

1st Inter-PEN Contract Meeting

November 4-5, 2011

St. Louis, MO



Research Presentations Strengthen Collaborative Efforts

By Kari Alca and Terry Sharp

On November 4-5, 2011, Washington University School of Medicine hosted the 1st Inter-PEN Contract Meeting. The event kicked off with a Welcome Reception on Friday night which gave members of the four PENs a chance to meet and mingle prior to a full day of collaboration and camaraderie. Saturday's presentations were kicked off by a welcome from Evan D. Kharasch, M.D., Ph.D.; Vice Chancellor for Research at Washington University (pictured).

Dr. Kharasch's enthusiastic remarks were followed by research presentations by all of the PENs, Mount Sinai School of Medicine, Georgia Institute of Technology, Massachusetts General Hospital and Washington University School of Medicine. Breaks in presentations were met with exciting discussions between researchers. Many new interests and ideas were shared among attendees, and plans for future collaborations were discussed.



Our Mission

The goal of NHLBI Programs of Excellence in Nanotechnology is to develop nanotechnology – based tools for the diagnosis and treatment of heart, lung, and blood diseases, and to move the translation of these technologies towards clinical application. The program will bring together multi-disciplinary teams from the biological, physical, and clinical sciences for the focused development and testing of nanoscale devices or devices with nanoscale components, and apply them to cardiovascular, hematopoietic, and pulmonary diseases. The program will also develop investigators with the interdisciplinary skills to apply nanotechnology to heart, lung, and blood disease problems.

A Word From Your Project Manager

By Kari Alca

I would like to begin by saying how truly wonderful it was to meet most of you in person at the 1st Inter-PEN Contract Meeting. It was a fun and informative weekend and I hope you all enjoyed it as much as I did.

You will notice a change in the arrangement of this quarter’s newsletter. The information I gained at the Inter-PEN Meeting made me very aware of the importance of collaborative efforts taking place in these NHLBI funded contracts. Great efforts are being made to encourage communication between PENs regarding research endeavors. In an effort to help bolster this effort, the Winter 2011 edition of the Inter-Pen newsletter is arranged by PEN. My hope is that after learning about research happening at other PEN sites, you will be able to quickly reference these research projects. This, after all, should be the most important goal of this newsletter. I hope you find the information contained in these pages helpful. Have a wonderful holiday season and a Happy New Year!

Kari Alca
 PEN Project Manager
alcak@mir.wustl.edu

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PEN Spotlight On... Omid Farokhzad, M.D.



Dr. Omid Farokhzad is an Associate Professor at Harvard Medical School (HMS) and a physician-scientist in the Department of Anesthesiology at Brigham and Women's Hospital (BWH). Dr. Farokhzad completed his post-doctoral clinical and research trainings, respectively, at the BWH/HMS and MIT in the laboratory of Dr. Robert Langer. He received his M.D. and M.A. from Boston University School of Medicine.

Dr. Farokhzad directs the Laboratory of Nanomedicine and Biomaterials at BWH. The research at the Laboratory of Nanomedicine and Biomaterials is highly interdisciplinary with a translational focus. The group has developed novel nanomaterials and nanotechnologies for a range of medical applications, including the treatment of cancers, in addition to immunological and cardiovascular diseases.

Dr. Farokhzad has made contributions to the field of nanotechnology for medical applications and has extensive expertise in the development of therapeutic nanoparticle technologies; most notably, he is credited for innovations leading to the clinical translation of two distinct polymeric nanoparticle technologies for medical applications.

Dr. Farokhzad is a faculty member of the BWH Biomedical Research Institute (BRI) Cancer Research Center. He is also a member of the Dana Farber/Harvard Cancer Center Programs in Prostate Cancer and Cancer Cell Biology and a Research Affiliate in the Harvard-MIT Division of Health Sciences and Technology (HST).

