompatibility of MSV, effect of geometry and targeting moieties on MSV biodistribution, and evaluation of MSV systems as near-infra red and MRI imaging agents. During the three years of her postdoctoral research, Dr. Godin Vilentchouk

published numerous book chapters, and peer-reviewed articles and reviews in top journals including Nature Nanotechnology and Cancer Research. She also received awards for her work at international conferences. In October 2010, Dr. Biana Godin Vilentchouk joined the Department of Nanomedicine at the Research Institute.

Jun Wang is a professor of Life Sciences and Polymer Chemistry of University of Science and Technology of China, an adjunct professor of Hefei National Laboratory for Physical Sciences at the Microscale and CAS Key Laboratory of Innate Immunity and Chronic Disease. He received his B.Sc. degree in Chemistry and Cell Biology in 1993 and a Ph.D. degree in Polymer Chemistry and Physics in 1999 from Wuhan University, China. From 1999 to 2004, he worked as a postdoctoral fellow at Johns Hopkins Singapore and Johns Hopkins School of Medicine. In 2004, he joined the faculty of University of Science and Technology of China as a full professor. His main research interests cover novel drug delivery systems and nanomedicine. He published 120 peer-reviewed papers and 8 patents. In 2013, he received the First prize of Natural Science Award of the Ministry of Education of China. He was selected as the awardee of “One Hundred Talents” of Chinese Academy of Science in 2005 and “National high-level personnel of special support program” in 2013. He received “Outstanding Young Scholar Award” of National Science Foundation of China in 2011.

Anthony Hickey is Distinguished RTI Fellow, Program Director in Inhaled Therapeutics in the Center for Aerosol and Nanomaterials Engineering at the Research Triangle Institute, Emeritus Professor of Molecular Pharmaceutics of the Eshelman School of Pharmacy (2010-present, Professor 1993-2010), and Adjunct Professor Biomedical Engineering in the School of Medicine, at the University of North Carolina at Chapel Hill. He obtained Ph.D. (1984) and D.Sc. (2003) degrees in pharmaceutical sciences from Aston University, Birmingham, UK. Following postdoctoral positions, at the University of Kentucky (1984-1988) Dr. Hickey joined the faculty at the University of Illinois at Chicago (1988-1993). In 1990 he received the AAPS Young Investigator Award in Pharmaceutics and Pharmaceutical Technology. He is a Fellow of the UK Society of Biology (2000), the American Association of Pharmaceutical Scientists (2003) and the American Association for the Advancement of Science (2005). He received the Research Achievement Award of the Particulate Presentations and Design Division of the Powder Technology Society of Japan (2012) and the Distinguished Scientist Award of the American Association of Indian Pharmaceutical Scientists (2013). He has published numerous papers and chapters in the pharmaceutical and biomedical literature, one of which received the AAPS Meritorious Manuscript Award in 2001. He has edited five texts on pharmaceutical inhalation aerosols and co-authored

three others on ‘Pharmaceutical Process Engineering’, pharmaceutical particulate science and ‘Pharmaco-complexity’. He is founder (1997, and formerly President and CEO, 1997-2013) of Cirrus Pharmaceuticals, Inc., which was acquired by Kemwell Pharma in 2013; founder (2001, and formerly CSO, 2002-2007) of Oriel Therapeutics, Inc, which was acquired by Sandoz in 2010 and founder and CEO of Astartein, Inc. (2013-present); member of the Pharmaceutical Dosage Forms Expert Committee of the United States Pharmacopeia (USP, 2010–2015, Chair of the sub-committee on Aerosols) and formerly Chair of the Aerosols Expert Committee of the USP (2005-2010). He is Principal Investigator for the ‘NIH Nanomaterial Registry’ (a multi-institute program). Dr. Hickey conducts a multidisciplinary research program in the field of pulmonary drug and vaccine delivery for treatment and prevention of a variety of diseases.



Suzie H. Pun received her Chemical Engineering Ph.D. degree in 2000 from the California Institute of Technology. She then worked as a senior scientist at Insert Therapeutics/ Calando Pharmaceuticals for 3 years developing polymeric drug delivery systems before joining the Department of Bioengineering at University of Washington (UW). She is currently the Robert J Rushmer Professor of Bioengineering, an Adjunct Professor of Chemical Engineering, and a member of the Molecular Engineering and Sciences Institute at UW. Her research focus area is in biomaterials and drug delivery and she has published over 75 research articles in this area. Current application areas for her group include biologics delivery to the central nervous system and cancer. For this work, she was recognized with a Presidential Early Career Award for Scientists and Engineers in 2006, the 2014 Inaugural Biomaterials Science Lectureship, and the 2014 Young Investigator Award from the Controlled Release Society.

Simona Mura gained the degree in Pharmaceutical Chemistry and Technology in 2005. In 2009, she was awarded her PhD in Chemistry and Technology of Drug at the University of Cagliari, Italy, working on the design and in vitro evaluation of novel vesicular systems for the topical delivery of drugs. In 2008 she joined the group of Pr. Elias Fattal (UMR CNRS 8612) at University Paris-Sud, Châtenay-Malabry, France, as a post-doctoral research assistant to study the lung toxicity of biodegradable nanoparticles designed for pulmonary administration of drugs. In 2011 she was appointed Associate Professor within the framework of the « CNRS-Higher Education chairs » program, in the group of Pr. Patrick Couvreur (UMR CNRS 8612) at University Paris-Sud, Châtenay-Malabry, France. Her background is in formulation/characterization and in vitro and in vivo evaluation of drug delivery systems. Her research focuses on the development of in vitro and in vivo models for the assessment of the therapeutic efficacy of novel nanoscaled drug delivery systems and biopolymers for the treatment of severe neoplastic diseases.

Horacio Cabral received his Ph.D. under the supervision of Prof. K. Kataoka in Materials Engineering from the University of Tokyo in 2007. He worked as an assistant professor at the Division of Clinical Biotechnology, Graduate School of Medicine, the University of Tokyo until 2009. Since 2010, he has been an associate professor at the Department of Bioengineering, the University of Tokyo. His main research interests relate to smart nanodevices for the diagnosis and therapy of cancer

Gaurav Sahay is an Assistant Professor in the College of Pharmacy at Oregon State University/Oregon Health Science University in Portland, Oregon. Dr. Sahay completed his postdoctoral research with Prof. Robert Langer and Prof. Daniel Anderson at Koch Institute for Integrative Cancer Research at MIT. He received his PhD from the Lab of Dr. Alexander Kabanov at the University Of Nebraska Medical Center (UNMC). He holds a Masters in Pharmacology from UNMC and Bachelors in Pharmacy from University of Pune. His work is on the interface of nanotechnology, cell biology and drug delivery. Dr. Sahay's lab is interested in unlocking the molecular mechanisms involved in the transport of nanoparticles to deliver small molecules, nucleic acids etc. inside cells. Most nanoparticles utilize intracellular trafficking to enter cells and even the most of efficacious amongst them remain trapped inside endosomes and the productive sites of escape remain elusive. At MIT using a combination of high-throughput confocal imaging, small molecule library that impacts endocytosis, cell signaling and autophagy he uncovered that exocytosis of lipid nanoparticles (LNPs) from the late endosomes was a major rate limiting step for siRNA delivery. His current interest lie in understanding nanoparticle mediated delivery of modified mRNA for protein production inside cells, with focus on therapeutic production of proteins in the treatment of lysosomal storage disorders. The long term goals are to gain mechanistic insights that provide a blueprint for development of new materials and targets for nanotechnology based therapies. He has 21 publications in top tier journals including Nature Biotechnology, Nature Nanotechnology, PNAS, Advanced Materials, ACS Nano etc and has one of the most cited review article in the Journal of Controlled Release from his graduate school days with Prof. Kabanov. He is the winner of 2013 AAPS Postdoctoral Fellow Award and Nature's Sci-Bx Innocentive challenge.

Kathryn A. Whitehead is an Assistant Professor in the Department of Chemical Engineering at Carnegie Mellon University, with a courtesy appointment in the Department of Biomedical Engineering. The interdisciplinary research interests of her lab include the chemical and biophysical analysis of biomaterials as well as their use in delivering macromolecular therapeutics to diseased tissue. Prof.

Whitehead obtained both her B.S. and Ph.D. in chemical engineering, the former from the University of Delaware in 2002 and the latter from the University of California, Santa Barbara in 2007. Her doctoral work with Samir Mitragotri focused on the engineering of oral delivery systems for macromolecules. From 2008-2012, Prof. Whitehead conducted her postdoctoral work on RNA interference technologies with Robert Langer and Daniel Anderson in the Koch Institute for Integrative Cancer Research at MIT. She has received awards from the Controlled Release Society and the American Diabetes Association as well as several fellowships, including an NIH NRSA postdoctoral fellowship. Several of her patents have been licensed and are currently being developed for reagent and therapeutic use.

Stephen Zale, has served as Vice President, Development at BIND Therapeutics since 2006. Previously, Dr. Zale was Vice President of Injectable Products R&D at Alkermes, where he led the group responsible for formulating Alkermes' biodegradable polymer-based microsphere products, including Nutropin Depot, Risperdal CONSTA, Bydureon and Vivitrol. Prior to joining Alkermes, Dr. Zale led the Bioseparations R&D group at Sepracor Inc. Dr. Zale received a Ph.D. in Biochemical Engineering from MIT and a B.S. in Chemistry, also from MIT.

Benjamin Yerxa is a founding officer of Envisia Therapeutics and is the CSO for both Liquidia and Envisia. Dr. Yerxa joined Liquidia from Clearside Biomedical. Prior to Clearside, Dr. Yerxa was the Executive Vice President and Chief, Research & Development of Inspire Pharmaceuticals, a top ranked publicly traded biotech company acquired by Merck & Co., Inc. During his time at Inspire, Dr. Yerxa helped the company build and commercialize a portfolio of innovative new products and provided critical support through multiple rounds of financing, including the company's initial public offering (IPO). Throughout his 20-year career in the pharmaceutical and biotechnology industry, Dr. Yerxa has been involved with the discovery and development of several investigational new drugs (INDs), phase 3 clinical programs, new drug applications (NDAs) and drug approvals. His experience spans a variety of therapeutic areas including ophthalmology, pulmonary, cardiovascular and HIV. Dr. Yerxa has more than 50 U.S. patents to his name, led a variety of licensing deals including technology transfers and manufacturing agreements and has built several R&D and corporate functions from inception. Dr. Yerxa serves on the board of directors of the North Carolina Biotechnology Center, the Nanomedicines Alliance and Sharefish.

Ralph Lipp is an accomplished innovator and leader in the life sciences area with over 20 years of experience in innovation-based value creation. At Lipp Life Sciences LLC he provides advice to clients from various sectors of the life sciences industries, including Top 5 pharma, drug delivery, and biotech companies, both in the US and Europe. Recent projects include innovation process re-design, product design, IP analysis, and biotech M&A strategy development. Ralph also serves as Founding Advisory Board Member for the Catalent Applied Drug Delivery Institute. Before he founded Lipp Life Sciences LLC, Ralph served for more than two decades in various research and development leadership roles in the biopharmaceutical industry, including Vice President Pharmaceutical Sciences R&D at Eli Lilly and Company and Head Pharmaceutical Development at Schering AG. His more than 120 publications in the life sciences sector include over 20 patents, covering five marketed medicines. Ralph is a pharmacist, holds a Ph.D. in Medicinal Chemistry, and graduated from INSEAD's International Executive Program as well as from Harvard Business School's Advanced Management Program.

Neal Fowler is the CEO of both Liquidia Technologies and Envisia Therapeutics. He joined Liquidia in 2008 after seven successful years at Johnson & Johnson (J&J). While at J&J, Neal served as President of Centocor, Inc., a multi-billion dollar subsidiary focused on development and commercialization of industry leading biomedicines used in the treatment of chronic inflammatory diseases. At Centocor, Mr. Fowler prepared the company for the launch of two potential blockbuster products and continued the company's legacy of double-digit growth for their flagship product REMICADE® (infliximab). Prior to Centocor, Mr. Fowler was president of Ortho-McNeil Neurologics Inc. and vice president of the central nervous system franchise at Ortho-McNeil Pharmaceuticals. Mr. Fowler joined J&J after a successful 13-year career at Eli Lilly and Company where he held a variety of sales, marketing and business development roles with increasing responsibilities in both the pharmaceutical and medical divisions. Mr. Fowler is a native of Raleigh, NC and received a Bachelor of Science degree in Pharmacy and Masters of Business Administration from the University of North Carolina at Chapel Hill (UNC-CH).

Andrew J. Geall, is the RNA Vaccine Platform Leader at Novartis Vaccines, Inc. (Cambridge, USA). He has undergraduate degrees in Chemical Engineering and Pharmacy and completed his Ph.D. in gene delivery at the University of Bath U.K. in 1999. Before joining Novartis in 2006, Dr. Geall was manager of the Pharmaceutics department at Vical (San Diego). Here, he led the formulation development of the company's DNA vaccine program and

was responsible for production of the gene delivery systems for clinical trials. He joined Novartis Pharmaceuticals AG to lead the siRNA delivery efforts and in 2008 moved to the Vaccines Research division to establish the RNA Vaccine Platform. He has made major contributions to the patent estate and publications for this novel technology.

Scott Minick has served as President and Chief Executive Officer of BIND Therapeutics since January 2010. From 1998 to January 2010, Mr. Minick was a Managing Director of ARCH Venture Partners, a venture capital firm, and was instrumental in the startup, development and financing of numerous ARCH portfolio companies, including BIND Therapeutics. From 1995 to 1998, Mr. Minick was Director, President and Chief Operating Officer of SEQUUS Pharmaceuticals, Inc., a biopharmaceutical company that was acquired by ALZA Corporation. He received his postgraduate training in neurobiology at the Salk Institute, an M.B.A. from Northwestern University and a B.A. from the University of California at San Diego.

Don Rose is Director of UNC's Carolina KickStart Program, a life science and biomedical commercialization program within the UNC TraCS Institute. In addition, he is an Adjunct Professor at UNC's Kenan-Flagler Business School. Prior to that, he held senior leadership roles at a number of life science startups: Metabolon (metabolomics, RTP), Deerac Fluidics (nanoliter dispensing, Dublin), and DataCentric Automation (high-throughput crystallography, Nashville). Before that, he was a general partner with Catalysta Partners (now Hatteras Venture Partners), a seed stage venture fund specializing in information technology and biotechnology start-ups. During this time, Don was co-founder and CEO of Phase Bioscience, a biomaterials start-up, spun out of Duke University. Prior to Catalysta, he was co-founder and VP of Research and Development for Cartesian Technologies, a leader in instrumentation for DNA microarrays and nanoliter dispensing. Prior to Cartesian, Dr. Rose was a research scientist at Glaxo Wellcome (now GSK) where he developed and promoted a number of technologies in bioanalytical chemistry, combinatorial chemistry and high-throughput screening. Prior to GW, Dr. Rose developed various aspects of capillary electrophoreses instrumentation at Hewlett-Packard Laboratories. Dr. Rose received his Ph.D. in Analytical Chemistry and BS in Nutrition from the University of North Carolina. He has published eight papers, two book chapters, and holds six US patents.

Clay B. Thorp is an entrepreneur turned venture capitalist. Since 1995, Clay has co-founded seven companies in the life science arena and co-founded Hatteras in 2001. Since co-founding Hatteras, Clay has been instrumental in building the firm and has led investments in a range of life science companies, including biopharmaceutical, medical device, diagnostics, and research informatics.

Clay was the lead investor and led the strategic transaction process for ArtusLabs that resulted in its sale to PerkinElmer in 2011. He was a co-founder and served as CEO and Chairman of Synthematix, Inc., a chemistry informatics company that was acquired in April 2005 by Symyx Technologies (SMMX). Clay is a co-founder and former Chairman of PhaseBio Pharmaceuticals, Inc. He was the co-founder and head of corporate development for Novalon Pharmaceutical Corporation, where he led financing efforts and was head of business development from inception until Novalon's sale to Karo Bio in May of 2000. Prior to Novalon, Clay was the co-founder and president of Xanthon, Inc., a bioinformatics company with electro-chemical detection technology for direct analysis of DNA, RNA and proteins.

Clay currently serves as Chairman of GeneCentric Diagnostics and Lead Director of Pathfinder Therapeutics. He is also on the boards of PhaseBio Pharmaceuticals, Clearside Biomedical, and G1 Therapeutics. He holds a Masters of Public Policy from Harvard University and a B.A. in mathematics and history from the University of North Carolina at Chapel Hill.

Ashutosh Chilkoti is the Theo Pilkington Chair in Biomedical Engineering at Duke University. Prof. Chilkoti was awarded the CAREER award by the National Science Foundation in 1998, the 3M non-tenured faculty award in 2002, and was awarded the Distinguished Research Award from the Pratt School of Engineering at Duke University in 2003 and in 2005. He was awarded a senior researcher award by the Alexander Von Humboldt Foundation in 2010, the Clemson Award for Contributions to the Literature by the Society for Biomaterials in 2011, and the 2013 Robert A. Pritzker Distinguished Lecture award by the Biomedical Engineering Society. He is currently the Director of the Center for Biologically Inspired Materials and Materials Systems at Duke University. His areas of research include Biomolecular Engineering with a focus on stimulus responsive biopolymers for applications in protein purification and drug delivery, and Biointerface Science, with a focus on the development of clinical diagnostics and plasmonic biosensors. He has co-authored over 250 publications, has been cited ~15,000 times, has an H-index of 72, and has 18 patents awarded and 43 in process. He is the founder of two start-up companies: PhaseBio Pharmaceuticals that has raised $65 million in venture capital funding and is taking drug delivery technology that he developed into clinical trials, and Sentilus, that is developing point-of-care clinical diagnostics based on a polymer brush technology developed in his laboratory. He serves on the Editorial Board of five journals, and is a reviewer for over 20 other journals.

André Nel is a Professor of Medicine and Chief/founder of the Division of NanoMedicine at UCLA. He is the Director of the UC Center for the Environmental Implications of Nanotechnology (UC CEIN) and also directs the NIH-funded UCLA Center for Nano Biology. Dr. Nel obtained his medical and Doctorate of Medicine (M.D.) degrees at Stellenbosch University in Cape Town, South Africa, and did Clinical Immunology training with board certification in the U.S. Dr. Nel is peer-selected member of Best Doctors of America since 1998, and has been the recipient of the John Salvaggio Memorial Award recognizing his outstanding service to the specialty and science of Allergy and Immunology. He is a recipient of the Harry Truman Award from Sandia National laboratories, and on behalf of UC CEIN received the Governor's Economic and Environmental Leadership Award in California in 2013. He is serving as a panel member for the U.S. President's Council of Advisors for Science and Technology (PCAST) for the review on the National Nanotechnology initiative (NNI), and has represented the NIH and the NNI in cooperative research agreements with Japan, the Chinese Academy of Sciences and Russia. He is an Honorary Foreign Professor in the Chinese Academy of Sciences, and is Associate Editor of ACS Nano. Dr. Nel's chief research interests are: (i) Nanomedicine and nanotherapeutics; (ii) Nanobiology with particular emphasis on nanomaterial interfacial properties and quantitative structure-activity relationships; (iii) Nanotechnology environmental health and safety, with particular emphasis on predictive toxicological modeling, high throughput safety screening, and safe implementation of nanotechnology in humans and the environment. His research is funded by personal (RO1) and center grants from the National Institute of Environmental Health Sciences, National Cancer Institutes, the EPA and the NSF. Dr. Nel and his collaborators have developed several nanotechnology patents and he is an advisor to PCAST, industry, U.S. federal agencies and EU nanosafety centers.

Yi Yan Yang is a group leader and principle research scientist with A*STAR Institute of Bioengineering and Nanotechnology, Singapore. She holds a Ph.D. in Chemical Engineering, Tsinghua University (China), 1990. She is currently also an Adjunct Associate Professor with the Department of Pharmacy, National University of Singapore. She has 157 peer-reviewed journal publications (e.g. Nat. Mater., Nat. Nanotech., Nat. Chem., Nat. Commun., Adv. Mater., Adv. Funct. Mater., Angew. Chem. Intl. Ed., J. Control. Release, Biomaterials, Adv. Healthcare Mater.) and 38 international patent applications. Dr Yang has received many invitations to hold lectures and seminars in Singapore and overseas. In addition to her scientific contributions, Dr Yang has organised and chair-ed a number of symposiums and seminars over the years. In 2009, Singapore Women's Weekly Magazine awarded her the Great Women of Our Time Award, Science and Technology Category. Dr Yang's other professional activities include member of Material Research Society (MRS) in Singapore and MRS (USA), and member of American Chemical Society (ACS). Her research interests

lie in drug/gene delivery, cancer therapy, antimicrobial polymers and peptides, and biomaterials.

Russel Mumper joined the University of Georgia as its vice provost for academic affairs in August 2014 following a national search. He was previously vice dean and John A. McNeill Distinguished Professor at the University of North Carolina-Chapel Hill Eshelman School of Pharmacy. He has received nearly $30 million in research grants and contracts and has published more than 300 peer-reviewed scientific manuscripts and abstracts in areas such as nanotechnology, drug delivery and plant-based anti-cancer and anti-inflammatory compounds. He is a named inventor on 45 patents or patent families, many of which have been licensed to companies, and has co-founded five companies as a faculty member. In addition, he has started and led or co-led several multidisciplinary research, development and manufacturing centers. Mumper began his academic career in 1999 at the University of Kentucky College of Pharmacy, after spending nearly a decade working in the pharmaceutical and biotechnology industries. He has received several research and education honors throughout his career. In 2007, the University of Kentucky Alumni Association recognized him as one of six “Great Teachers” at UK, the oldest continuously given award for teaching at the institution. In 2013, he received the Distinguished Teaching Award for Post-Baccalaureate Instruction, a campus-wide award at UNC.

Piotr Grodzinski is a Director of NCI Alliance for Nanotechnology in Cancer at the National Cancer Institute in Bethesda, Maryland. He coordinates program and research activities of the Alliance which dedicates around $150M over funding period of 5 years to form interdisciplinary centers as well as fund individual research and training programs targeting nanotechnology solutions for improved prevention, detection, and therapy of cancer. Dr. Grodzinski graduated from the University of Science and Technology (AGH) in Krakow, Poland and continued his studies at the University of Southern California in Los Angeles, where he researched novel semiconductor materials used in low threshold lasers. In mid-nineties, Dr. Grodzinski left the world of semiconductor research and got interested in biotechnology. He built a large microfluidics program at Motorola Corporate R&D in Arizona. The group made important contributions to the development of integrated microfluidics for genetic sample preparation with its work being featured in Highlights of Chemical Engineering News and Nature reviews. After his tenure at Motorola, Dr. Grodzinski was with Bioscience Division of Los Alamos National Laboratory where he served as a Group Leader and an interim Chief Scientist for DOE Center for Integrated Nanotechnologies (CINT). At the National Institutes of Health (NIH), in addition to his programmatic responsibilities, he co-chaired Trans-NIH Nanotechnology Task Force, which is coordinating the nanotechnology efforts across 27 institutes of the agency with the budget over $300M/year.

Dr. Grodzinski received Ph.D. in Materials Science from the University of Southern California, Los Angeles in 1992. He is an inventor on 17 patents and published 58 peer-reviewed papers and 10 book chapters. Dr. Grodzinski has been recently elected a Fellow of the American Institute for Medical and Biological Engineering.

J. Zach Hilt is the William T. Bryan Associate Professor of Chemical Engineering in the Department of Chemical and Materials Engineering at the University of Kentucky. Prof. Hilt received his bachelor degrees in Chemistry and Physics from Miami University (Ohio). He completed his Masters degree in Chemical Engineering from Purdue University and his Doctor of Philosophy in Chemical Engineering from the University of Texas at Austin. Prof. Hilt's research laboratory focuses on the design of novel nanocomposite materials, the development of novel methods to synthesize and characterize these advanced materials at the micro- or nanoscale, and the application of these nanocomposites as biomaterials, in cancer treatment, environmental remediation, etc. In addition to numerous referred publications and book chapters, he is co-editor of "Nanotechnology in Therapeutics: Current Technology and Applications" which is published by Horizon Scientific Press and is one of the first books dedicated to the topic of nanotechnology in drug delivery. Prof. Hilt is a co-investigator and Steering Committee member of the NIH-funded UK Cancer Nanotechnology Training Center, and is a co-investigator and Executive Committee member of the UK NSF IGERT Program on Bioactive Interfaces and Devices. He is also co-Director of the UK NSF REU Program on Bioactive Interfaces and Devices. He has taken an active role in various professional societies, including AIChE where he is currently serving as the chair of the AIChE Nanoscale Science and Engineering Forum.

Michael Jay is the Fred Eshelman Distinguished Professor and Chair, Division of Molecular Pharmaceutics in the Eshelman School of Pharmacy, University of North Carolina at Chapel Hill. Dr. Jay's research has been at the interface of the pharmaceutical and nuclear sciences. This includes the application of pharmaceutical approaches to solve problems related to nuclear medicine and therapy, and the use of radioanalytical approaches to solve problems encountered in the development of novel formulations and drug-delivery systems. His current work involves the development of orally-bioavailable radionuclide decorporation agents for use following a nuclear terrorism event, and the use of neutron-activated nanoparticles as radiotherapeutic agents. He is the recipient of the Berson-Yalow Award from the Society of Nuclear Medicine, the Mendell Award for Scientific Excellence in the Pharmaceutical Sciences, and is a Fellow of the American Association of Pharmaceutical Scientists. He is also the Chief Scientific Officer for two companies he co-founded and actively consults for the pharmaceutical industry.

Alexander Kabanov is a Mescal Swaim Ferguson Distinguished Professor, Director, Center for Nanotechnology in Drug Delivery, and Co-Director, Carolina Institute for Nanomedicine, University of North Carolina at Chapel Hill since July 2012. Prior to this appointment he served for nearly 18 years at the University of Nebraska Medical Center where he was a Parke-Davis Professor of Pharmaceutical Sciences and Director of the Center for Drug Delivery and Nanomedicine, which he founded in 2004. Dr. Kabanov received Ph.D. degree in chemical kinetics and catalysis in 1987 at Moscow State University, USSR. He has conducted pioneering research on polymeric micelles, DNA/polycation complexes, block ionomer complexes and nanogels for delivery of small drugs, nucleic acids and proteins that considerably influenced current ideas and approaches in drug delivery and nanomedicine. His work led to first-in-man polymeric micelle drug (SP1049C) to treat cancer, which successfully completed Phase II clinical trial and is under further evaluation. He co-founded Supratek Pharma, Inc. (Montreal, Canada), which develops therapeutics for cancer and Neuro10-9, Inc. (Omaha, NE and Chapel Hill, NC) focusing on diseases of the central nervous system. Dr. Kabanov published over 250 scientific papers, and has over 100 patents worldwide. His work was cited over 18,000 times (Hirsh index 73). His cumulative research support in academia as Principal Investigator has been over $45 M (and over $95 M total). His inventions attracted nearly $60M in private, foundation, and company-sponsored R&D funding in industry. He founded Nanomedicine and Drug Delivery Symposium series (2003-) and co-chaired Gordon Research Conference on Drug Carriers in Medicine and Biology (2006).

Jinming Gao is a Professor of Oncology and Pharmacology in the Simmons Cancer Center and Department of Pharmacology at UT Southwestern Medical Center. He also holds an adjunct professorship in Chemistry and Bioengineering at UT Dallas. Dr. Gao has published over 100 peer-reviewed papers in cancer nanotechnology and nanomedicine. He has received several awards including the Outstanding Scientist Award from the Society for Experimental Biology and Medicine. He is currently serving as the Chair of Gene and Drug Delivery study section at the NIH. He co-founded OncoNano Medicine, a startup company focusing on the translation of tumor-activatable nanoprobes for image-guided cancer surgery. Using a highly-interdisciplinary approach, Dr.Gao's lab is developing and applying new nanotechnology tools for cancer-targeted imaging and therapy.

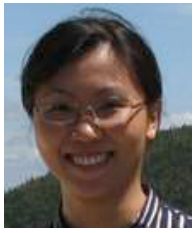
Rui Zhang is a postdoctoral fellow in Prof. Jindřich Kopeček's lab at the Department of Pharmaceutics and Pharmaceutic Chemistry at University of Utah. He received his B.S. in biotechnology and M.S. in biochemistry degrees at Zhejiang University in 2002 and 2006, respectively, and Ph.D. in biomedical science from the University of Texas MD Anderson Cancer Center in 2011. His research focuses on using multiple imaging technologies to study drug delivery systems.

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

She is a leader in the field of nanomedicine and angiogenesis (cancer and vascular biology). She serves as advisor to several Israeli and International biotech companies, is President of the Israeli Society for Controlled Release, and is on the editorial boards of several biological and chemical journals. She has published more than 65 papers, 12 book chapters, edited 2 books, is named inventor on 23 patents, and was awarded numerous prestigious grants and prizes, among them Fulbright, Rothschild, Wingate, Alon, Young Investigator Award of the European Association for Cancer Research, 50 most influential women in Israel (Globes, Calcalist, TheMarker, Forbes), Juludan Prize for the Advancement of Technology in Medicine, the 2013 Teva Pharmaceutical Industries Founders Award for the Discovery of new molecular mechanisms and targets that would lead to new therapeutic approaches. Recently, she received the European Research Council (ERC) Consolidator Award and the Saban Family Foundation-Melanoma Research Alliance (MRA) Team Science Award.

Chady Stephan, Manager, Global Applications – Nanotechnology, have a Ph.D. in analytical chemistry from University of Montreal, Canada. Joined PerkinElmer in 2009 as an inorganic product specialist supporting AA, ICP-OES and ICP-MS products coming from QSAR Risk Assessment Service where he worked as a project manager overseeing human health and ecological risk assessment projects associated with the potential effects of chemicals, radionuclides and pathogens in soil, water, air and sediments.

Clinton Hupple's work has primarily focused on ultrasound, photoacoustic and molecular imaging methods in cancer research. As a graduate student he focused on using ultrasound and microbubbles to study endothelial cell death in tumors in response to high dose radiotherapy. As part of this project, he developed software tools to help quantify tumour vascular response using power Doppler ultrasound. After receiving his masters he moved to the University of South Australia to learn and develop optical molecular imaging techniques for quantification and visualization of compound biodistribution in vivo. For the past several years Clinton has been working as an application scientist, working with MRI, optoacoustic and ultrasound imaging.



Sing Yian Chew is an Associate Professor at the Lee Kong Chian School of Medicine and the School of Chemical & Biomedical Engineering, Nanyang Technological University (NTU), Singapore. She obtained her Ph.D. at Johns Hopkins University under the sponsorship of the NTU Overseas Scholarship. After joining NTU, she continues to embark on scientific learning and exchanges by serving as visiting scholar/professor to INSERM (U698 and U791), France; University of Paris 13; University of Nantes; Jinan University in Guangzhou, China; and Wyss Institute at Harvard.

Dr. Chew's research interest lies in understanding the combined effects of nanotopography and biochemical signaling in directing cell fate. Specifically, her lab engineers nanofiber platforms for long-term delivery of biochemicals. Currently, Dr. Chew's works focus on scaffold-mediated non-viral delivery of small non-coding RNAs for long-term gene silencing applications. These biofunctional platforms are used for understanding and directing neural tissue regeneration post-traumatic injuries, stem cell fate and host-implant integration.

James Moon is the John Gideon Searle Assistant Professor in the Department of Pharmaceutical Sciences and Biomedical Engineering at the University of Michigan, Ann Arbor. Dr. Moon is also a member of the Biointerfaces Institute and Michigan Nanotechnology Institute for Medicine and Biological Sciences at the University of Michigan. His interdisciplinary research program aims to develop novel biomaterials-based strategies to advance fundamental understanding of the immune system and to modulate immune functions, with the ultimate goal of improving vaccines and cancer immunotherapies. He is the recipient of the 2014 New Investigator Award in Pharmaceutics from the American Association of Pharmaceutical Scientists, the 2012 NIAID Research Scholar Development Award, and the 2011 IEEE-EMBS Harvard Wyss Institute Award for Translational Research. Dr. Moon received his Ph.D. from Rice University, and he completed his postdoctoral training with Prof. Darrell Irvine (HHMI) at MIT.

J. Andrew MacKay received his S.B. in chemical engineering and biology from the Massachusetts Institute of Technology in 1999. A Howard Hughes Medical Institute Predoctoral Fellow, he completed his Ph.D. at the University of California at San Francisco and Berkeley in the joint graduate group in Bioengineering in 2005. As a Kirschstein National Research Service Award Postdoctoral Fellow, Dr. Mackay studied at Duke University in the Department of Biomedical Engineering. In 2008 Dr. MacKay joined the faculty at the University of Southern California as an assistant Professor. Dr. MacKay is the author on 41 peer-reviewed publications, is an inventor on 4 patents under prosecution by USC, and is involved with efforts to commercialize biomedical inventions made at USC. Dr. MacKay's research group develops next generation drug carriers that target disease microenvironments, including those in cancer, the liver, and the eye. His group specializes in the use of genetic engineering to produce protein polymers that: (i) drive self-assembly of proteins and drugs into multivalent, nanoparticles (10-200 nm in diameter); (ii) enable diagnostic imaging of distribution in the body; and (iii) promote selective activity or accumulation at target locations. The nanoparticles his group develops are highly innovative, as they are composed entirely from polypeptides. Biologically inspired by the human gene for tropoelastin, these elastin-like polypeptides are biodegradable, biocompatible, switchable, and may be seamlessly fused with an array of biopharmaceuticals. Dr. MacKay studies the biophysics of these systems and utilizes modeling to understand and tune their behavior for activity in biological environments, such as the blood, tumor, or cytosol.

Christian Kastrup is an Assistant Professor in the Michael Smith Laboratories and Department of Biochemistry & Molecular Biology at the University of British Columbia, joining UBC in 2011. He is a member of the Centre for Blood Research and an associate member of the Biomedical Engineering Program. He did his postdoctoral fellowship in Professor Robert Langer's laboratory at MIT, where he specialized in engineering biomaterials for drug delivery. He received his PhD from the University of Chicago, working with Professor Rustem Ismagilov, where he specialized in chemical biology, microfluidics, and blood coagulation. His lab at UBC utilizes biochemical engineering to solve problems related to coagulation and cardiovascular disorders. They investigate, utilize, and mimic the biochemistry and biophysical dynamics of coagulation to create innovative materials that perform new functions inside of blood vessels. He recent accolades include a New Investigator Award from the Canadian Institutes of Health Research and a Rising Stars in Global Health Award from Grand Challenges Canada.

Nanoparticle Remodeling of Tumor Microenvironment to Improve Therapy

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We have developed several different nanoparticle (NP) formulations to deliver siRNA, pDNA or small drugs to tumors. These NPs are used as tools to study the role of tumor microenvironment (ME) as barriers for drug delivery as well as suppression for immuno therapy. A stroma-rich human basal bladder cancer has been established in nude mice. Similar to clinical situation, the model is sensitive to combination therapy of gemcitabine (G) and cisplatin (C), especially when these drugs are presented as NP formulations. Combo G+C efficiently targets to tumor associated fibroblasts (TAFs) and down-regulates the stroma structure to improve subsequent NP penetration into the tumor. However, killing TAFs induces drug resistance of tumor cells. It also enhances epithelial-to-mesenchymal transition (EMT) via Wnt16 secretion from the injured TAFs. Wnt16 siRNA delivered by NP effectively blocks the cross-talk between TAFs and tumor cells. Repeated administration of Wnt16 siRNA and cisplatin NP shrinks, but does not eliminate, large tumors (>1 cm). The residual tumors grow back after dosing is stopped, suggesting the existence of other protective mechanisms in the tumor. The immune suppressive ME can also be down-regulated by delivering TGF-β siRNA to the tumor which significantly enhances the efficacy of a NP cancer vaccine.

Work supported by NIH grants CA129835, CA129421, CA151652, CA151455 and CA149363.

Bubble-Generating Carrier Systems for Localized Controlled Release

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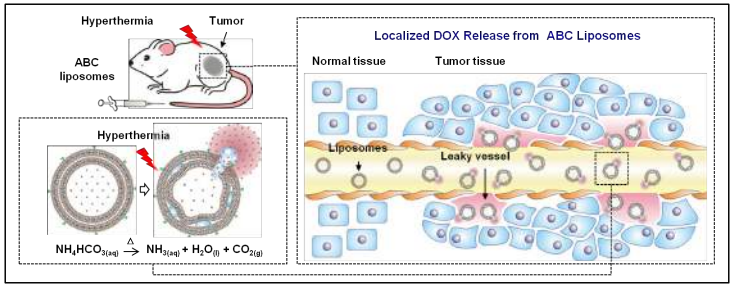
In this work, two bubble-generating agents, ammonium bicarbonate (ABC) and sodium bicarbonate (SBC) that can generate CO_2 bubbles, are separately encapsulated in carrier systems for actively triggering drug release locally. Widely recognized for their ability to increase intratumoral accumulation, PEGylated liposomes are employed as stable vehicles for carrying doxorubicin (DOX; Doxil®). However, the slow and passive drug release from the Doxil® formulation significantly inhibits its antitumor efficacy. To resolve this problem, our group develops a thermoresponsive liposomal formulation. As the key component of this liposomal formulation, its encapsulated ABC creates the transmembrane gradient needed for a highly efficient DOX encapsulation. Moreover, at a high temperature of roughly 42°C, ABC decomposition generates CO_2 bubbles, subsequently creating permeable defects in the lipid bilayer and ultimately inducing a rapid DOX release to instantly increase the drug concentration locally. The feasibility of using this thermoresponsive bubble-generating liposomal system for tumor-specific chemotherapy under mild hyperthermia is investigated. The in vitro drug-release profiles are quantified from test liposomes under mild hyperthermia conditions. Their in vivo biodistribution, pharmacokinetics, drug accumulation, and antitumor activity against locally heated tumors are examined as well. We also develop hollow microspheres (HMs) that can deliver anticancer drug into tumor cells and quickly release the drug in an acidic organelle such as lysosome. The HMs are fabricated from PLGA using a double-emulsion method, with the aqueous core containing DOX and SBC. In acidic environments, SBC reacts with



the acid to quickly generate CO_2 bubbles, triggering the shell of the HMs to disrupt, thereby quickly releasing DOX locally and causing the cells to die. These highly stimuli-responsive carrier systems contribute to efforts to establish effective tumor-selective chemotherapy.

Funding: This work was supported by a grant from the National Science Council, Taiwan

Microenvironment affects delivery of nanovectors in primary tumors and tumor metastasis

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Apart from genomic or proteomic factors at the nano-scale (e.g. upregulation of proteins, overexpression of efflux pumps), tumor therapeutic response may be profoundly influenced by elements at coarser physiological scales in the tumor microenvironment, such as diffusion gradients of the drug. These bio-barriers reside at multiple physical scales, spanning from the subcellular nano- to the tumor tissue and whole body scales. The bio-barriers are highly dependent on the nature of the organ and thus, for the same tumor, may differ drastically between primary tumor and metastatic lesion's loci as exemplified in Figure 1. Systemically administered molecules and nanovectors must first flow through the vasculature towards the tumor site, and then either attach to the tumor vascular endothelium or extravasate and diffuse through the tumor tissue. Alternatively, the nanovectors could be designed to be uptaken by the cells and to release drug intracellularly. There is a need to find the factors that may enable rational design of nanotherapeutics to efficiently localize in the tumor tissue. Since in the case of tumors the majority of nanotherapeutics are intended for intravenous administration, the main bio-barriers that affect homing of nanotherapeutics to the disease site are the blood flow dynamics in the lesion and the intactness of the blood-tumor barrier (including fenestrations in the blood vessel walls and basal membrane). The aim of this presentation is to describe a few markers, which can be useful in personalization of nanotherapeutics design, based on the nature of the lesion.

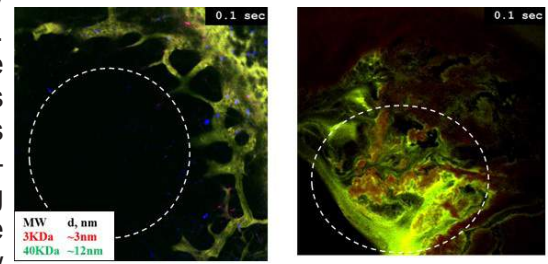

Figure 1: Intravital micrographs showing differences in the functional vasculature of 4T1 liver metastasis (left) and primary tumor in the mammary fat pad (right). Dextran tracers having MW of 3KDa (red) and 40KDa (green) were injected intravenously. Marked area represent tumor sites.

Funding: NIH U54CA143837 Physical Sciences and Oncology grant and NIH 1U54CA151668-01 Cancer Centre for Nanotechnology Excellence grant.

Tumor Microenvironment-Targeted Cancer Therapy with Nanoparticular Drug Delivery System

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The tumor microenvironment consists of a collection of multiple cells (e.g. immune cells, cancer-activated fibroblasts, and endothelial cells), extracellular matrix, signaling molecules, and so on. It can promote neoplastic transformation, support tumor growth and invasion, protect the tumor from host immunity, and foster therapeutic resistance. With the assistance of nanoparticle-based drug delivery system, the tumor microenvironment can be more efficiently targeted for cancer therapy. We will introduce extracellular pH- and enzyme-activated nanoparticles that can modulate the surface properties at the tumor site. Compared to the conventional specific tumor cell surface targeting, this approach is considered to be more general because the common existence of the acidic and specific enzyme microenvironment in solid tumor. The nanoparticles are relatively inert at physiological environment, but once accumulating at the tumor site, they are activated in response to the tumor microenvironment. With this concept, differential anti-cancer drug delivery can also be achieved by nanoparticles sensitive to artificial tumor microenvironment, which is established by a genetically engineered lipase-secreted bacterial accumulation in tumor. We will also introduce nanoparticle-mediated drug delivery for cancer stem cell therapy and immunotherapy. We demonstrate that nanoparticle-mediated drug delivery can be beneficial to the elimination of cancer stem cells in the treatment, and down-regulation of CTLA4 in T cells with nanoparticle-mediated drug delivery can inhibit immune system tolerance to tumors and thereby provides a potentially useful strategy for cancer therapy.

Funding: This work was supported by the National Basic Research Program of China, the National High Technology Research and Development Program of China and the National Natural Science Foundation of China.

The Nanomaterial Registry

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As the body of research in nanomedicine rapidly grows, the need for collaborative discoveries becomes clearer. There is currently an informatics effort, started by NIH, to address this challenge in a community-driven manner. The Nanomaterial Registry project, at RTI International, has developed a publicly available repository of nanomaterial data that includes physico-chemical characteristics, biological and environmental study data. This repository has been, and continues to be, populated through robust curation from many data sources and offers reliable collated data to the community via a central location (www.nanomaterialregistry.org). Through the project, minimal information about nanomaterials (MIAN), including 12 physico-chemical characteristics has been established and is used for curation of nanomaterial data. Contributions of data from the research community are vital to the success of collaborative discovery, as only the growth of the repository can enrich the database in a manner suitable to answer research questions and support further discovery.

RTI International would like to thank the National Institutes of Health (NIH) for funding this work, under contract HHSN268201000022C. The particular agencies advising this work are the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the National Institute of Environmental Health Sciences (NIEHS), and the National Cancer Institute (NCI).