Dr. Farokhzad is an active member of the MIT-Harvard Center for Cancer Nanotechnology Excellence and his research is supported by the Program of Excellence in Nanotechnology (PEN) through the National Heart Lung and Blood Institute, the US National Cancer Institute and the Prostate Cancer Foundation.

Dr. Farokhzad has authored more than 70 papers and is an inventor of more than 60 issued or pending US and worldwide patents. The combinatorial nanoparticle self-assembly technologies that Dr. Farokhzad has developed formed the foundation for the launch of three venture-backed biotechnology companies, BIND Biosciences, Selecta Biosciences, and Blend Therapeutics. These companies are translating the aforementioned academic innovations through clinical development. He serves as Director on the boards of BIND Biosciences, Blend Therapeutics and as Director and Vice Chairman on the board of Selecta Biosciences.

Dr. Farokhzad was named among the Nano50 winners of 2007 by the NASA Nanotech Briefs, which awards the most innovative people and design ideas that will revolutionize nanotechnology. In 2009 he was among the 15 recipients across all industries to receive the All Star Distinguished Achievement Award from the Mass High Tech (MHT) Journal for his contributions to the Life Sciences industry.

For more information, please visit: <http://www.dfcc.harvard.edu/membership/profile/member/468/0/>

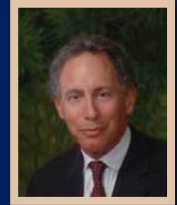
The "Spotlight" rotates between the PENs. If someone on your PEN has made a significant contribution to your PEN's success and you would like to recognize their work, please send a brief biography to Kari Alca at alsak@nar.usstl.edu.

MSSM, MIT, BWH, Columbia, NYU

Translational Nanomedical Therapies for Cardiac and Vascular Diseases



Principal Investigator – Zahi A. Fayad, Ph.D.
Co-Principal Investigator – Robert S. Langer, Ph.D.

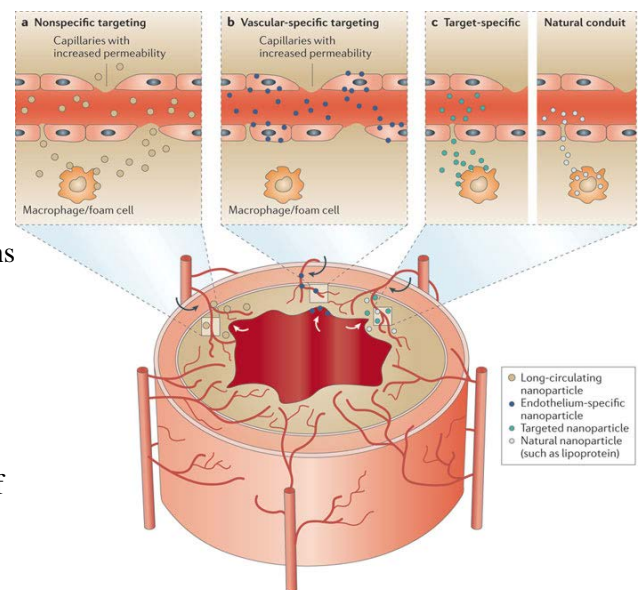


Perspectives and Opportunities for Nanomedicine in the Management of Atherosclerosis

Mark E. Lobatto, Valentin Fuster, Zahi A. Fayad, Willem J. M. Mulder

Key processes in atherosclerosis development

Atherosclerosis remains the major cause of morbidity and mortality in the field of cardiovascular disease, and represents a substantial economic burden¹. The build-up of an atherosclerotic plaque starts at lesion-prone areas in large and medium-sized arteries. Dysfunctional endothelium at these sites cause increased permeation of macromolecules such as lipoproteins and adhesion molecules, and the resulting enhanced recruitment and accumulation of monocytes. These monocytes subsequently differentiate into macrophages, which can then be transformed into foam cells by ingesting low-density lipoprotein (LDL). The retention of lipoproteins and immune cells may result in plaque progression, cell apoptosis and neovascularization over a period of several years or decades². Advanced atherosclerotic lesions may eventually contain a large volume of lipids and necrotic cells, referred to as the lipid or necrotic core.



Nature Reviews | Drug Discovery

Fig. 1. The vessel walls of larger arteries are supplied with nutrients by the lumen and the vasa vasorum — a network of small microvessels. In the lesioned vessel wall the vasa vasorum undergoes angiogenic expansion, with neovessels reaching into the base of the plaque, which is accompanied by the upregulation of cell-surface receptors and increased permeability of the endothelium. The upregulation of receptors and the increased permeability also affect the endothelium on the luminal side of the plaque. The main targeting principles can be classified into nonspecific targeting of the plaque (part a), specific targeting of the vasculature (part b) and specific targeting of components (part c) of the plaque (for example, the extracellular matrix or macrophages) with either synthetic nanoparticles or via interaction through a natural conduit. The targeting of the plaque occurs via both the vasa vasorum and the main lumen at lesioned sites, and is exemplified on the figure with corresponding arrows. Depending on the targeting principle applied, the cellular distribution of nanoparticles in the plaque will vary considerably.

In normal vessels the vasa vasorum, a network of microvessels, supplies nutrients to the outer component of the vessel wall. As an atherosclerotic plaque develops hypoxia induces neovascularization as a compensatory defense mechanism to restore nutrient supply to the vessel wall³. The microvessels that arise from neovascularization originate from the vasa vasorum in the adventitia and extend into the base of the plaque (Fig. 1). Plaque neovessels are fragile structures that are also prone to leakage and rupture.

Atherosclerotic plaques can rupture as a result of the breakdown of the fibrous cap that covers the lipid core via inflammatory processes, which can consequently lead to thrombotic occlusions and clinical events⁴. Lesions that are most susceptible to rupture are characterized by active inflammation, thin fibrous caps with large lipid cores, endothelial denudation with superficial platelet aggregation, fissured plaques or luminal stenosis exceeding 90%.

Nanoparticle targeting in atherosclerosis

The processes described above and their accompanying molecular and cellular events create numerous opportunities for targeting the atherosclerotic plaque using nanoparticles. Nonspecific targeting can be exploited owing to the previously mentioned permeability of the luminal endothelium, as well as the microvascular permeability and leakiness of the neovessels of the vasa vasorum.

By attaching antibodies, proteins, peptides or other ligands to its surface, a nanoparticle can be targeted to single or multiple receptors that are expressed on the surface of (or inside) an atherosclerotic plaque. Vascular targeting can be accomplished using nanoparticles that have been functionalized with specific ligands to adhesion molecules, selectins or integrins, as these adhesion molecules are expressed on the activated endothelium of the luminal wall or on the endothelium of newly formed microvessels⁵. In addition, cellular and non-cellular components within plaques, such as the extracellular matrix and lipids, allow for specific targeting of plaques with functionalized nanoparticles. Although this is a form of active targeting, nanoparticle accumulation (at the diseased site) is primarily dependent on vascular permeability as the nanoparticle must first extravasate from the circulation.

Specific nanoparticle accumulation can also be accomplished via inherent targeting of natural nanoparticles. Lipoproteins, including high-density lipoprotein (HDL) and LDL, interact with plaques through a natural conduit. Exploiting or mimicking this inherent plaque affinity of lipoproteins has been shown to be a powerful approach for targeting plaques effectively. These key plaque-targeting mechanisms are summarized in Fig. 1.

Atherosclerosis — imaging and nanomedicine

Traditionally, clinical investigations that were used to detect atherosclerotic plaques focused on the degree of stenosis in the blood vessel lumen. However, with the advent of the concept of vulnerable plaques, the early 1990s marked a change in the understanding of the underlying pathophysiology of atherosclerosis in relation to plaque instability. This resulted in a shift towards visualizing plaque morphology and biology using novel imaging techniques, either stand-alone or — more recently — combined⁶. Developments in the field of molecular imaging allow the characterization of biological processes in cardiovascular disease at the cellular and molecular level. This approach heavily relies on the development and application of contrast-loaded probes — often nanoparticles — that are specifically designed to target cell types and epitopes of interest. Reversely imaging techniques themselves can advance the development of nanoparticles (Fig. 2).