Phage panning, Peptides and Polymers: From ligand identification to in vivo medical applications

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Peptides are an attractive class of biomolecule that can provide chemical and biological diversity, specificity of target interaction and ease of production. We have recently synthesized brush-like, peptide-based copolymers by controlled polymerization techniques. These hybrid materials possess the biological functions contributed by their peptide components with the scalable synthesis of synthetic polymers. In this presentation I will describe several examples where we are using this material platform for applications in medicine. In one example, we first identify a peptide specific to anti-inflammatory M2 macrophage present in the tumor microenvironment using phage display screening. This peptide is used for targeted depletion of tumor-associated macrophage. Multivalent display of this peptide in the peptide-based polymers increases the avidity for target cells. In a second example, polymers containing multiple peptide sequences for DNA binding, endosomal release and cell targeting are synthesized for non-viral gene transfer to neural progenitor cells. We study the effect of polymer architecture and peptide composition on gene transfer efficiency. We further demonstrate effective gene transfer into brains of mice using these materials. Finally, I will discuss some new applications that we are investigating for these bioactive polymers.

Funding: NIH NINDS, NIH NCI, NSF DMR

Peptide-functionalized Nanoparticles for Selective Targeting of Pancreatic Tumor

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[2] Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, 9 via Pietro Giuria 10125 Torino, Italy
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Pancreatic cancer represents the fourth leading cause of cancer-related death in Europe and North America. The median survival is less than 6 months mainly because most patients are diagnosed at an unresectable, advanced and metastatic stage for which, at the moment, only palliative treatments are available. Despithe weak response rate and the modest overall survival benefit, gemcitabine remains the first-line treatment in the clinical practice. Its limited efficacy is due to the rapid blood metabolization and the insurgence of resistance phenomena. In addition, the formation of a dense stroma, the limited tumor tissue vascularization and the heterogeneity of pancreatic cancer cells dramatically hamper drug efficacy and bioavailability in the tumor. Functionalized nanocarriers able to specifically target receptors overexpressed on cancer cells and relatively down regulated on healthy ones, represent an attractive therapeutic alternative.

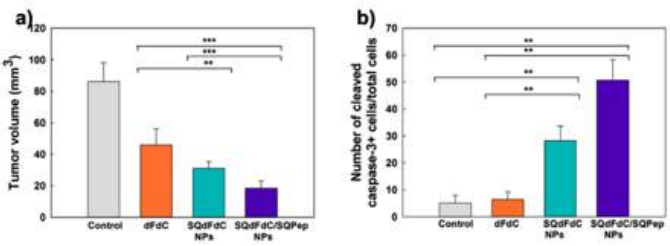

Fig.1 Anti-tumor activity (a) and apoptotic index (b).

In this context, we have constructed a novel targeted nanomedicine for pancreatic cancer treatment by using squalene as carrier material, gemcitabine as anticancer drug and a new peptide, identified by phage display screening, as ligand. Remarkably, this peptide was found capable of homing progenitor angiogenic cells as well as pancreatic stroma and cancer cells.

Cytotoxicity and cell internalization studies clearly demonstrated that peptide functionalization enabled the specific targeting of pancreatic cancer cells while decreasing interactions with the healthy ones. *In vivo*

studies in RIP-Tag2 mice, a model of spontaneously arising pancreatic cancer, demonstrated the higher efficacy of peptide-decorated NPs, which resulted from a dual activity on cancer and tumor vasculature cells. (Fig.1) Due to its specific targeting of the Wnt-2 signaling pathway, recently correlated to pancreatic tumorigenesis, this peptide may be considered as a novel efficient homing device within the pancreatic pathological microenvironment. To our knowledge, this approach is the first successful example of pancreatic cancer targeted nanomedicine with unique selectivity and multiple mechanism of action.

Funding: European Research Council under the European Community's Seventh Framework Programme FP7/2007-2013 (Agreement N°249835), the AIRC (Agreement N°11600) and the Fondazione Piemontese per la Ricerca sul Cancro-ONLUS (Intramural Grant).

Targeting intractable tumors and metastasis by using supramolecular nanodevices

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Polymeric micelles, *i.e.* self-assemblies of amphiphilic block copolymers consisting of a hydrophobic core and a poly(ethylene glycol) (PEG) hydrophilic shell, have demonstrated outstanding features for constructing tumor-targeted nanodevices. Micellar nanodevices can selectively accumulate in solid tumors through the enhanced permeability and retention (EPR) effect characterized by leaky blood vessels and impaired lymphatic drainage in tumor tissues. We have recently observed that the size of these nanodevices affect their extravasation, penetration and final accumulation in solid tumors, particularly in intractable cancers, such as pancreatic adenocarcinoma and scirrhous gastric cancer, which show dense fibrotic stroma. A comparable effect of the size of micelles was also found in models of liver metastasis of pancreatic cancer and lymph node metastasis of scirrhous gastric cancer. It is worth noticing that micelles reached the lymphatic metastasis after systemic administration through the vascular route in lymph nodes, while larger nanocarriers, such as liposomes, failed to extravasate from the blood compartment into the metastases. Micelles also accumulated in preangiogenic micrometastases in liver despite nanocarriers usually use malignant neovasculature for enhancing their tumor accumulation. This targeting was correlated with the inflammatory conditions of the preangiogenic micrometastases, which improved the penetration and retention of the micelles in the metastatic niche. Moreover, as the surface of micelles can be readily modified with ligands capable of recognition of cell-specific surface receptors, the effect of various ligands was confirmed to improve the targeting efficiency by increasing the cellular uptake and the retention of micelles in primary tumors and metastasis. Interestingly, micelles having cRGD peptides on their surface not only enhanced the cellular internalization by cancer cells, but also improved the extravasation and penetration of the micelles in a model of glioblastoma, which is notorious for its poor permeability due to the blood–brain tumor barrier, suggesting an active extravasation pathway for cRGD-installed micelles.

Funding: Grants-in-Aid from the Japan Society for the Promotion of Science (JSPS) and Center of Innovation (COI) Program from Japan Science and Technology Agency (JST)

Endo/lysosomal transport of lipid nanoparticles in-vitro and in-vivo.

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The molecular mechanisms involved in the subcellular delivery of nanoparticles to target cells remain largely undefined. To this end, we have identified Niemann Pick Type C-1 (NPC-1), a late endosome/lysosome transporter as a crucial determinant for subcellular delivery of lipid based nanoparticles *in-vitro* and *in-vivo*. A combination of high-throughput confocal microscopy, small libraries and novel trafficking tools reveal that siRNA delivery is substantially reduced as ≅70% of the internalized siRNA undergoes exocytosis through NPC1-dependent egress of LNPs from late endosomes/lysosomes. NPC1-deficient cells show enhanced cellular retention of LNPs inside late endosomes and lysosomes, and increased gene silencing of the target gene. Electron microscopy techniques were used to compare LNP trafficking in NPC1 deficient and wild type mice. Interestingly, enhance cellular retention of LNPs was observed in the endo/lysosomes of the NPC1 deficient mice which translated to improved hepatocyte specific gene silencing. Intracellular delivery of mRNA for therapeutic production of proteins in lysosomal disorders will be discussed.

Funding: This work was supported by National Cancer Institute Grant U54CA151884, 5R01HL107550-03 grant and Program of Excellence in Nanotechnology (PEN) Award, Contract #HHSN268201000045C from the National Heart, Lung, and Blood Institute, National Institutes of Health and by the Skolkovo Foundation Grant as part of the Center for RNA therapeutics and biology.

Lipid Nanoparticles to deliver siRNA to immune cells

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Despite the progress made in recent years towards the delivery of siRNA to specific cellular targets (e.g. hepatocytes), the transfection of non-adherent cells at clinically viable doses has remained a challenge. In particular, lymphocyte populations are notoriously difficult to transfect with siRNA and require very potent delivery systems. In general, immune cell populations are attractive targets for RNA interference therapy, as they have been implicated in various aspects of disease initiation and progression, including inflammation and autoimmune responses. Malignant immune cells such as leukemia and lymphoma may also benefit from gene silencing treatments. To address the delivery challenge to these cells, we have developed a class of degradable lipid-like materials termed 'lipidoids' that can be formulated along with siRNA into lipid nanoparticles (LNPs) with diameters of less than 100 nm. Upon IV injection to healthy mice, LNPs mediated high levels of gene silencing in CD11b+ and CD11c+ populations (monocyte/macrophages and dendritic cells, respectively) harvested from the peritoneal cavity and the spleen. *In vitro*, we have achieved up to 90% silencing at the mRNA level in malignant B cell populations at a dose as low as 10 nM. Together, these results indicate that lipidoid materials can achieve potent, specific and non-toxic siRNA delivery to various immune cell subsets and have potential to be used to induce gene silencing *in vitro* and *in vivo* for both therapeutic purposes as well as genetic study.

Funding: NIH award number EB009623

BIND Accurins: Targeted Nanomedicines Through Medicinal Nanoengineering

Steve Zale

BIND Therapeutics, Cambridge, MA 02139, USA

The challenge for all drugs is to maximize the net clinical benefit by increasing the desired therapeutic effect and reducing adverse effects. This is especially difficult in cancer, where the goal is to destroy or inhibit growth of cancer cells without damaging similar healthy cells. Accurins™ are polymeric nanoparticles that incorporate a therapeutic payload and are designed with specified physical and chemical characteristics to target specific cells or tissues and concentrate a therapeutic payload at the site of disease to enhance efficacy while minimizing adverse effects on healthy tissues.

BIND-014 is a prostate-specific membrane antigen, or PSMA, targeted Accurin that contains docetaxel. PSMA is a clinically-validated tumor marker expressed on prostate cancer cells and the blood vessels of many types of non-prostate solid tumors, including non-small cell lung cancer. In our Phase 1 clinical trial, of the 28 patients who received BIND-014 once every three weeks, to date there have been one complete response in a patient with cervical cancer and three partial responses in patients with NSCLC, mCRPC and ampullary cancer. Five additional patients had stable disease lasting longer than 12 weeks. BIND-014 is in Phase 2 clinical trials for non-small cell lung cancer and metastatic castrate-resistant prostate cancer.

We have engineered Accurins that incorporate therapeutic payloads with a range of physicochemical properties. For example, we have incorporated the microtubule inhibitor vincristine and the proteasome inhibitor bortezomib into Accurins and observed differentiated pharmacokinetics and improved net clinical benefit compared to the respective parent forms of the therapeutic payloads. In addition, with collaborators (announced collaborations with Amgen, Pfizer and AstraZeneca) we have incorporated multiple kinase inhibitors into Accurins and observed differentiated pharmacokinetics and improved net clinical benefit in preclinical models compared to the parent forms of the therapeutic payloads.

Commercializing PRINT® Technology

Benjamin R. Yerxa

Envisia Therapeutics and Liquidia Technologies, Research Triangle Park, NC, USA

Particles are central components to many products and industries, and various methods of manufacturing have been developed over the years to meet the demand for bulk particles of various sizes and compositions. Recently, through advances in the microelectronics industry and polymer science, Liquidia developed a way to design and engineer particles with precise control using a technology called PRINT (Particle Replication In Non-Wetting Templates). The PRINT platform enables not only control of size and composition, but also shape, flexibility and porosity, allowing for a very broad range of performance characteristics. Developing this technology with an eye towards commercializing it has required solving many problems along the way, including answering key questions such as: What can you make? Does it matter? Can you scale it up? How are you going to build a business?

Solving Drug Delivery Problems by Genetically Engineered Nanoparticles

Ashutosh Chilkoti

Center for Biologically Inspired Materials and Materials Systems and Department of Biomedical Engineering, PO Box 90281, Duke University, Durham NC 27708-0281, USA

I will summarize three new drug delivery systems that we have developed for drugs that range from small molecules to proteins. The first—attachment-triggered self-assembly of recombinant peptide polymers—packages hydrophobic small molecule cancer drugs into soluble nanoparticles of a peptide polymer, and increases the solubility, plasma half-life, and tumor accumulation of the drug, which translates to vastly improved efficacy of the nanoparticle formulation as compared to free drug. The second delivery system—Protease Operated Depot (POD)—is an injectable delivery system based on thermally sensitive polypeptides for the sustained and tunable release of peptide drugs from a subcutaneous injection site that enables once-a-week delivery of peptide drugs that have a short duration of action in their native, unmodified state. The third technology—Instealth™—enables the in situ growth of a PEG-like polymer from the N-or C-terminus of a peptide or protein drug, and yields a site-specific and stoichiometrically well-defined protein-polymer conjugate with greatly improved circulation and biodistribution as compared to the unmodified protein.

Development of multifunctional nano carrier platforms for cancer treatment

André Nel[1] and Huan Meng[1]

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I will introduce our multifunctional mesoporous silica nanoparticle (MSNP) platform to show how discovery at the nano/bio interface and iterative design can be used to provide improved nanocarriers for cancer treatment. One example is overcoming multidrug resistance by designing MSNP surfaces that allows co-delivery of synergistic anticancer drugs or drugs plus siRNA into drug-resistant cancer cells. The functionalization of the particle surface allows electrostatic binding of the chemotherapeutic agent, doxorubicin (Dox), to the porous interior. Phosphonate modification allows exterior coating with polyethyleneimine (PEI), which endows the carrier with the ability to bind and deliver P-glycoprotein (Pgp) siRNA that silences the expression of the efflux protein. Following the establishment of a Dox-resistant MCF-7 breast cancer xenograft model in nude mice, we demonstrated that a 50 nm MSNP functionalized by a PEI-polyethylene glycol (PEI-PEG) copolymer provides protected delivery of Dox and Pgp siRNA to the tumor site. This design was chosen for its effective biodistribution properties, reduced reticuloendothelial uptake, and the ability to have 8% of the injected dose retained at the tumor site. Compared to free Dox or the carrier loaded with drug alone, dual delivery resulted in synergistic inhibition of tumor growth. Dox encapsulation by the carrier was associated with reduced systemic side effects, including cardiotoxicity. However, analysis of the tumor biopsies demonstrated heterogeneous Pgp knockdown, resulting in effective Dox killing only at where the drug resistance gene was knocked down. The variable distribution of the carrier is due, in part, to heterogeneous vascular access across the tumor matrix and stroma. Since one of the major factors leading to heterogeneous carrier distribution is interference in vascular access due to pericyte coverage, we turned our attention to pancreatic ductal adenocarcinoma (PDAC), in which pericytes in the dysplastic stroma exerts a major effect on drug uptake and carrier availability. In order to deal with this challenge, we developed a dual wave therapeutic approach in which one MSNP carrier delivers a small molecule that interferes in pericyte coverage in the vasculature, while a 2nd nanocarrier (liposomes) introduced gemcitabine, resulting in a synergistic treatment effect. In further advances of technology, we are developing MSNP carriers in which synergistic drug combinations agent will address the effect of the tumor stroma, as well as delivering sufficient amounts of chemotherapeutic agents that promotes tumor killing.

Funding: NCI R01 CA133697; NSF and EPA Cooperative Agreement (DBI-1266377); NIEHS U19 ES019528

Targeted drug delivery towards cancer stem cells using polymeric nanostructures stabilized *via* non-covalent interactions

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Cancer stem cells comprise a small sub-population within tumors with enhanced capacity for tumor generation, and they possess several fundamental attributes similar to normal adult stem cells. The existence of cancer stem cells increased resistance to conventional chemotherapy. A number of genetic and cellular adaptations have been found to be related to the resistance, such as relative dormancy/slow cell cycle kinetics, efficient DNA repair, the expression of multidrug-resistance transporters, and resistance to apoptosis. Although conventional chemotherapies kill the majority of cancer cells, the surviving cancer stem cells could re-initiate the tumor, leading to relapse and recurrence.

In this talk, I will discuss micellar nanoparticles formed from amphiphilic block copolymers *via* several self-assembly strategies for co-delivery of anticancer therapeutics that target both cancer cells and cancer stem cells. The copolymers were synthesized with controlled molecular structure and functionalities *via* organocatalytic living ring-opening polymerization. Various functional groups were designed to provide non-covalent interactions within the micellar core for increasing drug loading capacity, enhancing *in vivo* stability and improving anti-tumor efficacy. The nanoparticles were employed to deliver thioridazine/phenformin (able to kill cancer stem cells) and conventional anticancer drugs doxorubicin/gemcitabine simultaneously. A strong synergistic effect in reducing cancer stem cell population and suppressing tumor growth was demonstrated both *in vitro* and *in vivo* (xenograft breast and lung cancer mouse models). In addition, design of multi-functional cationic polymers for co-delivery of siRNA and anticancer drugs will be discussed for the prevention of cancer metastasis.

Funding: Institute of Bioengineering and Nanotechnology (Biomedical Research Council, Agency for Science, Technology and Research, Singapore)

Nanomedicine and Education: Flipping the Way We Train the Next Generation of Scientists

Russell Mumper

Dr. Mumper had developed and taught, by lecturing, the majority of a Pharmaceutics course to pharmacy students in a PharmD program for over a decade (2000-2011). The Pharmaceutics course dealt with the science of delivery of drugs to the body by all routes of administration via complex, specialized and novel dosage forms including nanomedicines.

Dr. Mumper noticed that students were increasingly more distracted and the majority of his interaction with students was about core, foundational content. Faced with a mountain of content, not enough time and distracted students, Dr. Mumper was dissatisfied with the results and decided it was time for a change. Motivated by how engaged his students were in discussions outside of class and during group work, Dr. Mumper decided to flip his class in 2012. By flipping the class, in-class time was used to implement active learning strategies. Dr. Mumper created a library of recordings of critical core content for students to view prior to coming to class.

Over a three year period from 2011 to 2013, student engagement, perception and performance was tracked in the traditional lecture class (2011) and the flipped class (2012 and 2013). This talk will describe the rationale and motivation for the flipped classroom, how it was developed and executed, and qualitative and quantitative outcomes, and lessons learned. In addition, the talk will highlight how this experience is contributing to new educational models to train PharmD and PhD students as well as industry scientists desiring contemporary training in pharmaceutics and nanomedicine through either annual workshops or professional/executive MS/PhD training.

UPS Nanoparticles for Cancer Imaging and Therapy

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Stimuli-responsive nanomaterials are increasingly important in a variety of applications such as biosensing, molecular imaging, drug delivery and tissue engineering. For cancer detection, a paramount challenge exists in search of methods that can illuminate tumors universally regardless of their genotypes and phenotypes. Here we capitalized on the acidic tumor microenvironment to achieve broad detection of tumor tissues in a wide variety of mouse cancer models. This was accomplished using ultra-pH sensitive (UPS) fluorescent nanoprobes that have tunable, exponential fluorescence activation upon encountering subtle, physiologically relevant pH transitions. These nanoprobes were silent in the circulation, then dramatically activated (>300 fold) inside tumors. Recently, we adapted the UPS technology to indocyanine green and clinical camera (SPY Elite®). The nanoprobes were effective in the delineation of tumor margins and sentinel lymph nodes, which permits an accurate resection of head and neck cancers and peritoneal metastasis.

Funding: National Institutes of Health (RO1 EB013149) and Cancer Prevention and Research Institute of Texas (CPRIT RP120094).

Image-guided Drug Delivery for Cancer Treatment

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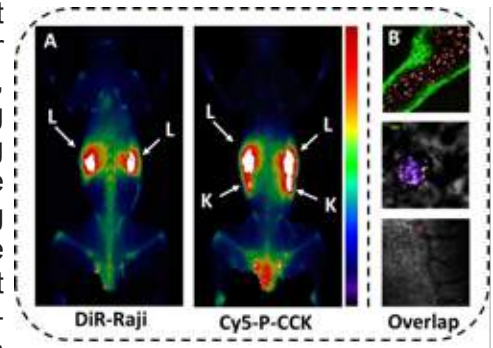
For treatment of non-Hodgkin's lymphoma, we have developed a new two-step-targeting drug-free macromolecular therapeutics, which is composed of (a) anti-CD20 Fab' fragment covalently linked to a peptide CCE (Fab'-CCE), and (b) linear poly[*N*-(2-hydroxypropyl)methacrylamide] (P) grafted with multiple copies of the complementary peptide CCK (P-CCK). This new therapeutic system specifically triggers apoptosis in lymphoma cells by cross-linking of (non-internalizing) CD20 antigens at B lymphoma cell surface mediated by the biorecognition of two complementary coiled-coil peptides (CCE and CCK). To obtain a better understanding of this two-step targeting system, we designed fluorophore-labeled conjugates (FITC-Fab'-CCE, Cy5-P-CCK) and used multiple imaging strategies to investigate their behavior: 1) At the cellular level, 3-dimensional fluorescence microscopy showed that the two conjugates FITC-Fab'-CCE and Cy5-P-CCK co-localized on the Raji cells surface and subsequently created clusters of lipid rafts. 2) At whole-body level, fluorescence molecular tomography imaging showed that 2nd-step conjugate Cy5-P-CCK co-localized with DiR-labeled Raji cells previously inoculated in mice, especially in bone and spine (Fig. A), indicating effective targeting *in vivo*. 3) At tissue level, high-resolution confocal microscopic images visualized co-localization of two conjugates (FITC-Fab'-CCE, Cy5-P-CCK) at the PE-labeled Raji cells in major tissues (i.e., calvarium, lung, spleen, and liver) (Fig. B), suggesting specificity of the two-step targeting system. The overall results indicate feasibility of the two-step-targeting paradigm on the basis of the biorecognition of peptide motifs at cell surface. This concept of drug-free macromolecular therapeutics possesses a potential for treatment of other diseases by using different components in its design.



Funding: This work was supported in part by NIH grant GM95606 from the National Institute of General Medicine (to JK) and by the University of Utah Research Foundation.

Identifying molecular signatures for tumor dormancy as a basis for the development of theranostic nanomedicines

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Tumor progression is dependent on a number of sequential steps, including initial tumor-vascular interactions and recruitment of blood vessels (i.e., the “angiogenic switch”), as well as an established interaction of tumor cells with their surrounding microenvironment and its different immune, endothelial and connective cellular and extra-cellular components. Failure of a microscopic tumor, either primary, recurrent or metastatic, to complete one or more of these early stages may lead to delayed clinical manifestation of the cancer (i.e., tumor dormancy). Micrometastasis, dormant tumors, and residual tumor cells – referred to as minimal residual disease, contribute to the occurrence of relapse, and constitute fundamental clinical manifestations of tumor dormancy that together are responsible for the vast majority of cancer deaths. However, although the tumor dormancy phenomenon has critical implications for early detection and treatment of cancer, it is one of the most neglected areas in cancer research and the associated biological mechanisms are still mostly unknown.

We have created several models of patient-derived xenografts mimicking pairs of dormant vs fast-growing, primary vs metastatic and drug-sensitive vs resistant cancers. We investigated the molecular and cellular changes in tumor-host interactions that govern tumor dormancy. Those led to the discovery of novel targets and provided important tools for cancer theranostics (therapy and diagnostics). Based on the acquired knowledge, we designed a new strategy to improve treatment outcomes of patients with bone neoplasms, glioblastoma, brain metastases, melanoma, breast and prostate cancers. We have identified molecular signatures that, following selective delivery into their target cells, can potentially induce a dormant-like phenotype. This goal was achieved by utilizing polymeric nanomedicines and guidance by high resolution, intravital non-invasive imaging techniques.

A better understanding of tumor dormancy and the availability of relevant markers will most likely change the way we diagnose and treat the disease using novel combined theranostic nanomedicines.

Funding: European Research Council (ERC) consolidator grant, Israel Science Foundation, Swiss Bridge Award, The Association for International Cancer Research (AICR), German-Israel Foundation (GIF), Israeli National Nanotechnology Initiative (INNI), The Leona M. and Harry B. Helmsley Nanotechnology Research Fund.

The Use of Single Particle-ICP-MS in Nanomedicine and Drug Delivery Systems

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Single Particle-ICP-MS is a new operating mode in ICP-MS that is dedicated to the analysis of engineered nanoparticles (ENPs) commonly used in Nanomedicine and drug delivery systems. Single Particle (SP-ICP-MS) allows the differentiation between ionic and particulate signals, quantitation of both ionic and particulate fractions, measurement of particle composition, concentration (part/mL), particle size, and size distribution, and assists in monitoring agglomeration.

The present work explores the use of SP-ICP-MS as a metrology tool for the analysis of engineered nanoparticles in biological matrices (blood, urine). Preliminary results show that Au ENPs (30 and 60 nm) are stable in diluted blood and urine. No change in the size of Au ENPs and/or concentration was noticed during the 24h study period.

SP-ICP-MS is rapidly becoming a **key analytical instrument** in assessing the fate, behavior and distribution of (ENPs) in several types of matrices[1,2] including biological fluids such blood and urine.

References:

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Dynamic Evaluation of Nanoparticle Pharmacokinetics and Biodistribution using Multispectral Optoacoustic Tomography (MSOT)

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A wide range of nanoparticles differing in size, shape and surface characteristics are available for therapeutic and diagnostic applications. To ensure optimal design of nanoparticles, it is essential to obtain quantitative data of pharmacokinetics and biodistribution in small animal models. Here Multispectral Optoacoustic Tomography (MSOT) was employed to evaluate nanoparticle pharmacokinetics and biodistribution in mice with orthotopic tumors. MSOT combines high-resolution real-time ultrasound detection with the specificity of optical contrast by utilizing the photoacoustic effect. Absorption of pulsed near infrared (NIR) laser light (680-980 nm) leading to the subsequent generation of acoustic waves detected using an ultrasound transducer (5MHz) allows for the detection of both intrinsic (e.g. oxygenated and deoxygenated hemoglobin, melanin) and extrinsic (fluorescent dyes, nanoparticles and fluorescent proteins) optical absorbers in tissue.

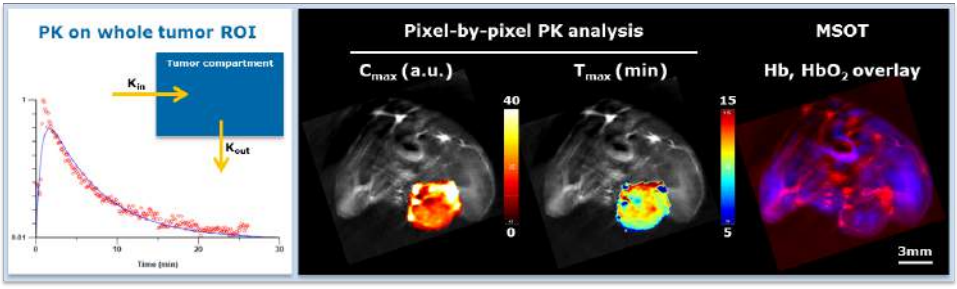

Figure 1. Parametric maps reveal heterogeneity with regard to maximum concentration (C_{max}) and time of maximum concentration (T_{max}). Tumor areas with a lower C_{max} and longer T_{max} correlate with areas of relative hypoxia within the tumor.

Here MSOT was utilized to track nanoparticle biodistribution and pharmacokinetics throughout the whole body of the mouse. Nanoparticles containing NIR fluorescent dyes were injected intravenously into mice and the accumulation and clearance of the nanoparticles over time was observed. Regions of interest placed within the liver, spleen, kidneys, brain, heart and vasculature allowed for the visualization and quantification of fast uptake kinetics, while longitudinal data acquisition allowed the determination of the

differential pharmacokinetic properties of each compound.

By combining MSOT imaging with Dynamic Contrast Enhancement (DCE-MSOT), detailed differential uptake in various organs and tumors could be evaluated at sub-tissue resolution. Areas of relative normoxia were visualized and correlated with particle delivery (Figure 1). In summary, MSOT provides the ability to aid the rational design process of nano-materials by enabling whole-body *in vivo* visualization of nano-formulation biodistribution.

Abstract title. Nanofiber-mediated gene-silencing for neural tissue engineering

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Nanofiber substrates closely mimic of the architecture of the natural extracellular matrix. These constructs represent a novel class of materials in regenerative medicine. Fibrous topographical cues can direct cellular response and stem cell fate. Combined with the incorporation of biochemicals such as small non-coding RNAs, these substrates provide synergistic topographical and biochemical cues to seeded cells. We will discuss our recent findings on the roles of nanofiber topography on nerve regeneration after spinal cord injury. The advantages of sustained scaffold-mediated gene silencing in directing the differentiation of progenitor cells (neural, oligodendrocytes) and axonal regeneration *in vitro* will also be discussed.

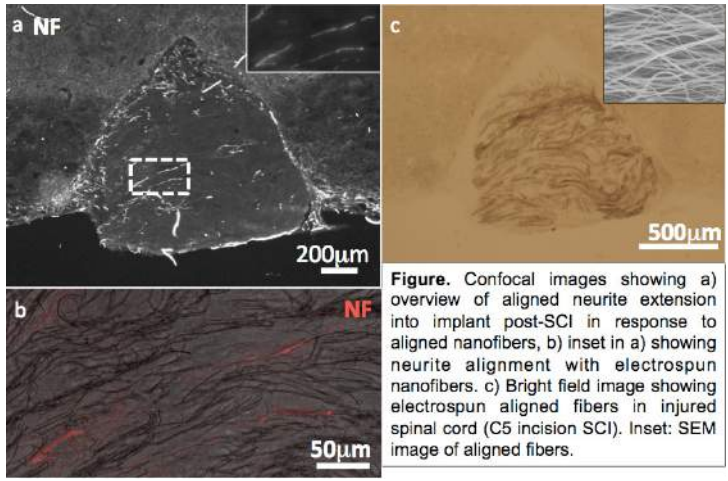

Figure. Confocal images showing a) overview of aligned neurite extension into implant post-SCI in response to aligned nanofibers, b) inset in a) showing neurite alignment with electrospun nanofibers. c) Bright field image showing electrospun aligned fibers in injured spinal cord (C5 incision SCI). Inset: SEM image of aligned fibers.

Funding: NMRC CBRG (NMRC/CBRG/0002/2012); MOE AcRF Tier 1 (RG75/10)

Elicitation of robust cellular and humoral immunity with vaccine nanoparticles

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Licensed vaccine adjuvants in clinics lack the capacity to elicit potent cellular and humoral immune responses against intracellular pathogens or tumor cells. To address this major challenge in vaccine technology, we have developed a new synthetic vaccine platform, called interbilayer-crosslinked multilamellar vesicles (ICMVs) that can stably deliver antigens to draining lymph nodes and elicit robust cellular and humoral immune responses against infectious pathogens and tumor cells. ICMVs were formed by fusing liposomes into multilamellar vesicles and subsequent crosslinking of adjacent lipid headgroups across lipid bilayers within multilamellar vesicles. These lipid-based nanoparticles exhibited substantially enhanced protein antigen loading and extended drug release kinetics compared with traditional drug delivery vehicles (e.g. liposomes and PLGA nanoparticles). Crosslinking of lipid bilayers enhanced stability of particles in serum, allowing efficient delivery of cargo antigens to antigen-presenting cells in draining lymph nodes. ICMVs encapsulating a model antigen and FDA-approved adjuvant elicited potent antibody and CD8+ T cell responses in vivo, comparable to those elicited by strong viral vector vaccines. ICMVs carrying a malaria antigen elicited robust humoral immune responses with high-avidity antibody titers lasting more than a year. In addition, pulmonary vaccination with ICMVs loaded with a gag HIV peptide induced a significantly higher frequency of antigen-specific $CD8^+$ T cells in lungs, lymph nodes, spleen, gut, and reproductive tract and conferred substantially enhanced protective immunity against viral challenge than vaccination with soluble antigens. Furthermore, our initial results indicate that ICMVs formulated with tumor-associated antigens can elicit robust CD8+ T cell responses against melanoma cells. These results suggest that ICMVs are a promising vaccine platform for induction of cellular and humoral immune responses against infectious pathogens and tumor cells.

Funding: This study was supported by the Michigan Institute for Clinical & Health Research (MICHR) Pilot Grant Program and by the National Institute of Health grant 1K22AI097291-01.

Protein polymer nanomedicines for ocular drug delivery

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Age-related macular degeneration, dry eye disease, and corneal wound healing are three conditions that would benefit from improvements in ocular drug delivery. Age-related macular degeneration manifests as decreased central vision acuity associated with loss of cells in the retina, which can be clinically accessed by intra-vitreal administration. Dry eye disease presents as an insufficiency of the tear film, which can result from dysfunction of the tear producing lacrimal gland. Corneal injuries result from dry eye disease, trauma, and refractive surgeries. Challenges in treating these sites include rapid local clearance (vitreous, fluid, plasma) and limitations in targeting cell-surface receptors. To modulate retention and efficacy in the eye, we have explored elastin-like polypeptides (ELPs) for their ability to assemble nanoparticles targeting: i) the retinal pigment epithelium via intra-vitreal administration; ii) the diseased lacrimal gland following systemic administration; and iii) the corneal epithelium upon topical administration. Three cargos have been evaluated, which include an anti-apoptotic short heat shock protein chaperone derived from alphaB crystallin, a fusion protein capable of carrying an immunosuppressant macrolide called rapamycin, and a mitogenic peptide modulator of the corneal epithelium called lacritin. Each of these peptides was fused to ELPs that either remain soluble or serve as an assembly scaffold. These fusions assemble structures for which we characterized their phase behavior, size, morphology, *in vitro* activity, and *in vivo* efficacy using suitable murine models. In each case, these ELP nanoparticles demonstrate unique and potentially beneficial behaviors not observed for soluble drug controls. The general trend observed across all three studies is that ELP-mediated assembly significantly alters both cellular internalization and efficacy. These findings suggest that ELP-mediated assembly may have applications to deliver a range of cargo to targets throughout the eye.

Funding: NIH RO1EY01545, RO1EY017293, NIH RO1EY017293-04S1, R21EB012281, P30CA014089, RO1EY03040, RO1EY01545, and the USAMRMC/TATRC W81XWH1210538.

Self-propelling particles that deliver coagulants and other cargos deep into wounds.

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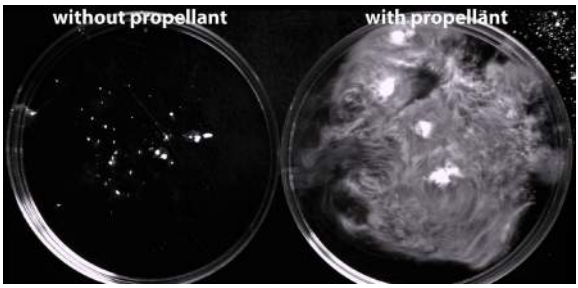

Topical delivery of drugs into wounds is difficult because flowing blood forces the therapeutic outward. Severe bleeding is a problem faced by many people because coagulants cannot be topically delivered deep into wounds. Severe bleeding complicates trauma, which is the number one killer of young people worldwide, and complicates postpartum hemorrhage (PPH), which leads to extremely high maternal death rates (>1% of all births) in low-income regions. Traditional agents for controlling bleeding cannot penetrate though blood and thus cannot clot deep inside the wound at the sites of vascular damage. We have developed simple self-propelling particles that can travel upstream through blood flow and clot blood at the source of damaged vessels. Many systems of self-propelling particles have been previously reported, but they have not been functional in vivo. Our simple particles are formulated with low-cost materials that are already used in other areas of the clinic, and can be loaded with a wide range of cargos. In vitro, the particles travel through blood at velocities comparable to blood flow in microvasculature and wounds. In vivo, when applied to an area of bleeding, the particles propelled up to a centimeter into the microvasculature around the wound. Compared to coagulants that do not propel, the propelled-particles are able to clot blood flowing 50 times faster. In two mouse models of severe hemorrhage, the propelling particles were significantly better at halting bleeding than coagulants currently used in the clinic to treat intraoperative bleeding.

Funding: Grand Challenges Canada (0098-01), Canadian Institutes of Health Research (MOP-119426 and MSH-130166), Natural Science and Engineering Research Council (418652-2012), and Canadian Foundation for Innovation (31928).

Poster abstracts

#1

HA-coated nanoparticles for CD44-mediated anti-inflammatory therapy.

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Inflammation, a cascade of biochemical responses to harmful stimuli, can result in debilitating pathologies such as rheumatoid arthritis and inflammatory bowel diseases. It is characterized by hypervascularisation of the inflamed tissue, recruitment of inflammatory cells and production of potent cytotoxic cytokines and free radicals. Inflammation is mainly mediated by macrophages, chief producers of inflammatory cytokines. During inflammation, macrophages heavily express CD44, a proteoglycan transmembrane receptor. Together with its principal ligand hyaluronic acid (HA), CD44 plays a critical role in a wide range of inflammatory diseases. We have previously shown that macrophages uptake of nanoparticles where HA is displayed on the surface is a CD44 mediated process. Therefore, such nanoparticles are good candidates for actively targeting macrophages and efficiently deliver specific payloads for possible downregulation of inflammatory cytokines production. Moreover, high molecular weight HA was shown to exert a CD44 dependent anti-inflammatory effect, which could in principle allow synergic anti-inflammatory action. In the current ongoing study, we are investigating the feasibility of the aforementioned approach using HA-coated nanoparticles. Electrostatic interactions were exploited to entrap TNF-α silencing small interfering RNA (siTNF) within chitosan (CS)-TPP nanostructures and to subsequently coat the surface with HA. We have confirmed soluble HA increasing anti-inflammatory character with increasing molecular weight. However, HA concentrated on the nanoparticles surface (360KDa), at an analogous concentration to soluble HA, significantly increased the anti-inflammatory effect. Different organization and possibly different crowding and mobility of HA chains may give rise to this significant effect on macrophages inflammatory activation. Despite the successful loading of siTNF in HA-coated nanoparticles (loading efficiency ≥ 90%), no additive effect on inflammatory cytokines production was detected. This indicates that the anti-inflammatory character of the nanoparticles themselves, possibly arising from clustered binding to HA receptors such as CD44, overwhelmed any further action due to RNA interference to TNF-α expression.

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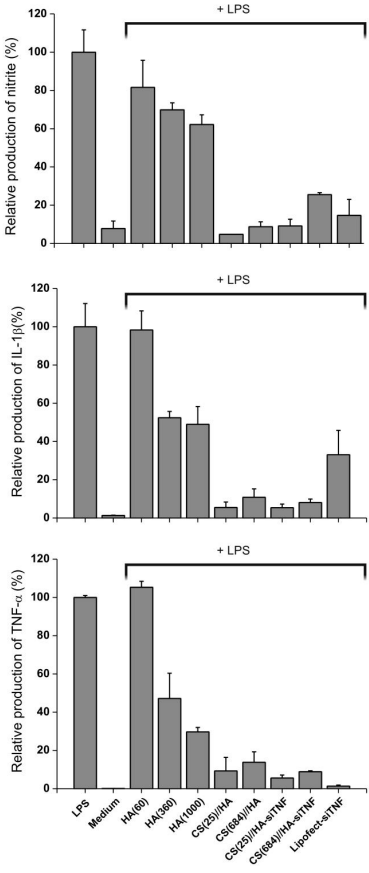

Figure. Effect of chitosan (CS)-TPP//HA nanoparticles on the production of nitrite, TNF-α and IL-1β in lipopolysaccharides (LPS)-activated (1 μg/mL) RAW 264.7 macrophages. Cells were exposed to 1 μg/mL LPS for 24 h co-administered with free HA at 125 μg/mL or nanoparticles at 250 μg/mL (roughly corresponding to the same HA concentration). siTNF was entrapped in nanoparticles or Lipofectamine™ and incubated at 200nM/well. LPS and plain medium were respectively used as positive and negative controls. The numbers between brackets indicate polymer molecular weight expressed in KDa

Funding:Marie Curie Industry-Academia Partnerships and Pathways (IAPP) ''Replixcel'' project (No 251420); King Abdulaziz City for Science and Technology (KACST).

#2

Tuning pH sensitivity of acetalated dextran nanoparticles

Riyad Alzhrani, Ali H. Alhasan, Caroline de Gracia Lux, Aws Alshamsan, and Adah Almutairi

Acetalated dextran (Ac-Dex) is an acid-sensitive material that allows tuning of pH sensitivity by varying the ratio of cyclic to acyclic acetals; cyclic acetals degrade slower than acyclic acetals. While Ac-Dex seems relevant to a broad range of drug delivery applications, prior studies have employed microparticle formulations, whose utility is limited to local delivery. Therefore, we sought to identify the size and percentage of cyclic acetals that yielded selective release at mildly acidic pH (~6). While ~110 nm particles (formulated by single emulsion) composed of 56% cyclic Ac-Dex yield similar release of Nile Red at either pH 6 or pH 7.4, either increasing the particle size to ~250 nm or increasing the percentage of cyclic acetals to 64% improved pH sensitivity, reducing release at pH 7.4. We also examined the effect of size on phagocytic cell uptake by flow cytometry; 1000 nm Ac-Dex nanoparticles (formulated by electrospray) were taken up by Raw264.7 macrophages within 1 hr, while ~110, 250, and 600 nm particles were not. This work informs future development of Ac-Dex nanoparticles for systemic delivery applications.

Mechanistic Insight Into Receptor Specific Gene Delivery by Cationic-β-Cyclodextrin: HyaluronicAcid- Adamantane Host: Guest pDNA Nanoparticles

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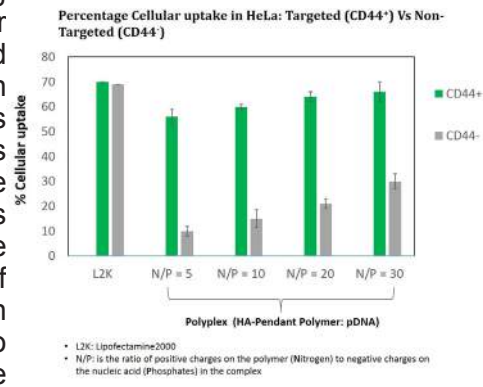
Targeted delivery is one of the key element in improving the specificity, efficiency and safety of non-viral vectors for gene therapy. We recently have developed a new hyaluronic acid (HA)-based pendant polymer system capable of forming complexes with cationic β-cyclodextrins and pDNA. These compounds have shown promising transfection efficiency and cell viability. However the detail investigation of time and location dependent cellular events happening through the transfection pathway such as receptor binding, route of cellular entry, endosomal/ lysosomal escape and nuclear entry remain uncovered. Using confocal microscopy and multicolor flowcytometry studies, spatial and dynamic tracking of transfection complexes in *in vitro* system was analyzed. Moreover the initial studies involving in vivo tracking of these complexes using IVIS techniques is also demonstrated. Here, we have elucidated a mechanism of transfection in CD44 cell line, which can be of immense importance to cancer research community for the design of even improved transfection agent.



Funding: NIH GM087016, Purdue University Center for Cancer Research.

#4

Amphotericin B Bound Iron Oxide Nanoparticles and Their *in vitro* Characteristics

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Purpose: Iron oxide nanoparticles (IONPs) were developed as nano-scale drug carriers that can be surface modified to bind poorly water-soluble drugs such as amphotericin B (AmB) and their *in vitro* characteristics were investigated.

Methods: The AmB bound IONPs were prepared by conjugation of amino groups on AmB to oleic acid coated surface modified IONPs (15nm). The particles were then coated with bovine serum albumin (BSA) by incubating the particles in a BSA solution at ambient temperature. Finally, AmB was bound to the particles by incubation in AmB solution. The amount of AmB was estimated with a validated HPLC method. Size and zeta potential of NPs were estimated by dynamic light scattering and morphology of NPs were determined with transmission electron microscopy (TEM). The *in vitro* antifungal activity of the AmB bound IONPs (AmB-IONPs) and AmB deoxycholate (commercial product) were evaluated against *Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis, and Candida tropicalis.*

Results: The mean particle size and zeta potential of AmB-IONPs was found to be 35nm and -32mV, respectively. TEM confirmed the mono dispersity of the spherical NPs. The amount of AmB per mg of BSA coated IONPs was found to be 10.5µg. Antifungal activity *in vitro* of AmB-IONPs against all tested *Candida* species was better than the commercial AmB deoxycholate formulation. The minimum inhibitory concentrations indicated at least 3-fold improvement over conventional drug AmB deoxycholate.