Besides diagnosing subclinical atherosclerosis, nanoparticles carrying contrast-generating material can be used to track drug delivery or enable the quantification of expression of cellular markers after treatment.

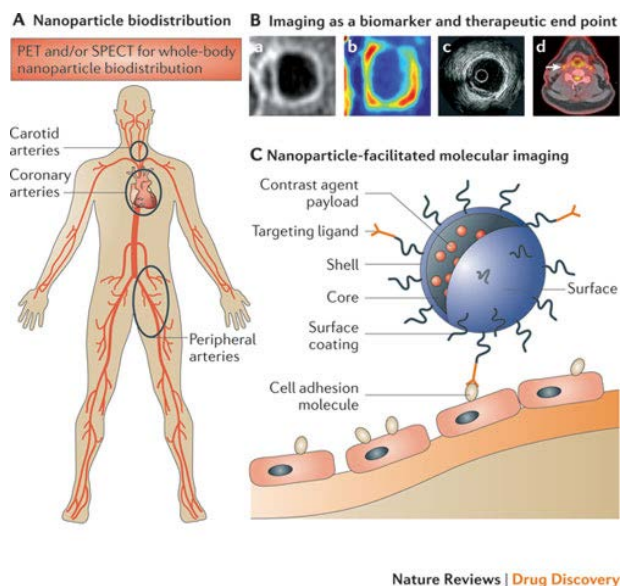


Fig. 2. This figure provides examples of the ways in which imaging is associated with nanomedicine. A | The figure depicts the systemic arterial vasculature, highlighting the carotid arteries, coronary arteries and peripheral arteries, which are prone to developing vulnerable lesions that can be visualized by imaging. B | State-of-the-art imaging techniques that are used to visualize plaque morphology and plaque processes are shown. Ba | The image shows a T1-weighted contrast-enhanced magnetic resonance imaging (MRI) scan of a carotid artery, showing thickening of the arterial wall. MRI can provide information on anatomical features and can be used to differentiate between the fibrous cap and lipid core. Bb | The image depicts the same carotid vessel illustrated in part Ba, which has been assessed using dynamic contrast-enhanced MRI; this imaging technique provides information on vascular permeability and neovascularization. Bc | An intravascular ultrasound image is shown in the figure; intravascular ultrasound imaging can be used to obtain information on plaque area within coronary arteries in response to treatment. Bd | A fused image, obtained using ^{18}F -fluorodeoxyglucose imaging in combination with positron emission tomography (PET) and computed tomography imaging, is shown. The white arrow in the image points to the right carotid artery, showing a high amount of signal, which is indicative of metabolic activity within an atherosclerotic plaque. C | The figure shows a nanoparticle attaching to an endothelial cell lining the atherosclerotic plaque. The contrast payload within the nanoparticle can render it detectable by an imaging modality of choice. SPECT, single-photon emission computed tomography. Image Bc is modified, with permission, from Ref. 63 © (2006) American College of Cardiology.

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Hot Topic

This quarter's Hot Topic for the Mount Sinai School of Medicine comes from Willem Mulder's laboratory:

Site-specific targeting of nanoparticles requires the conjugation of ligands to their surface. However, the exposure of targeting ligands in circulation may shorten the nanoparticle's circulation half-life and can cause immune responses. To deal with this issue we have successfully created nanoparticles for which the targeting molecules are actively shielded by long polyethylene glycol (PEG) polymers. In the presence of matrix metalloproteinase-2 (MMP-2) the long PEG moieties are cleaved which results in the exposure and availability of the targeting ligands. This nanoparticle platform will be used to accomplish efficient accumulation in atherosclerotic plaques via its long circulation properties and subsequent targeting of macrophages when ligands become available after PEG removal by MMP-2 inside the plaque.