Conclusions: Drug-bound albumin coated IONPs can be utilized to entrap poorly water-soluble drugs and enhance therapeutic efficacy. This system is also a potential theranostic agent for simultaneous imaging and therapy of deadly diseases like invasive fungal infections. We will further evaluate the efficacy of this theranostic agent in *in vivo* models.

Ormeloxifene loaded PLGA nanoparticles: A novel nano-approach for cervical cancer therapeutics

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Ormeloxifene (ORM) is a non-steroidal Selective Estrogen Receptor Modulator (SERM) used as an oral contraceptive in humans. Recent investigations suggest that Ormeloxifene exhibits potent anti-cancer activity against various types of cancers. Nanoparticulates offer targeted delivery of anti-cancer drugs and promise newer approaches for cancer diagnosis and treatment. Tumors due to their enhanced permeability and retention (EPR) effect allow nanoparticles specifically to enter and accumulate in them. Site specific delivery of anti-cancer drugs using nanotechnology provides very minimal or no toxicity. Therefore, nanotherapy approach outstands over traditional chemotherapy which is not site specific and often associated with various side effects. Thus, pursuing this novel nanotherapy approach, our lab recently has developed ORM loaded PLGA (poly [lactic-*co*-glycolic acid]), an FDA approved biodegradable polymer, nanoparticle(s) to achieve the targeted drug delivery and improved bioavailability. ORM encapsulated PLGA nanoparticles (PLGA-ORM) were developed employing nanopreciptation method and stabilized by using poly vinyl alcohol (PVA) and Poly (L-lysine) stabilizers. Physiochemical characterization of PLGA-ORM nanoformulation was determined by analyzing particle size, drug loading and drug release by employing dynamic light scattering (DLS), transmission electron microscopy (TEM) and high performance liquid chromatography (HPLC) techniques. Additionally, anti-cancer efficacy of PLGA-ORM nanoformulation was tested in SiHa and Caski HPV 16 positive cervical cancer cells. Improved inhibition of cellular proliferation and clonogenic potential was observed with the treatment of PLGA-ORM when compared with free ORM. Further studies are in the progress for preclinical evaluation of PLGA-ORM using an orthotropic mouse model for cervical cancer. Taking together, our findings suggest that PLGA-ORM nanoformulation has a great potential for repurposing it; as a novel modality for cervical cancer treatment and needs to be developed as a lead therapy approach with appropriate pre-clinical/clinical investigations.

Funding: This work is supported by NIH UO1 (CA162106-01A1) grant awarded to Meena Jaggi and Subhash C. Chauhan.

#6

Identification of IGF2R specific peptides using phage display

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Liver fibrosis is characterized by the excessive accumulation of extracellular matrix (ECM) in the liver. Hepatic stellate cells (HSCs) are the main producers responsible for the excessive production of ECM and profibrogenic cytokines in fibrotic liver. Therefore, development of HSC-specific delivery systems is essential for the success of antifibrotic therapeutics. The purpose of this study is to identify peptide ligands targeting IGF2R, which is overexpressed on HSCs. We expect to use the peptide ligands for the development HSC-specific nanocarriers.

Protein-based and whole-cell-based phage display biopannings were conducted to identify peptide phage candidates. Phage ELISA, cellular uptake and cell viability assay were employed to evaluate the binding affinity and specificity to IGF2R and HSCs. IGF2R siRNA was used to silence the IGF2R expression in human hepatic stellate cells (LX-2) to evaluate the specificity of the identified peptides.

Several phage candidates were identified using phage display. Phage ELISA showed that these selected phages have higher binding affinity with the IGF2R protein compared with the control phage. Among these peptide candidates, the peptide-431 shows the highest binding affinity and specificity to IGF2R. The K_d value of the peptide-431 is 6.05 μM for LX-2 cells and 11.83 μM for rat HSCs (HSC-T6). Cellular uptake of the peptide-431 in LX-2 cells is significantly reduced after silencing IGF2R. The peptide-431 also enhances the uptake of a proapoptotic peptide (KLA peptide) in LX-2 and HSC-T6 cells, indicating that the peptide-431 can be used as a targeting ligand to deliver antifibrotic agents into HSCs.

This work was supported by the NIH grant (1R01AA021510)

Photothermal stable gold/mesoporous silica hybrid nanoparticle as a theranostic platform for cancer therapy

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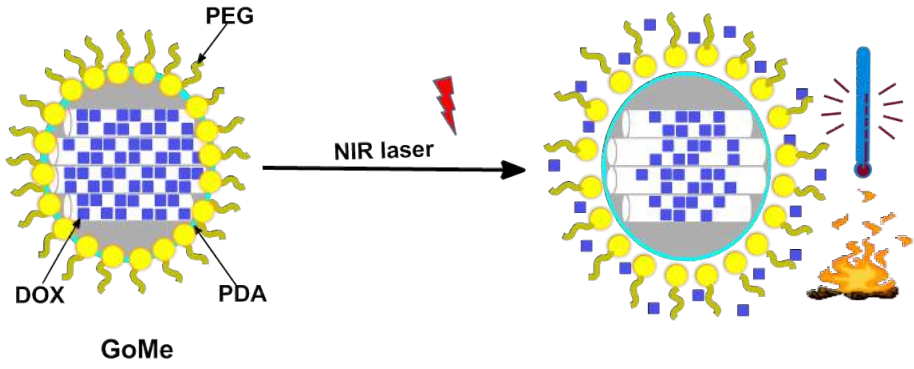
Gold nanoparticles (gold nanorods, gold nanoshells, and gold nanocages) have been extensively explored for the photothermal therapy attributable to their photothermal converting capacity in the near-infrared (NIR) window. However, due to poor photothermal stability, their photothermal capacity quickly diminished upon repetitive NIR irradiation. In addition, these gold nanoparticles are not good drug carrier either due to low drug loading capacity or poorly controlled drug release kinetics. Hereby, we developed a gold/mesoporous silica hybrid nanoparticle based theranostic platform (GoMe) for disease detection, drug delivery, photothermal therapy. The photothermal stability study revealed that GoMe is extreme stable upon repetitive NIR irradiation. Doxorubicin could be easily encapsulated into GoMe with high drug loading content (28%). Furthermore, the release of the doxorubicin from GoMe can be remotely controlled by NIR irradiation. Cell viability assay revealed that GoMe could effectively kill cancer cells due to the integration of photothermal therapy and chemotherapy.



Funding: The ASPIRE award from the Office of the Vice President for Research of The University of South Carolina, the Center for Colon Cancer Research (5P20 RR017698), and the Center for Targeted Therapeutics (1P20 GM109091) from the National Institutes of Health.

Mucus-penetrating cisplatin nanoparticles for the local treatment of lung cancer

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Local delivery of chemotherapeutics to the lung is an attractive strategy for treating lung tumors; however, inhaled free chemotherapeutics are rapidly removed from the lung via systemic absorption and the natural clearance mechanisms of the lung. Local delivery of chemotherapeutics in polymer nanoparticles may serve to prolong the retention of drug in the lungs and provide sustained release to tumor cells, while mitigating systemic and local toxicities. Here, we formulated cisplatin (CDDP)-loaded polymeric nanoparticles from safe materials and evaluated the potential of these carriers for inhaled delivery to lung tumors. Optimized particles (CDDP-MPP) exhibited CDDP loading up to 60% by weight and provided sustained release of CDDP for more than 3 days. The released drug exhibited cytotoxic effects in cell cultures of lung cancer cells and in primary subcutaneous xenografts, eradicating tumors in 80% of mice following intratumoral injections while causing no observed systemic side effects. CDDP-MPPs rapidly penetrated human respiratory mucus *ex vivo* and were well distributed in cryosections of the mouse conducting airways when inhaled. Inhaled CDDP-MPP drastically improved the lung PK of CDDP, increasing the lung AUC by 40- and 10-fold compared to IV CDDP (the current standard of care for lung cancer) and inhaled free CDDP, respectively. Further, inhaled CDDP-MPP reduced the systemic drug exposure leading to decreased nephrotoxicity compared to IV CDDP treatment. Inhalation of CDDP-MPP significantly reduced the pulmonary toxicity observed in inhaled free CDDP-treated mice, allowing for safe administration of higher drug doses. Finally, inhaled CDDP NPs provided

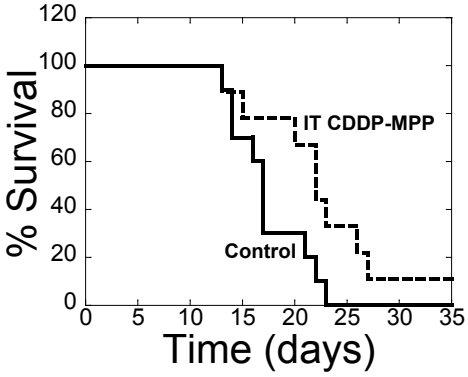

Figure 1. Kaplan-Meier survival analysis of mice bearing orthotopic 3LL/LLC tumors. Mice were inoculated with 3LL/LLC tumor cells intratracheally and subsequently given a single dose of CDDP-MPP intratracheally (IT CDDP-MPP). The survival analysis of n=9-10 mice per group was then compared to control mice. Statistical significance was determined using the Log-Rank Test with a 95% confidence interval. IT CDDP NP statistically increased survival compared to control (p=0.039).

improved efficacy of CDDP in an orthotopic mouse model of lung cancer (Fig.1). The results from these studies suggest these particles are very promising for local treatment of lung tumors and warrant further preclinical efficacy testing of this formulation against lung tumors, compared to standard treatments.

NCI CCNE Grant U54 CA151838

Design and Analysis Hybrid Onconase Nanocarriers for Mesothelioma Therapy

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Onconase (ONC) belongs to a ribonuclease superfamily that has cytostatic activity against malignant mesothelioma (MM). The objective of this investigation was to develop BSA-chitosan based hybrid nanoformulations for efficient delivery of ONC to MM while minimizing exposure to normal tissues. Taguchi Orthogonal Array L9 type design was used to formulate ONC loaded albumin nanocarriers (ONC-ANC) with average particle size of 15.78±0.24 nm (ζ= –21.89±0.11 mV). The ONC-ANC surface was hybridized using varying chitosan concentrations ranging between 0.100-0.175% w/v to form various ONC-hybrid nanocarriers (HNC). The obtained data set was analyzed by principal component analysis (PCA) and principal component regressions (PCR) to decode the effects of investigated design variables. PCA showed positive correlations between investigated design variables like BSA, ethanol dilution, and total ethanol with particle size and entrapment efficiency (EE) of formulated nanocarriers. PCR showed that particle size depends on BSA, ethanol dilution, and total ethanol content, while EE was only influenced by BSA content. Further analysis of chitosan and TPP effects used for coating of ONC-ANC by PCR confirmed their positive impacts on the particle size and surface charge as well as prolongation of drug release than uncoated ONC-ANC. PCR analysis of preliminary stability studies showed increased particle size and surface charge at low pH. However, particle size, zeta potential, and EE of developed HNC were ≤63 nm, ~+31 mV, and ≥96%, respectively, indicating their stability under subjected buffer conditions. Out of the developed formulations, HNC showed enhanced inhibition of cell viability with low IC50 values performed in human MM-REN cells than ONC and ONC-ANC. These studies indicated that this nanotherapeutic approach might aid in reducing the therapeutic dose of ONC against MM and reduce adverse effects by limiting exposure of ONC to normal tissues, and may help in the development of new therapeutic forms and routes of administration.

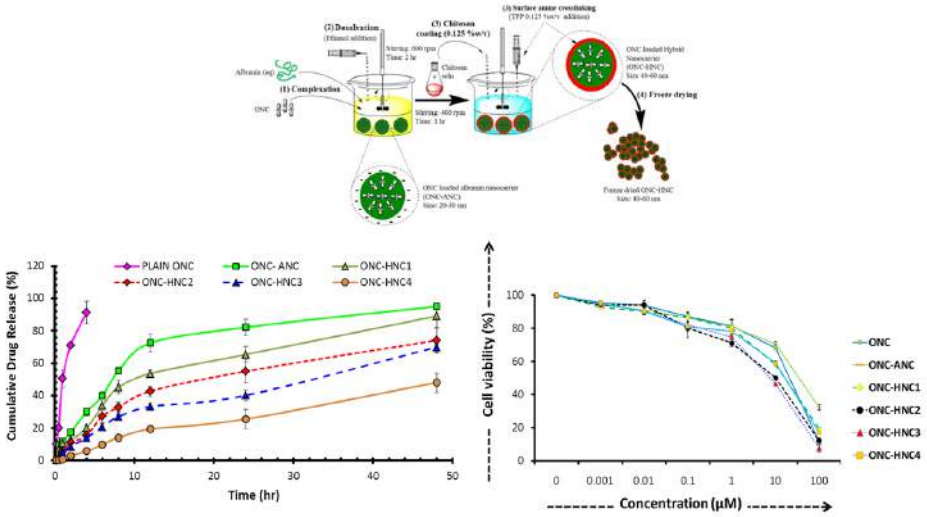

Figure 1. Hybrid Nanocarrier Preparation and Schematic.

Funding: The authors would like to acknowledge Leahi Foundation and the support of College of Pharmacy, University of Hawaii at Hilo and University of Hawaii Cancer Center, Honolulu, Hawai‘i.

AR-12 Acetalated Dextran Microparticles as Host-Mediated Therapy to Control *Francisella tularensis* and *Salmonella enterica* serovar Typhi

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AR-12 was originally used as an anti-cancer drug but has recently shown potent host-mediated clearance against a broad spectrum of bacteria. AR-12 has previously been shown to have activity in vitro against *Salmonella enterica* serovar Typhmiurium and *Francisella* species through host immune pathways mainly autophagy induction. Treatment of S. Typhmurium-infected mice with AR-12 resulted in a 10-fold reduction in bacterial loads within the spleen and liver, and an increased survival period. The treatment however, could not protect the mice from death likely due to poor formulation. In the current study, AR-12 was encapsulated in microparticles (MPs) of the novel biodegradable polymer acetalated dextran (Ace-DEX). Ace-DEX encapsulated AR-12 was evaluated for its anti-bacterial activity in human monocyte-derived macrophages (hMDMs) and mice. Our findings showed that hMDMs efficiently internalized MPs and the re-formulation significantly reduced host cell cytotoxicity compared to unencapsulated AR-12 resulting in greater clearance of intracellular *S*. Typhi and *F*. *tularensis*. Additionally, intranasal administration of *F. tularensis* (SCHU S4) and Ace-DEX MPs resulted in significantly reduced mortality when co-administered with sub-optimal levels of gentamicin. These studies provide support that Ace-DEX encapsulated AR-12 may be a promising formulation which has the ability to clear intracellular bacterial pathogens of macrophages more efficiently while reducing the associated toxicity of the unencapsulated drug.

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White adipose tissue but not enlarged livers are sinks for nanoparticles in diet-induced and genetic mouse models of obesity

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Since almost 70% of the U.S. population is overweight and 30% is obese, with much of the western world following suit, many patients that will potentially be administered circulating nanoparticles designed to localize to tumors and avoid non-target areas will have significant amounts of white adipose tissue (WAT), enlarged livers, and additional metabolic complications such as type 2 diabetes. However, studies on nanoparticle biodistribution and efficacy take place almost without exception in lean rodents. Here, we determined the biodistribution of model nanoparticles – neutral filomicelles and charged polystyrene spheres carrying near infrared (NIR) dye - as a function of mouse diet, weight, and metabolic condition. In obese ob/ob mice lacking the hormone leptin, which regulates appetite control, nanoparticles accumulated in the WAT in higher amounts than in the spleen, which was previously the focal point of macrophage-induced nanoparticle clearance (Figure 1). However, the localization of nanoparticles to WAT seems to be a passive mechanism since the NIR signal per WAT weight was much lower than for the liver and spleen. Surprisingly, nanoparticle accumulation in the liver remained constant even in obese mice with liver mass four-times that of lean mice. Both of these localization results were independent of nanoparticle geometry and charge. From these observations we postulate that the metabolic condition of the patient will drastically change the efficacy of current nanoparticle technologies.

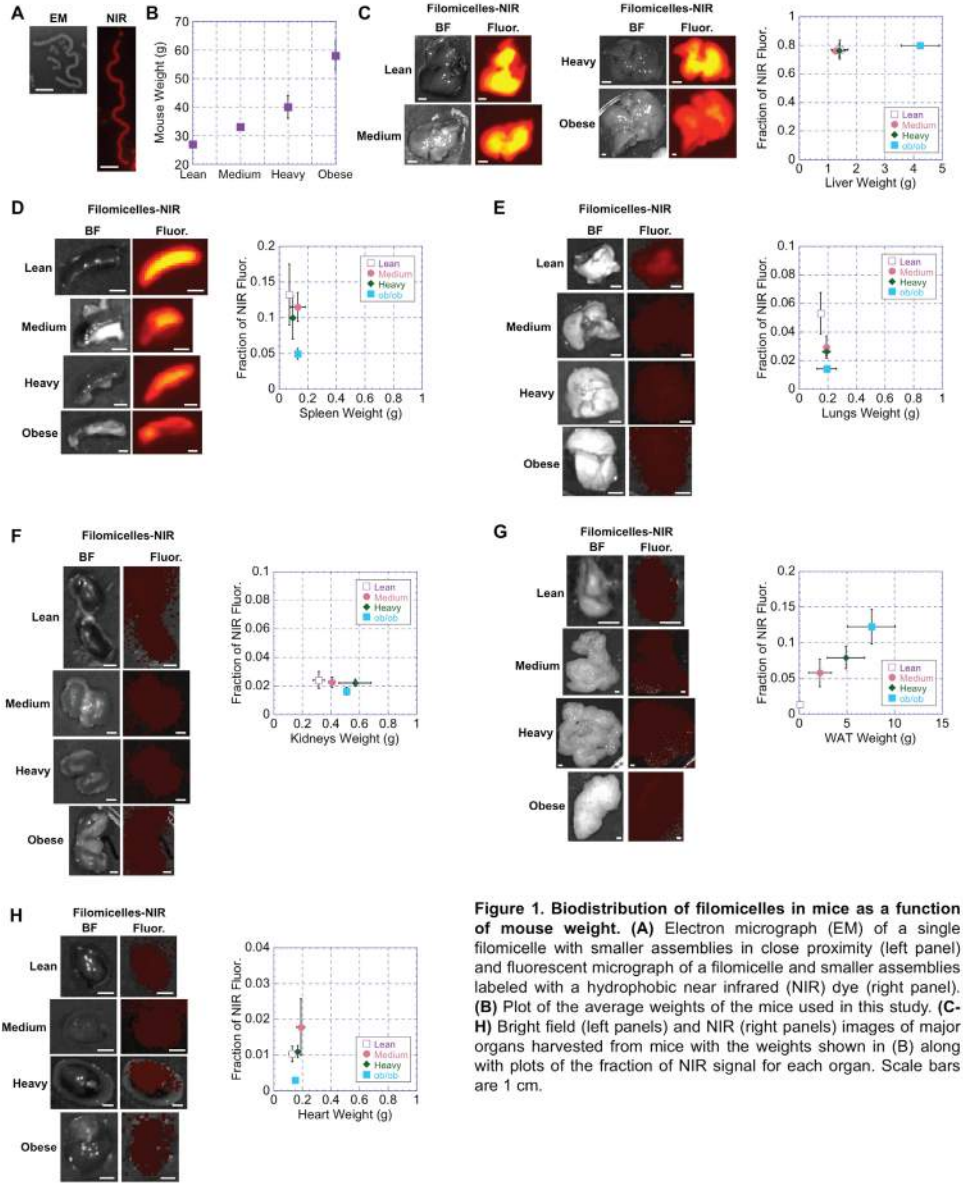

Figure 1. Biodistribution of filomicelles in mice as a function of mouse weight. (A) Electron micrograph (EM) of a single filomicelle with smaller assemblies in close proximity (left panel) and fluorescent micrograph of a filomicelle and smaller assemblies labeled with a hydrophobic near infrared (NIR) dye (right panel). **(B)** Plot of the average weights of the mice used in this study. **(C-H)** Bright field (left panels) and NIR (right panels) images of major organs harvested from mice with the weights shown in (B) along with plots of the fraction of NIR signal for each organ. Scale bars are 1 cm.

Structure-Relaxivity Relationships of Well-Defined Polymer-Iron Oxide Clusters for Magnetic Resonance Imaging and Drug Delivery

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Multifunctional nanocarriers that integrate imaging agents and drugs into a single system have attracted considerable interest for tracking drug biodistribution. We report the fabrication of colloidally-stable clusters of polymer-iron oxide clusters with hydrophobic cores, controlled sizes, and compositions. Discrete ~ 8 nm nanoparticles of primarily magnetite were synthesized by reducing iron (III) acetylacetonate in benzyl alcohol and their surfaces were subsequently modified to be hydrophobic by coating with oleic acid. A poly(ethylene glycol)-b-poly(D,L-lactide) block copolymer [mPEG(5 kDa)-b-PDLLA(10 kDa)] was synthesized by ring-opening polymerization of D,L-lactide in the presence of monofunctional PEG. Hydrophobic-core clusters were prepared by Flash Nanoprecipitation using an amphiphilic poly(D,L-lactide)-poly(ethylene oxide) diblock copolymer stabilizer. Nanoparticles with intensity-average hydrodynamic diameters in the range of 100-150 nm and polydispersity indices less than 0.2 were obtained, as measured by dynamic light scattering. The transverse and longitudinal NMR relaxivities of the iron oxide-loaded nanoparticles were ~ 200 s^-1 mM Fe^-1 and ~ 1 s^-1 mM Fe^-1 respectively. The transverse relaxivities enabled sensitive T2-weighted MRI and the low longitudinal relaxivities are consistent with the hydrophobic nature of the cores of the nanoparticle clusters. We have developed a model that predicts the transverse relaxivity of these particles typically to within ±15% with no adjustable parameters so that can now design MRI T2-contrast agents. Nanoparticles were also made with the antiretroviral drug, ritonavir, complexed with the mPEG(5 kDa)-b-PDLLA(10 kDa)] diblock and also with the diblock copolymer poly(ethylene oxide-b-butylene oxide). The particles had intensity average hydrodynamic diameters <100 nm. Ritonavir drug loadings greater than 20 wt% and encapsulation efficiencies ~ 70-80% were attained. These nanoparticles were colloidally stable under simulated physiological conditions (phosphate buffered saline containing 1% bovine serum albumin, T = 37°C) for more than 24 hours.

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Formulation, characterization and evaluation of Paclitaxel loaded solid lipid nanoparticles.

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Solid lipid nanoparticles (SLNs) are spherical lipid particles in the nanometer size range having large surface area with the capability to incorporate poorly water soluble drugs. This research aims to advance the formulation technique of SLNs by preparing glyceryl monostearate (GMS) nanoparticles using a temperature modulated solidification technique developed and optimized in our laboratory. The most important advantage of this technique is that it does not use any organic solvents during formulation and the SLN properties are highly reproducible.

METHODS: Paclitaxel was incorporated in the SLNs. An HPLC method was developed for Paclitaxel and validated. Drug concentrations of 50mg, 75mg and 100mg were utilized to determine the entrapment efficiency. Characterization of SLNs was done by particle size analysis, zeta potential analysis, atomic force microscopy, transmission electron microscopy, and *in vitro* drug release studies.

RESULTS: Entrapment efficiencies of 75 and 100mg formulations were found to be similar and was approximately 62%. The particle size of the SLN dispersion was found to be less than 100 nm. Zeta potential revealed the presence of a negative surface charge of about -25 mV. TEM and AFM images of the blank SLNs confirmed the spherical shape of the particles. *In vitro* drug release studies demonstrated that the encapsulated drug was released from the SLNs from 15 minutes up to three days.

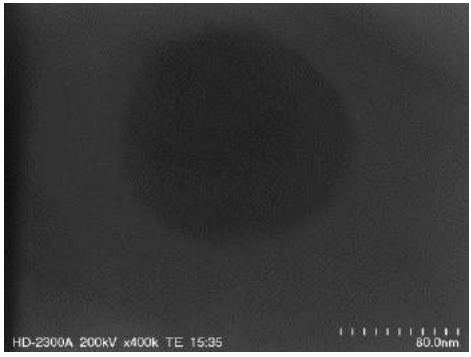

Figure: TEM image of a blank SLN

CONCLUSION: Paclitaxel SLNs were formulated successfully and confirmed to have appropriate particle size and well-defined periphery. *In-vitro* drug release studies confirmed the sustained release of the drug. Hence, the formulated SLNs may be potentially utilized in an anticancer drug therapy.

Ultrasound-Triggered Noninvasive Regulation of Blood Glucose Levels Using Microgels Integrated with Insulin Nanocapsules

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Diabetes mellitus, a disorder of glucose regulation, is a global burden affecting 382million people across the world. An artificial microgel system able to be stimulated by focused ultrasound to mimic pancreas activity and release insulin in response to glucose level changes has the potential to improve patient compliance and health. Herein we develop an ultrasound-mediated release strategy for insulin delivery using injectable and acid-degradable polymeric microgels. Formed by electrospray chitosan microparticle encaupsulated with poly(lactic-co-glycolic) acid PLGA insulin loaded nanoparticles, the microgels can be dissociated and subsequently release insulin in a hyperglycemic state through stimulation of focused ultrasound (**Figure 1**). By serving as a synthetic insulin reservoir, the microgel integrated with insulin loaded PLGA nanoparticles significantly promoted insulin release upon intermittent FUS triggers. *In vitro* insulin release can be modulated in a pulsatile profile according to focused ultrasound triggering. *In vivo* studies validated that these formulations provided for improved glucose control in Type 1 diabetic mice subcutaneously administered with insulin-loaded micro-nanoballs. A single injection of the developed micro-nanoballs facilitated stabilization of the blood glucose levels in the normoglycemic state (< 200 mg/dL) for over two weeks. We have developed a novel means of ultrasound-triggered controlled drug delivery based on the use of an injectable microgel. It can be effectively triggered to release insulin upon FUS-mediated administration. This system provides an unprecedented useful tool for noninvasive, rapid and pulsatile regulation of BG levels for diabetes treatment. It also can be extended to deliver other drugs, therapeutic proteins, or peptides in an intermittent and spatiotemporal release fashion. Furthermore, this method can be integrated with an ultrasound imaging system for noninvasively monitoring degradation of the drug-contained formulation and facilitating the subsequent administration.

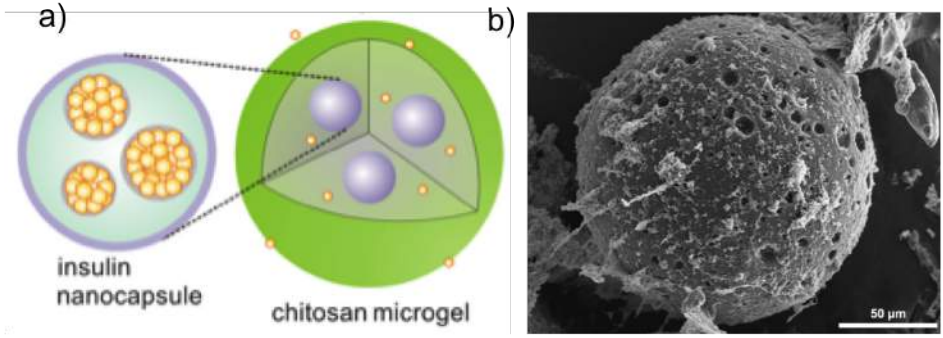

Figure 1. Characterization of microgel integrated with insulin loaded nanocapsules. a) Schematic of chitosan microgel integrated with insulin loaded PLGA nanocapsules. b) The SEM image of formed microgel structure. Scale bar: 50 µm.

Funding: This work was supported by the Junior Faculty Award of the American Diabetes Association (ADA), grant 550KR51307 from NC TraCS, NIH's Clinical and Translational Science Awards (CTSA) at UNC-CH (1UL1TR001111), the NC State Faculty Research and Professional Development Award, and the start-up package from the Joint BME Department of UNC-CH and NCSU to Z.G.

Autonomic Modulation as a paradigm for Cardiovascular Treatments

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Objective: Consider physiological interventions of the autonomic nervous system (ANS) related to treatments for cardiovascular diseases, especially arrhythmias, and note a developing theme for *autonomic modulation*.

Summary: Increasingly, experimental and clinical data on ANS denervation or stimulation are reporting therapeutic effects: a) Regional radiofrequency catheter ablation of atrial ganglionated plexi (GP) restored sinus rhythm in 71% of patients with atrial fibrillation (AF); b) Low level electrical stimulation of the vago-sympathetic trunks significantly suppresses AF inducibility in the canine; c) our demonstration that polymeric magnetic nanoparticles delivering a neurosuppressant payload while extravasated and targeted by an external magnetic field to GP, suppressed/prevented AF inducibility; d) Vaso-vagal syncope (intrinsic cardiac ANS dysreflexia) had no recurrence in patients with partial GP ablations; and e) Low level transcutaneous electrical stimulation of the auricular branch of the vagus nerve at the tragus of the ear suppresses AF in patients. Forty patients with paroxysmal AF about to undergo radiofrequency ablation for AF were randomized into two groups. Pacing –induced AF duration was significantly decreased by low level stimulation of the tragus.

Conclusion: Together these observations suggest that selective ANS denervation, stimulation or suppression, delivered by devices, electrodes or magnetically targeted nanoformulations represent a paradigm of ANS modulation that might present as future therapeutic cardiovascular interventions.

Funding: Magnetic Targeting Studies: NanoMed Targeting Systems Inc., Oklahoma City

Nanomedicine for gastrointestinal drug delivery: mucoadhesion, friend or foe?

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It is believed that mucoadhesive particle formulations are the most effective for enhancing drug delivery to the gastrointestinal (GI) tract. Mucoadhesive nano- and microparticle systems are typically compared to unencapsulated therapeutics or to moderately mucoadhesive particles, and certain experimental methods may compromise the mucus barrier. Thus, it is difficult to separate the benefits of encapsulation from the effects of mucoadhesion. To explore this paradigm, we orally and rectally-administered nanoparticles with strongly mucoadhesive or non-mucoadhesive surfaces. We found that mucoadhesive particles (MAP) aggregated far from the absorptive epithelium. In contrast, water absorption by the GI tract uniformly transported non-mucoadhesive mucus-penetrating particles (MPP) through mucus to the epithelial surfaces between villi in the small intestine. However, when using high gavage fluid volumes or injection into ligated intestinal loops, common methods for assessing oral drug and nanoparticle absorption, we found that both MAP and MPP were well-distributed. Thus, it appears as though these common methods compromise the mucus barrier, and thus may overstate the benefits of mucoadhesive particle formulations. The normal function of the mucus barrier is to trap and remove foreign particulates, which is likely to limit the effectiveness of MAP, particularly when taken as a pill or capsule rather than in large volumes of fluid. In contrast, MPP took advantage of normal fluid absorption processes in the GI

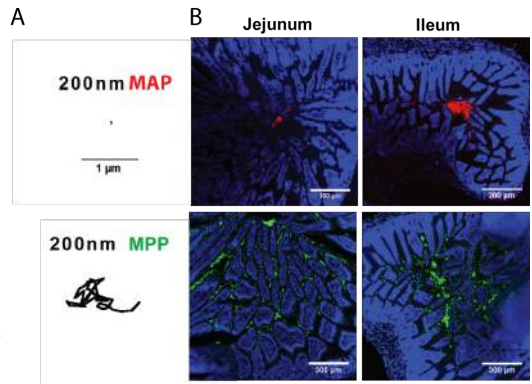

Figure 1. Comparison of MAP and MPP in the mouse small intestine. (A) Trajectories representative of 3 s of movement of 200 nm MAP and MPP in mucus on freshly excised mouse small intestine tissue. (B) Distribution of fluorescent 200 nm MAP or MPP in the healthy mouse jejunum or ileum after low volume oral gavage. Images are representative of n ≥ 3 mice.

tract to distribute and reach all absorptive epithelial surfaces. We observed similar improvements in MPP distribution throughout the colorectum upon administration in an enema vehicle that induced fluid absorption by the epithelia (hypotonic). In a mouse model of ulcerative colitis, the non-mucoadhesive MPP were also able to penetrate into ulcerated tissue regions. In contrast, MAP remained adherent in the luminal mucus layers. Such widespread and uniform distribution of MPP in the GI tract is likely to improve local and systemic delivery of therapeutics.

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Engineered nanoparticles that mimic bacterial pathogens for the treatment of breast cancer

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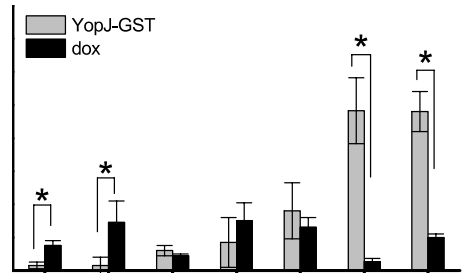
Bacterial pathogens have evolved mechanisms to control the fate of host cells during infection. Some Gram-negative bacteria use needle-like structures, known as the type III secretion system (T3SS), through which they inject effector proteins directly into the cytoplasm of host cells. These effector proteins have high potential as cancer therapeutics because they are capable of efficiently subverting a variety of eukaryotic signaling pathways. In breast cancer, the NF-κB and MAPK pathways are known to be dysregulated and critical in the development and progression of the disease. Effector protein YopJ, from *Yersinia Pestis,* simultaneously down-regulates MAPK and NF-κB pathways to induce apoptosis only in specific cell types during infection. However, bacterial delivery of effector proteins for therapeutic purposes is not likely to be a viable option.

In order to deliver the protein to cancer cells, we have expressed YopJ as a fusion protein with glutathione S-transferase (GST), to form self-assembled protein nanoparticles with diameters of 100nm. YopJ-GST nanoparticles efficiently deliver protein to cells, replacing the T3SS mechanism. These nanoparticles induce 97% cell death within 72 h of treatment in SKBR-3 breast cancer cells, significantly more than the same molar dose of doxorubicin (Figure). Similarly, studies with a panel of breast cancer cell-lines showed that YopJ-GST nanoparticles induce cell death in doxorubicin-resistant SKBR-3 cells, in MCF-7, MDA-MB-468, and MDA-MB-231 (Figure). YopJ-GST nanoparticles were not cytotoxic to healthy fibroblast cells (NIH/3T3) or cervical cancer cells (HeLa), which are not sensitive to direct MAPK/NF-kB inhibition. In addition, sub-lethal doses of YopJ-GST nanoparticles decreased SKBR-3 cell migration *in vitro* and down-regulated the MAPK ERK 1/2 pathway, which has been correlated to metastasis. Therefore, this effector fusion protein nano-assembly demonstrates potential as a novel breast cancer therapeutic with potent and selective cytotoxicity, and the capacity to decrease metastatic markers in surviving cells.



Funding: This work was supported by the National Science Foundation (NSF)

Next generation multivalent PRINT nanoparticle vaccine targeting pneumococcal disease

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Background and Aims: A new generation PRINT nanoparticle Pneumococcal vaccine matrix has been designed to mimic size and structural aspects of bacterial pathogens to efficiently present and deliver polysaccharide and protein antigens to elicit robust immune responses. These compositions have been repeatedly characterized by physicochemical and bioanalytical assays to quantify polysaccharide and protein content. To imitate bacterial size, 80x320nm rods were used to formulate particle compositions consisting of CRM_{197} or mutant Pneumolysin protein (PLD) as a carrier protein/immunogen combined with purified Pneumococcal polysaccharides (PnPs) 1,5,6A,14 and 19A. All particle vaccines demonstrated strong immune responses in BALB/c mice.

Methods: *In vitro* antibody-based ELISA, DIONEX and BCA assays have been developed and optimized to quantify the amount of polysaccharide and protein in PRINT nanoparticles. Antigen-specific IgG (for 5 PnPs strains, and PLD) and cellular assays (IL-17, IFN-**γ**) have been developed to quantitate the immune response in mice. PRINT particles were manufactured using roll-to-roll PRINT manufacturing process and were purified by Tangential Flow Filtration (TFF). Using the TFF process, PRINT suspensions afforded stable nanoparticle formulations with average particle size of ~250 nm and >70% post TFF recovery yield. The particles were further characterized using SEM, DLS, Zeta Potential, sterility, pH, Osmolarity.

Results: Five of the Prevnar 13 serotypes have been successfully formulated and quantified in PRINT particles with strong reproducibility. Antigen-specific IgG responses were observed for all five serotypes and were found to be ≥ Prevnar 13. This response driven by particulated delivery of PnPs was independent of adjuvant components. The level (IgG) and quality (neutralizing) of antibody responses produced by particulated delivery of PLD protein demonstrated superiority as compared to soluble antigen and equivalent to soluble antigen plus adjuvent. These findings demonstrate the ability of PLD not only to serve as an alternative carrier protein to CRM_{197} but also act as an effective immunogen in PRINT

formulations.

Conclusions: Incorporation of five of the most prevalent Pneumococcal Polysaccharides and multiple proteins as carriers in PRINT nanoparticles has demonstrated a wide-ranging multi-antigenic formulation that could allow for broadened efficacy and be adaptable to different regions through incorporation of relevant serotypes.

Development of dichloroacetate biomaterials for prevention of postoperative adhesions.

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Injury to the peritoneum during surgery often leads to the development of postoperative adhesions (PAs). PAs are a significant health problem and source of morbidity, including pelvic pain, bowel obstruction, and infertility. The mechanism of PA development is incompletely understood. Despite significant progress, currently used methods of PA prevention methods rely on simple physical barriers between the tissues. Limited attention has been paid to the development of materials that would provide local, sustained release of drugs. A drug with potential ability to target physiological processes involved in the formation of PAs is dichloroacetic acid (DCA). DCA has multiple effects on the expression of factors involved in PA development, including matrix metalloproteinase-1 (MMP-1), MMP-1 tissue inhibitor, and transforming growth factor-β. Its anti-fibrotic and related effects support the potential of DCA in reducing PA development. DCA is a small, water-soluble molecule that diffuses rapidly after encapsulation in traditional polymeric biomaterials. As an alternative to simple encapsulation, our goal was to develop DCA-based biomaterials exhibiting sustained release of DCA. We selected two widely used polyols with a proven record of safety in biomedical applications: hydroxyethyl starch (HES), a natural polysaccharide used as plasma expander, and polyvinyl alcohol (PVA), a synthetic polymer employed in a wide variety of pharmaceutical applications. HES and PVA were covalently modified with DCA (Figure 1) via direct esterification of the hydroxyl groups. Polymers properties, degradation rate and rate of DCA release were examined. The developed DCA-polymer conjugates represent innovative biomaterials with the potential to effectively prevent postoperative adhesions.

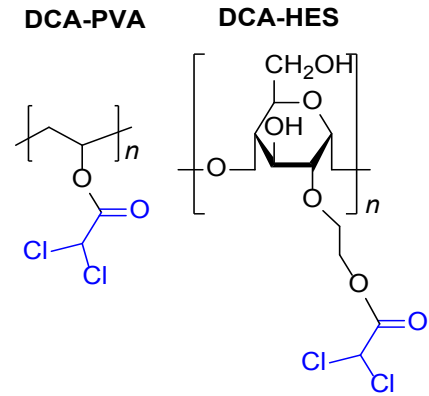

Figure 1. Structures of PVA and HES covalently modified with DCA (DCA-PVA and DCA-HES respectively).

Funding: Nebraska Research Initiative.

Delivery of nanoformulated enfuvirtide across the blood-brain barrier

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In HIV management, eradication of virus by sanctuary sites remains a main challenge. In fact, although current antiretroviral (ARV) therapies suppress plasma HIV below detectable levels in a consistent proportion of subjects, total virus eradication is still beyond our possibilities. An important barrier to achieve such goal is related to the suboptimal concentrations of ARVs within the HIV sanctuaries. The central nervous system (CNS) is a key example of sanctuary where viral replication occurs despite of a complete viral suppression in the peripheral blood. In recent years, nanotechnology has provided great promise in the eradication of HIV from CNS. However, this is the first time in which a complex and heavy peptide like enfuvirtide (Enf), which normally does not penetrate in the CNS, is found to cross the blood brain barrier (BBB) of mice, by conjugation with a nanoconstruct. We demonstrated that iron oxide nanoparticles coated with an amphiphilic polimer (MYTS),

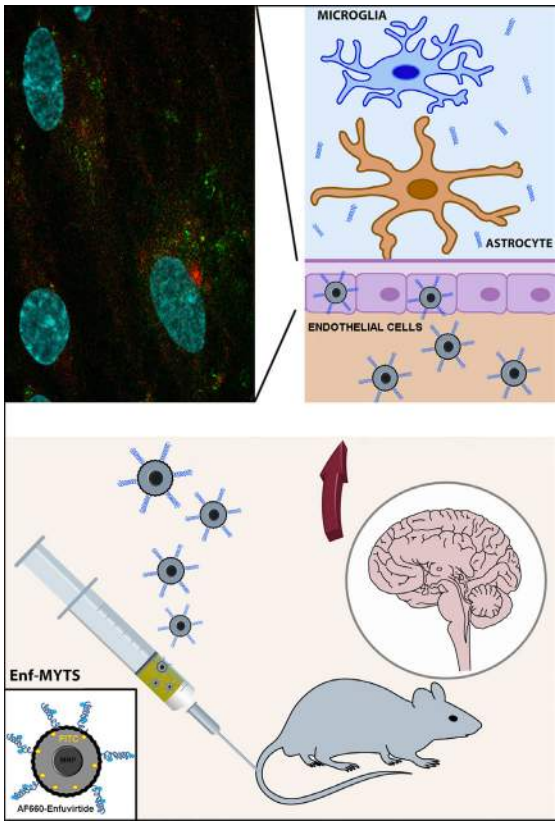

Figure 1: Enfuvirtide-conjugated MYTS, intravenously injected in mice, reach the brain microvascular endothelium and are internalized by the endothelial cells to release the drug in brain parenchyma. Confocal image show AF660-Enf (red) and FITC-MYTS (green) in endothelial cells of a BBB *in vitro* model. Nuclei are stained with DAPI (cyan). AF660 and the FITC fluorescence pixels were mostly non-overlapping.

labeled with FITC, increased AF660-Enf translocation across BBB *in vitro*, using a validated BBB model composed of rat BMVECs and astrocytes, and in mice i.v. injected with the nanoformulated Enf. We described a delivery mechanism involving the uptake of MYTS-Enf in the endothelial cells, the nanocomplex dissociation and the release of the peptide, which is eventually excreted by the cells in the brain parenchyma (Figure 1). Transmission electron microscopy demonstrated that MYTS internalization in the BMVECs occurred by the fusion of the amphiphilic coating with the cell membrane. Histopathological analysis of brain and other organs dissected from mice exposed to MYTS showed the lack of any systemic toxicity of the circulating nanoconjugates.