MGH, BWH, BI, Harvard, MIT

Translational Program of Excellence in Nanotechnology

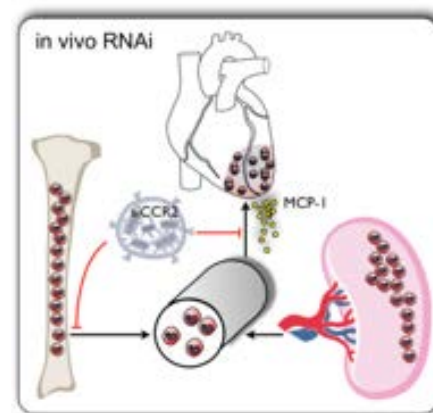
Principal Investigator – Ralph Weissleder, Ph.D.

Therapeutic siRNA silencing in inflammatory monocytes in mice

Matthias Nahrendorf and Ralph Weissleder

Our overall understanding of the key role played by inflammatory monocytes and macrophages in the development and progression of cardiovascular disease has expanded considerably over the years. However, harnessing this insight into the development of concrete and viable therapeutic strategies is a goal that has yet to be realized. One major hurdle towards achieving this objective has been our inability to "surgically" eliminate the disease-promoting effects of the immune system whilst preserving its protective functions. Owing to a synergistic relationship between our Program at MGH/BWH/BI/Harvard/MIT (PI Ralph Weissleder) and the Program at Mount Sinai/MIT/BWH/Columbia/NYU (PI Zahi Fayad and Robert Langer), we have thus far been successful in targeting monocyte subsets in inflammation and healing. Indeed, now, as a result of a truly collaborative effort led by Matthias Nahrendorf (MGH) and Daniel Anderson (MIT), we demonstrate how, using RNA interference in conjunction with nanotechnology, specific subsets of innate immune cells can be targeted with nanoparticles. This work has since motivated a collaborative effort between MGH, MIT and Alnylam Pharmaceuticals. Researchers at these institutions have been focused on silencing the CCR2 receptor protein, a receptor that is responsible for the migration of inflammatory (but not reparative) monocytes towards the chemokine MCP-1 in both mice and humans.

A 70-80nm nanoparticle was injected intravenously into mice and used to deliver silencing RNA (siRNA) into immune cells. The nanoparticle was formulated using C12-200 lipid, distearylphosphatidyl choline, cholesterol, 1-(monomethoxy polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG) and siRNA. By fluorescently tagging siRNA, the nanocarrier and its load could thus be tracked following its systemic delivery into mice. Interestingly, the injected nanoparticles were found to rapidly distribute to splenic reservoir monocytes, a monocyte pool recently discovered at the Center for Systems Biology at MGH (2). This cell pool within the spleen, identified as a "rapid response team", is swiftly recruited to sites of injury such as infarcted myocardium. However, in situations where inflammation is the propagator of disease, it is likely these cells are more harmful than helpful. Other immune cells located in the blood and bone marrow were likewise observed to absorb the nanoparticles. Once internalized, each nanoparticle delivered its load to the cytosol, where effective CCR2 mRNA was quickly neutralized. Production of the CCR2 receptor protein was thus inhibited, an effect that was surprisingly efficient at stopping monocyte recruitment in several major diseases, including atherosclerosis, heart failure, and transplant rejection. Most notably, siRNA treatment was able to reduce ischemia reperfusion injury in the heart as well as reduce the recruitment of inflammatory cells to atherosclerotic lesions in apolipoprotein (apo) E-deficient mice. Future work will now be focused on finding nanomaterials that can silence immune cells at lower doses, multiple targets (i.e. using a siRNA cocktail), and in large animals. The above-described technology is capable of silencing any gene target within immune cells, including



Nanoparticles targeted to inflammatory monocytes silence the chemokine receptor CCR2.

proteins (e.g. transcription factors and/or cytokines) involved in proliferation, maturation, differentiation and antigen presentation.