Funding: “NanoMeDia” project financed by Lombardia District (Italy)

Synthesis and Characterization of Chemically Modified Immunostimulatory Polysaccharide Serving Dual Function as Adjuvant and Protein Antigen Delivery Vehicle

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The only adjuvants currently Food and Drug Administration (FDA)-approved for subunit vaccines are aluminum salt-based (alum). However, alum formulations have several drawbacks: antigens with certain surface charges and hydrophobicity are required for binding, adsorption can lead to undesired antigen denaturation, it has limited cold-chain storage options, and it typically results in a polarization towards an antibody-based Th2 response. Optimal vaccines should create long-term adaptive immunity against the target pathogen through a combined humoral (antibody) and cell-mediated (CD8+ and Th1) response, because many pathogens exist intracellularly. Other adjuvants that elicit a Th1 response have been considered but are often too cytotoxic to use clinically (e.g. Freund's complete adjuvant). One alternative option to alum or other toxic Th1 adjuvants is through the use of an immunostimulatory polysaccharide. Here, we chemically modified a naturally occurring inulin polysaccharide, which has recognized immunostimulatory properties, into an acid-sensitive biopolymer. The acetalated inulin polymer (Ace-IN) was formed into microparticles (MPs) by a solvent evaporation method, and we showed MP degradation is tunable based on the polymer synthesis conditions. RAW macrophages were passively targeted with cytocompatible Ace-IN MPs based on size and showed tunable release of a Th1 cytokine correlated with the MP degradation profile. The Ace-IN MPs were loaded with a model antigen, ovalbumin (OVA), and in vivo immunization with the antigen led to production of anti-OVA IgG antibody levels comparable to an alum-based formulation. Our work demonstrates the potential for this modified immunostimulatory inulin polysaccharide to serve a dual function as a subunit vaccine adjuvant and antigen delivery vehicle.

Construction of T7 phage-displayed random peptide library for ligand discovery used in targeted nanoparticle (NP) delivery

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Purpose: Phage display is a powerful tool in ligand discovery for targeted NP delivery. Current phage display almost exclusively uses linear M13/fd phage (~ 7 x 800 nm) that is incompatible with the common endosome (~ 100 nm) involved in intra/trans-cellular NP delivery. T7 phage is a natural spherical NP (~ 65 nm). However, compared to M13/fd phage display, random peptide library construction in T7 phage is much more difficult. As a result, there are no commercial libraries available and only one linear 12-mer random library reported. That library samples less than one millionth of the library's theoretical sequence space. We seek to make our own Cysteine-constrained 9-mer library with a complexity of ~ 5 x 10^9 that covers 1% of its theoretical sequence space, or five-order of magnitude higher than the reported library.

Methods: An NCI lab kindly provided the reported T7 random peptide library, from which the BamH1/HindIII-cut phage vector backbone was made. Tri-nucleotide scheme that completely eliminates stop codon and codon degeneracy was employed to design the DNA oligomers for the insert that encodes the random Cys-9-mer library. Standard molecular biology techniques were used to prepare the backbone and the insert, to package them into infectious T7 phages using a T7 phage packaging mix from Novogen, and to titter the resultant phages.

Results: CsCl/ultracentrifugation-purified vector DNA was made, digested with BamH1/HIndIII, phenol-extracted and ethanol-precipitated to result in the backbone. Two insert oligomers were synthesized by Genescript and annealed to form the ds-insert. Backbone and insert were packaged at different ratios. First pilot package attempt yielded as high as 10 million of live phages per 5 ml of packaging extract.

Conclusion: On scale up and at this yield, it is already possible to reach the desired library complexity. Such libraries should enable us to isolate NP-compatible ligands.

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Development of cathepsin S cleavable peptides to enhance the diagnostic and radiotherapeutic efficacy of ^{177}Lu-labeled HPMA copolymers

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Objective. The development of nanomedicine delivery systems offers several advantages over traditional drug delivery including the ability to control both the rate and location of drug release. However, one of the primary disadvantages of these micro- and nanoparticle based systems has been opsonization and uptake by macrophages (e.g., Kupffer cells) of the mononuclear phagocyte system (MPS). So far, MPS accumulation, particularly in the liver and spleen, remains an important obstacle in the clinical translation of many nanomedicines. Here we describe the application of ^{177}Lu labeled HPMA copolymers incorporating different cathepsin S cleavable linkers (CSLs) for human pancreatic adenocarcinoma (PDAC) diagnostic imaging and radiotherapy. The cleavable copolymers are expected to enhance the diagnostic and therapeutic efficacy of nanomedicines platforms by reducing MPS accumulation.

Methods. Three cathepsin S cleavable peptide linkers with linking groups of different length (0, 6 and 13 atoms) were utilized to link DOTA chelated ^{177}Lu to a Mw 109 kDa HPMA copolymers prepared by RAFT. These conjugates were evaluated by in vitro cleavage studies and in vivo biodistribution studies in mouse xenograft models of human pancreatic adenocarcinoma.

Results. In vitro enzymatic studies revealed that the longest linking group (13 atoms) led to faster cleavage by cathepsin S. The radiolabeled HPMA copolymers with CSLs incorporated demonstrated significantly higher levels of excretion and a significant decrease in long-term hepatic and splenic retention of radioactivity, relative to the non-cleavable control. In vivo biodistribution studies showed that the length of the linking group had minimal impact on the non-target clearance. However, the HPMA copolymer with CSL bearing the null (0 atom) linking group demonstrated significantly higher levels of tumor retention relative to other CSLs.

Conclusions. Our results demonstrate that the CSLs can substantially reduce the non-target retention of radioactivity from ^{177}Lu-labeled HPMA copolymers thereby increasing their clinical potential.

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Optimization of Liposomal Formulations for Convection Enhanced Delivery in the Treatment of Glioblastoma

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Glioblastoma multiforme (GBM) is the most common and aggressive malignant primary brain tumor in humans. It is a type of astrocytoma, and therefore shows the abnormal histological features of astrocytes. Although the incidence rate of GBM is low, the median survival without treatment is only about 5 months, and 15 months with the standard radiotherapy and chemotherapy. Due to this aggressiveness, there have been many attempts to treat GBM in new ways such as drug delivery through blood vessels. However, the strong and intrinsic defense system, the blood-brain barrier (BBB), impedes the deep penetration of drugs in the brain tissue, thus reducing the therapeutic efficacy. A convention enhanced delivery (CED) method, which is a delivery technique to infuse drugs directly into tissue using a catheter with continuous positive pressure, was recently developed to solve the delivery issue in the brain. However, although the CED improves the drug penetration, the interstitial and cerebrospinal fluids drain out the infused drugs shortly from the brain tissue. Here, we optimized a liposomal formulation to simultaneously improve penetration and retention of drugs infused using the CED method in the brain tissue. We prepared various liposomal formulations with different surface chemistries, which were loaded with fluorescent dyes. Then, we injected them directly into the brain tissue *via* the CED and examined their time-dependent distribution using fluorescent microscopy. As a result, a liposomal formulation containing both cationic and PEGylated lipids remained relatively long in the brain tissue compared with other liposomal formulations including the PEGylated one, although their tissue penetration appeared similar. We believe that the optimized distribution of cationic and PEGylated lipids on the liposomal surface determines the retention in the brain tissue. These results suggest that optimized liposomal formulation can further enhance the therapeutic effect of drugs administered *via* the CED for glioblastoma treatment.

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Genomic DNA nanoparticles rescue rhodopsin-associated retinitis pigmentosa phenotype

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Conflict of Interest: Mark Cooper is an employee of Copernicus Therapeutics and holds stock in the company. No other authors have a conflict of interest.

Gene delivery strategies routinely use cDNA-based vectors, however non-coding components of genomic DNA can regulate gene expression. Genomic sequences can preserve the stability of the message obtained from RNA splicing as well as improve translational yield and produce physiologically appropriate levels of gene expression. In the current report, we test the hypothesis that inclusion of introns improves the efficacy of transgene expression in a mouse model of retinitis pigmentosa. We utilize compacted DNA nanoparticles, which have the ability to transfer large genes, to deliver murine rhodopsin cDNA or genomic DNA to the rhodopsin knockout eye. We show functional and structural improvements for up to 8 months after NP-mediated genomic DNA but not cDNA delivery. These results suggest that inclusion of native DNA elements, such as introns, can significantly enhance rhodopsin gene expression and therapeutic efficacy and may become an option in the array of available gene delivery tools.

Funding: This work was supported by the National Eye Institute (R21EY024059-ZH, EY018656-MIN and EY22778-MIN), the Oklahoma Center for the Advancement of Science and Technology (ZH), and the Knights Templar Eye Foundation (ZH).

Tumor-nanoparticle interaction of intraperitoneally delivered neutron activatable nanoparticles *in vivo* and in a 3D tumor spheroid model

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Purpose: To establish an *in vitro* OVCAR-8 3D spheroid model and investigate the tumor specific accumulation of holmium-166 loaded mesoporous silica nanoparticles (MSNs) on avascular, metastatic peritoneal tumor tissues after intraperitoneal administration.

Methods: MCM-41 type mesoporous silica nanoparticles (MSNs) were synthesized and loaded with either fluorescein isothiocyanate (FITC), or a stable holmium-165 (^{165}Ho) acetylacetonate complex. ^{165}Ho loaded particles were subsequently irradiated to yield MSNs containing ^{166}Ho, a beta-emitter with therapeutic nuclear properties (Emax = 1.84 Mev; t½ = 26.8 h). In order to determine the mechanism of the tumor-nanoparticle interaction and best mimic avascular peritoneal tumor metastases, uniform OVCAR-8 3D tumor spheroids (400µm geometric mean diameter) were cultured for 2 weeks in 96 well plates using a liquid overlay technique. These spheroids were then treated with various

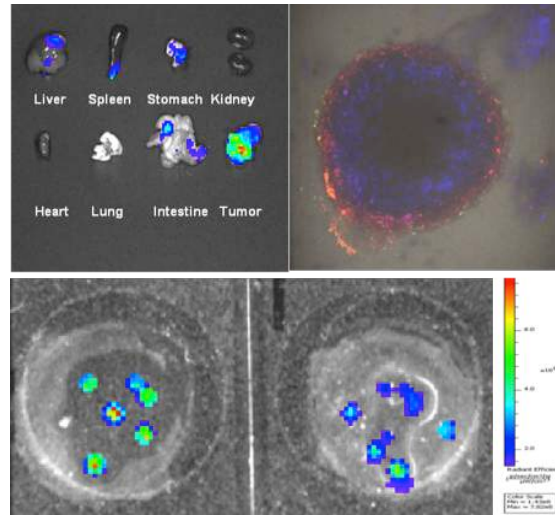

Figure 1: (Top Left) Optical images of ex vivo tissues of tumor bearing mice 24 hours after intraperitoneal delivery of Cy5.5 labeled MSNs. MSNs showed high accumulation only on tumor tissues. (Top Right) Depth color coded image of an OVCAR-8 3D tumor spheroid incubated with FITC labeled MSNs taken using confocal microscopy. (Bottom) Optical images taken with the IVIS Spectrum system of OVCAR-8 spheroids (n=7) incubated with FITC labeled MSNs for 2 hours. The spheroids on the right were treated with 1mg/mL collagenase in PBS for 18 hours and exhibited a statistically significant decrease in nanoparticle adhesion when compared to the no treatment group.

concentrations of collagenase to remove the extracellular matrix (ECM). Spheroids with and without ECM were then incubated with FITC-MSNs and MSN adhesion was determined using optical imaging.

Results: The predominant accumulation of the MSNs in tumor tissues was demonstrated using SCID mice bearing OVCAR-8 and Mia-PaCa peritoneal tumors. Confocal microcopy showed the ability of FITC-MSNs to penetrate from the surface (around 30 μm in 24 h) to significant tumor depths (more than 300 μm in one week). A uniform 3D tumor spheroid model was established using the OVCAR-8 cell line. Preliminary data showed increased MSN accumulation in tumors with a fully intact ECM over spheroids in which the ECM was removed by collagenase treatment.

Conclusions: Specific tumor accumulation, significant tumor penetration, and the ability to make MSNs radioactive after preparation make them a promising brachytherapy agent. Tumor ECM plays a significant role in the adhesion of MSNs to tumor tissues, which may be a driving force for the tumor specific accumulation of MSNs after intraperitoneal administration.

Funding: Connecticut Institute for Clinical and Translational Science

Silencing Rcn2 gene in endothelial cells by exosome-delivered siRNA.

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Exosomes, one subpopulation of nanosized extracellular vesicles derived from multivesicular bodies, ranging from 30-150 nm in size, emerged as promising carriers for siRNA delivery, as they are capable of transmitting molecular messages between cells through carried small non-coding RNAs, mRNAs, DNAs and proteins. In this work we investigated the capability of novel endothelial exosmes carrying exogenous siRNA against RCN2 to abrupt the atherogenic pathway Ath29-RCN2, a novel target we recently identified, in endothelial cells of B6 apoE-/- mice in vitro. Exosomes were isolated and purified from cultured mouse endothelial cells by sequential centrifugation and ultracentrifugation. The resulted endothelial exosomes were nanometer sized vesicles examined by both Nanosight instrument and TEM. ELISA analysis confirmed the expression of two exosomal markers, CD9 and CD63. The uptake of green fluorescent dye labeled exosomes into endothelial cells was investigated in permissive and restrictive temperatures by ImageStream^X flow cytometry. Our results showed that endothelial exosomes were homing back efficiently to parent cells likely through a receptor-ligand mediated uptake. Finally, exosomes transmitted siRNA/RCN2 was able to significantly reduce RCN2 in Western blot analysis and decrease the downstream MCP1/VCAM1 in ELISAs (Figure 1). In conclusion, we have demonstrated that endothelial exosomes have the capability to accommodate and deliver short foreign nucleic acids into endothelial cells and silence the target Rcn2 gene and further development of this novel exosomes to deliver siRNA against Rcn2 gene in mouse model are warranted.

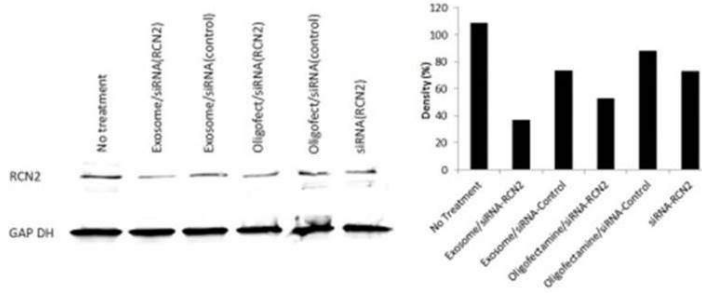

Figure 1: Image represents Western blot analysis of RCN2 protein (~50 kDa) levels expressed by exosome-siRNA/RCN2 treated endothelial cells and control groups. Glyceraldehyde 3-phosphate dehydrogenase (GAP-DH) is used as a control of total protein expression (~25 kDa). Line graph depicts intensities of RCN2 levels expressed by Western blot.

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Engineering Poly(2-oxazoline) Micelles to Deliver New Generation Taxoid SB-T-1214 against Resistant Metastatic Breast Cancer

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Introduction: Third generation Taxoid SB-T-1214 (SB) has shown remarkable in vitro activity against multidrug resistance (MDR) cancer cells including several human breast, ovarian, and pancreatic cancer cell lines which express MDR related genes or carry mutated tubulins. However, the solubility of this promising compound has been a major roadblock in the development of clinically suitable formulation of the drug. Previous studies utilized Cremophor EL/Ethanol or Tween as excipients which often causes severe side effects and undesired drug distribution.

Methods: Here, the low toxicity amphiphilic Poly(Oxazoline)s (POx) triblock copolymers are used to formulate water-insoluble new taxoid SB. The resulting polymeric micelles (nanoformulation) was manufactured, characterized and evaluated in vivo.

Results: Defined polymeric micelles are high capacity sub-100 nm micellar formulation of SB (almost 1/1 (w/w) POx/SB) which exhibits excellent stability at room temperature for at least a week without changes in particle size and polydispersity. In vitro MTT assay indicated that POx/SB formulation is 20 to 40 times more potent than commercial paclitaxel formulation against resistant breast cancer cells. In an orthotopic mouse model of resistant and metastatic LCC6-MDR breast cancer, POx/SB micelles revealed significantly better tumor inhibition and less incidence of metastasis as compared to Cremorphor/SB or Taxol® commercial formulation. Furthermore, POx/SB also achieved superior antitumor activity and survival benefits with less toxicity as compared to Taxol® and Abraxane® in a particularly faithful and aggressive T11 orthotopic syngeneic transplant model (claudin-low

subtype of triple negative breast cancer).

Conclusion: The promising preclinical data on POx/SB nanoformulation showcase the need to investigate new excipients and is a robust basis to translate into clinical trials.

A New Strategy for Cancer Therapy: Combination of Chemotherapy and Oxidative Stress-induced Autophagy in A549 Lung Cancer Cells Using Redox-responsive Nanohybrids

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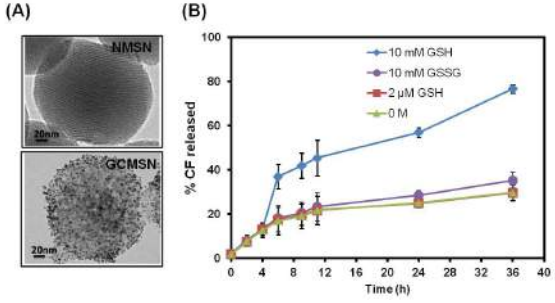
A combination of various therapeutic approaches has emerged as a promising strategy for cancer treatment. A safe and competent nano-delivery system is thus in urgent demand to facilitate the simultaneous transport of various therapeutic agents to cancer cells and a tumor region to achieve synergistic effect. Gold nanoparticles (GNPs) and mesoporous silica nanoparticle (MSNs) were fabricated herein as potential candidates for drug delivery. Serving as gatekeepers, GNPs (5 nm in diameter) were attached onto the amino-functionalized MSNs (denoted as NMSNs) via a relatively weak gold-nitrogen bonding. The resulting nanohybrids (denoted as GCMSNs) were uptaken by cells, and the detachment of GNPs and subsequent intracellular drug release from NMSNs were achieved by competitive binding of intracellular glutathione to GNPs. In addition to the function of gatekeeping, GNPs also play another role as the oxidative stress elicitor. Our in vitro studies revealed that GCMSNs induced higher oxidative stress in lung cancer cells (A549) than in normal cells (3T3-L1). This growth inhibitory effect found in the cancer cells was likely induced by mitochondria dysfunction originated from the GCMSN-induced, oxidative stress-triggered mitochondria-mediated autophagy. The redox-responsive nanohybrids were further loaded with camptothecin and the intensified synergistic therapeutic effects were observed associated with combined chemotherapy and oxidative stress strategy. The results clearly demonstrate that such unique nanohybrids hold great promise for selective and effective cancer treatments.



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Tumor Delivery of Polymersome-Encapsulated Myoglobin Results in Rapid Intratumoral Hemorrhaging

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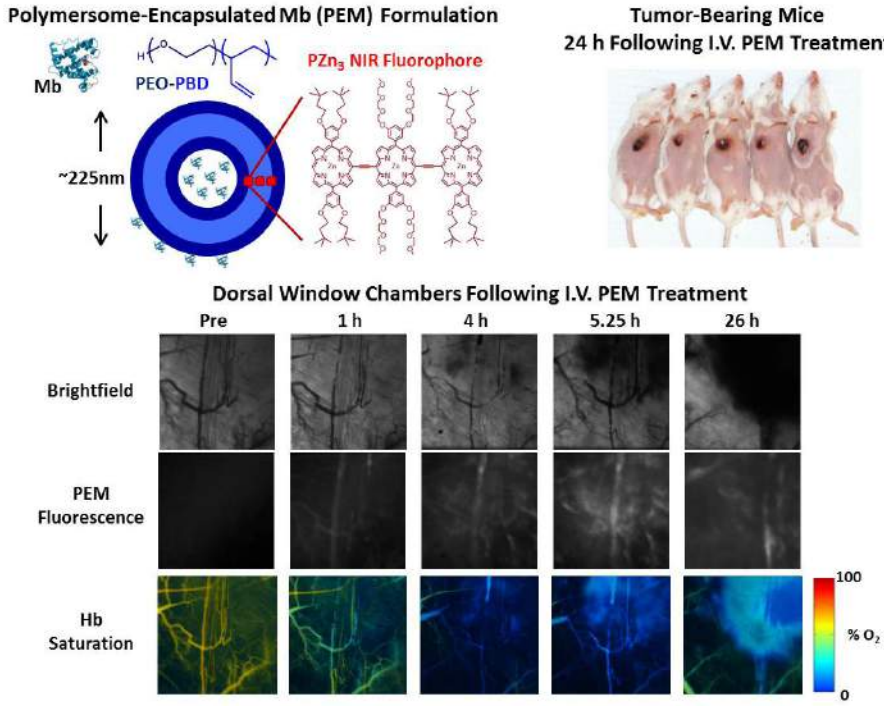
Polymersomes, nanoscale polymeric vesicles, were formulated to contain myoglobin (Mb) for delivery to tumors. It was expected that Mb, an oxygen-carrying protein, would release oxygen into hypoxic tumors, resulting in improved radio- and chemotherapy. The polymersomes encapsulated with Mb (PEMs) were loaded with a near-infrared (NIR) emissive fluorophore to enable *in vivo* imaging of vesicle biodistribution. Upon tail vein injection in



breast tumor-bearing mice, the PEMs accumulated within tumors due to the enhanced permeability and retention effect. Surprisingly, we found that within 3-6 h, all mice treated with PEMs displayed a dramatic tumor effect, with the tumors turning a dark red color, consistent with hemorrhage. Histology revealed necrosis within the center of tumors 24 h following treatment, as well as decreased perfusion and a rim of hypoxia surrounding the necrotic tissue. Window chamber studies confirmed the accumulation of PEMs, significant hemorrhaging, decreased blood flow, and decreased oxygenation (as measured by hemoglobin saturation) at the tumor site. These data suggest a never-before-seen rapid and significant tumor-specific effect of PEMs, most likely due to vascular damage.

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The chemosensitization effect of Pluronic block copolymers in multiple myeloma cancer cells

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Multiple myeloma (MM) is a hematological malignancy of plasma cells that are predominantly located in bone marrow (BM). Despite recent improvements in MM treatment by introduction of several novel agents including bortezomib and thalidomide, MM remains incurable and almost all patients eventually relapse or become refractory to the current treatment regimens. So the current challenge of MM treatments is to maintain treatment response, prevent relapse and eventually prolong survival. Here we show that Pluronic block copolymers ((Pluronic L61 : Pluronic F127 = 1 :8 w/w, SP1017) can significantly increase cytotoxicity of proteasome inhibitors (Bortezomib or Carfilzomib) in a panel of MM cells. Specifically, SP1017 (0.005%) co-treatment triggered 2-fold increase in drug cytotoxicity to MM cells. Lower concentrations of SP1017 (0.002%) showed lower, but still significant cytotoxicity when combined with proteasome inhibitors. Augmented inhibition of chymotrypsin-like proteasome activity, much higher levels of ubiquitinated proteins and enhanced apoptosis were observed in MM cells treated with bortezomib/SP1017 combination than in cell treated with drug alone. The combined treatment-induced apoptosis was associated with activation of caspases via both mitochondria-dependent (loss of mitochondrial membrane potential, cytochrome C release, and activation of caspase-9/3) and mitochondria-independent (activation of caspase-8/3) mechanisms. Importantly, SP1017 co-treatments restore drug sensitivity in bortezomib/carfilzomib-resistant MM cells. Further studies have revealed that SP1017 co-treatment could also sensitize MM cells in co-culture models of the BM microenvironment, which triggers cytokine- and adhesion-mediated MM drug resistance. These results provide support for the design of therapeutic strategies aimed to counteract the acquired drug resistance mechanisms in MM. Future studies will investigate the effectiveness of this combined treatment in animal MM model.

Synthesis of phosphonate monomers and polymers

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Phosphorus-containing polymers have recently elicited much interest in biomedical applications because of their excellent coordination and binding properties. Herein we describe the synthesis of ammonium bisdiethylphosphonate acrylate and methacrylate monomers by an aza-Michael addition of 3-aminopropanol or 3-aminohexanol across the double bond of bisdiethylphosphonate, followed by reaction with (meth)acryloyl chloride. Free radical copolymerizations of the monomers with an acrylate-functional poly(ethylene oxide) (PEO) macromonomer produced graft copolymers. Quantitative deprotection of the alkylphosphonate groups, then adjustment of the pH to 7.74 ± 0.03, yielded graft copolymers with zwitterionic ammonium bisphosphonate backbones and PEO grafts. The zwitterionic copolymers spontaneously assembled into aggregates in aqueous media, likely as a result of their zwitterionic nature. The zwitterionic copolymers were used as carriers for both MRI imaging agents and anticancer drugs. The resulting complexes exhibited excellent anti-proliferative effects against breast cancer cells.

A new acrylamide phosphonate monomer was recently designed to probe structure-property relationships among the polymers and their complexation properties. This monomer appeared is of interest due to the improved chemical stability compared to the (meth)acrylate analogues. An anionic phosphonic acid polyacrylamide homopolymer was prepared using similar conditions to those used for the (meth)acrylate phosphonate polymers. Solubility studies showed that the anionic homopolymer was soluble in multiple solvents, including water (pH 7), DMSO, and methanol. This acrylamide phosphonate-based copolymer was complexed with magnetite nanoparticles for MRI imaging. The complex showed excellent stability under physiological pH and high transverse relaxivity which might lead to further exploration for its application in contrast agents and drug delivery systems

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Triple-responsive expansile nanogel for tumor and mitochondria targeted photosensitizer delivery

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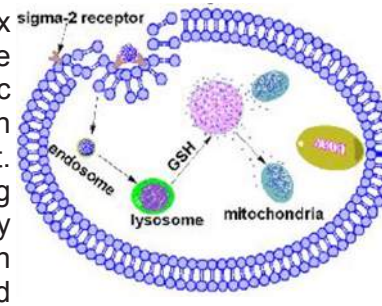

We developed a pH, thermal, and redox potential triple-responsive expansile nanogel system (TRN), which swells at acidic pH, temperature higher than its transition temperature, and reducing environment. Both TEM and dynamic light scattering techniques proved that TRN quickly expands from 108 nm to over 1200 nm (in diameter), achieving more than 1000-fold size enlargement (in volume), within 2 h in a reducing environment at body temperature. Pc 4, a silicon phthalocyanine photosensitizer was loaded into TRN, following the functionalization of TRN with a sigma-2 receptor targeting-ligand 4-Methoxybenzoic acid (MBA), to obtain a tumor targeted photodynamic therapy system (MBA-Pc 4-TRN). Confocal microscopy exhibited that MBA-Pc 4-TRN showed higher cellular uptake compared to free Pc 4 or TRN without targeting ligand (Pc 4-TRN) in the sigma-2 receptor overexpressed head and neck cancer cell line (UMSCC22A). Further cellular Pc 4 quantification revealed that MBA-Pc 4-TRN achieved as high as1.8-fold increase of Pc 4 uptake compared to Pc 4-TRN. Sub-cellular localization of MBA-Pc 4-TRN in UMSCC22A was further assessed by confocal microscopy and showed that TRN firstly entered lysosomes where it expanded and broke the lysosomes, following escaping and releasing Pc 4 to mitochondria. *In vitro* photodynamic therapy showed that MBA-Pc 4-TRN killed almost all UMSCC22A cancer cells 12 h post irradiation, while only 44.5% cells were killed in Pc 4-TRN treated group. Finally, biodistribution of MBA-Pc 4-TRN in head and neck cancer xenograft mice model revealed that TRN can effectively accumulate in tumor while few TNR was found in other tissues like liver and spleen after 72 h.

Funding: American Cancer Society Institutional Research Grant (ACS-IRG), the ASPIRE award from the Office of the Vice President for Research of The University of South Carolina, the Center for Colon Cancer Research (5P20 RR017698), and the Center for Targeted Therapeutics (1P20 GM109091) from the NIH.

Multiorgan Pharmacokinetic Analysis Using Multispectral Optoacoustic Tomography (MSOT)

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Conventional optical imaging techniques suffer from low spatial resolution, long acquisition times, and limited depth of penetration. Multispectral optoacoustic tomography (MSOT) combines ultrasound and optical imaging to detect ultrasound waves generated by the photoacoustic effect. MSOT offers real-time imaging of optical contrast with high spatial resolution at depth, and allows for the determination of individual absorbers in tissue. Examples of endogenous optical absorbers include oxyhemaglobin, deoxyhemaglobin and melanin. Examples of exogenous contrast agents include fluorescent dyes such as ICG, or nanoparticles such as gold. Multispectral unmixing allows for the separation of multiple injected probes at the same time and without the necessity of a baseline scan before administration. Verification of the results is achieved using ex vivo cryoslice imaging in a cryostat that is equipped with the possibility to take color and fluorescence photographs of the mouse body, creating cross-sectional images that are comparable with the ones acquired using MSOT.

Here we present a non-invasive *in vivo* study of kinetic processes in mice in multiple organs. Selected areas in kidneys, liver and spleen were examined to determine the clearance kinetics of the injected substances over time. Monitoring of selected regions of interest shows distinct pharmacokinetic behavior for each probe in excellent accordance with *ex vivo* fluorescence imaging.

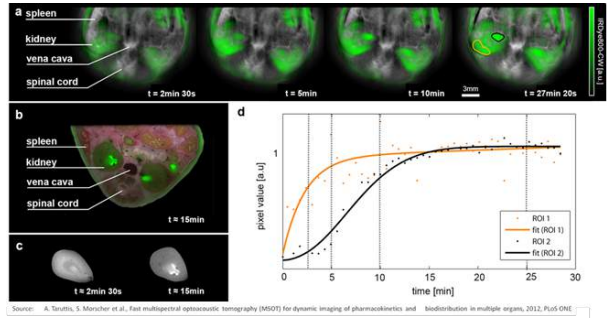

Figure 1: a) Multispectral unmixing of signals from IRdye800 are overlaid over an anatomical background image provided by signal from hemoglobin for multiple time point's after injection. b) Validation using cryoslice imaging reveals a distribution of IRdye800 consistent with multispectral unmixing results. c) Fluorescence cryoslice images of kidneys at two time points after injection. d) Quantification of IRdye800 signal over time for two regions of interest show differences in uptake of the agent within the kidney.

Oral gene delivery for treatment of Hemophilia B

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Current protein replacement therapies require multiple IV injections that are expensive, painful, and inconvenient. Oral gene therapy - where exogenous genes are administered orally for therapeutic effects - can overcome these problems. Viral vectors risk random integration and limit the size of the delivered gene; non-viral gene delivery, although achieving only transient and low gene expression levels, overcomes these drawbacks. To date, oral gene delivery using chitosan (Ch, a cationic, mucoadhesive, polysaccharide) nanoparticles for treatment of hemophilia A has only improved the disease from a severe state to a mild state [1, 2] therefore leaving room for improvement. Recently published work shows hFIX plasmid optimization for oral gene delivery to treat Hemophilia B [3], however, nanoparticle optimization for hFIX delivery was not done. We hypothesize that adding a small amount of protamine sulfate (PS), a highly cationic protein, to low molecular weight chitosan nanoparticles will improve *in vivo* transfection efficiency by stabilizing the nanocomplexes extracellularly while using lower MW components that can be more readily degraded intracellularly. Ternary complexes of Ch-PS-pFIX form nanoparticles with an average diameter of 456nm±90nm, with sizes ranging from 160nm to 1µm, and a zeta potential of 31mV±3mV. Particles were validated *in vitro* by transfecting Caco-2 and HEK293 cells using both pFIX and a luciferase plasmid (pLuc). Results show that the Ch-PS-DNA nanoparticles have significantly higher transfection efficiency than Ch-DNA nanoparticles. A pilot study of nude mice fed with a single dose of Ch-PS-pLuc nanoparticles showed luciferase expression in the

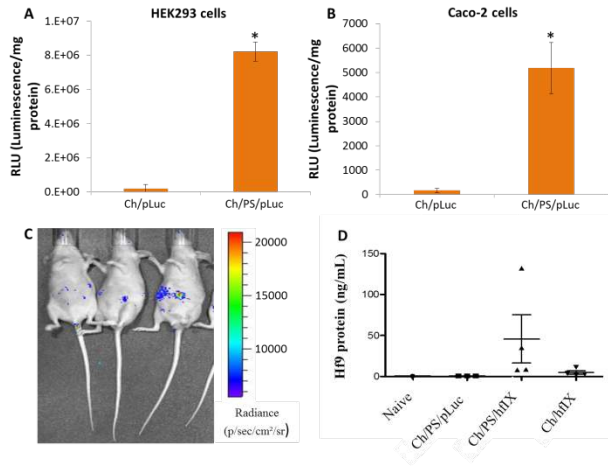

Figure 1: (A) & (B) In vitro transfection. (C) IVIS images after a single oral dose of Ch/PS/pLuc (200µg pLuc) from left to right: control, control, treated. (D) hFIX protein detection in Hemophilic B mice after nanoparticle delivery with a total of 600µg DNA dose.

stomach and the large intestine as compared to the untreated mice which indicated no significant luciferase expression. Currently, Ch-PS-phFIX nanocomplexes are being tested in Hemophilic B mice. Human FIX protein has been detected in 2 out of 4 mice that were given with Ch-PS-hFIX by oral gavage whereas all other treatment groups had no detectable FIX protein. Overall results indicate that Ch-PS-DNA nanoparticles have better transfection efficiency than Ch-DNA nanoparticles and therapeutic hFIX protein levels can be detected in Hemophilic B mice following oral delivery of Ch-PS-hFIX nanoparticles.

[1] K. Bowman, R. Sarkar, S. Raut, K.W. Leong, Gene transfer to hemophilia A mice via oral delivery of FVIII–chitosan nanoparticles, J Control Release, 132 (2008) 252-259. [2] S.S. Dhadwar, J. Kiernan, J. Wen, G. Hortelano, Repeated oral administration of chitosan/DNA nanoparticles delivers functional FVIII with the absence of antibodies in hemophilia A mice, Journal of Thrombosis and Haemostasis, 8 (2010) 2743-2750. [3] P. Quade-Lyssy, P. Milanov, D. Abriss, C. Ungerer, C. Konigs, E. Seifried, J. Schuttrumpf, Oral gene therapy for hemophilia B using chitosan-formulated FIX mutants, Journal of thrombosis and haemostasis : JTH, 12 (2014) 932-942.

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Recombinant MUC4 Nano-Vaccine for Pancreatic Cancer.

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Pancreatic cancer (PC) is a highly metastatic and therapy-resistant malignancy. Recently, immunotherapy has emerged as a viable alternative to conventional therapies due to relative safety and potential for long term response. Immunotherapy tends to reprogram the patient's immune system to fight cancer by proper recognition of specific antigens displayed on tumor cell surface, leading to an enhanced immune response. A major challenge in cancer immunotherapy is development of vaccine formulations that can elicit a cell-mediated immune response and overcome immunological tolerance to the tumor antigen. Overexpression and altered glycosylation of mucins in tumor cells is a hallmark of epithelial malignancies. We demonstrated that while MUC4 is undetectable in normal or inflamed pancreas, de novo MUC4 expression is observed in the earliest preneoplastic lesions (PanIN), and its expression increases progressively with disease advancement. Consecutive studies demonstrated functional contribution of MUC4 to PC pathogenesis and MUC4 association with poor prognosis. Preliminary studies also demonstrated the presence of circulating anti-MUC4 antibodies in MUC4 overexpressing cancer patients (pancreatic and lung). Evidence of humoral immune response against MUC4, supports that MUC4 is immunogenic in cancer patients, and with MUC4 overexpression in PC, advocates MUC4 as a promising vaccine candidate. Synthetic peptides exhibit limited efficacy as vaccine candidates due to less epitopes and inefficient processing. While intact proteins or recombinant protein fragments have larger epitope repertoire, formulation and delivery of current protein-based vaccines elicit suboptimal immune response due to rapid clearing and poor stability. Recent studies have demonstrated utilization of amphiphilic polyanhydrides as carriers of protein-based vaccines, as they provided ideal environment for protein stabilization, enhanced adjuvanticity and immunomodulation. The overall goal of project is to develop MUC4 based nanovaccine using amphiphilic polyanhydrides against pancreatic cancer and evaluate its efficacy for the immunotherapy of PC in genetically engineered murine models of pancreatic cancer.

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Enhanced Synergistic Effect of Anticancer Agents by Sequential and Site-Specific Deliver

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Combination therapy has been proved to be more effective than monotherapy in cancer treatment by achieving synergistic effects and reducing toxicity. We have developed a core-shell based “nanodepot” consisting of a liposomal core and a crosslinked-gel shell (designated gel-lipsome, Gelipo) for the sequential and site-specific dual-delivery (SSSD) of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and doxorubicin (Dox). Dox, a small-molecule anticancer drug that can intercalate the nuclear DNA, was encapsulated in the liposomal aqueous core, while TRAIL, which functions on the death receptors on the cell membrane, was entrapped in the outer crosslinked hyaluronic acid (HA) shell. The degradation of the HA shell by hyaluronidase, an enzyme rich in the tumor matrix, leads to the rapid extracellular TRAIL release and subsequent internalization of the liposomes. The parallel activity of TRAIL targeting the cell membrane and Dox targeting the nucleus shows enhanced synergistic anticancer efficacy. In addition, Gelipo, a programmed choreography, exhibits high tumor accumulation and significantly improves the inhibition of tumor growth in the MDA-MB-231 xenograft tumor mouse model. It was suggested to be an effective nanocarrier system that transport different anticancer therapeutics to distinct targets successively, which has great implications for cancer treatment.

Funding: This work was supported by the grant 550KR51307 from NC TraCS, NIH’s Clinical and Translational Science Awards (CTSA) at UNC-CH and the NC State Faculty Research and Professional Development Award to Z.G.

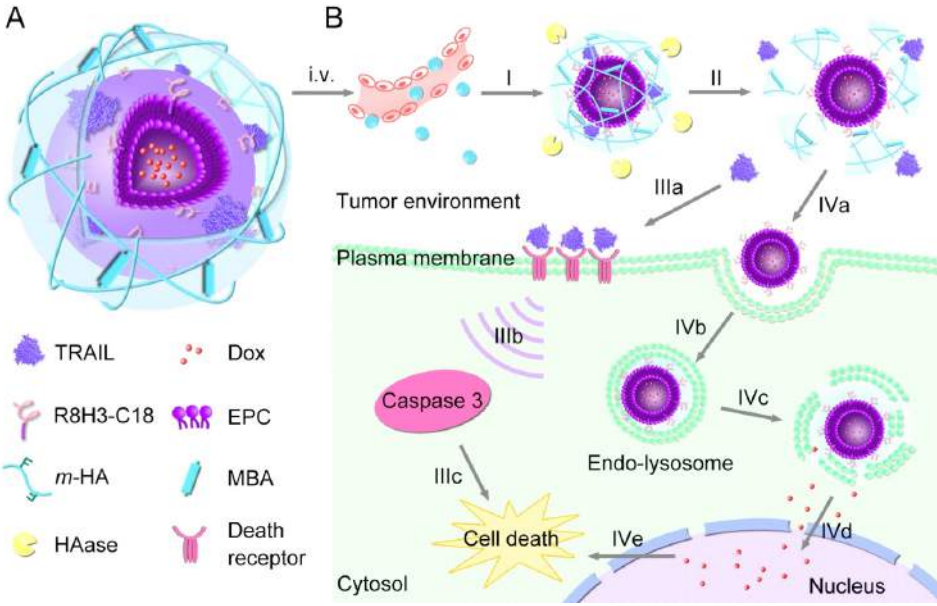

Figure 1. Schematic design of TRAIL/Dox-Gelipo for SSSD. (A) The main components of TRAIL/Dox-Gelipo: R8H3 modified liposomal core loading Dox and crosslinked HA gel based outer shell encapsulating TRAIL. (B) Sequential delivery of TRAIL to the plasma membrane and Dox to the nuclei by TRAIL/Dox-Gelipo for combination cancer treatment. I, accumulation of Gelipo (blue balls) at the tumor site; II, degradation of HA crosslinked shell by HAase; IIIa, released TRAIL binding onto the death receptors on the membrane; IIIb, activation of the caspase 3 signaling pathway; IIIc, induction of the cell death; IVa, R8H3 facilitating the internalization of Dox-R8H3-L; IVb, Dox-R8H3-L entering the cells; IVc, endo/lysosomal escape; IVd, accumulation of the released Dox into nucleus; IVe, intercalation of Dox on DNA.

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Nano-formulation of BDNF: A Potential Therapy for BDNF Associated Neurological Disorders

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Bain derived neurotrophic factor (BDNF) has been recognized as a potential therapeutic agent for a number of different neurological disorders including Rett syndrome (RTT), Alzheimer's disease (AD), Parkinson's disease, and stroke. However, to hold promise in treating patients, exogenous BDNF must be delivered to the brain in sufficient amounts via clinically feasible administration route. Brain delivery of protein therapeutics is a well-known challenge because of their poor serum bioavailability and limited capacity to cross the blood-brain barrier (BBB). To address this critical challenge in the delivery of BDNF to the brain, we propose a novel delivery system for BDNF composed of safe and biocompatible polymers that entrap the BDNF molecule in a nanoscale complex, named nano-BDNF. We show that formulating BDNF into this Nano-BDNF particle significantly prevents BDNF aggregation and results in a stable and narrowly distributed nanoparticle. Importantly, this Nano-BDNF demonstrates 1) similar serum clearance as BDNF (t1/2 6.6 min vs. 7.1 min); 2) greater blood to brain penetration in comparison to BDNF that is rapidly effluxed from brain to blood; and 3) that the % of the injected dose taken up by the brain (%Inj/g) exceeds that of BDNF. By the first 10 min, the area under the curve (AUC) for nano-BDNF is ~6 times higher than that of BDNF (2.96 vs 0.54). Together, we develop a novel, safe and stable nano-formulation of BDNF and provide preclinical pharmacokinetic evidence that systemically administered BDNF in this nano-formulation enters the brain in a greater amount than native BDNF. Therefore, continuous development of nano-BDNF as a potential therapy for a variety of neurological disorders will be of great interest.

A site selective and on-demand drug delivery depot for the treatment of proteolytic diseases

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Chronic proteolytic diseases, such as rheumatoid arthritis, inflammatory bowel disease and chemotherapy- or radiation-induced oral mucositis affect more than 50 million Americans. Conventionally used intravenous injections and oral pills result in low local drug concentration and high systemic toxicity. Use of conventional drug delivery platforms results in an initial rapid burst release, followed by sudden fall of drug concentration below the therapeutic window, which is undesirable.

We used an amphiphilic molecule, chosen from the FDA'a generally recognized as safe (GRAS) list, to develop a self-assembled nanofibrous gel, containing esterase labile linkages and negative surface charge. Upon local administration, this system preferentially adheres to positively charged inflamed regions and releases the encapsulated drug in response to the elevated levels of lipases, esterases and proteases (MMP-2 and MMP-9) at the site of lesion (Figure 1A). Besides possessing high drug encapsulation efficiency, this gel can also withstand shear forces in dynamic environments of mouth, joints and colon. The gel viscosity can be easily manipulated depending on the applications, ranging from a mouthwash for oral mucositis to a solid patch for dermatitis. Rapid and selective adhesion of gel to the inflamed oral mucosa and ulcerated colon was observed in radiation treated hamsters and ulcerative colitis induced mice, respectively (Figure 1B). Administered as an enema to ulcerative colitis induced mice, dexamethasone doped gel showed superior anti-inflammatory effect while significantly reducing plasma concentration in comparison to the free drug (Figures 1C and 1D). Overall, this approach offers a promising therapeutic platform for localized treatment of proteolytic diseases.