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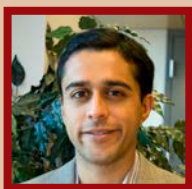
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Hot Topic

Stanley Shaw's laboratory at the MGH Center for Systems Biology is collaborating with cheminformatics experts to explore structure activity relationships of nanoparticles. In collaboration with two different computational biology groups, they are analyzing a unique dataset consisting of the cellular effects of 50 different nanoparticles, measured using several different cellular assays. Physical measurements such as zeta potential and particle size are also included in the models. Preliminary results suggest that these models can predict certain aspects of nanoparticle behavior or class membership, and point to ways that such information can inform decisions involving nanoparticle toxicity. The dataset generated by the Shaw laboratory is a unique resource in these early efforts to explore systematic SAR for nanoparticles, and is freely available to interested investigators.

Awards



Dr. Neal Devaraj, of Dr. Weissleder's research group, has accepted an Assistant Professor of Chemistry and Biochemistry and Bioengineering position at the University of California, San Diego.



Dr. Jered Haun, also of Dr. Weissleder's research group, has accepted an Assistant Professor of Biomedical Engineering position at the University of California, Irvine.



Dr. Stanley Shaw has recently become an Associate Member of the Broad Institute of Harvard and MIT



Georgia Tech, Emory, UC-Davis

Center for Translational Cardiovascular Nanomedicine

Principal Investigator – Gang Bao, Ph.D.

A Molecular Beacon-Based Approach to Assess Circulating miRNAs in Patients with Coronary Artery Disease

Charles D. Searles, M.D.

Recent studies have suggested that miRNAs are pivotal regulators of normal development and physiology, as well as disease. miRNAs are a class of short (19-25 nt), single stranded, noncoding RNAs that regulate an array of cellular functions through the degradation and translational repression of mRNA targets. Importantly, tissue levels of specific miRNAs have been shown to correlate with pathological development of disease¹. miRNAs have also been found in whole blood, serum, plasma and other body fluids in a stable form that is protected from endogenous RNase activity². Recent work from our group and others has indicated that circulating microRNAs (miRNAs) could be useful biomarkers for various human disease states, including cancer³, acute myocardial infarction⁴⁻⁷, heart failure⁸, and chronic vascular disease⁹⁻¹³. Thus, circulating miRNA expression signatures have a potential role in the diagnosis, prognosis and assessment of therapy for cardiovascular disease.

Although only the mature form of the miRNA is involved in posttranscriptional regulation of gene expression, quantifying the relative expression levels of mature miRNA and its precursors is important for understanding miRNA transcription, localization, and processing. Distinguishing between precursor and mature miRNAs is also important because their expression levels are not analogous^{15, 16}. *Due to limitations in current methods for assessing precursor and mature miRNAs, we have developed a molecular beacon-based approach that can readily distinguish mature- and pre-miRNAs, distinguish miRNA family members, and reliably quantify miRNA expression.*

Molecular beacons are oligonucleotide (DNA or RNA) stem-loop hairpin probes containing an anti-sense hybridization sequence that is flanked by two short, self-complementary sequences¹⁷. The termini of the probe are conjugated to a fluorescent dye and a suitable quencher for that dye. In the absence of complementary miRNA target, the probe forms a stem-loop structure that results in quenching of the fluorophore (Figure 1). Hybridization of the beacon with the complementary miRNA sequence, which is energetically more favorable, opens the hairpin probe, thus physically separating the fluorophore from the quencher, and allows the fluorophore to fluoresce upon excitation.