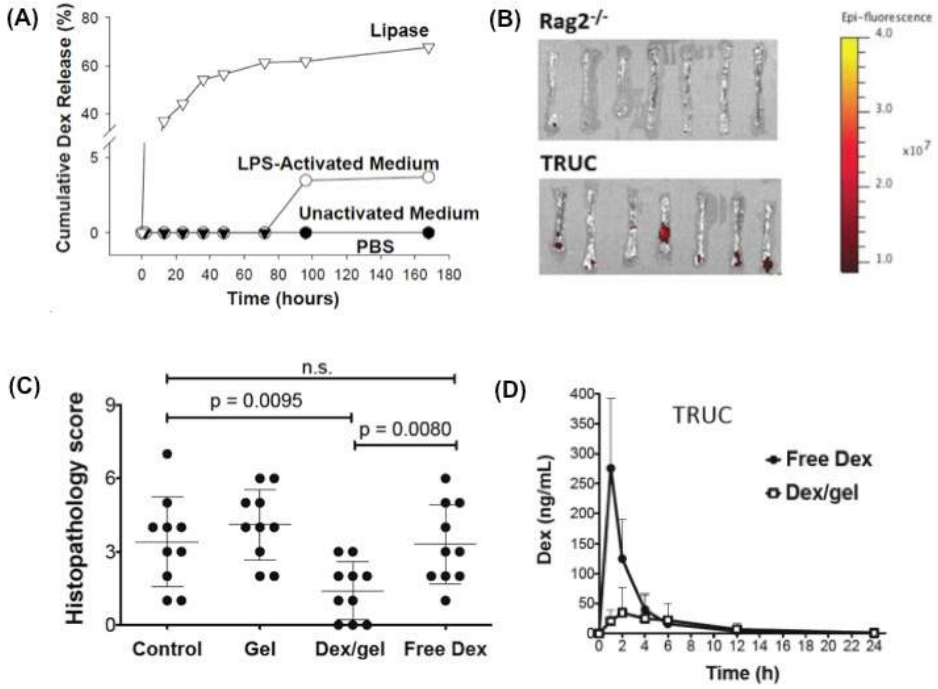

Figure 1: (A) On-demand release of dexamethasone from gels in response to lipase and medium from lipopolysaccharide (LPS) activated macrophages, respectively. **(B)** Selective adhesion of DiD loaded gels to ulcerated colon in Tbet-/- Rag2-/- ulcerative colitis (TRUC) mice. **(C)** Histopathology scores for individual mice (n=10 mice per group) shows improved anti-inflammatory efficacy of dexamethasone loaded gels (Dex/gel) in comparison to free dexamethasone (Free Dex), both administered as enema to TRUC mice. **(D)** Dexamethasone delivery via gel enema reduces systemic drug exposure in comparison to free dexamethasone.

Funding: Harvard Institute of Translational Immunology/Helmsley Trust Pilot grant, NIH grant DE023432, and US Department of Defense grant to J.M.K.

Title: In vivo induction of CD$^+$8 effector T cells via co-delivery of antigen and adjuvant by reduction sensitive PRINT® hydrogel subunit vaccine

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Purpose: Training our immune system with vaccines to fight against cancer is an attractive approach. Tumor-specific cytotoxic T cells (CTLs) such as CD8$^+$ effector T cells play a critical role in tumor control. However, vaccination aimed at eliciting a potent CD8$^+$ T cell responses with tumor-associated peptide antigens, are typically ineffective due to poor immunogenicity. We have co-conjugated ovalbumin-derived CTL epitope ($OVA_{257-264}$) and a TLR9 agonist (CpG ODNs) via reduction sensitive linkers to PEG hydrogel nanoparticles. Using this particulate vaccine, we have demonstrated induction of potent CD$^+$8 effector T cells.

Results: Co-delivery of $OVA_{257-264}$ peptide with CpG ODN by reduction sensitive hydrogel NPs has significantly raised the frequency of IFN-gamma producing CD8$^+$ effector T cell in mice, which is ~15 fold higher as compare to soluble antigen and adjuvant. Moreover, subunit vaccine-induced effector T cells were able to kill up to 90% of antigenic peptide-loaded target cells adoptively transferred into mice.

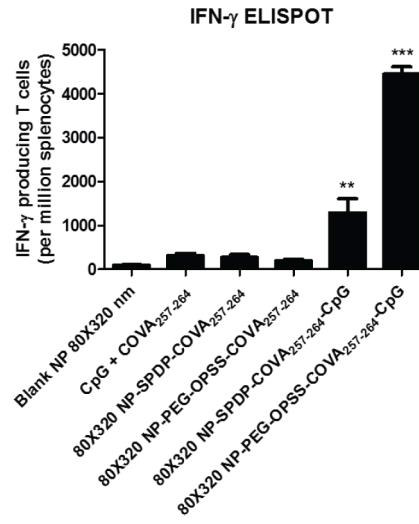

Figure-1: Generation of INF-γ secreting antigen specific CD8+ T cells.

Conclusion: CTLs are essential for killing target cancer cells and preventing tumor growth. These results demonstrate that the particulate vaccine design is very efficient in eliciting a potent CTL response. Currently, this nanoparticulate system is being utilized to develop a melanoma vaccine using, tyrosinase related protein ($Trp2_{180-188}$). The effectiveness of this vaccine will be evaluated in mice for tumor growth inhibition.

Acknowledgement: NIH C-CCNE grant, UNC University Cancer Research Fund, and Liquidia Technologies, Inc.

CD44-Targeted Biodegradable Nanoparticles for Image-Guided Surgery

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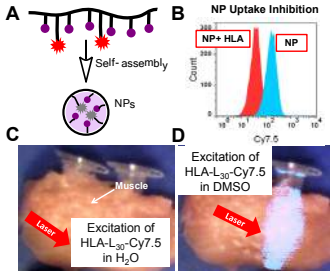
Surgery is a primary course of treatment for more than 50% of the patients with some form of cancer. Presence or absence of residual cancer cells during the surgery around the area surrounding the tumor resection, known as surgical margin, impacts tumor recurrence, follow-up treatment, and ultimately, patient survival. Targeted imaging agents that can specifically highlight tumor cells during surgery, can potentially improve visualization of surgical margins when using an appropriate image-guided surgery system.

We report the design and development of a panel of targeted imaging agents using a near-infrared (NIR) dye, Cy7.5, directly conjugated to one of two molecular weights of hydrophobically-modified (with 5-beta cholanic acid, 5βCA) hyaluronic acid (HLA) (10kDa and 100kDa). HLA is a ligand for the receptor CD44, which is a common biomarker present on many primary tumor cells, cancer-initiating (stem) cells, and tumor-associated fibroblasts. In addition, a family of enzymes that degrade HLA, called hyaluronidases (HYAL), are also overexpressed with increased activity in many tumors.

Amphiphilic HLA-5βCA-Cy7.5 conjugates with varying amount of hydrophobic ligand (0-30%) self-assembled in water to form NPs with a hydrophobic core as schematically depicted in Fig. 1A. These nanoparticles showed high uptake in the CD44-expressing breast cancer cell line, MDA-MB 231. Binding of the contrast agent to CD44 was inhibited in the presence of excess HLA, i.e. the competitive binding ligand (Fig. 1B). When nanoparticles were placed underneath a muscle tissue (chicken breast; slice thickness = 5 mm) and studied with fluorescence image-guided surgery system (FIGSS) (Fig. C and D); fluorescence was detected only from NPs activated by DMSO. These results demonstrate feasibility that activatable NIR fluorescence emission could facilitate specific fluorescence detection. Studies are on-going to evaluate the effect of hydrophobic modification of HLA and CD44 interaction and tuning fluorescence activation specifically to HYAL.



Funding- NIH (R00 CA153916 to AMM), Wake Forest – Virginia Tech School of Biomedical Engineering and Sciences, and the Wake Forest Institute for Regenerative Medicine.

Targeted Delivery of Anti-Inflammatory Drugs to Macrophages Using Mannose-Conjugated PLGA Nanoparticles

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Acute inflammation is the immediate and protective response of the immune system. However, although it is a natural body reaction common in various invasive circumstances, anti-inflammatory treatments are necessary to reduce pain and collateral damage of normal tissues. Generally, non-steroidal anti-inflammatory drugs (NSAIDs) are prescribed for inflammation to alleviate pain and vigorous immune responses at the site of inflammation. They induce the anti-inflammatory effect by inhibiting cyclooxygenase (COX) activity, resulting in suppressed prostaglandin E2 synthesis. Additionally, since macrophages are known to produce the prostaglandins primarily among inflammation-associated cells, targeted intracellular delivery of NASIDs to macrophages would enhance anti-inflammatory effects in the injured tissues. Here, we developed mannose-conjugated poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles to improve the intracellular delivery and therapeutic efficacy of anti-inflammatory drugs in macrophages. First, we synthesized PLGA nanoparticles with different sizes to see size effects on the cellular uptake in macrophages. PLGA nanoparticles with the size of ~100 nm showed higher cellular uptake compared with ones with the size of ~200 nm. Then, we incorporated anti-inflammatory drug ketoprofen into the PLGA nanoparticles, and assessed the release profile and anti-inflammatory effect *in vitro*. PLGA nanoparticles with the size of ~100 nm released ketoprofen over 3 days in the physiological condition. Macrophages treated with ketoprofen-loaded PLGA nanoparticles produced less prostaglandin E2 than ones treated with free ketoprofen. Lastly, we conjugated mannose to the surface of PLGA nanoparticles to further improve the delivery of anti-inflammatory drugs because macrophages express large number of mannose receptors on the surface. The mannose-conjugated PLGA nanoparticles showed greater cellular uptake in macrophages compared with bare PLGA nanoparticles. Collectively, these results suggest that the mannose-conjugated PLGA nanoparticles with the size of 100 nm can be used to localize anti-inflammatory drugs efficiently to macrophages at the site of inflammation.

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Exosome-Encapsulated Water-Insoluble Small Molecule Chemotherapeutics for the Treatment of Pulmonary Metastases

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Exosomes are naturally occurring membrane-derived extracellular vesicles ~100nm in size; they are produced by many cell types and are involved in intracellular communication by delivering cargo (e.g. proteins, nucleic acids) to recipient cells. Exosomes have recently generated interest as possible drug delivery vehicles due to their ability to be loaded with various cargo (e.g. siRNA, curcumin) and because of the absence of toxic excipients (e.g. Cremophor EL in the commercial formulation of paclitaxel, Taxol). Here, we have endeavored to load exosomes derived from macrophages with a potent chemotherapeutic, paclitaxel (PTX). PTX, a water-insoluble small molecule chemotherapeutic, was incorporated into exosomes to increase its solubility and enhance its therapeutic efficacy against pulmonary metastases. We found that sonication provides the greatest loading of PTX into exosomes (exoPTX), achieving a loading capacity of 28.29 ± 1.64%. ExoPTX demonstrated significantly greater cytotoxicity against 3LL-M27 cells (13.57 ± 1.33 ng/mL) as compared to either paclitaxel or Taxol (120.81 ± 28.58 ng/mL and 23.16 ± 1.88 ng/mL, respectively). Furthermore, incorporation of PTX into exosomes averted resistance to PTX in Pgp+ cells (MDCK MDR1); the exact mechanism behind this phenomenon remains to be elucidated. ExoPTX were taken up by 3LL-M27 Lewis Lung Carcinoma cells significantly greater than either liposomes or polystyrene nanoparticles *in vitro*. Intranasally administered exosomes were shown to travel to the lungs in mice where they co-localized with pulmonary metastases; no organ toxicity from exosomes was observed. Furthermore, exoPTX exhibited a greater antineoplastic effect than Taxol in a C57BL/6 mouse model of pulmonary metastases, resulting in near complete eradication of pulmonary metastases. Taken together, these findings indicate that exosomes hold great promise for use as a novel and effective drug delivery vehicle of low molecular weight water-insoluble chemotherapeutics, such as PTX, for the treatment of pulmonary metastases.

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Liposomes and polymeric nanoparticles as delivery vehicles for the treatment of lung diseases

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Reactive oxygen species (ROS) are greatly involved in important physiological and pathophysiological processes. Overproduction of most toxic ROS - superoxide radicals - leads to a variety of detrimental health conditions including cardiovascular disease, neurodegenerative disorders, and extensive oxidative inflammation. Among others, lung tissue is especially susceptible to oxidative insult because it is in direct contact with oxygen from the air. Inhaled toxic agents such as dust or smoke could stimulate the generation of ROS, which in turn provokes chronic inflammation and development of chronic obstructive pulmonary disease (COPD). To combat these unrelenting conditions, lung tissue is protected by a variety of antioxidant mechanisms. Superoxide dismutases (SOD) are a group of antioxidant enzymes responsible for conversion of superoxide radicals to much less reactive hydrogen peroxide. We propose their use to alleviate lung conditions such as COPD. Major obstacle in drug delivery to the lung is rapid clearance of the drug by respiratory epithelium. To overcome these limitations we propose using delivery vehicles such as liposomes and polymeric nanoparticles

Targeted nanoparticle-based drug delivery to the lungs is an emerging area of interest for both scientists and medical workers because it allows better drug retention in the lungs and can provide prolonged drug release. In our study we investigated the potential of using liposomes and polymeric nanoparticles surface modified with a specific antibody as drug carriers for targeted drug delivery to the lungs. Normal human bronchial epithelial (NHBE) cell culture was used as cell culture model in In vitro study of targeting effect. These nanoparticulate systems have shown to have better targeting in comparison to the plain NPs and samples with control antibodies. Nanoparticles were also loaded with antioxidative drugs (SOD/SOD mimic) to show the protective efficacy against ROS damaging production.

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Shiftosomes: liposomes containing liquid perfluorocarbon nanodroplets that convert to gas and release entrapped dye in response to activation by ultrasound.

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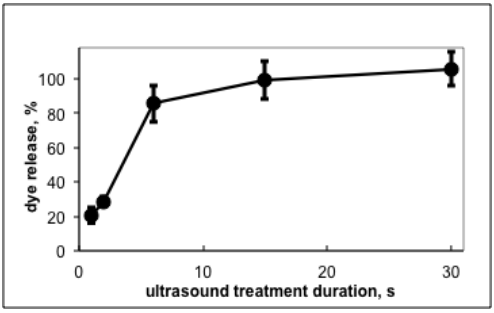
Stimuli-responsive drug release from pharmaceutical carriers is widely investigated as a next-generation targeted delivery approach. Ultrasound as a trigger for drug release from the carrier micro- or nanoparticles in vivo offers numerous advantages: medical ultrasound equipment is portable and inexpensive; ultrasound can be tightly focused; therapeutic ultrasound is used in image-guided therapy. Here we report a "shiftosome" concept: liposomes entrap liquid perfluorocarbon nanoparticles (e.g., decafluorobutane has -2°C boiling point) in superheated state: perfluorocarbon liquid nanoparticles of submicron size are stable even at 37°C yet achieve shift to gas phase, i.e., convert to gas bubbles in response to ultrasound. We report preparation of liposomes with decafluorobutane liquid nanoparticles that co-entrap calcein dye, and demonstrate ultrasound-triggered dye release.



Liposomes were prepared by a proliposome method. Briefly, DOPC/ethanol/aqueous calcein (1:1:1 mass ratio) solution was prepared; to 0.15 ml of this mixture 10 ml of decafluorobutane gas was added, pressurized to achieve gas conversion to liquid, and mixture emulsified by a repeated passage through 0.4 um Nuclepore membrane. Fluorocarbon-ethanol-water emulsion was rapidly diluted with 20x excess of normal saline, resulting in liposome formation. Unentrapped calcein was removed by repeated centrifugation (sedimentation) in normal saline; liposomes co-entrapping decafluorobutane nanoparticles were then diluted with normal saline and stored on ice. Fluorescence increase was used to monitor calcein release from liposomes; Triton X-100 was used to achieve complete dye release. A physical therapy ultrasound apparatus (Birtcher Megason) providing continuous 1 MHz ultrasound was used for insonation. Liposome samples in microtubes were embedded in ultrasound gel on the transducer probe. Conversion of liquid perfluorocarbon to gas during insonation was observed as turbidity increase. At maximum ultrasound power, over 80% dye release was achieved within 6 s of

insonation.

Overall, a shiftosome perfluorocarbon liquid nanoparticle liposome may provide ultrasound-triggered liposome drug delivery platform with a wide variety of applications.

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DNA Three Way Junctions Stabilized by Hydrophobic Interactions for Creation of Functional Nanostructures

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Nucleic acids based nanotechnology provides the capability to fabricate nanostructures carrying multiple therapeutics having well-defined structure and stoichiometry. The construction of nanomaterials from oligonucleotides by modular assembly invariably requires the use of branched nucleic acid architectures such as three way and four way junctions (3WJ and 4WJ). We describe the stabilization of DNA 3WJ by using non-nucleotide lipophilic spacers to create a hydrophobic pocket within the junction space. Stabilization of nucleic acid junctions is of particular importance when constructing nanostructures in the 'ultra-nano' size range (< 20 nm) with shorter double stranded regions. UV thermal melting studies show that lipophilic spacers strategically placed within the junction space significantly increased thermal stability. For a three way junction with 8 base pair arms, thermal stability was increased from 30.5 °C for the unmodified junction up to a maximum stability of 55 °C. The stability of the junction can be modulated within this temperature range using the appropriate combinations of spacers. Tripartite DNA nanoassemblies were constructed with 10 nt overhangs for hybridization to splice switching antisense oligonucleotide (SSO) that modify the splicing of a reporter gene (luciferase) in tumor cells in culture. Our initial studies demonstrate enhanced cell uptake and biological activity for multivalent nanostructures conjugated to the bivalent RGD peptide for targeting αvβ3-expressing tumor cells.

Oxidative stress amplifying polymeric prodrug micelles as novel anticancer therapeutic agents

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"Oxidation therapy" has arisen as a promising anticancer strategy, which can be achieved by inducing the generation of cytotoxic level of reactive oxygen species (ROS) or inhibiting the antioxidant systems in tumor cells. BCA (benzoyloxy cinnamaldehyde) is an analogue of cinnamaldehyde, which is a major component of cinnamon and exerts anticancer activity through ROS-mediated apoptosis. ZnPP (zinc protoporphyrin) is one of endogenous metalloporphyrins and is known to inhibit antioxidant heme oxygenase-1 (HO-1), which is overexpressed in many human cancer cells and protects cancer cells from oxidative stress by generating potent antioxidant bilirubin. Inhibition of HO-1 is, therefore, a potential therapeutic strategy to kill cancer cells specifically. In this regard, we developed oxidative stress amplifying nanoplatforms as novel anticancer therapeutics, which is able not only to suppress antioxidants but also to generate ROS simultaneously in tumor microenvironments. The oxidative stress amplifying nanoplatforms are composed of dual pH-sensitive PBCAE copolymer, polymeric prodrug of BCA and HO-1 inhibiting ZnPP. PBCAE was designed to incorporate ROS-generating BCA in its backbone *via* acid-cleavable acetal linkages and self-assemble to form micelles that encapsulate ZnPP. *In vitro* proof-of-concept studies revealed that ZnPP encapsulated in PBCAE micelles suppressed HO-1 to make cancer cells more vulnerable to BCA-induced ROS, leading to enhanced apoptotic cell death. In addition, ZnPP-loaded PBCAE micelles significantly suppressed the tumor growth in human cancer xenograft mouse models. We believe that oxidative stress amplifying micellar nanoparticles have great potential as novel redox anticancer therapeutics.

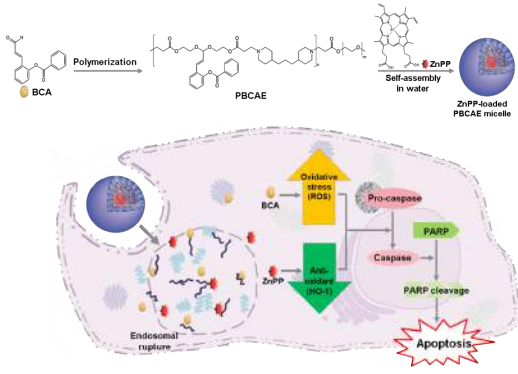

Fig. 1. A schematic diagram of an oxidative stress amplifying anticancer nanoplatform that is formulated from ROS-generating PBCAE and antioxidant scavenging ZnPP.

Treatment of metastatic breast cancer by dual-function polymeric CXCR4 antagonists delivering STAT3 siRNA

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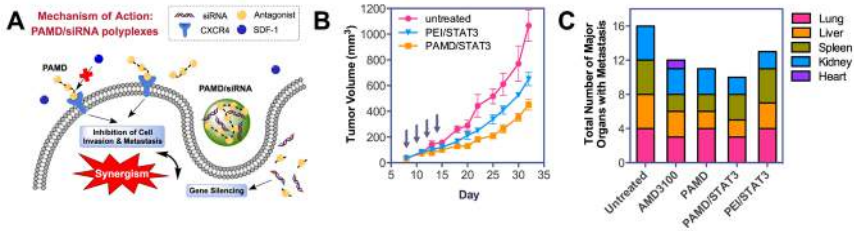
Breast cancer is the second leading cause of cancer morbidity and mortality among women in the United States. Among the patients that die of breast cancer, the cause of death is generally not the primary tumor, but instead metastases to distant organs. Mounting clinical and preclinical evidence highlights the critical role of chemokine receptor CXCR4 and its ligand stromal cell-derived factor 1 (SDF-1) in the process of tumor invasion and metastasis. Blocking CXCR4/SDF-1 interactions using antagonists like Plerixafor (AMD3100) reduces breast cancer metastasis and controls growth of the primary tumor. We will present a novel nanomedicine strategy that combines RNAi therapy with CXCR4 antagonism to inhibit tumor metastasis in single polyplex formulation (**A**). Polycations have been traditionally viewed as pharmacologically inert components of delivery systems. We have synthesized a poly(amido amine) named PAMD by incorporating CXCR4 antagonist AMD3100 via direct Michael polyaddition reaction with a bisacrylamide linker. PAMD exhibited high CXCR4 antagonism after polymerization with EC_{50} at submicromolar range. The antimetastatic efficiency of the self-assembled PAMD/siRNA polyplexes was further confirmed by cell invasion assay. In addition, PAMD showed high siRNA delivery efficiency. Anticancer activity of the PAMD/siRNA polyplexes was evaluated in orthotopic 4T1 murine model of spontaneous metastatic breast cancer. Our results showed that treatment with PAMD/STAT3 siRNA polyplexes significantly inhibited primary tumor growth compared with mice treated with PEI/STAT3 siRNA (**B**). More importantly, significantly reduced metastasis in various major organs in PAMD treated animals was observed (**C**). In conclusion, we have developed a synthetic polycation that functions as a polymeric CXCR4 antagonist capable of delivering therapeutic siRNA and mediating gene silencing while simultaneously limiting metastasis in vivo. Such strategy represents simple and efficient combination drug-nucleic acid nanotherapy for treating metastatic breast cancer.



Polymeric carriers for the oral delivery of Biopharmaceuticals

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The field of protein therapeutics is growing fast, thus driving the search for optimal delivery systems. In terms of chronic treatment, the oral route is the favored. However, oral protein delivery is subject to two problems: (1) The acidic and enzymatic environment in the gastrointestinal intract; (2) The low permeability through the epithelium. The first problem can be addressed by the use of coated pills/capsules, which prevent protein from degradation. The second problem remains to be solved. The aim of this research is to design a delivery system that would improve the efficiency of protein permeability through epithelial cells. Our group has identified an endogenous transcytosis pathway used by certain bacterial toxin protein to cross epithelial cells. Non-toxic domains responsible for transport proteins can be used for transport biopharmaceuticals. We hypothesize these non-toxic domains can also be used to transport nanoparticles. In this study, different preparations of nanoparticles have been explored: polyelectrolyte complex, nano-emulsion, and calcium phosphate nanoparticles. These methods are based on biopolymer chitosan, alginate, and tripolyphosphate, which are generally regarded as safe. Chitosan-alginate and chitosan-tripolyphosphate complex were selected for further study. The particle sizes were measured as 198.5±1.58nm and 401.2±43.39nm, and encapsulation efficiencies of bovine serum albumin are 42% and 28% respectively.

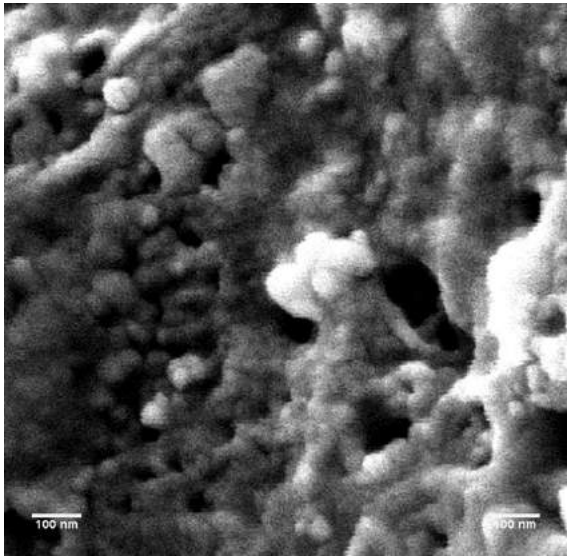

Fig. 1 Scanning Electron Microscope image of the chitosan-alginate complex

Interleukin-13-$Gd_3N@C_{80}(OH)_x(NH_2)_y$: A Targeting MR Imaging Contrast Nanoparticle for Enhanced Glioblastoma Mutiforme Tumor Detection

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Water-soluble derivatives of gadolinium-containing metallofullerenes have been synthesized and tested as excellent candidates for magnetic resonance imaging (MRI) contrast agents because of their high relaxivity and robustness. It is expected that nanoparticles with a positive charge will bind more efficiently to negatively charged cell phospholipid bi-layer cellular surfaces and will more readily undergo endocytosis. Although numerous gadolinium-containing metallofullerenes diagnostic MRI contrast agents have been reported, the metallofullerene cage surface in most cases consist of negatively charged carboxyl or hydroxyl groups that limits attractive forces with the cellular surface. In this paper, we report the preparation of a new functionalized trimetallic nitride endohedral metallofullerene, $Gd_3N@C_{80}(OH)_x(NH_2)_y$ with a cage surface consisting of positively charged amino groups. This new nanoparticle was characterized by X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS), reflection-absorption IR spectroscopy (RAIRS), NMR, and exhibit excellent ^1H relaxivity. Previous studies have clearly demonstrated that the cytokine, interleukin-13 (IL-13) effectively targets glioblastoma multiforme (GBM) cells which are known to overexpress IL-13 receptors (IL-$13R_\alpha$) sites. We now report that this Gd-nanoplatform when subsequently conjugated with an IL-13 peptide, (IL-13-$Gd_3N@C_{80}(OH)_x(NH_2)_y$)exhibits enhanced targeting of U-251 GBM cell lines. These results support the hypothesis that positively charged nanoparticles provide enhanced cell surface charge attraction for GBM cellular endocytosis.

Funding: Childrens National Hospital and Virginia Tech Carilion Research Institute, “Antibody-Conjugated Endohedral Metallofullerene B7-H3 and IL-13 Nanoparticles for Targeted Neuroblastoma Diagnostic and Therapeutic Applications”

PRINT NANOPARTICLE VACCINE CARRYING BACTERIAL POLYSACCHARIDE AND PROTEIN ANTIGENS INDUCES ENHANCED B- AND T-CELL (IL-17) IMMUNITY

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Pneumococcus is a common organism causing invasive bacterial disease, especially in children < 2 years and elderly adults. Using nanoparticles in vaccine formulations allows for improved antigen stability and immunogenicity, but also targeted delivery and slow release. A nanoparticle vaccine consisting of pneumococcal polysaccharides (PnPs) and protein antigens (toxoids, surface proteins) has the potential to offer broader and enhanced protective immunity (antibody/cellular) against invasive pneumococcal disease and carriage/colonization. In partnership with PATH, Liquidia is developing a next generation multivalent nanoparticle polysaccharide protein vaccine for *Streptococcus pneumoniae* based on roll-to-roll PRINT technology. Specifically, a PRINT® nanoparticle multivalent vaccine developed co-delivers 2 components – (i) key capsular polysaccharides (PnPs 1, 4, 5, 14, 6A, 19A, 23F) and (ii) pneumococcal carrier protein. Conjugation of antigens onto NPs can allow presentation of the immunogen to the immune systems in much the same way that it would be presented by the pathogen, leading to potent and specific antibody (IgG) and cellular (IL-17) immune responses against target antigens.

Preclinical vaccination studies in mice and rabbits with single and multivalent non-adjuvanted PRINT formulations produced polysaccharide-specific functional antibody (IgG and OPK) responses greater than or equivalent to Prevnar-13. Additionally, existing PRINT particle formulations were translated to all of Prevnar-13 serotypes and multiple proteins (CRM_{197}, pneumococcal specific surface proteins and toxoids, TT), further demonstrating the wide-ranging multi-antigen formulation capability.

Antigenic multivalent PRINT nanoparticle formulations leverage precise control of size, shape and composition, sterile filterability and scalable cGMP roll-to roll manufacturing to offering a low cost and simplified manufacturing advantage over traditional conjugate vaccines. Moreover, the broad flexibility of the current PRINT approach has been applied to other antigen targets such as typhoid, and H. influenzae. The expectation is that PRINT particulate vaccines will provide potent antigen-specific humoral and cellular immune responses and will allow development of next generation vaccines against a range of infectious diseases.

Self-Assembled Nano/Micro Bubbles for Oral Insulin Delivery

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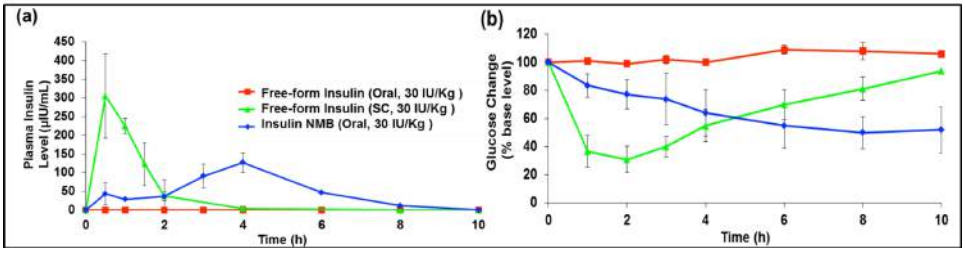
A variety of approaches have been studied in the past to overcome the problems encountered with the oral delivery of insulin, but with little success. In this study, an oral insulin delivery system of self-assembled nano/micro bubbles (NMBs) is prepared by blending sodium dodecyl sulphate (SDS), sodium bicarbonate (SBC), insulin, and diethylenetriamine-pentaacetic (DTPA) diahydride together in an enteric-coated capsule. Results of the small angle X-ray scattering and molecular dynamic simulation indicate that SDS surrounds the gas core and forms an inverted liposomal structure, and the insulin molecules are embedded in a gap between the bilayer structures. The in vitro confocal microscopy indicates that the transport of insulin across Caco-2 cell monolayers by the inverted liposomal NMBs via both paracellular and transcellular pathways. In the in vivo dissolution study, the enteric-coated capsules remain intact while in the stomach; the capsule is completely dissolved in the proximal segment of the small intestine and the loaded contents are then rapidly released. Single-photon emission computed tomography images performed in the biodistribution study clearly show the ^{123}I-insulin orally delivered by NMBs in the systemic circulation. The in vivo pharmacodynamic/pharmacokinetic (PD/PK) study conducted in a diabetic rat model shows that the insulin loaded in test NMBs is absorbed into the systemic circulation and provides a prolonged reduction in blood glucose levels in rats (Figure 1). These results indicate that the developed NMBs can be a promising carrier for oral administration of insulin.



(a) Plasma insulin level vs. time profiles; (b) Blood glucose change vs. time profiles of the diabetic rats following the administration of different insulin formulations.

Funding: National Science Council of the Republic of China, Taiwan (Contract No. NSC 101-2120-M-007-015-CC1)

Ring-Opening Polymerization of Prodrugs: A Versatile Approach to Prepare Well-Defined Drug Loaded Nanoparticles

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We report a new methodology for the synthesis of polymer-drug conjugates that are designed to self-assemble into nanoparticles and release drug in response to a physiologically relevant stimulus from “compound”—all in one—prodrug monomers that consist of a cyclic polymerizable group that is appended to a drug through a cleavable linker (Figure 1a). We show that organocatalyzed living ring-opening polymerization (ROP) can polymerize these compound monomers into biodegradable polymer prodrugs in a single polymerization step. This new method is compatible with structurally diverse drugs and allows different drugs to be copolymerized with quantitative conversion of the monomers. The drug loading of the polymer prodrugs can be easily controlled by adjusting the monomer(s) to initiator feed ratio and drug release can be encoded into the polymer by the choice of linker. Initiating ROP of these prodrug monomers from a polyethylene glycol (PEG) macroinitiator yields amphiphilic diblock copolymers that spontaneously self-assemble into spherical nanoscale micelles in which the drug is sequestered in the core of the micelle with a PEG corona that imparts a long plasma circulation to the micelle that is optimal for systemic therapy of solid tumors. Herein, anticancer drugs including chlorambucil, camptothecin, and paclitaxel were selected as examples because of the existence of a carboxyl or hydroxyl functional group that was able to link to the polymerizable cyclic carbonate by a bio-responsive linker (Figure 1b). The drug loading of the obtained polymer-drug conjugates were controlled from ~3 to 50 wt% by adjusting the molar feed ratio. Due to the amphiphilicity,

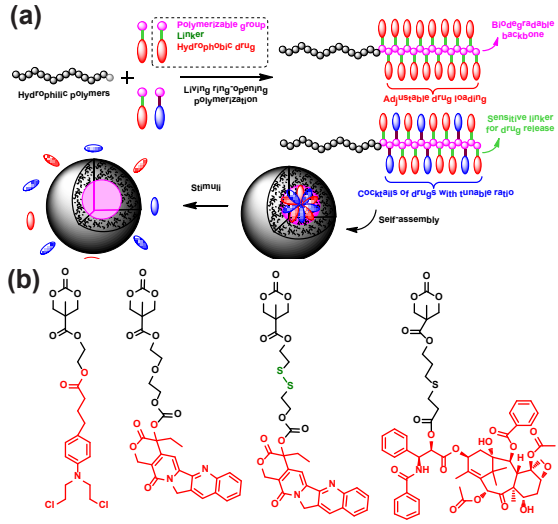

Figure 1. (a) Schematic illustration of the design and synthesis of polymer prodrugs with tunable drug loading and drug release by living ROP of prodrugs containing a cyclic polymerizable moiety, a customizable linker and a drug, as well as their self-assembly into nanoscale micelles. (b) The detailed structure of four different types of polymerizable prodrugs based on chlorambucil, camptothecin and paclitaxel, respectively.

these polymer-drug conjugates self-assembled into nanoparticles in aqueous solution with hydrodynamic radius from ~20 to 75 nm. In vitro experiments demonstrated the highly anti-proliferation efficacies of these polymer-drug conjugates against several different tumor cell lines.

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Polypeptoids as New Precision Materials Platform for Drug Delivery

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Polypeptides are accessible from nature, via biotechnology, through stepwise, solid-phase synthesis and by ring-opening polymerization (ROP) of N-carboxyanhydrides (NCA). Natural and synthetic polypeptides are of limited use as biomaterials as they have a tendency to form secondary structures, are often insoluble in organic media and water when uncharged. In addition, depending on the amino acids employed, immunogenicity is a potential limitation of polypeptides. N-Substituted polypeptides, poly(peptoid)s (POI) are a diverse group of polymers, which have shown significant potential as biomaterials and do not suffer from many limitations of polypeptides. Polypeptoids can be water- and organo-soluble, have no tendency for secondary structures unless desired and are very stable against proteases. At the same time, synthetic problems that have plagued polypeptide synthesis are reduced and we and others could show recently that well-defined block copolypeptoides are accessible by nucleophilic living ROP (NuLROP) of N-substituted NCAs, which allows for extraordinary control over the polymerization and its products, which is attributed to the nature of the propagating chain end, a comparably stable secondary amine. Thus, highly complex yet defined polymers such as multiblock copolymers are rather simple to realize, compared to living ionic polymerizations. We also demonstrated that the NuLROP is also possible from solid substrates and solid-phase synthesis resins, which allows free combination with the synthesis of sequence specific peptides or peptoids for targeting or other pharmacological tasks. Moreover, our data show that the polymers are not only well tolerated by mammalian cells but are degradable under physiologically relevant conditions. In summary, the combination of synthetic control and biological properties of polypeptoids make these materials unique among all synthetic, biological and biomimetic biomaterials

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Temperature-triggered self-assembly of nanoparticles with pro-apoptotic peptide drug cargo creates a digital cytotoxic switch

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Systemically delivered anti-cancer therapeutics face many barriers before achieving therapeutic outcomes in the tumor. Cellular uptake is one such challenge for drugs with an intracellular therapeutic target and for which the cell membrane is an impenetrable barrier. Drug carriers can facilitate cellular uptake of drug cargo and can target uptake to the tumor. Active targeting of intracellular delivery traditionally relies on intrinsic tumor features (ex. upregulated receptors) that are heterogeneous among cancer types and patients. Alternatively, an extrinsic trigger can achieve active targeting, circumventing variability in cancer phenotypes. We hypothesize that an extrinsic stimulus can target intracellular delivery by controlling non-specific cellular uptake of cell-penetrating peptides (CPPs) with modulation of CPP density on self-assembled nanoparticles, where low CPP density limits internalization and high CPP density enhances internalization.

Extrinsic modulation of CPP density was achieved with elastin-like polypeptide diblock copolymers (ELP_{BC}s), which undergo temperature-triggered micelle self-assembly. A CPP (ex. Arg_8) was appended to the hydrophilic ELP_{BC} domain, such that self-assembly achieved high CPP density on the micelle corona. Assembly of Arg_8-ELP_{BC}s occurred at mild hyperthermia, such that Arg_8-ELP_{BC} was a unimer with low CPP density at 37°C, and assembled into micelles displaying high CPP density at 42°C. Minimized uptake of unimers occurred at 37°C (Figure 1A) and significant amplification of micelle uptake occurred at 42°C (Figure 1B). Therapeutic efficacy was evaluated with pro-apoptotic BH3 peptide drug cargo. Arg_8-ELP_{BC}-BH3 achieved controlled cytotoxicity, enhancing cell death at 42°C, while sparing cells at 37°C (Figure 1C). Modulation of CPP density, controlled by mild hyperthermia, can thereby potentially achieve targeted intracellular drug delivery while circumventing tumor heterogeneity.

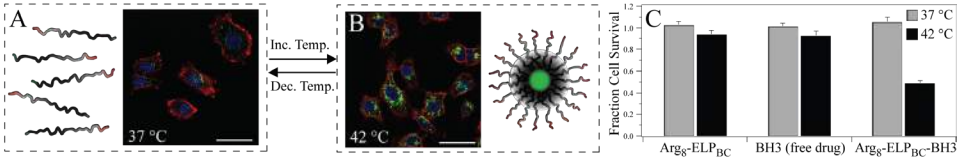

Figure 1. Arg_8-ELP_{BC} unimers exhibited less cellular uptake at 37°C (A), compared to micelles at 42°C (B). Arg_8-ELP_{BC}-BH3 enhanced cytotoxicity only at 42°C, compared to Arg_8-ELP_{BC} or BH3 alone. Red–membrane; Blue–nuclei; Green–Arg_8-ELP_{BC}; Bars–50 μm.

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Block copolymers guide macrophages to transfect skeletal muscle cells.

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Purpose: Despite various chemical or biological advances in therapeutic gene delivery to skeletal muscle by direct intramuscular injection *(i.m.)*, this application is hindered by very low transfection efficiencies. Non-ionic block copolymers, Pluronic P85 and SP1017 (mixture of L61 and F127), when mixed with pDNA, enhance skeletal muscle transfection in immunocompetent (BALB/c and C57BL/6) mouse and not immunodeficient (nu/nu) mouse. Therefore, we studied the mechanism of muscle transfection and developed a pluronic pre-injection scheme to further enhance the levels of gene expression.

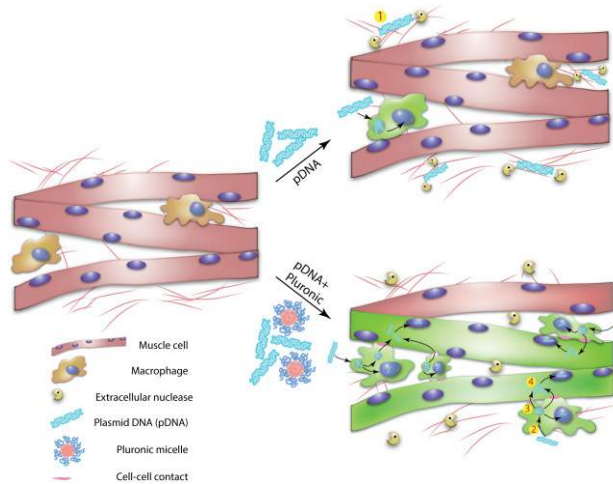

Figure: Graphical representation of muscle transfection after *i.m.* injection of pDNA formulated with block copolymer aka Pluronics.

Method: pDNA alone or pDNA+Pluronic were injected into *tibialis anterior* (TA) muscle in presence or absence of local inflammation. The cellular immune response upon direct *i.m.* pDNA injections was characterized by immunophenotyping. *In vitro* muscle inflammation model was developed by co-culturing macrophages (MPs) with muscle cells to see the effect of P85 on muscle gene delivery. Constitutive (CMV) and muscle specific (Desmin) promoter driven gene expression constructs were used to determine DNA transfer from MPs to muscle cells.

Result: Local-inflammation drastically enhanced DNA, RNA and gene expression levels when pDNA was co-delivered with P85 or SP1017 and not pDNA alone. GFP expression in pDNA+P85 group co-localized with

anti-inflammatory MPs ($CD11b^{+}Ly6G^{-}F4/80^{-/int}Ly6C^{lo}$) and not neutrophils ($CD11b^{+}Ly6G^{hi}$ $F4/80^{-/int}Ly6C^{+}$) or T cells ($CD45^{+}CD3e^{+}Cd19^{-}$) or B cells ($CD45^{+}CD3e^{-}Cd19^{+}$). Upon treatment of co-culture (pDNA transfected MPs with muscle cells) with P85, total and muscle specific gene expression increased ca. 30 and 120 times respectively. The latter, confirmed pDNA transfer from MPs to muscle cells. Finally, pre-injection with P85 on day 1.5 and not day 5 before pDNA+P85 injections further enhanced the gene expression levels.

Conclusions: Pluronic block copolymers utilize immune cells (anti-inflammatory macrophages) to deliver genes to otherwise very hard to transfect skeletal muscle fibers and hence constitute a novel class of biological response modifiers or a platform technology to deliver genes.

Funding: Eshelman Gift Trust-Research and Training Enterprise, Center of Biomedical Research Excellence (CoBRE) Nebraska Center for Nanomedicine-1P20RR021937 and Department of Defense (DoD)-W81XWH-09-1-0386.