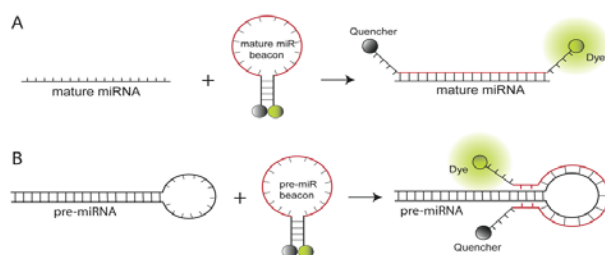


Figure 1. Schematic illustration of molecular beacon hybridization assays. (A) Hybridization of the mature beacon to the mature miRNA target. (B) Hybridization of the precursor beacon to the pre-miRNA target. This beacon hybridizes to the loop sequence of the pre-miRNA hairpin structure.

We performed a microarray analysis of whole blood samples from 5 male subjects with significant CAD (one major vessel >50% vessel stenosis) and 5 healthy controls, matched for age, gender and race. Subsequently, we isolated miRNAs from whole blood of an additional 30 subjects and performed TaqMan-based assays of microRNAs that were identified by microarray to be highly expressed in blood. We identified 11 circulating miRNAs downregulated in CAD subjects compared to healthy controls.

We have designed molecular beacons complementary to either the mature sequence of circulating miRNAs or the hairpin loop region of their precursors. We have found that molecular beacons with DNA, RNA and combined LNA-DNA backbones can all detect miRNAs of low (< 1 nM) concentrations *in vitro*, with RNA beacons being most sensitive (Figure 2). We also found that molecular beacons are able to distinguish miRNA family members that differ by one nucleotide (Figure 3). These results suggest that our approach to assess miRNA expression and distinguish mature and precursor miRNA species is quite robust.

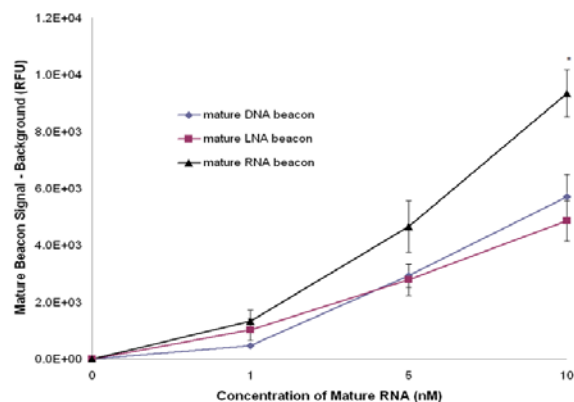


Figure 2. Differences in sensitivity among molecular beacons with varying backbone chemistries that target miR-21. Molecular beacons were incubated with miR-21 RNA (0 – 10 nM) at 37°C for 60 minutes. For each experiment, background fluorescence was subtracted from beacon signal and each data point represents mean \pm SEM of three separate experiments. * $p = 0.006$ RNA beacon vs DNA beacon.

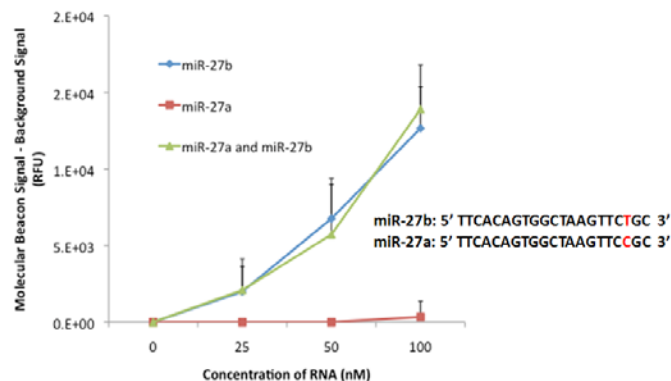


Figure 3. Binding of the mature miR-27b molecular beacon to miR-27b or miR-27a (differ by one nucleotide) at 55°C. The mature miR-27b DNA molecular beacon was incubated with mature miR-27a RNA (blue line), mature miR-27b RNA (red line), or a 1:1 mixture of both (green line) at concentrations of 25 nM, 50 nM, and 100 nM. Background fluorescence was subtracted from each data point. Each data point represents mean \pm SEM of three separate experiments.