Systemic targeting of lymph node metastasis by controlled design of drug-loaded polymeric micelles

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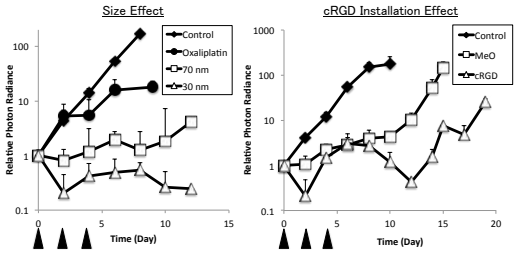
Metastasis is one of the most threatening aspects in cancer, and especially the one to the regional lymph node is concerned with distant metastasis or poor prognosis. Although surgery and radiotherapy are the main therapeutic approach, they are not always effective, especially when lymph node metastasis (LNM) is in early stage or multiple metastatic lymph nodes are already formed. In that respect, systemic injection of anticancer drugs will solve that problem by treating LNM and preventing its recurrence, but this is difficult to achieve due to the need of high doses with risk of side effects. Thus, effective targeting of LNM by intravenously injected nanocarrier could be a promising strategy for developing efficient therapies. Herein, we used (1,2-diaminocyclohexane)platinum(II) (DACHPt)-loaded polymeric micelles to study their ability to target LNM after intravenous injection. The LNM were developed spontaneously in the brachial lymph nodes of mice after inoculation of B16F10-luc murine melanoma cells into their forepaws. By using 30- and 70-nm polymeric micelles, we found that the efficacy of the micelles against LNM was affected by their size, with 30-nm micelles being more effective. Furthermore, we focused on cRGD peptide-installed DACHPt-loaded micelles, which can selectively bind to avb3- or avb5-integrins overexpressed on tumor endothelial cells and cancer cells, to evaluate the ligand-targeting effect. These cRGD-installed micelles showed higher antitumor efficacy against LNM than non-targeted micelles, probably because of their higher accumulation in metastatic tissue and walls of tumor vessel, as determined by in vivo confocal laser microscopy. Our findings suggests the potential of small polymeric micelles for developing early diagnosis or effective therapeutic methods against lymph node metastasis, and the enhancement of their targeting efficiency by installation of tumor-directed ligands.



Size effect and ligand installation effect of DACHPt-loaded polymeric micelles to antitumor efficacy against LNM

Funding: Graduate Program for Leaders in Life Innovation, The University of Tokyo Life Innovation Leading Graduate School from MEXT, Japan.

Half-Antibody conjugated Hybrid Nanoparticles of Lipid Shell and Albumin Core for Targeted Delivery of an EGFR-Tyrosine Kinase Inhibitor in Lung Cancer Cells

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Hybrid nanoparticles consisting of polymeric core enveloped by phospholipid layer(s) have emerged as a multifunctional nano-carrier system alternative to liposomes and polymeric nanoparticles. In this study, hybrid nanoparticles (HNPs) consisting of phospholipid shell and albumin core, loaded with Erlotinib [ETB, an epidermal growth factor receptor(EGFR)-tyrosine kinase inhibitor], and functionalized with EGFR-specific monoclonal half-antibody (hAb) were developed with the aim of achieving increased therapeutic efficacy and reduced toxicities of ETB for non-small cell lung cancer (NSCLC). ETB loaded HNPs were prepared by two-steps method using albumin, ETB, and dipalmitoylphosphatidylcholine, cholesterol, lipid-PEG2000 and/or lipid-PEG2000-maleimide. For hAb-ETB-HNPs preparation, EGFR monoclonal antibody was reduced to half-antibody (hAb) fragment and attached to HNPs *via* maleimide-thiol conjugation (Figure 1). Various formulation and process parameters were optimized. HNPs were evaluated for: i) physicochemical properties (size, zeta potential, drug loading efficiency, drug release, serum and storage stability) and ii) *in vitro* biological properties (cellular uptake, mechanisms of uptake, trafficking, and cell viability) in human lung cancer cells, A549 (wild-type EGFR) and HCC 827 (mutant EGFR). HNPs were successfully prepared with size range of 190-210 nm, zeta potential of -10 to -30mV and drug loading of 1-2%. SDS-PAGE confirmed the formation of hAbs at 100-fold molar excess of disulfide reducing reagent. The hAb-ETB-HNPs exhibited colloidal stability in bovine serum for 12h and shelf-life stability of at least 30 days at 2-8^{o}C. Flow cytometry results showed the increased uptake of fluorescent lipid-loaded HNPs in cells *via* both clathrin and caveolae mediated endocytotic pathways. Significant reduction in cell viability was observed for hAb-ETB-HNPs compared to control groups in both cell lines. The analysis of IC_{50} demonstrated that hAb-ETB-HNPs could be about 6 to 8-fold more efficacious than free ETB. The results suggest that hAb-ETB-HNPs have potential therapeutic advantages compared to free ETB for the treatment of EGFR overexpressing NSCLC.

Funding: Plough Center for Sterile Drug Delivery Systems at University of Tennessee Health Science Center, Memphis, TN 38163.

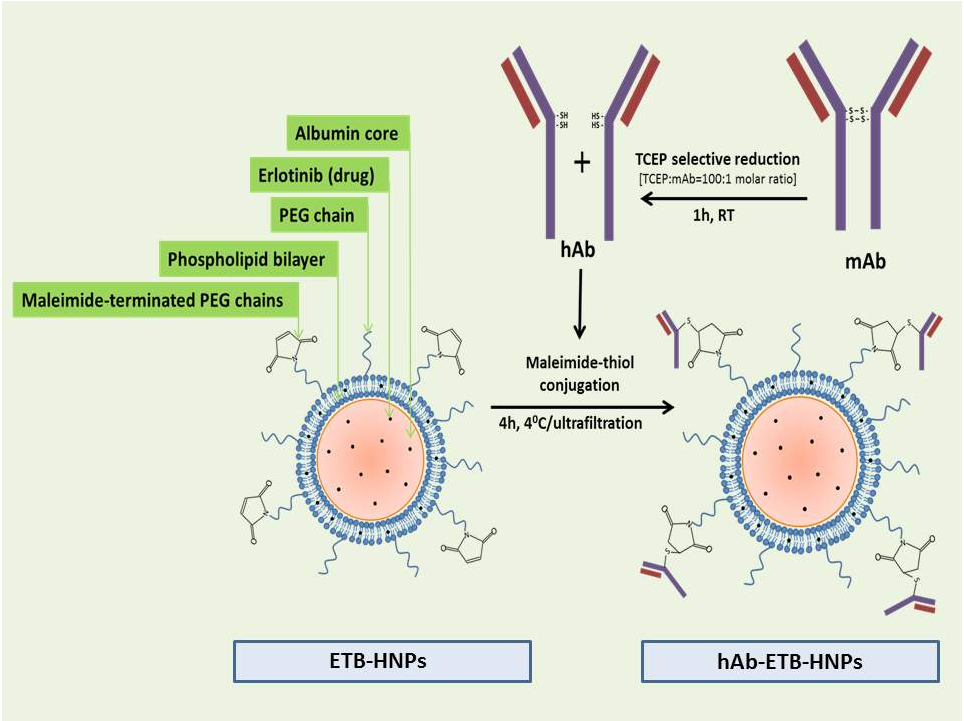

Figure 1: Scematic of the preparation of targeted hAb-ETB-HNPs [Note: TCEP ; Tris-(2-Carboxyethyl)phosphine]

Thermally Responsive Multivalent Fusion Proteins as TRAILR-2 Superagonists for Cancer Therapy

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TNF-related apoptosis-inducing ligand receptor 2 (TRAILR-2) activates the extrinsic death pathway in a range of human cancer cells. TRAILR-2 has been targeted using its natural ligand, TRAIL, and novel agonists, resulting in promising anti-tumor activity in preclinical models. These successes have not translated well to patients in the clinic, and the reasons for this are not well understood. In 2013, Swers et al. tested the hypothesis that engineering a more potent TRAILR-2 agonist could bridge the gap between success in preclinical models and survival in cancer patients. The group engineered a fibronectin type III domain for high affinity binding to TRAILR-2, and created multivalent constructs by connecting the scaffold proteins to one another using flexible linkers. Despite the subpicomolar EC50 of these multivalent proteins and their ability to induce apoptosis in some TRAIL-resistant cell lines, the half-life of these proteins is only half an hour. We created thermally sensitive versions of these superagonist proteins using elastin-like-polypeptides (ELPs) and showed that the ELP does not affect the potency of the proteins. We then characterized the protein-ELP fusions using spectrophotometry to obtain the transition temperatures and choose an appropriate candidate for testing an intratumoral drug depot in mice with human Colo205 xenografts.

Funding: This work is supported by the NIH through grant RO1EB007205

Novel Shikonin-Loaded, Antibody-Armed Nanoparticles for Endosialin-Targeted Drug Delivery in Tumor Microenvironment

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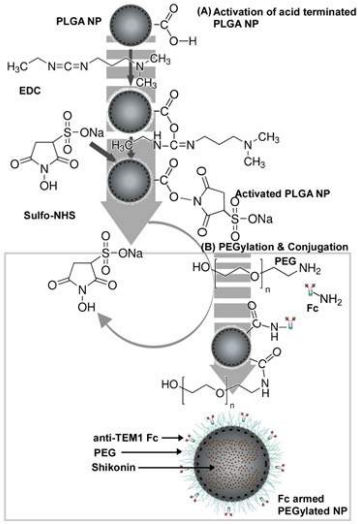
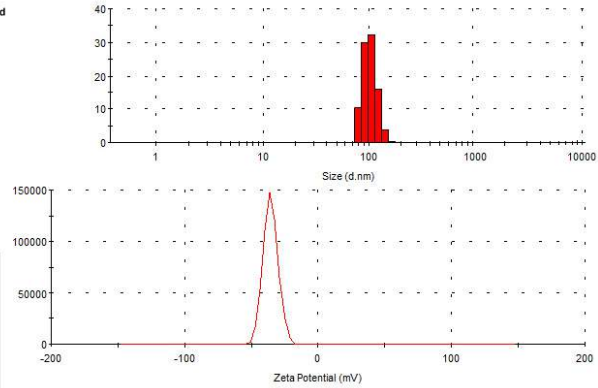
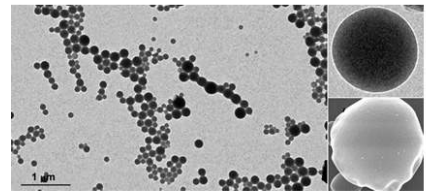
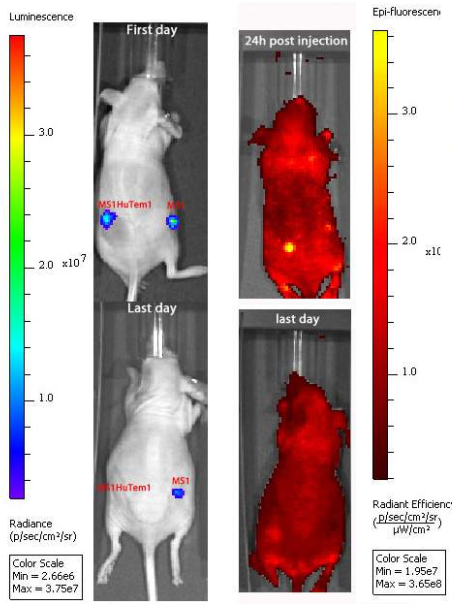
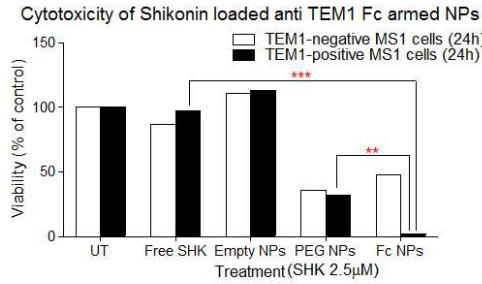
Endosialin/Tumor Endothelial Marker 1 (TEM1) is expressed by tumor-associated pericytes, myofibroblasts and vascular endothelial cells in various human cancers. Endosialin plays role in promoting tumor angiogenesis, proliferation, migration and metastasis. Importantly, mice without functional Tem1 gene present a striking reduction in tumor growth, invasiveness, and metastasis after tumor transplantation to abdominal sites. Thus, targeting TEM1 for diagnostic and/or therapy could provide a valuable strategy against cancer. Shikonin (SHK) is a potent pharmaceutical substance with, among others, antitumor activity. Recently, SHK was also found to cause unique necrapoptosis to tumor cells resistant to chemotherapeutic drugs. In order to deliver SHK in the tumor microenvironment, biodegradable nanoparticles (NPs) of poly(lactic-co-glycolic acid) (PLGA) were formulated and further modified with polyethylene glycol and anti-TEM1 antibody fragment (78Fc) through carbodiimide/N-hydroxysuccinimide chemistry. Dynamic light scattering and electron microscopy showed smooth spherical shape, with size of ~120nm and ζ potential of –30mV. Upon modification, drug entrapment efficiency was ~92%, while, bioconjugation efficiency reached ~90%. SHK showed a sustained-release profile, in PBS and serum, which fitted various kinetics models. To address regulatory and clearance concerns, a biodistribution study was performed *in vivo*, where indocyanine green loaded PLGA NPs did not retain in liver/spleen, while most of the NPs were excreted via urine track after 24h. The *in vivo* targeting efficacy of these NPs was firstly studied using a MS1 xenograft mouse model. SHK loaded, 78Fc armed NPs showed higher accumulation at the TEM1-positive tumor site and higher killing efficacy compared to free drug/unarmed SHK-loaded NPs. TC1 murine lung carcinoma subcutaneous and intravenous (lung metastasis) models were selected to evaluate the targeting efficiency in aggressive tumor models. In these models, the armed NPs increased detrimentally SHK cytotoxicity compared to unarmed NPs/free drug. Based upon these findings, we propose SHK-loaded, 78Fc-armed PLGA NPs as

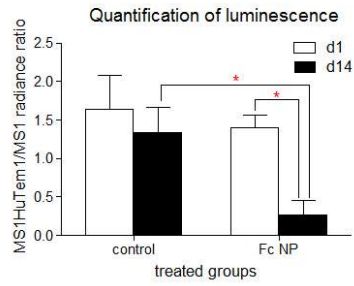












novel nanomedicine for tumor targeted therapy.

Funding: NIH R01-CA-156695 (GC), SPORE in Ovarian Cancer Pilot P50 CA083638-14 (CL), Sandy Rollman Ovarian Cancer Foundation (CL).

Engineered Apoferritin Nanocages for Self-triggered Nuclear Delivery of Drugs at Cancer Cells

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Engineered apoferritin nanoparticle (HFn) was developed to achieve a cumulative self-triggered nuclear delivery of antitumor drugs in cancer cells (CC) with subcellular precision. The rationale of our approach is based on exploiting the natural arsenal of defense of CC to stimulate them to recruit large amounts of HFn loaded with doxorubicin (DOX) inside their nucleus in response to a noxious stimuli, which leads to decrease of viability and cell death. After demonstrating the selectivity of HFn for representative cancer cells compared to healthy fibroblasts, DOX-loaded HFn were used in CC treatment. Our results proved that loading of DOX in HFn increased the nuclear delivery of the drug, thus enhancing DOX efficacy (Figure 1A, B, C). DOX-loaded HFn acts as a “Trojan Horse”: HFn were internalized in CC more efficiently compared to free DOX, then promptly translocated into the nucleus following to the DNA damage caused by the partial release in the cytoplasm of encapsulated DOX. This self-triggered translocation allowed the drug release directly in the nuclear compartment, where it exerted its toxic action probably bypassing the action of the Glycoprotein-P. This approach was reliable and straightforward providing an antiproliferative effect with high reproducibility. The particular self-assembling nature of HFn nanocage makes it a versatile and tunable nanovector for a broad range of molecules suitable both for detection and treatment of CC.

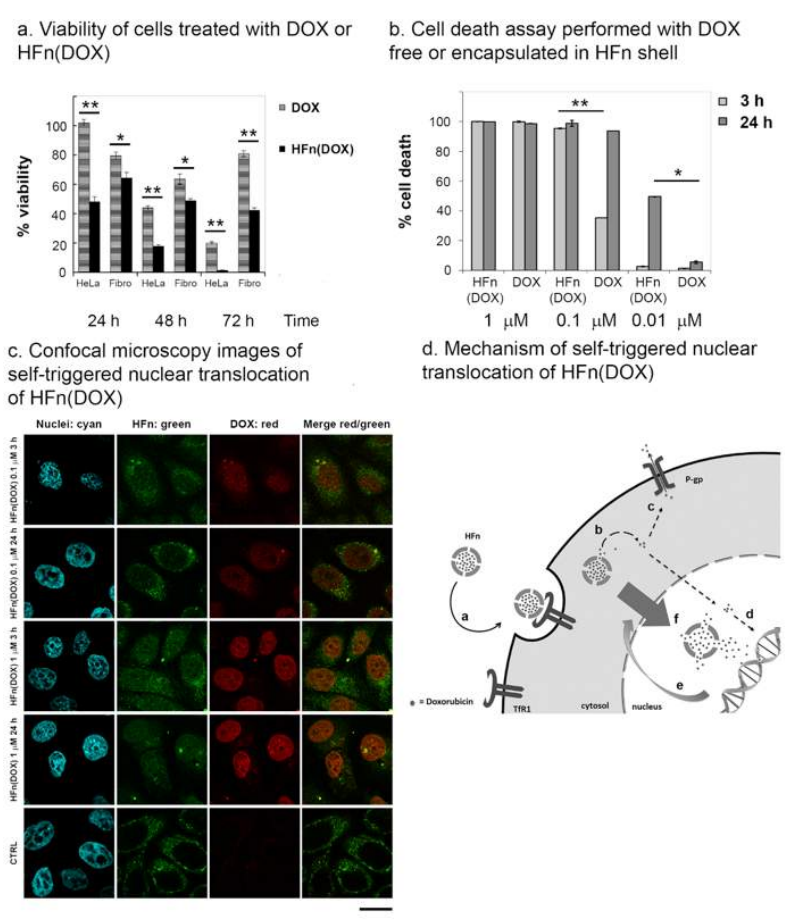

Figure 1. (a) Viability of cells treated with free DOX or HFn(DOX). Fibroblasts and HeLa cells were treated with 0.1 μM of DOX or HFn(DOX) for up to 72 h. Viability was assessed by measuring the conversion of MTT into formazan. Reported values are the mean of six replicates ± s.e., normalized on cell proliferation of untreated fibroblast or HeLa cells, respectively * P<0.005; ** P<0.0005 (Student's t-test). (b) Cell death assay with DOX free or encapsulated in HFn shell. HeLa cells were treated with 1, 0.1 and 0.01 μM of DOX or HFn(DOX) for 3 or 24 h. Cell death was assessed on the basis of the exposure to Annexin V, evaluated by flow cytometry. Untreated cells were used to set region of positivity. Reported values are the mean of three replicates ± s.e. * P<0.01; ** P<0.005 (Student's t-test). (c) Self-triggered nuclear delivery of DOX in HFn(DOX) nanoparticles. Confocal microscopy images of HeLa cells incubated for 3 and 24 h at 37 °C with 0.1 μM of HFn(DOX). HFn(DOX) was labeled with FITC (HFn; green) on the shell and then loaded with DOX (red). Nuclei were stained with DAPI (cyan). Scale bar: 10 μm. (d) Schematic representation of self-triggered nuclear delivery of HFn(DOX). HFn were internalized upon the interaction with TfR1 by receptor-mediated mechanism without incurring lysosomal degradation (a). Encapsulated DOX was partially released in the cytoplasm through hydrophobic channels of its architectucture (b). Then DOX is pumped out of the action of P-glycoprotein (c), or diffuses into the nucleus where it causes the DNA damage (d), which triggers the nuclear translocation of HFn(DOX) (e), and the massive release of DOX in the nuclear compartment (f).

Modeling Nano-Scale Diffusion through Complex Fluids: Implications for Treatment of Pulmonary Diseases

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Every organ of the human body not covered by skin is coated with a mucosal layer that protects the underlying organ from pathogens and other foreign insults. The mucosal layer also inhibits the diffusive passage of nano- and micro-scale drug carrier particles (DCPs) as they dissolve or decompose, providing a significant influence on the concentration-time profile of the embedded active pharmaceutical ingredients. The impact of the mucosal layers on the bioavailability and distribution of a DCP is a complex function of DCP surface chemistry and size as well as the physical properties of the mucosal layer itself. The design of DCPs for controllable transit of API through the mucosal layer requires statistical and mathematical tools to analyze experimental data, perform model selection, fit model parameters, and forecast outcomes. Furthermore,

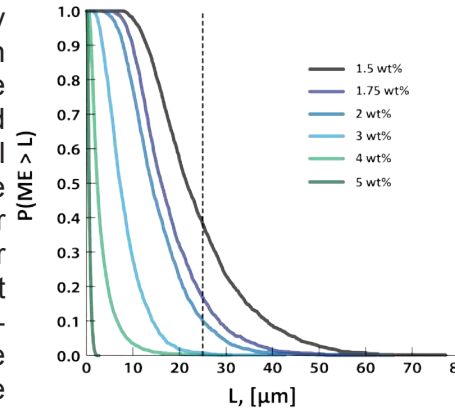

Figure 1. Probability that a 1μm carboxylated polystyrene particle will diffusively penetrate a mucus barrier of width L within 20 minutes (an estimated clearance time of particles embedded in the mucus layer of the upper airways). The vertical dashed line indicates a typical mucus layer thickness in the upper airways.

no current approaches adequately address the variability in the properties of the mucosal layer that can significantly influence DCP diffusion and API uptake. As a further complication, the diffusion of DCPs through biological fluids is often anomalous – the dynamics are not consistent with standard Brownian diffusion, invalidating a central assumption of most pharmacokinetic models. To address these issues, we have developed a theoretical-experimental protocol to more accurately model the diffusion of DCPs. We demonstrate these techniques by analyzing the movement of nano-scale particles through pulmonary mucus and, using novel simulation methods, investigate the impact of pulmonary disease progression on the bioavailability and transit times of inhaled DCPs.

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Nanoparticle delivered Wnt16 siRNA remodels tumor microenvironment to enhance therapeutic outcome of cisplatin in bladder cancer

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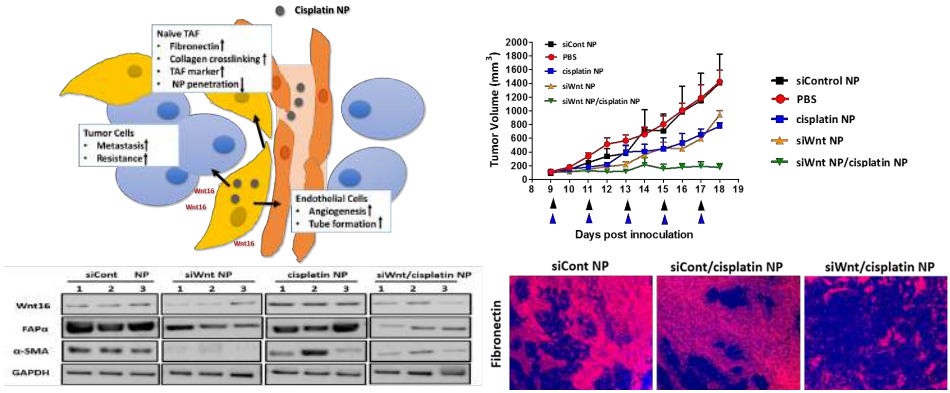
The cisplatin nanoparticle (NP) composed in our lab have shown potent efficacy against stroma-rich bladder cancer. However, acquired resistance has limited the therapeutic outcome. Components of the tumor microenvironment (TME) confer the acquired resistance. Results have shown that 22% of the NP accumulated in the tumor were distributed to tumor associated fibroblasts (TAF), leading to upregulation of Wnt16. Wnt16 has been reported to induce resistance in prostate and breast cancer. Given the high specificity of siRNA, we use liposome-protamine-hyaluronic acid NP to deliver siRNA against Wnt16 (siWnt NP), and propose the use of siWnt NP in combination with cisplatin NP as a potential strategy to augment antitumor outcome. Cisplatin NP and siWnt NP were prepared according to previous protocols. Mouse specific Wnt16 siRNA was chosen to target the mouse fibroblast, not the tumor cells. Our results shown that cisplatin induced secretion of Wnt16 in TAF. The secreted Wnt16 worked paracrinely on adjacent tumor cells, fibroblasts and endothelial cells to activate the canonical Wnt pathway and decrease cancer cells and the fibroblasts' sensitivity towards cisplatin. The combination of cisplatin NP and siWntNP sensitized cisplatin treatment in vitro and inhibited tumor growth in both small and large tumor models in vivo. Moreover, knocking down Wnt 16 using siWnt NP alone didn't inhibit TAF growth, but decreased the expression of TAF marker, α-SMA, inhibited fibronectin secretion,



prohibited collagen crosslinking and increased the overall NP penetration. Down-regulation of Wnt16 also inhibited angiogenesis. Overall, our finding indicates Wnt16 as one of the key players in regulating the interaction between tumor cells and TME, and suggests that formulating a single NP formulation with one payload of a chemodrug and the other that targeting DNA damage induced stromal protein (e.g. Wnt16) as a potential strategy to improve the overall therapeutic outcome.

Funding: This work was supported by NIH grants CA151652 and CA149363

Nanoparticle Formulations of Histone Deacetylase Inhibitors for Effective Chemoradiotherapy in Solid Tumors

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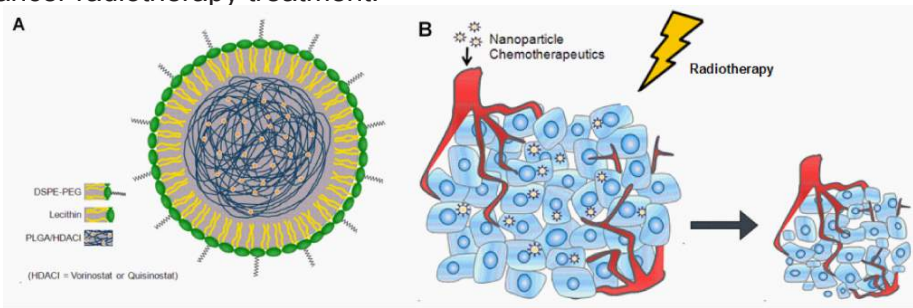
Abstract: Histone deacetylase inhibitors (HDACIs) represent a class of promising agents that can improve radiotherapy in cancer treatment. However, the full therapeutic potential of HDACIs as radiosensitizers has been restricted by limited efficacy in solid malignancies. In this study, we report the development of nanoparticle (NP) formulations of HDACIs that overcome these limitations, illustrating their utility to improve the therapeutic ratio of the clinically established first generation HDACI vorinostat and a novel second generation HDACI quisinostat. We demonstrate that NP HDACIs are potent radiosensitizers in vitro and are more effective as radiosensitizers than small molecule HDACIs in vivo using mouse xenograft models of colorectal and prostate carcinomas. We found that NP HDACIs enhance the response of tumor cells to radiation through the prolongation of γ-H2AX foci. Our work illustrates an effective method for improving cancer radiotherapy treatment.



Scheme 1. NP HDACs used as radiosensitizer to enhance the efficacy of radiotherapy

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Formulation Optimization and Testing of CD22 Targeted AD198 Loaded Liposomes

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Purpose: For the treatment of hematological malignancies we have tested the novel drug, AD198 that bypasses resistance which doxorubicin is susceptible to, and is cardioprotective rather than cardiotoxic. However, the systemic administration of AD198 manifests dose related toxicities such as neutropenia and thrombocytopenia. These can be limited by encapsulating AD198 in liposomal systems which can be targeted to certain receptors such as CD22, overexpressed on the malignant B cells. The objective of this study was to optimize the formulation of CD22 targeted AD198 loaded liposomes and test them *in vitro*.

Method: Liposomes were prepared using the Bangham method. Parameters such as EPC and HSPC composition and other excipient ratios which affect the physico-chemical parameters of the liposomes were optimized. For preparing targeted liposomes, the thioether bond was utilized for coupling the CD22 mAb to the maleimide derivatized liposomes. Particle size, ζ -potential, drug loading, dissolution and conjugation were analyzed by DLS, electrophoretic mobility, RP-HPLC, SDS-PAGE respectively. In vitro testing was performed in CD22+ Daudi and CD22- Jurkat cells. Cellular uptake and cytotoxicity were studied by cell cytometry and MTT assay respectively.

Results: HSPC was the phospholipid of choice. Total phospholipid was optimized at 75 mM and theoretical AD198 concentration at 2mg/mL. DSPE-PEG_{2000} and cholesterol were optimized at 2 mole% and 10 mole%. Approximately 30% AD198 was released in 72 hours. Cellular uptake results suggest preferential uptake by CD22+ Daudi cells and maximum saturation at 4 hours. MTT assay results suggest an IC_{50} of 0.25 μM and >3.0 μM targeted liposomal AD198 in Daudi and Jurkat cells.

Conclusion: In this study we have optimized the various parameters affecting the physico-chemical properties of the liposomal system. *In vitro* results suggested selectivity of the targeted liposomes towards CD22+ Daudi cells which would be beneficial in alleviating adverse effects due to non-specific drug activity.

Funding: Plough Center for Sterile Drug Delivery Systems, Department of Pharmaceutical Sciences, University of Tennessee Health Science Center.

ATP-Triggered Anticancer Drug Delivery

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Stimuli-responsive drug-delivery systems (DDSs) are playing an increasingly crucial role in a variety of applications for cancer treatment. We herein report an ATP-responsive nanogel consisting of three distinct functional components: an ATP-responsive DNA scaffold with doxorubicin (Dox), protamine and a hyaluronic acid (HA)-crosslinked shell (Figure 1a). The DNA scaffold was composed of the ATP aptamer and its complementary single-stranded DNA (cDNA),which also provided faithful loading sites for Dox. When exposed to ATP, the dissociation of the DNA duplex resulting from the formation of the ATP/ATP aptamer complex caused the intercalated Dox to be released from the duplex. Cationic protamine was utilized to complex the DNA motif, which had enhanced cell penetration, endosomal escape and nuclear targeting effects. Anionic HA was coated on the complex core followed by UV irradiation-mediated photocrosslinking to form a protective crosslinked gel shell, and also serves as ligands to bind the CD44 receptors that are overexpressed on the cell surface of many cancers. This ATP-responsive nanocarrier showed an enhancement in the chemotherapeutic inhibition of tumor growth using xenograft breast tumor-bearing mice. In addition, a liposome-based co-delivery system, composed of a fusogenic liposome encapsulating the Dox-loaded ATP-responsive DNA scaffold and an ATP-loaded liposome, was further developed for ATP-mediated drug release triggered by liposomal fusion (Figure 1b). Directly delivery of extrinsic liposomal ATP facilitated the Dox release from the fusogenic liposome in the acidic intracellular compartments by a pH-sensitive membrane fusion and therapeutic efficacy was enhanced both *in vitro* and *in vivo*.

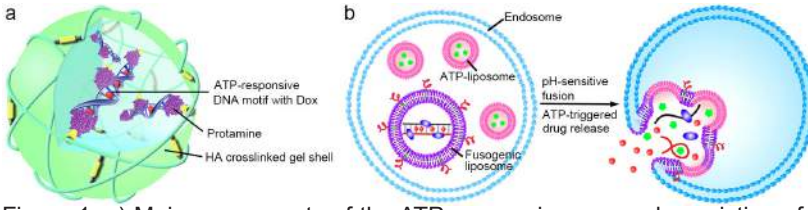

Figure 1. a) Main components of the ATP-responsive nanogel consisting of Dox/NG: ATP-responsive DNA motif with Dox, protamine and a HA-crosslinked gel shell. b) Schematic illustration of the liposome-based co-delivery system composed of a fusogenic liposome and ATP-liposomes.

Funding: 550KR51307 from NC TraCS, NIH's Clinical and Translational Science Awards at UNC-CH (1UL1TR001111) and the NC State Faculty Research and Professional Development Award, and start-up package from the Joint BME Department of UNC-CH and NCSU to Z.G.

Therapeutic Modalities of Squalenoyl Nanocomposites in Colon Cancer: An Ongoing Search for Improved Efficacy

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Drug delivery of combined cytotoxic and antivascular chemotherapies in multidrug nanoassemblies may represent an attractive way to improve the treatment of experimental cancers. Here we made the proof of concept of this approach on the experimental LS174-T human colon carcinoma xenograft nude mice model. Briefly, we have nanoprecipitated the anticancer compound gemcitabine conjugated with squalene (SQ-gem) together with isocombretastatin A-4 (isoCA-4), a new isomer of the antivascular combretastatin A-4 (CA-4). It was found that these molecules spontaneously self-assembled as stable nanoparticles (SQ-gem/isoCA-4 NAs) of ca. 142 nm in a surfactant-free aqueous solution. Cell culture viability tests and apoptosis assays showed that SQ-gem/isoCA-4 NAs displayed comparable antiproliferative and cytotoxic effects than those of the native gemcitabine or the mixtures of free gemcitabine with isoCA-4. Surprisingly, it was observed by confocal microscopy that the nanocomposites made of SQ-gem/isoCA-4 distributed intracellularly as intact nanoparticles whereas the SQ-gem nanoparticles remained localized onto the cell membrane. When used to deliver these combined chemotherapeutics to human colon cancer model, SQ-gem/isoCA-4

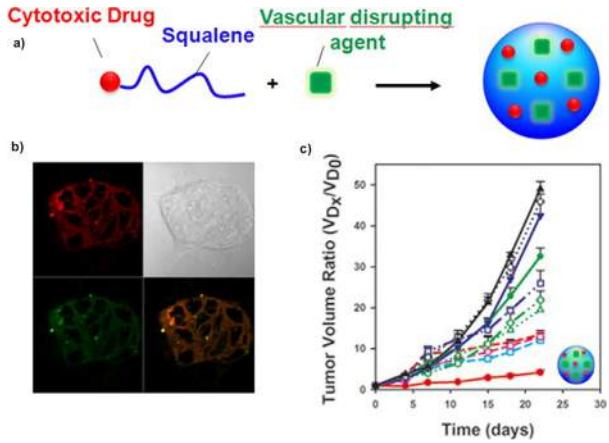

Fig.1. Design of squalene-based nanocomposites (a), cell internalization (b) and tumor growth inhibition after multidrug treatment (c).

nanocomposites induced complete tumor regression (by 93%) and were found superior to all the other treatments, whereas the overall tolerance was better than the free drug treatments. This approach could be applied to other pairs of squalenoylated nanoassemblies with other non-water-soluble drugs, thus broadening the application of the “squalenoylation” concept in oncology.

Funding: The research leading to these results has received funding from the European Research Council under the European Community’s Seventh Framework Programme FP7/2007-2013 Grant Agreement No. 249835. UMR 8612 Couvreur’s team is a member of the laboratory of excellence NANOSACLAY, and BioCIS UMR 8076 is a member of the Laboratory of Excellence LERMIT supported by a grant from ANR (ANR-10-LABX-33)

A unique squalenoylated and nonpegylated doxorubicin nanomedicine with systemic long-circulating properties and anticancer activity

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We identified that the chemical linkage of the anticancer drug doxorubicin onto squalene, a natural lipid precursor of the cholesterol's biosynthesis, led to the formation of squalenoyl doxorubicin (SQ-Dox) nanoassemblies of 130-nm mean diameter, with an original "loop-train" structure. This unique nanomedicine demonstrates: (i) high drug payload, (ii) decreased toxicity of the coupled anticancer compound, (iii) improved therapeutic response, (iv) use of biocompatible transporter material, and (v) ease of preparation, all criteria that are not combined in the currently available nanodrugs. Cell culture viability tests and apoptosis assays showed that SQ-Dox nanoassemblies displayed comparable antiproliferative and cytotoxic effects than the native doxorubicin because of the high activity of apoptotic mediators, such as caspase-3 and poly(ADP-ribose) polymerase. In vivo experiments have shown that the SQ-Dox nanomedicine dramatically improved the anticancer efficacy, compared with free doxorubicin. Particularly, the M109 lung tumors that did not respond to doxorubicin treatment were found inhibited by 90% when treated with SQ-Dox nanoassemblies. SQ-Dox nanoassembly-treated MiaPaCa-2

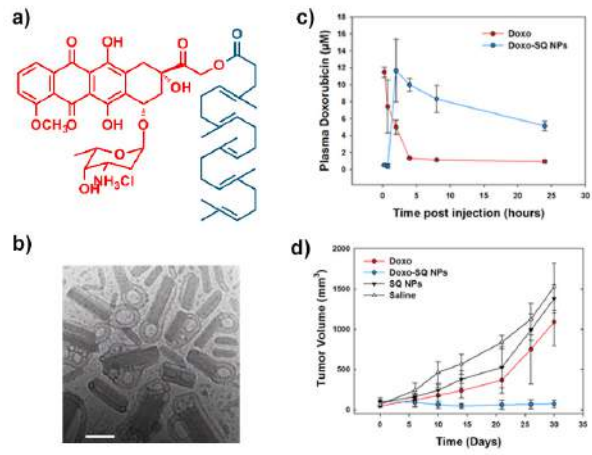

Fig.1 Chemical structure of SQ-Dox(a) and Cryo-TEM appearance of the SQ-Dox NAs(b). Plasma pharmacokinetics (c) and antitumor activity (d) of the SQ-Dox NAs.

pancreatic tumor xenografts in mice decreased by 95% compared with the tumors in the saline-treated mice, which was significantly higher than the 29% reduction achieved by native doxorubicin. Concerning toxicity, SQ-Dox nanoassemblies showed a fivefold higher maximum-tolerated dose than the free drug, and moreover, the cardiotoxicity study has evidenced that SQ-Dox nanoassemblies did not cause any myocardial lesions, such as those induced by the free doxorubicin treatment. Taken together, these findings demonstrate that SQ-Dox nanoassemblies make tumor cells more sensitive to doxorubicin and reduce the cardiac toxicity, thus providing a remarkable improvement in the drug's therapeutic index.

Funding: The research leading to these results has received funding from the European Research Council under the European Community's Seventh Framework Programme FP7/2007-2013 (Agreement N°249835),

Development of an Interleukin-13 Metallofullerene Targeting Nanoparticle for Glioblastoma Diagnosis and Treatment

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Purpose: Glioblastoma multiforme (GBM) is one of the most deadly cancers. With aggressive therapies such as surgical resection, ionizing radiation, and chemotherapy, the 5-year survival rate is only 5%. Although the high mortality of GBM is primarily attributed to the therapeutic resistance, ambiguity in GBM diagnosis often delays the treatment, provides non-efficient data for surgical resection, as well as fails to monitor disease progression after treatment. For example, certain non-cancerous lesions caused by brain infections, inflammation, or stroke present MRI results similar to tumor lesions, which demands more complicated imaging analysis and laboratory tests. Therefore, more definitive targeting modalities that facilitate GBM diagnosis and treatment are needed.

Methods: Trimetallic nitride cluster metallofullerene (TNT EMFs) nanoparticles have attracted considerable attention because of their unique electronic and structural features making them more stable in the body and less toxic. In previous studies, we have shown that the intracranially injected Gd-containing IL-13-conjugated to the EMF, Gd3N@C80 (IL-13-f-Gd-EMF) was primarily distributed in the brain, lungs, liver, spleen and kidneys using inductively coupled plasma mass spectrometry (ICP-MS). [1] In this study, we have examined the biodistribution of intravenously injected TAMRA-IL-13-f-Gd-EMF (TAMRA, fluorescence tag) in GBM mice and MRI imaging.

Results: We have found that a significant quantity of nanoparticles were in the murine brain, indicating that they are able to cross the blood-brain barrier (BBB). This unique feature makes this type of nanoparticle extremely valuable for diagnosis and therapeutics of neurological disorders. Remarkably, the accumulation of nanoparticles in brain tumor cells was further supported by the ~5-fold higher Gd concentration (determined by ICP-MS) in the GBM-bearing hemisphere than that in the contralateral hemisphere. MRI also shows tumor specific enhancement.

Conclusion: The results of this study demonstrate that IL-13 conjugated gadolinium (Gd)-containing nanoparticles can be delivered intravenously and exhibits excellent brain penetrance with tumor specific targeting.

Identifying Molecular Signatures of Tumors Using Novel Fluorescence Resonance Energy Transfer Networks

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Fluorescence microscopy is one of the most widely used assays in biological systems. However, the technique suffers from limited multiplexing capability with previous attempts at detecting more than 11 fluorophores simultaneously resulting in barcodes that are too big for in vivo analysis, expensive and involve time-consuming detection schemes. Here, we introduce DNA self-assembled FRET networks that provide a unique, unpredictable optical output when probed by a series of light pulses. Markov and entropy modeling of the FRET sensors show that 125 fluorophores can be observed simultaneously. Experimental analyses of over 1200 time-resolved fluorescence signatures on 300 prototypical networks show that the optical responses are highly repeatable and minor variations between FRET networks can be discriminated resulting in a total of 10^{375} unique responses. The increase in spatial information density enabled by the novel FRET networks allowed us to identify molecular signatures in lung and breast cancer tumors.

It is now known that the presence of aberrant secondary structure in the regulatory regions of genes involved in cell proliferation, cells growth and apoptosis can lead to cancer. The FRET sensor we designed, self-assembles DNA probes labeled with acceptor fluorophores to the target DNA/RNA secondary structure forming an optical network. A DNA strand labeled with a donor fluorophore triplex binds to a unique sequence adjacent to the secondary structure. When the donor fluorophore is excited, the optical network results in a different optical signal based on the presence of the wild-type or the aberrant secondary structure, through which we identified lung and breast cancer markers with high specificity. The use of DNA probe strands allows access to highly condensed regions in the nucleosome and can be loaded with cargo, such as photosensitizers, for therapy. The small size of fluorophores results in molecular scale spatial resolution while the optical sensing mechanism enables the *in vitro* and *in vivo* characterization of structure at picosecond time resolution.

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Optimization of M2 macrophage-binding peptide (M2pep) for targeted drug delivery to tumor-associated macrophages

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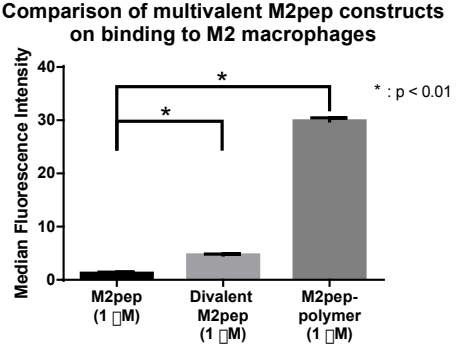
Tumor-associated macrophages (TAMs) are tumor-promoting immune cells that facilitate tumor proliferation, angiogenesis, and metastasis. Influenced by the tumor microenvironment, these TAMs mainly possess phenotypes characteristic to M2 macrophages. Our lab has previously discovered an M2 macrophage-binding peptide (M2pep) which can selectively bind to and be internalized by murine M2 macrophages. Intravenous administration of a fusion peptide of M2pep and the KLA pro-apoptotic peptide (M2pepKLA) has been shown to delay tumor growth in CT-26 colorectal tumor mouse model. However, the use of this peptide as a targeted therapeutic is limited by its poor serum stability and weak binding affinity to M2 cells. In this study, we investigated N-terminal acetylation as a strategy to improve serum stability of M2pepKLA. M2pepKLA and acetylated M2pepKLA (AcM2pepKLA) were incubated in normal mouse serum and analyzed for degradation over time by HPLC and MALDI-TOF MS. Acetylation of M2pepKLA helps prevent N-terminal degradation; however, the endolytic cleavage of M2pep at the "WW" position was found to be a more predominant factor in the overall peptide stability in serum. To improve affinity, we also explored multivalent displays of M2pep in order to improve binding avidity to M2 macrophages. Divalent M2pep and M2pep-grafted polymer (about 21 M2pep peptides per polymer chain) were synthesized and tested for binding to M2 macrophages by flow cytometry. Increased multivalency of M2pep resulted in a greater binding to M2 macrophages. Together, these results suggest multivalent display of M2pep as a promising strategy to improve targeted delivery of therapeutics to TAMs.



Funding: National Institute of Health Grant R01 HL067267 and Anandamahidol Foundation Fellowship

VEGF-targeted drug delivery systems for brain tumor therapy

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Introduction. Many approaches of the conventional therapy are not effective in case of brain tumor because of the rapid progression, intense infiltrative growth, with high resistance and less penetration activity for drugs. One of potent targets for antitumor drug delivery is vascular endothelial growth factor (VEGF), since its over-expression in brain tumors. The purpose of this study was to design cisplatin-loaded delivery systems (nanogel and liposome) conjugated with monoclonal antibodies to VEGF for treatment of glioma *in vivo.*

Results and discussion. Two types of nanocontainers - stealth liposomes and nanogels - were prepared, conjugated to mAbVEGF with high retained activity (75% of initial affinity) and loaded with anticancer drug cisplatin (CDDP). The fluorescent-labeled targeted nanocontainers had enhanced internalization in comparison with untargeted nanocontainers or non-specific IgG-targeted nanocontainers in glioma C6 cells. This led to a considerable increase of cytotoxicity of CDDP-loaded targeted nanocontainers in VEGF-over-expressed cells. VEGF-targeted liposomes accumulated in the brain tumor after *i.v.* injection with elevated penetration into malignant cells in comparison with untargeted and IgG-targeted liposomes. Nanogel accumulation in the brain had the similar pattern (nanogels < IgG-nanogels < VEGF-nanogels), however at the less extent. Also, we evaluated the antitumor activity of CDDP-loaded nanogels and revealed that VEGF-targeting groups promote therapeutic efficiency of anticancer drug. Free CDDP showed a similar antitumor activity in comparison with mAb-nanogel/CDDP, however revealed profound loss of body weight (systemic toxicity) and neurotoxicity (dilatation of the subarachnoid space and ventricle of brain).

Conclusions. VEGF-targeted nanoparticles penetrated through abnormal blood vessels of disturbed BBB into the extracellular space of glioma, where antibodies selectively recognize expressed VEGF. This leads to efficient accumulation of targeted nanoparticles in the brain tissues and enhanced therapeutic performance of CDDP-loaded nanocontainers. Thus, VEGF-targeted nanoparticles could be are prominent candidates for delivery of

drugs and diagnostic agents into brain tumors.

Funding: This work was supported by Russian Federation Government grant 11G34.31.0004, RSF grant 14-15-00698, and RFBR grants 12-04-31731 and 13-04-01383.

The Potential of Alkoxylphenacyl-Based Nanoparticles in Light- Responsive Drug Delivery

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We evaluated the potential of alkoxylphenacyl-based polycarbonates (APP) as a novel polymeric platform for light-responsive drug delivery. APP is a new class of light-responsive polymer that was constructed to contain the chromophoric unit within the polymer backbone. As such, APP polymer has the capacity to undergo light-activated chain scission (light stimulus wavelength 280-320 nm). Nanoparticles (NPs) were prepared from APP co-polymer with polycaprolactone (APP-PCL, 10% w/w) using the nanoprecipitation method. NPs were prepared for assessment of delivery of doxorubicin (Dox) as a model drug. NPs were characterized based on size, size distribution, zeta potential, stability as well as biocompatibility in BALB/c mice. Release of entrapped Dox from APP- NPs was carried out with or without pre-exposure to the light stimulus. Cardiotoxicity studies in BALB/c mice were employed to assess the extent of dose-dumping of entrapped Dox upon exposure to the light stimulus. Dox-loaded NPs were spherical with mean sizes between 80-120 nm depending on APP polymer concentration. Dox entrapment occurred with high efficiency (>70%). Exposure to the light stimulus disrupted the surface morphology of APP-NPs while leaving the core intact as monitored by transmission electron microscopy. Both the rate and extent of Dox release from APP-NPs were altered by exposure to the light stimulus. The APP concentration and frequency of light exposure markedly influenced the release of Dox from APP-NPs. Sequential exposure of APP-NPs to the light stimulus (2-3 exposure cycles) was required to sustain release of Dox from APP-NPs. APP-NPs were not subjected to dose-dumping of entrapped Dox as indicated from the release studies as well as evaluation of cardiotoxicity

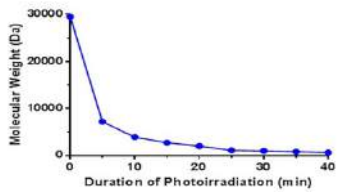

Fig. 1: Characterization of APP polymer responsiveness to the light trigger based on loss of molecular weight after various photoirradiation times.

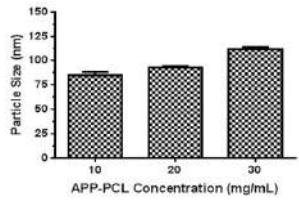

Fig. 2: Particle sizes of NPs prepared at various concentrations of APP-PCL polymer.

studies in BALB/c mice. Overall data suggest that the light-responsive Dox release from APP-NPs is most likely dictated by a multifaceted mechanism involving light-activated surface erosion, pore formation/growth and hydrolytic degradation.

Funding: College of Pharmacy, NEOMED Priority Project Funding

Ultraviolet and Infrared Lights-Induced Synergistic Theranostics Using Multifunctional Gold Nanorods

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Gold nanomaterials have demonstrated their applications in cancer research due to established protocols for surface functionalization, optical properties and biocompatibility. These novel characteristics make gold-based nanomaterials attractive for utilization in simultaneous diagnostic and therapeutics in cancer treatment. In this research, we have developed a multifunctional gold nanorod (AuNR) complex for cancer cell imaging, drug delivery, photothermal therapy. This AuNR- based complex exploits the light-scattering properties of gold for dark-field imaging of cancer cells and the synergistic effects of ultraviolet (UV) light-triggered drug release and infrared (IR) light-induced hyperthermia causing cancer cell apoptosis as shown in Figure 1. The AuNRs were synthesized using the seed-mediated method and functionalized with 11-mercaptoundecanoic acid (MUA-AuNR) and (2-hydroxypropyl)-β-cyclodextrin (CD-AuNR) to afford loading of doxorubicin (DOX) at the hydrophobic core of the CD. UV-active dextran-phenyl-azo-benzoate (DexAzo) was used to coat the CD-AuNR while folic acid-terminated ligand (FA) was incorporated as cancer cell targeting moiety. Intracellular delivery and DOX release was demonstrated by incubation of the DOX-loaded AuNR

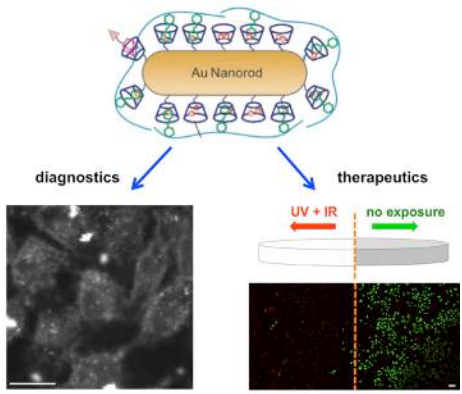

Figure1. Schematic of the multifunctional AuNR complex and its applications in cancer cell diagnostics using dark-field imaging (scale bar = 10 μm) and the synergistic effects of UV and IR treatment for drug release and photothermal therapy, respectively, as shown in the fluorescence microscopy image (scale bar = 20 μm).

complex with HeLa cells followed by UV treatment, which resulted in less than 30% cell viability after 24 h. Photothermal therapy was demonstrated by IR treatment of HeLa cells with red laser at 800 nm at 5 W/cm2 resulting in cell death as shown by trypan blue staining. Furthermore, the synergistic effects of UV and IR treatments using multifunctional AuNR complex in cancer cells were exhibited using live/dead cell assay. This AuNR-based complex offers a combined diagnostic and therapeutic tool for cancer treatment.

ECO Delivery of β3 Integrin-Specific siRNA Alleviates Breast Cancer Metastasis

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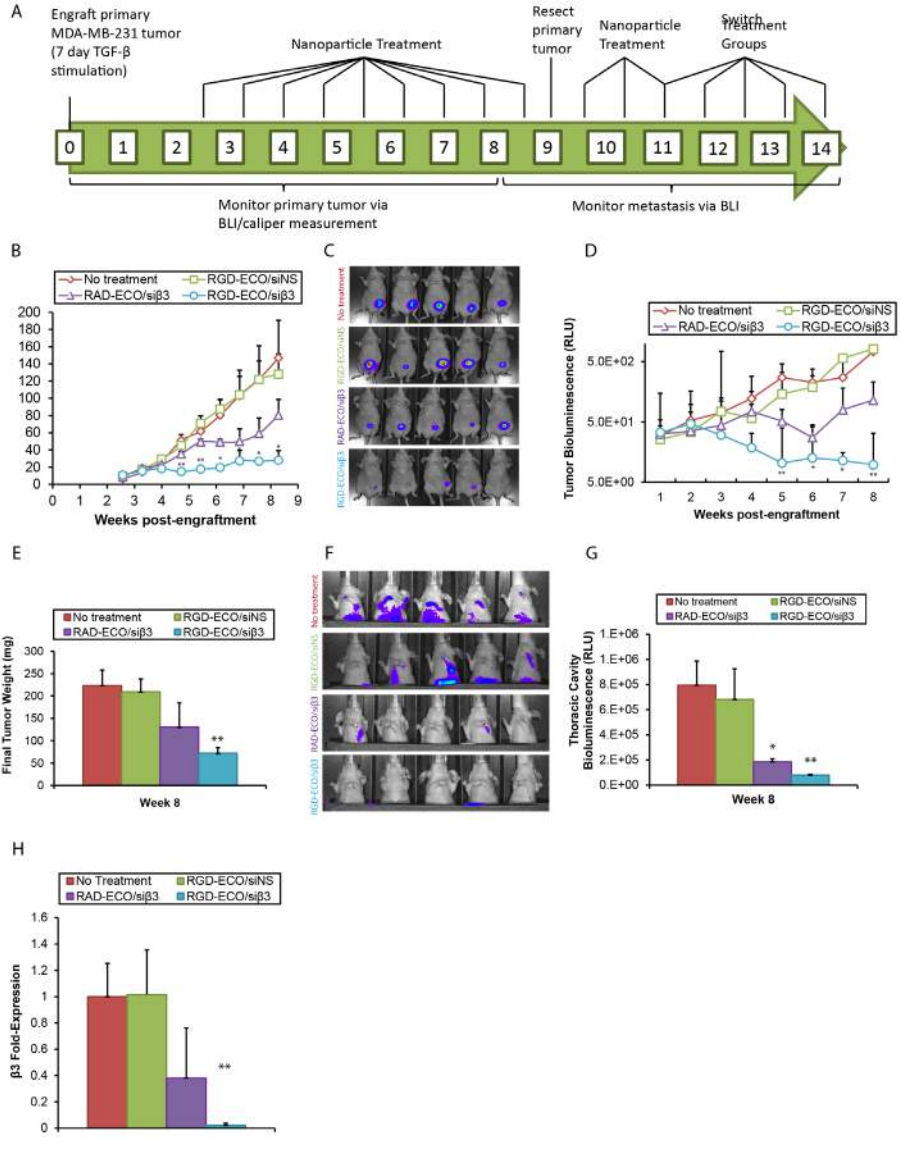
*These authors contributed equally to this research

β3 integrin has been implicated to play seminal roles in TGF-b-mediated epithelial-mesenchymal transition (EMT) and metastasis in breast cancer. Functional disruption of b3 integrin has been demonstrated to alleviate the oncogenic properties of TGF-b, and as such, eliciting this event on therapeutic settings by siRNA-mediated gene silencing is an attractive approach. In efforts to translate RNAi-based technologies into the clinic, we recently developed a multifunctional cationic lipid-based carrier, ECO, which efficiently delivers siRNA to mediate RNAi. We demonstrate in both mouse NME and human MDA-MB-231 breast cancer cells that ECO nanoparticle delivery of b3 integrin-specific siRNA (ECO/sib3) sustained gene silencing for up to 7 days *in vitro*. Importantly, ECO silencing of b3 integrin attenuated TGF-b-mediated EMT, invasion, proliferation, and organoid outgrowth in 3-Dimensional (3D) culture systems. To improve tumor targeting *in vivo*, ECO nanoparticles were modified with an RGD peptide, which enhanced cellular uptake by post-EMT cells. Importantly, mice bearing MDA-MB-231 primary tumors exhibited significantly reduced primary and metastatic tumor burden when they were treated with RGD-targeted ECO/sib3 nanoparticles in comparison to control groups. Collectively, these findings clearly demonstrate the effectiveness of ECO/sib3 nanoparticles as a promising therapeutic strategy to combat metastatic breast cancer.

Funded by DoD Postdoctoral Fellowship.


A
Engraft primary MDA-MB-231 tumor (7 day TGF-β stimulation)
Nanoparticle Treatment
Resect primary tumor
Nanoparticle Treatment
Switch Treatment Groups
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Monitor primary tumor via BLI/caliper measurement
Monitor metastasis via BLI
B
No treatment
RGD-ECO/siNS
RAD-ECO/siβ3
RGD-ECO/siβ3
Tumor Volume (mm3)
Weeks post-engraftment
C
No treatment
RGD-ECO/siNS
RAD-ECO/siβ3
RGD-ECO/siβ3
D
No treatment
RGD-ECO/siNS
RAD-ECO/siβ3
RGD-ECO/siβ3
Tumor Bioluminescence (RLU)
5.0E+02
5.0E+01
5.0E+00
Weeks post-engraftment
E
No treatment
RGD-ECO/siNS
RAD-ECO/siβ3
RGD-ECO/siβ3
Final Tumor Weight (mg)
Week 8
F
No treatment
RGD-ECO/siNS
RAD-ECO/siβ3
RGD-ECO/siβ3
G
No treatment
RGD-ECO/siNS
RAD-ECO/siβ3
RGD-ECO/siβ3
Thoracic Cavity Bioluminescence (RLU)
1.E+06
1.E+06
8.E+05
6.E+05
4.E+05
2.E+05
0.E+00
Week 8
H
No Treatment
RGD-ECO/siNS
RAD-ECO/siβ3
RGD-ECO/siβ3
β3 Fold-Expression

Treatment of Experimental Autoimmune Encephalomyelitis by Co-delivery of MOG_{35-55} and Dexamethasone Acetalated Dextran Microparticles

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Multiple Sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system, thought to be caused partially by inflammatory T cells specific for myelin proteins. Current therapies suppress systemic T cell activity, thus ameliorating MS symptoms in a non-specific manner. However, non-specific suppression can result in cancer, osteoporosis, or chronic infections, whereas antigen-specific suppression can ameliorate symptoms without inhibiting protective immune responses. Previous work has shown intravenous administration of polystyrene or poly(lactide-co-glycolide) microparticles (MPs) with surface conjugated proteolipid protein ($PLP_{139-151}$), a disease-specific peptide, was capable of decreasing severity of experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Additionally, intravenous delivery of gold nanoparticles with disease-associated antigen, myelin oligodendrocyte glycoprotein (MOG_{35-55}), and a tolerogenic sequence adsorbed onto the surface, decreased EAE symptoms. Although these therapies ameliorated progression of EAE, it is important to optimize therapies using biodegradable MPs administered through more clinically desirable routes. To build on this work, we co-delivered MOG and dexamethasone (DXM), a compound known to induce immune tolerance, in biodegradable acetalated dextran (Ac-DEX) microparticles (DXM/ MOG/ MPs). DXM in Ac-DEX particulate carriers was shown to significantly decrease both nitric oxide and interleukin (IL)-6 production, both markers of inflammation. Subcutaneous administration of DXM/ MOG/ MPs, 18 days after disease induction, significantly lowered clinical scores in EAE, versus controls. After three injections of DXM/ MOG/ MPs, clinical scores decreased from 3.4 (severe hind limb weakness to paralysis) to 1.6 (minor to no hind limb weakness). In addition, DXM/ MOG/ MPs therapy significantly diminished production of IL-17 and GM-CSF, both disease-associated cytokines, compared with controls. Here, we show a novel biodegradable particulate system, deliverable through subcutaneous routes, for the treatment of MS.

Funding: NIH R21 NS072813-01

Linear-dendritic block copolymers based on poly (γ-propargyl L-glutamate) as pH-responsive biomaterials for drug delivery

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To design biocompatible, pH-responsive nanoparticulate drug delivery systems (Nano-DDS), we synthesized a linear-dendritic block copolymer by coupling hydrophobic poly (γ-propargyl L-glutamate) (PPLG) polypeptide block to a poly (ethylene glycol, PEG) conjugated bis (methyl propionic acid)-derived generation 4.0 dendron by highly efficient thiol-maleimide chemistry. The propargyl side-chains of the linear polypeptide segment were attached to a pH-responsive diethylamine moiety through azide-alkyne 1,3-dipolar cycloaddition. The copolymers self-assembled into nanoparticles with an average diameter of 147 nm in a buffer media of pH 8.0 due to mesoscale phase transition of the amine-substituted polypeptide block. The resulting nanoparticles assumed a vesicular structure as confirmed by Static light scattering and TEM, and were capable of withstanding physiological salt and serum conditions. A pH-dependent determination of critical association constant (CAC) showed the structural responsiveness of these nanoparticles to pH change. Circular dichroism (CD) experiment revealed that incorporation of heavy dendritic motif did not alter the α-helical conformation of the PPLG block. Such sterically stabilized and densely PEG-shielded block copolymer assemblies were able to encapsulate substantial quantity of both hydrophilic and hydrophobic anticancer agents, and were able to release them in a pH dependent pattern. When tested in triple negative breast cancer cell lines (MDA-MB 468), the copolymers did not show any level of cytotoxicity; neither did they compromise the anticancer activity of the encapsulated agents. The biological performance of the empty nanoparticles prepared from fluorescently labeled dendritic PPLG block copolymer with a diisopropylamine side chain was determined in BALB/C mice following systemic administration which showed that the particles persisted in the circulation for ~24h. Subsequent use of these responsive nanoparticles as Nano-DDS was investigated against MDA-MB-468 xenografts in NCR nude mice, whereby repeated intravenous administration of doxorubicin-loaded particles were found to suppress tumor growth for up to 40 days compared to the rapidly growing untreated control.

Funding: Novartis Institute for Biomedical Research, Inc.

Inhaled Particle Technology for Nerve Agent Inactivation

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To date, there is no efficient antidote for civilians and/or soldiers who are exposed to nerve gas. Nerve gas contains organophosphates, which are deadly due to their rapid disruption of the signaling process to major organs. Recently it was discovered that Butyrylcholinesterase (BuChE), a naturally occurring biologic produced in the liver, has the ability to scavenge or neutralize organophospate-derived nerve agents, therefore providing protection against nerve damage. We believe that by formulating BuChE particles for pulmonary delivery, we can develop a self-administered nerve gas antidote. Utilizing PRINT (Particle Replication in Non-wetting Templates) technology, we will formulate BuChE into particles and will explore the inhaled delivery of this therapeutic protein. Inhaled delivery will provide respiratory protection and avoid systemic toxicity associated with oral and injectable therapies. PRINT® is a top-down particle fabrication technology that allows the engineering of precisely defined particles. The PRINT technology is critical for the success of this project, as it allows for careful control of particle characteristics such as an unlimited array of size, shape, matrix and surface chemistry options. We hypothesize that by controlling particle properties the delivery of PRINT BuChE particles to the lung will result in higher delivery efficiencies of inhaled biologics. Our initial experiments focused on further understanding inhaled protein delivery and endogenous BuChE levels in animal models. Administered free BuChE is detectable post-instillation and does not impair immune cells; therefore we predict our ability to chemically tune the BuChE particles will provide increased residence times and efficacy for prophylactic treatment.

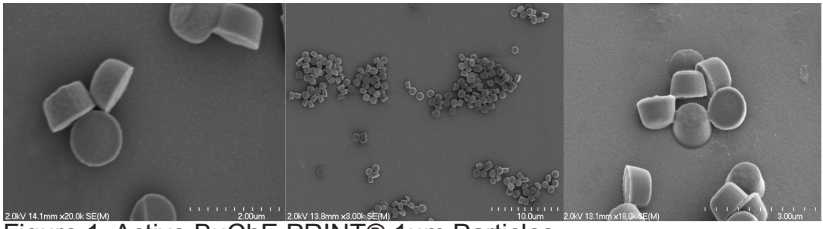

Figure 1. Active BuChE PRINT® 1µm Particles

Funding: This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program, the Defense Threat Reduction Agency (DTRA), and Liquidia Technologies.

Polymeric micelles and nanoemulsions as tumor-targeted drug carriers: insight through intravital fluorescence imaging

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The strong therapeutic effects (dramatically extended life span, suppression of metastases and ascites) achieved with drug-loaded micelles and/or nanoemulsions in preclinical studies of breast, ovarian, and pancreatic cancer have warranted studies of nanoparticle *in vivo* behavior in circulation using intravital laser fluorescence microscopy.

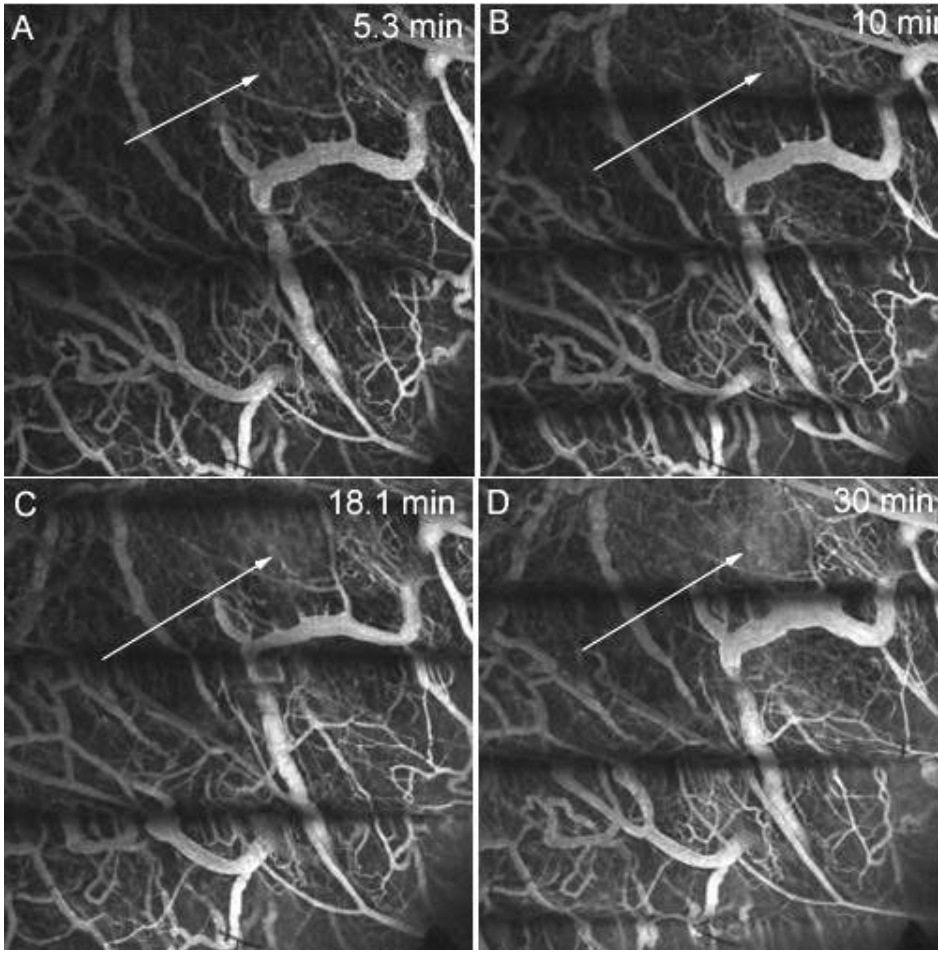
In the current study, we used fluorescently labeled micelles, nanoemulsions, or fluorescently labeled drug (green-fluorescent paclitaxel, F-PTX) to monitor vascular retention and extravasation rates upon intravenous injections to pancreatic tumor bearing mice. Two sets of experiments were performed. In the first set, extravasation and interstitial diffusion rates were compared for poly(ethylene glycol)-co-poly(D,L-lactide) (PEG-PDLA) micelles and PEG-PDLA-stabilized perfluorocarbon nanodroplets. In the second set, extravasation of nanodroplets was compared with that of the nanodroplet encapsulated drug, fluorescent paclitaxel (F-PTX).

Effective extravasation coefficients (E) were calculated using initial slopes of tumor accumulation curves normalized to a maximum fluorescence of blood vessels. Fluorescence curves were obtained using video files processed with ImageJ software.

The extravasation of PEG-PDLA micelles was relatively fast (E = 0.04 ± 0.01 min^{-1}), with micelles effectively diffusing from the extravasation sites throughout the tumor tissue. The extravasation of the nanodroplets proceeded significantly slower (E = 0.0085 ± 0.003 min^{-1}); nanodroplets were presumably localized near blood vessels but their extravasation and diffusion was significantly enhanced by ultrasound. Interestingly, no decrease of total fluorescence was observed for micelles and nanodroplets during the observation time suggesting a slow rate of the non-specific uptake.

The vascular and tissue fluorescence curves of nanodroplet-encapsulated drug were dramatically different from those of the nanodroplets; F-PTX vascular residence was much shorter and the extravasation coefficient higher (E = 0.0135 ± 0.005). A decrease of the total fluorescence during the observation time was observed. These data suggested a premature f-PTX release from the carrier in the vasculature. Corresponding video files will be demonstrated.

Funding: NIH R01 EB 001033 to NR and …to BON.



Intravital fluorescence microphotographs (taken as slices of the corresponding video file) of the PEG-PDLA micelle accumulation in the pancreatic tumor of a mouse upon the intravenous injection. Time points after the injection are indicated in the micrographs.

Targeted PRINT Hydrogels: Ligand density and nanoparticle size effects on cell association, biodistribution, and tumor accumulation

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Targeting nanoparticles (NPs) to interact with over-expressed surface receptors on cancer cells can promote selective cellular uptake, diminish systematic toxicity, and enhance therapeutic efficacy. In this account, we fabricated two hydrogel particle types (80 nm x 320 nm and 55 nm x 70 nm) with varying amounts of EGFR binding domain affibody and observed ligand density effects on cell uptake, biodistribution, and tumor accumulation of both particle types. In vitro studies revealed direct dependence of cell membrane association and cellular internalization as a function of NP size and ligand density. In vivo studies displayed a notable inverse relationship between circulation time and ligand density, as well, a direct correlation was observed for tumor accumulation. Overall, both targeting ligand density and NP size have major impacts on in vivo outcomes.

This work was funded by the University Cancer Research Fund, Liquidia Technologies, and the Carolina Center for Cancer Nanotechnology Excellence (U54CA151652).

Nanoformulated copper/zinc SOD is effective in reducing adiposity and adipose tissue inflammation in obesity

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Studies have indicated that aberrant inflammation in adipose tissue (AT) is intricately linked to metabolic disorders in obesity. An intimate association exists between oxidative stress and inflammation and therefore, we hypothesized that reducing AT oxidative stress will be effective in reducing AT inflammation which, in turn, can improve AT homeostasis. Wild type mice were fed a high fat (HF) diet for 8 wk followed by 2 wk treatment with native (Nat) or nanoformulated (Nano) copper/zinc superoxide dismutase (SOD). The mice were divided into 4 groups: 1) Chow diet (CD), 2) high fat (HF), 3) HF + Nat, and 4) HF + Nano. A striking finding of our study was that the HF + Nano treated mice showed a significant reduction in weight gain compared to HF diet-fed mice. In addition, these mice gained less gonadal AT mass than HF and HF + Nat groups. AT inflammatory markers, in particular, MCP-1 mRNA expression was greatly increased in HF compared to CD-fed mice. Nano but not native SOD significantly reduced the expression of MCP-1. Moreover, native SOD treatment by itself led to an increase in the expression of TNFα and ICAM-1. Analysis of macrophage-enriched stromal vascular cells from AT showed that the expression of MMP-12 was significantly decreased only in nano but not in native SOD treated mice. Finally, forskolin-stimulated AT lipolysis was significantly increased in HF + Nano but not in other groups. As stimulated lipolysis is implicated in the browning of white AT, which in turn, can reduce adiposity, our data indicate that nanoSOD treatment can increase fat burning in AT. Together, our data suggest that nanoSOD is not only effective in reducing AT inflammation but also in reducing weight gain likely via browning of white AT. Further analysis of brown fat markers in AT is needed.

Funding. This project is supported by the NIH-Nebraska Center for Nanomedicine/Center of Biomedical Research Excellence (2P20GM103480-NIH-NIGMS)

Nanogel Conjugates for Improved Stability and Cellular Permeability of Curcumin: Pharmacokinetics, Targeted Delivery and Antitumor Activity

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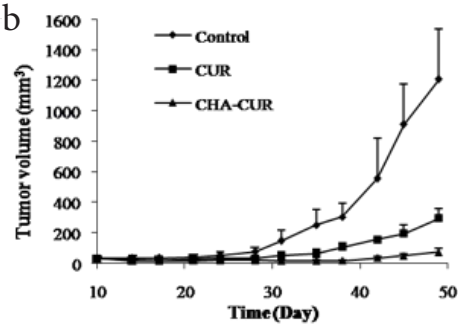
Curcumin (CUR) is natural compound with promising anticancer and anti-inflammatory activities. However, the therapeutic efficacy of curcumin was challenged in clinical trials, mostly due to its low bioavailability, rapid metabolism and elimination. Recently, we designed a nanodrug form of curcumin, which significantly increased its stability and enhanced cell targeting and permeability at systemic administration. Curcumin was conjugated as an ester to cholesteryl-hyaluronic acid (CHA) nanogel network, which demonstrated excellent solubility and sustained drug release in physiological conditions.CHA-CUR nanogels could target CD44-overexpressing drug-resistant cancer cells and inflammation. Modification of nanocarriers with brain-specific peptide (BP2) increased its accumulation in the brain in three-fold. CHA-CUR induced apoptosis in cancer cells targeting the same cellular targets (NF-κB, TNF-α, and COX-2) as the free curcumin. Modified BP2-CHA-CUR was also able to suppress inflammatory activity in microglial cells and protect neurons. PK/PD studies have revealed improved circulation parameters of CHA-CUR after various (oral, *i.p.* and *i.v.*) administration routes (Fig.1a). CHA-CUR treatment was well-tolerated and showed enhanced tumor accumulation by bioimaging.The nanodrug dosages resulted in up to 13-fold tumor suppression in human pancreatic adenocarcinoma MiaPaCa-2 xenograft (Fig.1b) and aggressive syngeneic murine mammary carcinoma 4T1 models, making it a promising candidate for cancer treatment (including brain cancers) and prevention.

a

Pharmacokinetic Parameters of CHA-CUR (CUR 7% by Weight)

Parameters	Curcumin		
	i.v.	*i.p.*	*p.o.*
Dose (mg/kg)	1.75	1.75	1.75
C_0 (ng/mL)	11620	-	-
C_{max} (ng/mL)	-	1871 ± 329	853 ± 96
T_{max} (h)	-	0.5	1.0
$T_{1/2}$ (h)	21.23 ± 0.16	24.07 ± 7.67	23.40 ± 7.46
MRT (h)	8.51 ± 0.52	8.58 ± 0.62	8.95 ± 0.51
AUC_{0-t} (h ng/mL)	12252 ± 770	12059 ± 838	7781 ± 449
$AUC_{0-\infty}$ (h ng/mL)	16830 ± 1111	16686 ± 1018	11053 ± 843
CL (mL/h)	2.08 ± 0.14	2.10 ± 0.13	3.17 ± 0.24

b



Funding: NIH CA136921 grant for S.V.

Magnetic field responsive Poly (2-oxazoline)-based Nano-ferrogels

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Current nanomedicine drug delivery systems are able to achieve sufficient loading and release of therapeutic agents. However, a commonly observed limitation of nanocarriers is that they are unable to control the release of their therapeutic payload. Several attempts such as light, ultrasound, and hyperthermia have been developed for remote actuation of nanocarriers. Herein, we will utilize a different external stimulus, a magnetic field, with responsive organic-inorganic nanoclusters (Nano-ferrogels) to control the release and delivery of hydrophobic drug: paclitaxel (PTX). We posit that under an applied magnetic field, Nano-ferrogels will change their structure as magnetic nanoparticles (MNPs) in the Nano-ferrogels realign along the fields, resulting in controlled delivery and release of drug. The two main components of Nano-ferrogels - MNPs and poly (2-oxazoline)-based polymeric micelles (POx) - were connected to each other by various anchors. First, MNPs were prepared by the thermal decomposition of Fe (III)-acetylacetonate ($Fe(acac)_3$). Amphiphilic block copolymers were synthesized by living cationic ring-opening polymerization. After conjugation of linker to the block copolymer terminal, PTX-loaded POx micelles were prepared by the thin film hydration method. Previous studies have shown that POx micelles have an extremely high loading capacity (45 %) with narrow size distribution (30 nm). MNPs and POx micelles were reacted with each other in aqueous media. In aqueous media, block copolymers formed stable micelles with a hydrophobic core containing solute (PTX) and anchor group-conjugated hydrophilic block shells connected to MNPs. Our MNP-POx Nano-ferrogels were stable for several days without any significant alteration of cluster size.

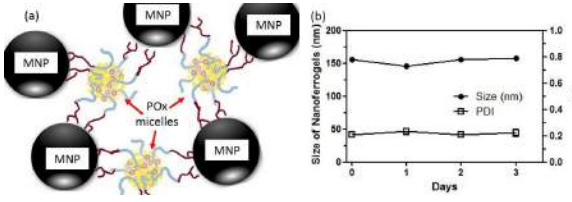

Fig. 1. (a) Illustration of Nano-ferrogels in aqueous media. (b) Daily stability of Nano-ferrogels under DI water (n=3).

Funding: This work was supported by the Seed program of the Triangle Materials Research Science and Engineering Center (MRSEC)

Transforming erythrocytes into near-infrared light targetable drug delivery vehicles.

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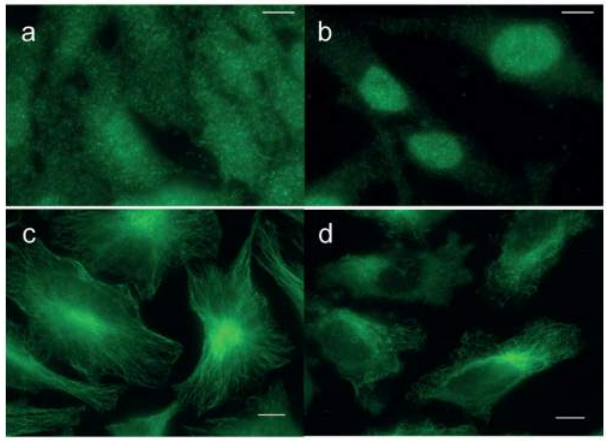
Light-activatable drugs offer the promise of controlled release with exquisite temporal and spatial resolution. However, light-sensitive prodrugs are typically converted to their active forms using short-wavelength irradiation, which results in cytotoxicity and an inability to illuminate through living tissue. We have developed erythrocyte contained assemblies of photolabile pro-drugs and NIR fluorescent antennae that combine to release bioactive molecules if and only if exposed to predetermined wavelengths of light. The activating wavelength of the constructs is assigned by selecting fluorophores with desired excitation wavelengths, allowing us to work within the optical window of tissue (600 – 900 nm). That excitation energy is then transferred to the pro-drug complex causing a chemical reaction that releases it from the red blood cell.



These systems are capable of delivering arbitrary therapeutic payloads in a light targeted manner. We have demonstrated with three different anti-inflammatory drugs (methotrexate, colchicine, and dexamethasone) that small molecules do not leave the erythrocyte, and hence do not interact with their biological target, until exposed to light. Furthermore we have demonstrated that drugs can be released from the interior of the red blood cell and from complexes bound to the erythrocyte membrane. In the latter case, fluorescent antennae and drugs need only be co-localized to the membrane itself in order to achieve energy transfer and drug release.

In the following figure we demonstrate that HeLa cells are unaffected

by erythrocytes loaded with dexamethasone or colchicine containing complexes in the absence of light (a and c respectively). However when exposed to 660 nm light (dexamethasone, b) and 780 nm light (colchicine, d) phenotypical changes occur. In the case of colchicine, tubulin staining reveals that photo-activated colchicine is cable of disrupting microtubule networks. Activated dexamethasone on the other hand causes a migration of its GRα receptor from the cytosol into the nucleus.

We thank the NIH for financial support (R01 CA79954)

Remotely Actuated Magnetic Liposomes for Cancer Therapy

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Background: This study employs mechanochemical actuation of superparamagnetic nanoparticles (SMNPs) in super - low frequency AC magnetic fields as a trigger for controlled release of drugs from liposomes to enhance their therapeutic potential. The realignment of the SMNPs along the field can lead to tensile, contracting, twisting and tangential forces in the materials ranging from ca. 5 to 500 pN. These forces can be captured and translated liposome membrane disruption and drug release from the liposomal inner cavity. The remote actuation would enable the liposomes to release their low molecular weight cargo at the right site and the right time once the liposomal drug accumulates in the tumor vessels. This paradigm is a drastic departure from traditional approaches that use SMNPs to generate heat following exposure to high frequency AC magnetic field and employ the temperature as release trigger.

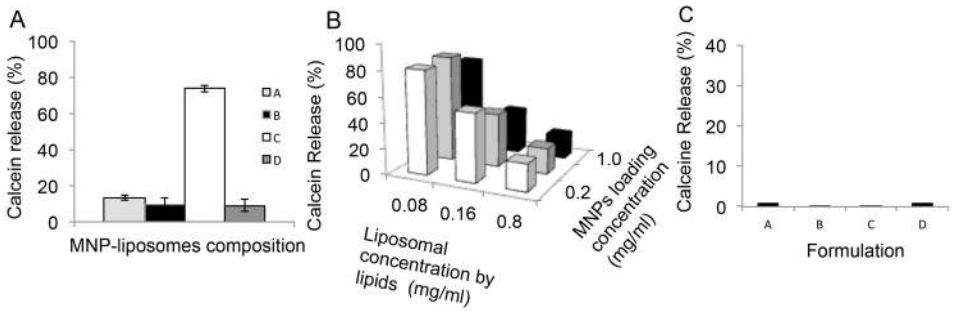

Figure 1: A) Effect of lipids composition and B) Effect of Liposomal and MNPs concentration on calcein release following exposure to 50kA/m, 50 Hz AC magnetic field (30 min). C) Calceine release following exposure to 50kA/m static magnetic field. Formulations: A) Egg Phospholipid B) Egg Phospholipid + cholesterol, C) DSPC, D) DSPC + cholesterol.

Study design: Liposomes with different lipid composition were prepared and loaded with 5nm Fe_3O_4 magnetic nanoparticles and self-quenched fluorescent dye calcein as model drug. Effects of lipid composition, magnetic nanoparticles loading, sample concentration as well as different field parameters (AC versus DC, field strength and frequency and duration of exposure were evaluated. The most responsive formulation as well as optimal exposure conditions were identified (Figure 1).

Conclusions: here we report that single exposure to super-low frequency, low amplitude non-heating magnetic field results in release of 80% of the accumulated calcein in concentration independent manner. Notably, the release is enhanced in lower liposomal concentrations. The responsive liposomal formulation was loaded with doxorubicin and is being farther evaluated.

Funding: the authors thank Carolina Center of Cancer Nanotechnology Excellence, Pilot Project Grant to MSP

Targeted Local Chemotherapy of Oral Cancers: Topical Iontophoresis for Mucosal Delivery of Cationic Liposomes

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Targeted local delivery of chemotherapeutics would be an ideal treatment for oral cancers. However, achieving therapeutic drug levels after topical application is a challenge. Topical iontophoresis is a non-invasive technique that uses a mild electric current to enhance drug penetration into biological membranes. It has seldom been used for topical delivery of chemotherapeutics since their physicochemical properties are often poorly suited to the technique. Here, we report a novel liposomal formulation for the simultaneous iontophoretic delivery of docetaxel and cisplatin for the management of oral cancers.

Cationic liposomes composed of DOTAP and Lipoid S-75 were prepared via the lipid film hydration method. Cisplatin was loaded in the aqueous core of the liposomes and docetaxel was distributed in the lipid bilayer. The formulated liposomes had diameters of 100-120nm with a positive zeta potential of 53mV (Figure 1a). The liposomes were then evaluated for their chemotherapeutic efficacy in HeLa cells. The cellular uptake of liposomal cisplatin and docetaxel was approximately 1.5- and 10-fold higher than that of free-form cisplatin and docetaxel, respectively. In addition, the liposomal formulation was more than twice as cytotoxic as the free-form combination of the drugs, demonstrating the benefits of co-formulation.

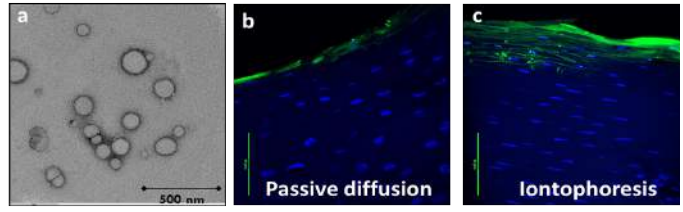

Figure 1 (a) transmission electron micrograph of liposomes; (b) and (c) confocal images showing the effect of iontophoresis on deposition of fluorescein-labeled liposomes (green) in porcine esophageal mucosa, the tissue sections were counter-stained with Hoechst blue to visualize the cell nuclei.

Mucosal transport of liposomes was evaluated in porcine esophagus, an *in vitro* model for buccal mucosa. The liposomes were unable to enter the epithelial barrier by passive diffusion. However, after anodal iontophoresis, significantly more liposomal cisplatin (120-fold increase) and docetaxel (140-fold increase) were deposited in the mucosa, without any noticeable increase in their transmucosal permeation. Confocal microscopic examination of mucosa treated with fluorescein-labeled liposomes further supported these findings (Figure

1b and 1c). Overall, the results suggested that combining a liposomal formulation with topical iontophoresis might selectively increase delivery of chemotherapeutics to oral cancers without increasing systemic absorption and associated risk of side-effects.

Funding: KS thanks the University of Geneva for a postdoctoral fellowship and we also thank the University of Geneva Bioimaging platform for access to the confocal imaging facilities.

Oxygen-Containing Nanoemulsions for Reducing Tumor Hypoxia

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Purpose: To demonstrate the reduction in tumor hypoxia by oxygen-containing perfluorooctyl bromide (PFOB) nanoemulsions using non-invasive optical imaging and immunohistochemistry.

Methods: 20% v/v PFOB nanoemulsion was prepared with phosphotidylcholine, cholesterol and Tween 60. The nanoparticles were characterized by TEM and Nanosight Particle Characterization System for size distribution and zeta potential. Optical imaging was carried out using Severe combined immunodeficiency (SCID) mice with human lung cancer A549 cell line implanted subcutaneously. The fluorescent hypoxia probe 2-nitroimidazole-ICG dissolved in 9% w/v sucrose aqueous solution (25 μM) was injected intravenously to 20 mice (100 μL/mouse). The tumor fluorescent signal was monitored within 48 hours using an IVIS imaging system. The mice were separated into 5 groups (4 in each group). Six days after the hypoxia probe injection, a second i.v. injection was followed to each group including (1) 2-nitroimidazole-ICG hypoxia probe; (2) 2-nitroimidazole-ICG hypoxia probe mixed with PFOB nanoemulsion (oxygenated); (3) 2-nitroimidazole-ICG hypoxia probe mixed with PFOB nanoemulsion (non-oxygenated); (4) PFOB nanoemulsion (oxygenated) followed 25 min later with 2-nitroimidazole-ICG hypoxia probe; (5) PFOB nanoemulsion (non-oxygenated) followed 25 min later with 2-nitroimidazole-ICG hypoxia probe.

Results: The mean particle size and zeta potential of PFOB nanoemulsion were characterized as 198 nm and -16.2 mV, respectively. Co-administration of oxygenated PFOB nanoemulsion and 2-nitroimidazole-ICG significantly reduced the accumulation of the hypoxia probe in tumors, indicating the successful

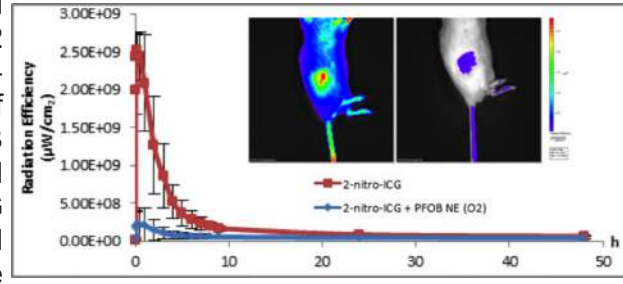

Figure 1. Co-administration of oxygenated PFOB nanoemulsion and 2-nitroimidazole-ICGsignificantly reduced the accumulation of hypoxia probe in tumor tissue.

reduction in tumor hypoxia. Non-oxygenated PFOB nanoemulsion showed no signal reduction from the hypoxia probe, which confirmed that oxygen plays the role but not PFOB nanoemulsion. These results were consistent with immunohistochemistry.

Conclusions: The PFOB nanoemulsion was able to deliver oxygen to tumors and reduce tumor hypoxia. The efficacy of chemotherapy and radiation therapy is expected to be improved by being co-administered with the oxygenated PFOB nanoemulsion.

Funding: University of Connecticut Research Foundation

Cocoon-like Self-Degradable DNA-Nanoclew for Anticancer Drug Delivery

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Abstract: Stimuli-responsive nanocarriers capable of minimizing the systemic toxicity associated with most free anticancer drugs have attracted extensive attention in modern chemotherapy, among which pH responsive drug nanocarriers can utilize the pH gradient in tumor environment to achieved controlled drug release under complex pathological conditions for maximized therapeutic efficacy. We report here a DNA and DNAse based formulation for biocompatible and controlled drug delivery. This bio-inspired self-degradable drug delivery system consists of a DNA nanoclew weaved by long chain single stranded DNA and an acid degradable polymeric nanocapsule with DNAse I caged inside. The DNA nanoclew was synthesized by rolling circle amplification with its surface negatively charged and doxorubicin (DOX) was loaded into the DNA core by intercalating into the GC-base pair sequences (DOX/NCl). The polymeric nanogel containing positively charged monomers was cross-linked onto DNAse I surface by acid degradable cross-linkers, which made the DNAse I nanocapsule (NCa) positively charged as well as acid degradable. NCa was embedded into NCl via electrostatic interaction and formed a cocoon like structure with NCl as the “cocoon matrix” and NCa as “hibernating worms”. The NCa was stable in physiological conditions while degraded in acid environment and exposed the encapsulated DNAse I to its substrate DOX/NCl, promoting the release of DOX. To further enhance the targetability of DOX/NCl/NCa, folic acid (FA) was conjugated to a DNA oligo complementary to NCl form DOX/FA-NCl/NCa. Rapid DOX/FA-NCl/NCa internalization and nucleus targeting in breast cancer cell line MCF 7 that overexpressed folate receptors was observed within 0.5 h. Embedding NCo into NCl reduced the half-maximal inhibitory concentration (IC50) of DOX from 2.3 μM in DOX/NCl to 1.2 μM in DOX/NCl/NCa, which was further reduced to 0.9 μM after the conjugation of folic acid.

This work was supported by the grant 550KR51307 from NC TraCS, NIH’s Clinical and Translational Sci-ence Awards (CTSA) at UNC-CH, the NC State Faculty Research and Professional Development Award, and the start-up package from the Joint BME Department of UNC-CH and NCSU to Z.G.

Biomimetic Delivery of Insulin Using Synthetic Glucose-Responsive Vesicles

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Inspired by insulin "vesicles" (or granules) of pancreatic β-cells, we have developed a new glucose-mediated insulin delivery system using a bilayer membrane-based vesicle, self-assembled by an amphiphilic block copolymer. As shown in Figure 1A, assembled by the mildly acid-sensitive diblock copolymer consisting of poly (ethylene glycol) (PEG) and Ketal modified polyserine (designated PEG-Poly(Ser-Ketal)) (Figure 1B), the polymersome has a nano-scaled vesicle structure. The core of the polymersome is loaded with insulin, glucose oxidase (GOx) and catalase (CAT). Glucose can passively transport across the bilayer membrane of the vesicle and be oxidized into gluconic acid by GOx, thereby causing a decrease in local pH. The acidic microenvironment causes the hydrolysis of the pH sensitive vesicle that in turn triggers the release o f Insulin in a glucose responsive manner. For *in vivo* application, these vesicles can be integrated with a thermoresponsive and injectable hydrogel-based matrix for the subcutaneous administration (Figure 1C). The amphiphilic PEG-Poly(Ser-Ketal) copolymer can assemble into polymersome vesicles with a diameter of around 320 nm. *In vitro* studies validated that the release of insulin from vesicle was effectively correlated with the external glucose concentration. *In vivo* experiments, in which diabetic mice were subcutaneously administered the vesicles demonstrate

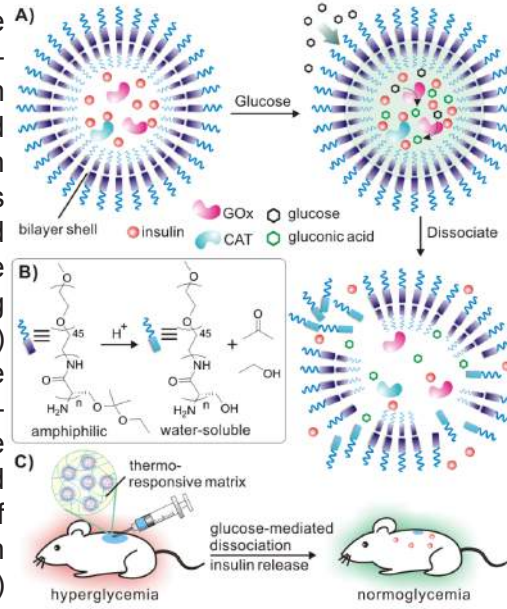

Figure 1. (A) The schematic of the glucose-responsive synthetic vesicle. **B)** The chemical structure of the pH-sensitive diblock copolymer PEG-Poly(Ser-Ketal). **C)** Synthetic vesicles can be integrated with the thermoresponsive hydrogel matrix and subcutaneously injected into the Type 1 diabetic mice for regulation of BG levels.

that, a single injection of the developed vesicle facilitated stabilization of the blood glucose levels in the normoglycemia state (<200 mg/dL) for up to at least 5 days. A novel insulin delivery strategy has been developed using glucose-responsive vesicles. The biomimetic design of synthetic vesicles provides a promising step forward in the development of the next-generation closed-loop insulin delivery systems for improving quality of life of diabetics.

Funding: This work was supported by the grant from the Junior Faculty Award of American Diabetes Association (ADA) and the grant from NC TraCS, NIH's Clinical and Translational Science Awards.

In vivo targeting efficiency of multifunctional nanoconstructs bearing antibody-derived ligands

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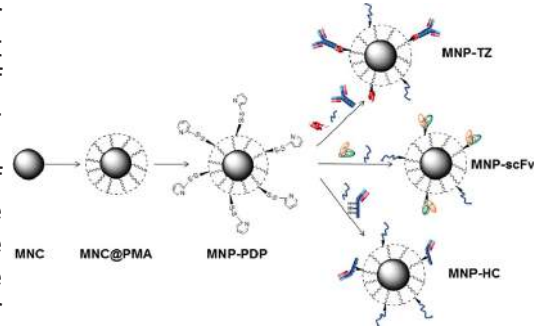
The identification of new strategies aimed to optimize the detection and treatment of primary breast cancer and metastases represents a great technical and medical challenge. Target-specific nanodevices may allow to combine specific tumor recognition with the capability to act as a drug reservoir for the selective delivery of chemotherapics to tumor sites. At present, the importance of surface functionalization of nanoparticles to improve their *in vivo* localization at the tumor is still controversial. Here, we have designed and developed a set of multifunctional nanoprobes, modified with three different variants of the model antibody trastuzumab (TZ), a widely used therapeutic agent for the management of HER2-positive breast cancer. We have performed a comparative study of internalization, trafficking, and metabolism in breast tumor cells of multifunctional nanoparticles (MNP) functionalized with either the entire TZ or alternative lower molecular weight variants of the monoclonal antibody, such as the half-chain (HC) and a single chain variable fragment (scFv). Then, we have estimated to what extent the structure of the surface bioligand could affect the targeting efficiency of the nanoconjugate in both *in vitro* and *in vivo* settings, and found that the highly stable MNP-HC is the best candidate for application in breast cancer detection. Our results provide evidence that a specific functionalization of nanoconstructs may afford long-term action in cancer cells *in vivo*. Furthermore, the longer period of accumulation of MNP-HC in the tumor makes this nanoparticle a promising candidate for future application in breast cancer diagnosis and treatment.



Funding: This work was supported by "Assessorato alla Sanità", Regione Lombardia, and Sacco Hospital (NanoMeDia Project), Fondazione Regionale per la Ricerca Biomedica (FRRB) and "Fondazione Romeo ed Enrica Invernizzi".

Pluronic-stabilized T_2-weighted potential theranostic agents prepared by flash nano-precipitation of magnetite nanoparticles

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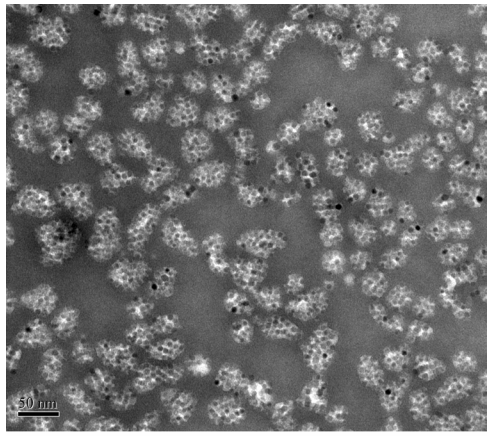
Magnetite nanoparticles are known to shorten transverse (T_2) relaxation times of water protons and thus find application in magnetic resonance imaging (MRI) as negative contrast agents. The FDA has already approved such contrast systems. The sensitivity of these magnetically susceptible contrast agents is expressed as transverse relaxivity (r_2; proton relaxation rate per millimolar iron), which depends on magnetization, size of the core magnetite nanoparticles (MNPs) and aggregation pattern of the MNPs into polymer stabilized clusters. It is known that the presence of MNPs in certain proximity of each other at nano-scale enhances r_2. We employed the flash precipitation or multi-inlet vortex mixing (MIVM) technique to fabricate stable magnetic nanoclusters from Pluronic F127 alone or its mixture with Pluronic P123. Flash precipitation of MIVM involves rapid nucleation, growth and kinetically induced polymeric stabilization of the nanoparticles during the rapid micromixing of the organic solvent stream containing dissolved polymer and MNPs into miscible anti-solvent streams at time scales in the order of 1-2ms. MNCs fabricated by MIVM display uniform spherical structure and have a narrow size distribution and very low polydispersity. By changing the Reynolds number (R_e), the cluster size and size distribution could be controlled easily. Given the higher HLB of pluronic F127, inclusion of a relatively hydrophobic pluronic P123 enabled



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successful encapsulation of Paclitaxel along with the MNPs. These resulting MNC formulations also have significantly higher relaxivity (r_2) values. Thus, we report the successful fabrication of a biocompatible theranostic system with potential applications in cancer therapy wherein the site-specific chemotherapeutic efficacy and visualization of tumor regression can be achieved simultaneous over an extended period of time.

A Simple and Non-smart Micellar Formulation of Paclitaxel with Superior Safety and Efficacy in vivo.

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Paclitaxel (PTX) is an anticancer drug with very little aqueous solubility and require significant amount of excipients to solubilize, such as Cremophor EL/ethanol, which cause toxicity and limit maximum tolerated doses (MTD). Poly (2-oxazoline) (POx) micelle is a new platform for higher PTX loading and better effect of tumor inhibition.

We use tri-block POx polymer to make micellar formulation of PTX as a high capacity delivery system for cancer therapy. MTD was tested in female nude mice. Anti-tumor activity was evaluated in A2780 human ovarian cancer xenograft and T11 orthotopic syngeneic transplants (OST) breast cancer models. Taxol and Abraxane were used as controls. Pharmacokinetics and biodistribution of the formulation were studied on healthy balb/c mice and A2780 tumor bearing mice.

Tri-block POx micelles are able to incorporate PTX with high loading capacities up to 50 wt.%. The formulation is stable in size (50nm) and drug loading for at least 2 weeks. The MTD of PTX loaded POx micelle is 150mg/kg, which is much improved comparing to Taxol (20mg/kg) and Abraxane (90mg/kg) receiving an every four days four-time in total tail vein injections (q4dX4). In the xenograft model PTX loaded POx micelles (MTD dose) shrink the tumor, and the formulation shows better tumor inhibition effect than Taxol on T-11 OST model. In conclusion, PTX loaded POx micelles provide a high drug loading, stable and effective platform of anticancer therapeutics.

Novel Bioinert Crosslinked Iron Oxide Nanoworms for Magnetic Resonance Imaging

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Superparamagnetic iron oxide (SPIO) nanoparticles have been actively investigated for magnetic resonance imaging (MRI), and several SPIO agents were clinically approved for MRI of liver, spleen or tumors. However, those MRI agents have been discontinued due to safety issues including complement activation and anaphylactic reactions. In our present studies, we aim to develop bioinert SPIO that can be potentially used for clinical trials and translational imaging. Dextran coated SPIO nanoworms were synthesized using modified Molday precipitation method. Dextran molecules on the nanoparticle surface were then crosslinked using 1-chloro-2, 3-epoxypropane (epichlorohydrin or ECH) with sodium hydroxide as a catalyst to form crosslinked iron oxide (CLIO) nanoworms. The SPIO and CLIO sizes were determined by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The transverse relaxivity measurements were performed using a 4.7T MRI scanner. The dextran modification after crosslinking was tested by anti-dextran antibody and conconavalin A binding. The complement component 3 (C3b/iC3b) binding to nanoworms in serum was investigated using dot immunoblotting assay. Macrophage uptake was tested on peritoneal macrophages in vitro and clearance half-life was measured in mice. Our results show that (1) crosslinking of dextran does not influence the size and shape and molar relaxivity of nanoparticles; (2) crosslinking can significantly reduce dextran immunoreactivity and thus significantly decrease C3b/iC3b binding on nanoworms; (3) CLIO showed significantly decreased cellular uptake by macrophages compared to non-crosslinked SPIO; (4) crosslinking of dextran can significantly prolong the circulation half life in mice up to 10 hours. In conclusion, the crosslinked dextran coated CLIO with improved immunocompatibility and blood circulation is a highly promising agent for MRI in the future clinical use.

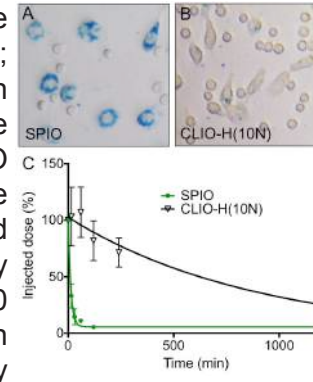

Figure. Macrophage uptake and blood circulation of dextran coated iron oxide nanoparticles

Funding: Simberg Startup at Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Denver.

Development of functional poly(amido amine) CXCR4 antagonists with the ability to transiently mobilize leukocytes and deliver nucleic acids

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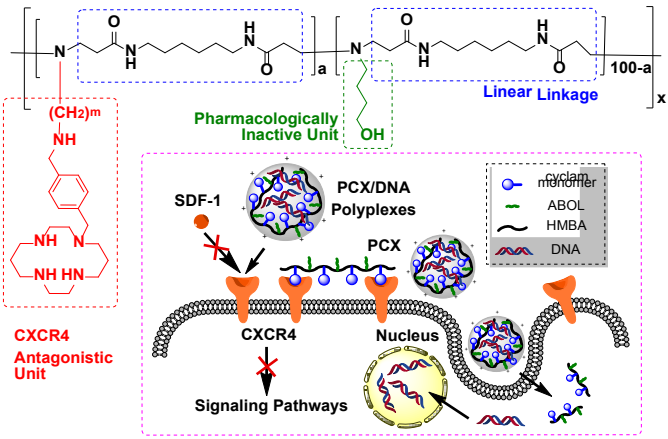

CXCR4/CXCL12 axis plays an important role in regulating cancer cell invasion and metastasis. The goal of this study was to develop functional polymeric CXCR4 antagonists with improved CXCR4 inhibition activity and simultaneous transfection of nucleic acids suitable for cancer therapy. A series of cyclam monomers was designed on the basis of a previously identified CXCR4-binding pharmacophore. Using two of the synthesized monocyclam monomers and Michael polyaddition, we prepared linear polymeric CXCR4 antagonists (PCX) with pendent CXCR4-binding side chains. CXCR4 antagonism of the synthesized polymers was determined using CXCR4 receptor redistribution assay. Inhibition of cancer cell invasion by the polymers was assessed using a Boyden-chamber method. Transient mobilization of peripheral blood leukocytes was examined using an automatic hematology analyzer. Transfection activity of DNA polyplexes formed with the synthesized polymers was evaluated in U2OS osteosarcoma and B16F10 melanoma cells. Our data demonstrate that placing the CXCR4-binding moiety in the polymer side chain results in improved presentation of the CXCR4 antagonist moiety in the PCX polycations. This resulted in enhanced CXCR4 inhibitory activity when compared with our previously reported polymers based on commercial CXCR4 antagonist AMD3100. Increasing the content of the cyclam units in PCX increased the ability of the polymers to inhibit cancer cell invasion. The best performing PCX showed activity similar to AMD3100. Leukocytosis associated with CXCR4 inhibition has been used as an

indicator of the mobilization of hematopoietic stem and progenitor cells. We found that PCX rapidly induced leukocytosis and that the mobilization activity was similar to the clinically used AMD3100. PCX/DNA polyplexes showed high transfection efficiency, which was comparable to PEI. Overall, we have optimized the design of PCX polymers by improving presentation and accessibility of the CXCR4 antagonist moiety for CXCR4 binding. This resulted in higher CXCR4 inhibition activity, while maintaining the ability to deliver nucleic acids and mobilize hematopoietic stem cells.

Cytotoxic Effect of Curcumin Encapsulated Hyaluronic acid-ADH-PLA Nanoparticles on Activated Hepatic Stellate Cells

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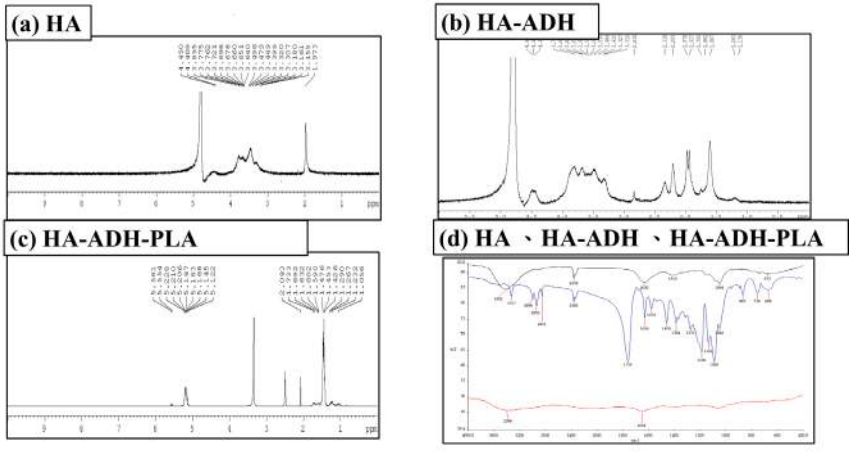
In this study, we synthesized novel hyaluronic acid nanoparticles (HA-NPs) to be used in drug delivery system. The HA receptor (CD44) have been revealed on the surface of cancer cells and activated hepatic stellate cells (aHSCs) than normal cells. HA-NPs bind to CD44 and be effectively internalized through endocytosis to release the encapsulated drugs. Thus HA-NPs are potentially to significantly improve drug efficiency. HA-NPs were constituted of HA and poly lactic acid (PLA). ADH linked the two biomaterials to form HA-ADH-PLA for acid hydrolysis. Spectroscopic results revealed the successfulness of the synthesis of HA-ADH-PLA NPs [IR spectra: 1614 cm^{-1}, the amide 1° of the HA; NMR: δ2.07, (3H, -NH-CO-CH_3) of the HA; δ1.42 & 1.45, (d, O-CO-CH (CH_3)-O-) of the PLA, δ5.1, (m,-O-CO-CH (CH_3)-O-) of the PLA spectral feature positions]. The average particle size was about 60~70 nm through the analysis by dynamic light scattering. Zeta potential from -30 to -40 mv suggested the stability of the particles. Particle shape and size were analyzed by AFM. Additionally *in vitro* results demonstrated that HA-ADH-PLA NPs bound to CD44 of aHSCs, and efficiently entering cells as shown by the fluorescent dye labeled cells. Moreover the value of IC_{50} of curcumin-NPs was 30-fold performance improvement than those of free drugs. Conclusively HA-ADH-PLA held a great potential to be used in hepatofibrosis amelioration due to the potent cytotoxic effect on aHSCs.



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Application of surface plasmon resonance for the characterization of the liposomes developed for targeted delivery

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Antibodies and other physiological ligands have been widely utilized as the targeting reagent of nano-sized drug delivery vehicles to achieve the localized drug delivery. However, limited studies have focused on the quantitatively evaluation of the targeting efficiency derived from these reagents for comparison and optimization. Here, in this study, a real-time and quantitative technique, Surface Plasmon Resonance (SPR), was utilized to investigate the targeting efficiency of antibody modified liposomes. For SPR analysis, antigens were immobilized on the SPR sensor chip, while the antibody modified liposomes was running in the test channels, mimicking the kinetic association and dissociation process in the physiological situation. The response unit in the sensorgram was positively related to the associated liposomes. The results demonstrated that the targeting antibody modified liposomes presented magnitudes of enhancement in apparent binding affinity compared with free antibody molecules, attributed to the avidity effect. Also, the targeting effect was found elevated as the increase of the surface density of the targeting antibody. However, this trend was hindered when surface density reached a threshold, and there is no significant increase of the targeting efficiency after it got saturated (Fig. 1). This threshold of surface density was quite consistent in spite of the liposome size or the type of targeting antibody. This threshold was quite essential to optimize the drug delivery system, maximizing the targeting efficiency from limited amount of the targeting reagents. Later, the traditional cell binding experiment further proved the threshold and the saturation phenomena.

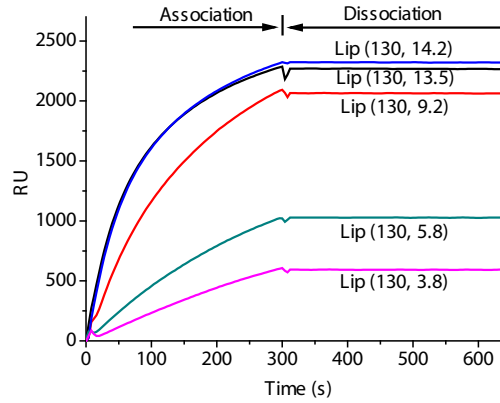

Figure 1. Sensorgrams of liposomes (130nm) with various target antibody surface density (3.8~ 14.2 antibody per liposome). (To precisely determine the dissociation rate, prolonged dissociation phase was performed but was not fully demonstrated.) The associated liposomes in 5 minutes were increased as the raise of surface density, but got saturated at around 10 antibody per liposome)

Engineering Pre-targeted, Immune-inert Nanoparticles for Cancer Therapy

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The coating of nanoparticles with polyethylene glycol (PEG) is routinely used to reduce uptake and clearance by immune cells, thus extending the circulation times of nanoparticle therapeutics. However, few studies have rigorously controlled the PEG grafting density to study the effectiveness of PEG's "stealth" properties. Similarly, targeting ligands conjugated to the nanoparticle surface are often utilized for specific delivery to cancer cells, but the effects of varying ligand densities on target/non-target cell uptake are poorly understood. As a first step towards addressing these challenges, we sought to determine the surface physiochemical characteristics that would enhance evasion of immune cell uptake to engineer immune-inert nanoparticles for systemic delivery. We covalently coated latex beads of well-defined sizes with different molecular weights and surface densities of PEG and characterized the PEGylated particles using an array of techniques, including direct and indirect fluorescence-based assays to quantify the PEG coating density. We analyzed particle uptake by a human macrophage cell line and by primary leukocytes and evaluated the circulation time and biodistribution of the PEGylated model particles *in vivo*. Particles with a high surface PEG density beyond that required for brush-conformation PEG exhibited dramatically reduced uptake by phagocytic cells *in vitro*, as well as significantly extended systemic circulation times. Less than 20% of these nanoparticles were cleared from the blood after 2 h in BALB/c mice, whereas slightly less densely PEGylated and uncoated control particles were both virtually eliminated within 2 h. Our results suggest that the stealth properties of PEG-coated nanoparticles are critically dependent on achieving PEG grafting at densities exceeding

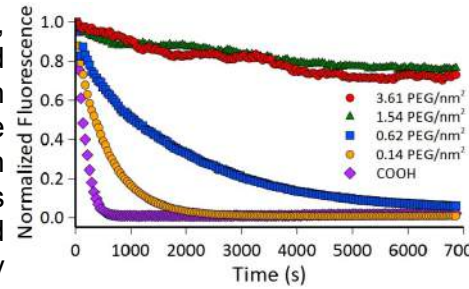

Figure 1. Quantification of the intravital imaging results for latex beads modified with varying densities of PEG_{5k}. Blood vessel fluorescence was normalized to the initial peak values after injection.

those required for brush conformation. We are currently synthesizing densely PEGylated nanoparticles with varying numbers of terminal biotin groups and exploring the effect of biotin density on lymphoma cell uptake using a pre-targeted nanoparticle system.

Funding: This work was supported by the Carolina Center for Cancer Nanotechnology Excellence (U54CA151652), Pilot Grants Program (S.K.L.), PhRMA Foundation Predoctoral Fellowship (Q.Y.), American Association of Colleges of Pharmacy New Faculty Research Award (S.K.L.), and startup funds from the Eshelman School of Pharmacy and Lineberger Comprehensive Cancer Center (S.K.L.).

Laser-Activated Delivery of Therapeutic Oligonucleotides

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Therapeutic oligonucleotides (ONs) hold mounting promise for tumor therapy, as they are more specific than small molecule drugs and can modulate undruggable targets. However, therapeutic activity of ONs had been constrained by the poor delivery to their intracellular sites of action because of non-productive trapping in endosomal compartments. We aim to synchronize delivery of therapeutic ONs and photodynamic therapy (PDT) agents to cancer cells using nanoparticles (NPs) and to promote endosomal release of ONs through laser-activated PDT effect. Thus, multiple morpholino ONs were linked to a single molecule of PAMAM dendrimer via a reductively responsive linkage, and the photosensitizer Ce6 was further conjugated to the dendrimer via NH_2-NHS chemistry. The resultant NPs showed uniform and monodispersed size distribution with a diameter of 28 nm. A single NP contains approximate 15 oligonucleotides as well as Ce6 molecules on the surface of dendrimer. The NPs demonstrated over 100-fold enhancement in cellular delivery of oligonucleotides compared to free ONs in A375 melanoma cells. We used confocal microscopy to examine the PDT-triggered endosomal release of ONs. Confocal imaging showed that the NPs were largely trapped in the endosomes after entering A375 cells. After the cells are exposed to a 660 nm laser, the image showed the cytosolic localization and the nuclear entry of ONs, indicating that laser-activated delivery leads to endosomal release of ONs. We tested functional delivery of the NPs using Bcl-x splice-switching ON as test ON. Laser activation of Ce6 enables functional delivery of the ON, which causes switching of Bcl-x from the anti-apoptotic Bcl-xL form to proapoptotic Bcl-xs. Apoptosis of A375 cells was also observed after treatment of the nanoparticles and laser exposure. As a result of their great cellular delivery, small size, and laser-activated delivery, the multifunctional NPs may provide an effective tool for targeting oligonucleotides to tumors.

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In vitro and in vivo characterization of Raw 264.7 macrophages-derived exosomes as brain delivery nanovectors

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Exosomes are 40-150 nm natural membrane-bounded vesicles that carry proteins and RNAs for intercellular communication within an organ or at a distance. The good stability and biocompatibility of exosomes have inspired their application as drug delivery nanovectors. We are interested in the potential use of exosomes derived from Raw 264.7 macrophages as brain delivery nanovectors. Herein, we report the physical chemical properties of these exosomes, their cellular uptake and endocytosis mechanisms within brain endothelial cells, and brain pharmacokinetics in mice.

Raw 264.7 macrophages derived exosomes were negatively charged spherical nanoparticles with size around 90 nm as characterized by dynamic light scattering, nanoparticle tracking analysis and transmission electron microscopy. Using western blot we confirmed Alix and Tsg 101, two exosomal markers expressed in the exosomes. To study the cellular uptake and endocytosis mechanism, exosomes were fluorescently labeled and incubated with human brain endothelial cells (hCMEC/D3) for flow cytometry and confocal microscopy analysis. Exosomes were actively internalized in a saturable manner and not via fluid-phase macropinocytosis. Furthermore, exosomal internalization might associate with exosomal surface integrin (LFA-1) and sugar moieties. Upon internalization, exosomal lipids did but luminal protein cargo did not colocalize with endo/lysosomes. Following intravenous injection to CD-1 mice, iodinated exosomes circulated in bloodstream as long and stable as albumin, and entered the brain at a slow but higher influx rate than albumin. Exosomes were mainly distributed in liver and spleen followed by lung and kidney.

In conclusion, Raw 264.7 macrophages derived exosomes had appropriate size and charge as drug delivery nanovectors. They were actively internalized and interacted with brain endothelial cells via exosomal sugar and integrin associated pathways. The long serum circulation, peripheral stability, and permeability at the BBB present the potential of macrophages derived exosomes as natural nanovectors to deliver therapeutics for treatment of brain diseases.

Improving the Therapeutic Relevancy of Cisplatin for Malignant Gliomas Using Nanotechnology

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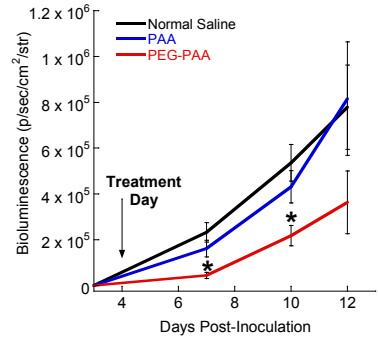
Introduction: Glioblastoma (GB) remains incurable largely due to the inability to focally remove all tumor cells which inevitably leads to tumor recurrence and patient death. Therefore, therapeutics capable of affecting a larger, more widespread population of pathological cells in the brain are necessary for improving outcome of GB patients. Cis-diammineplatinum (CDDP) is a promising chemotherapeutic given its potency but its applicability for central nervous system neoplasms is limited due to offsite toxicities. Therefore, ameliorating CDDP's inherent toxicity is paramount in its translatability. Our nanoparticle CDDP system, derived from a polyaspartic acid (PAA) peptide and conjugated with polyethylene glycol (PEG), rapidly diffuse in rodent brain and reduces CDDP toxicity, thereby improving the relevance of CDDP as a chemotherapeutic for GB.

Results and Discussion: Nanoparticles were characterized and tracked in freshly excised *ex vivo* rodent brain slices. PEG-PAA nanoparticles were synthesized with an average hydrodynamic diameter of 70nm, near neutral surface charge, a high weight percentage of cisplatin, and were confirmed to penetrate in *ex vivo* brain tissue. Intracranial surgeries targeted the rodent striatum for administrations of free CDDP or CDDP encapsulated in nanoparticle form. Tolerance of CDDP delivered in a nanoparticle was improved by 100% as compared to free CDDP. In addition, we observed a statistically significant delay in tumor growth when orthotopically implanted F98 glioma cells were treated with PEG-PAA CDDP as compared to both the saline treated and PAA CDDP groups (Figure 1). Therefore, we have developed a CDDP nanoparticle therapeutic demonstrating improved CDDP toxicity profile and delayed tumor growth in an orthotopic rodent glioma model.



Funding: NIH R01 CA164789

Novel Magnetite-Bisphosphonate Ionomer Nanocarriers for Dual Drug Delivery and Imaging

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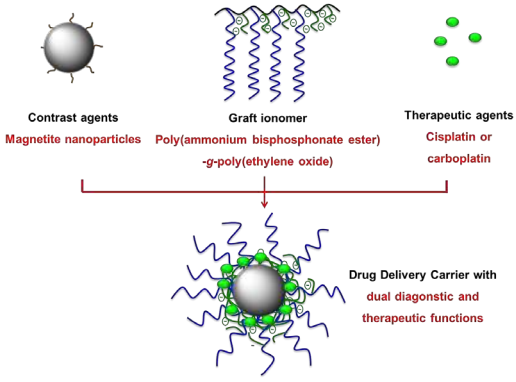

Development of novel polymer-based drug delivery carriers with dual diagnostic and therapeutic functions is of primary interest in our group. Herein we describe the preparation and characterization of novel magnetite-poly(ammonium bisphosphonate ester)-*g*-poly(ethylene oxide) complexes for drug delivery and magnetic resonance imaging. The ionic graft copolymer was synthesized via free radical polymerization followed by selective hydrolysis of phosphonate t-butyl ester groups. It was used to form complexes with magnetite nanoparticles that had an average diameter of 16 nm. The magnetite-graft ionomer complex (MGICs 16) was dispersible in aqueous media and the colloidal suspensions remained stable in phosphate buffer saline for at least 1 day. The complexes had narrow size distributions as measured by dynamic light scattering and they had high NMR transverse relaxivities of ~244 $s^{-1}(mM\ Fe)^{-1}$. Cisplatin and carboplatin were loaded into one of these complexes and the relaxivities increased to 409 and 335 $s^{-1}(mM\ Fe)^{-1}$, respectively. Sustained release of platinum drugs was also observed in comparison with the free drug release profile. Preliminary results indicated the nanoparticles generate heat when exposed to an alternating magnetic field, so triggered drug release might be achievable. The MGICs 16 complexes could be promising as dual diagnostic and therapeutic candidates for potential drug delivery applications.

Boronate crosslinked ATP- and pH-responsive nanogels for intracellular delivery of anticancer drugs

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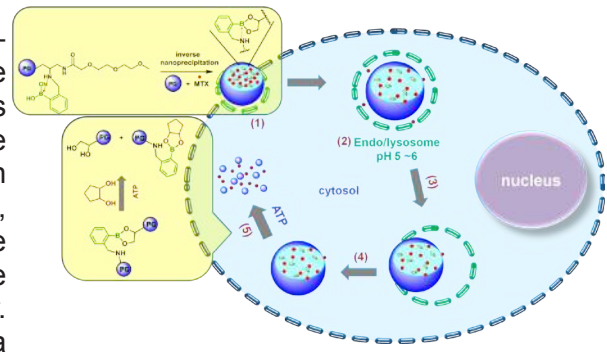
Stimuli-responsive carrier systems have been attracted enormous attention in controlled drug delivery due to their robotic response towards intracellular or extracellular stimuli such as pH values, redox conditions, enzymes, temperature, et al. However, single stimuli-responsive systems are often not enough to accomplish a high therapeutic efficacy. Dual-responsive systems combining two stimuli signals have been recently developed, including pH/redox, pH/temperature, and enzyme/pH. The combination of Adenosine-5'-triphosphate (ATP) and pH triggered drug delivery system has not been reported yet. ATP plays an important role in cellular metabolism and is considered as an essential immunogenic signaling molecule. The intracellular concentration of ATP is in the range of 1 to 10 mM, which is much higher than its extracellular concentration.



A novel ATP and pH dual-responsive degradable nanogel (NG) system was developed based on the complexation of 1,2-diols in dendritic polyglycerol (dPG), and boronic acids, which are conjugated with dPG as the macromolecular crosslinker. The NGs were formed by a mild and surfactant free inverse nanoprecipitation method. An anticancer drug, methotrexate (MTX) was co-precipitated with the macromolecular precursors and crosslinkers, forming MTX-encapsulated NGs (NG-MTX) with a loading capacity of 13 wt.%. The size of NG is controllable from 100 to 300 nm, which is suitable for the enhanced permeation and retention (EPR) effect, and can be degraded into small fragments that are within the clearance limitation in the presence of 5 mM ATP or at pH 4 after 24 h. The release of MTX was accelerated by increasing ATP concentrations and decreasing pH values of the release medium. Both the real-time cell analysis and MTT results showed no cytotoxic effect of NG and a dose-dependent effect of NG-MTX on HeLa cells. The cellular uptake of FITC-labeled NG was examined by confocal laser scanning microscopy, which presents a time-dependent internalization through endocytosis.

Funding: Chinese Scholar Council (CSC)

nanoEXPO

Over the next decade, nanotechnology has the potential to influence virtually every aspect of our lives, including our energy, food, water, buildings, and medicines. However, in order to realize this broad potential - robust, cost-effective, regulatory-friendly manufacturing technologies will be required. Through its novel technology platform and expansive intellectual property, Liquidia is poised to be a leader in the development of nanotechnology-based healthcare products and a catalyst for the growth anticipated across this industry.

By leveraging fabrication techniques from the semiconductor industry, Liquidia has the ability to rapidly design and manufacture precisely engineered particles of virtually any size, shape, or composition. This unique ability to precisely engineer particles enables scientists to explore new product frontiers that, until now, have otherwise been out of reach. Using the company's proprietary PRINT® Platform, Liquidia is creating precisely engineered vaccines and inhaled therapeutics that address critical unmet patient needs. Strong partnerships with GSK and PATH Vaccine Solutions (PVS) are key elements of Liquidia's strategy for developing and commercializing products around the world. To learn more about the PRINT technology, click here.

In 2013, Liquidia formed a new company based on promising preclinical work with ophthalmology related therapies. The newly formed ophthalmology company, named Envisia Therapeutics, is currently focused on areas of ocular disease with significant unmet medical needs such as glaucoma and age-related macular degeneration, where modes of administration and patient compliance can be a barrier to care.

Bind Therapeutics is a clinical-stage nanomedicine platform company developing Accurins™, the novel targeted and programmable therapeutics. Accurins are designed with specified physical and chemical characteristics to target specific cells or tissues and concentrate a therapeutic payload at the site of disease to enhance efficacy while minimizing adverse effects on healthy tissues. Their strategy is to leverage their medicinal nanoengineering platform to develop a pipeline of Accurins, initially in oncology, as well as Accurins in collaboration with biopharmaceutical companies. Bind's lead drug candidate, BIND-014, is in Phase 2 clinical trials for non-small cell lung cancer (NSCLC) and metastatic castrate-resistant prostate cancer (mCRPC). The company has collaborated or is collaborating with Amgen, Pfizer, AstraZeneca, and Roche to develop Accurins based on therapeutic payloads from their product pipelines.

Bind believes Accurins represent the next stage in the evolution of cancer therapy. Accurins are nanoparticles containing a therapeutic payload and are designed to target tumors at three levels: tissue, cellular and molecular. They combine this triple targeting with a prolonged circulation time to concentrate the therapeutic payload at the targeted disease site, where it is then released in a controlled and timely manner. Accurins have the potential to significantly increase the net clinical benefit of the therapeutic payload and result in efficacy and safety not currently achievable through other therapeutic approaches

PerkinElmer is a global leader focused on improving human and environmental health, for the better.

Single Particle Inductively Coupled Plasma Mass Spectrometry

Single Particle-ICP-MS, a new operating mode in ICP-MS that is dedicated to the analysis of engineered nanoparticles (ENPs) commonly used in Nanomedicine and drug delivery systems. Single Particle (SP-ICP-MS) allow the differentiation between ionic and particulate signals, quantitate both ionic and particulate fractions, measures particle composition, concentration (part/mL), particle sizes and explores agglomeration and size distribution.

The present work explores the use of SP-ICP-MS as a metrology tool for the analysis of engineered nanoparticles in biological matrices (blood, urine). Preliminary results show that Au ENPs (30 and 60 nm) are stable in diluted blood and urine. No change in the size of Au ENPs and/or concentration was noticed during the 24h study period.

SP-ICP-MS is rapidly becoming a key analytical instrument in assessing the fate, behavior and distribution of (ENPs) in several types of matrices1,2 including biological fluids such blood and urine.

Malvern

NanoSight NS300

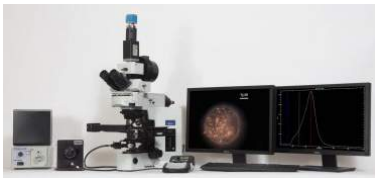
The Malvern NanoSight NS300 provides an easy-to-use, reproducible platform for nanoparticle characterization. The NS300 allows rapid, automated analysis of the size distribution and concentration of all types of nanoparticles from 10nm to 2000nm in diameter.

QUIDEL

MicroVue Pan-Specific C3 Reagent Kit

A novel approach to fill the gap of animal-specific Complement ELISAs. Quidel MicroVue products are a well-established name in biochemical bone makers, immune system monitoring and assays for the assessment of Complement activation. Quidel's product list is extensive, offering ELISA assays, depleted sera, proteins, monoclonal and polyclonal antibodies, antisera, antigens, controls, and special reagents. Looking to expand the methodical arsenal of Complement analysis in animals, Quidel marketed the Pan-Specific C3 Reagent kit (RUO product). These reagents convert the activity of C3 in an animal specimen to human SC5b-9 that is detectable with the MicroVue SC5b-9 Plus EIA kit. The method allows sensitive and quantitative measurement of C3 in animal plasma or serum, which, to the extent C3 has been consumed prior to the assay, also provides a measure of prior Complement activation. The assay enables preclinical immune toxicology testing of C-mediated (pseudoallergenic) adverse drug effects.

CytoViva®



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- ***In vivo tracking of nanoparticles***
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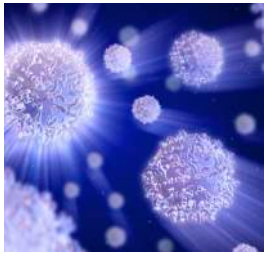

HemaCare BioResearch Products & Services is a leading blood products and services company serving the scientific community for over 35 years. HemaCare provides healthy and disease state human-derived primary blood cells and tissues derived from:

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