The biggest challenge that we currently face is limitations in sensitivity. Whereas we are able to use molecular beacons to accurately assess miRNA and pre-miRNA levels in tissues, we have found that circulating levels of many miRNAs are 10 to 100 fold lower than that in tissues. Problems with sensitivity are largely due to little flexibility in hybridization sequence design of the beacon, since the length of miRNAs is similar to the length of a typical beacon. Furthermore, this approach requires that molecular beacon design be optimized for each mature miRNA and pre-miRNA. In the coming year, we will examine different strategies to improve molecular beacon sensitivity, including metal-enhanced fluorescence.

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Hot Topic

This quarter's Hot Topic for the Georgia Institute of Technology comes from David Ku:

We have recently been able to demonstrate thrombus formation under very high shear conditions that create bonds almost 100 times faster than any previously reported biological bond. The realm of super fast kinetics opens up a new area of rapid multiple nanoscale bonding that has previously not been known and may provide a new mechanism of attacking sudden cardiac death.

Awards



David Sotto's Goizueta Foundation Fellowship was renewed for two more years on August 15, 2011 for the 2011-2012 and the 2012-2013 academic years. The Society of Hispanic Professional Engineers (SHPE) awarded David the Xerox Scholar - Highest Achieving Graduate Student Award last Spring on April 29th, 2011 for the 2011-2012 Academic Year.

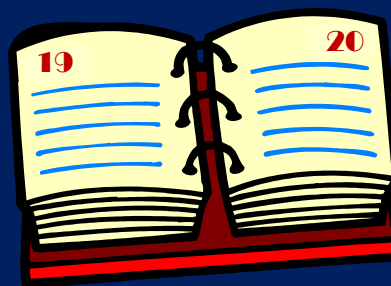
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2012 Inter-PEN Meeting

October 19-20, 2012

Hosted by:

Massachusetts General Hospital



WUSTL, TAMU, UCB, UCSB, UTSW

Integrated Nanosystems for Diagnosis and Therapy

Principal Investigator – Michael J. Welch

Co-Principal Investigator – Karen L. Wooley



Meet Our PEN

Washington University



Michael Welch



Robert Gropler



Steven Brody



Pamela Woodard



Samuel Achilefu



John-Stephen Taylor



Suzanne Lapi



Joseph Culver



Dana Abendschein



Mikhail Berezin



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Kari Alca



Susannah Grathwohl



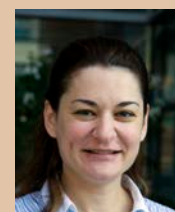
Suellen Greco



Sean Gunsten



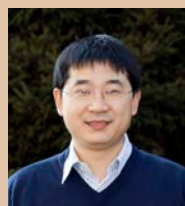
Tiffany Gustafson



Aida Ibrićević



Hannah Kutosky



Yongjian Liu



Alexander Loftis



Annie Nguyen



Ralph Nothdurft



Terry Sharp



Yufei Shen

Meet Our PEN (Cont'd)



Monica Shokeen



Jillian Smith



Zhenghui Wang



Natalia Zhegalova



Jie Zheng

Not Pictured: Pamela Baum, Sharon Bloch, Verdella Brink, Barb Donnelly, Kathleen Grapperhaus, Scott Harring, Richard Laforest, Donna Lesniak, Zifan Li, Suzanne Mazhuvanchery, Doug Moeckel, Richard Pierce, Tom Pilgram, Adam Salazar, Sally Schwarz, Joel Sher, Kooresh Shoghi, Jenny Sun, Rui Tang, and Rhonda Wilton

Texas A&M University



Karen Wooley



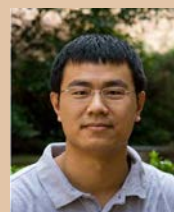
Jim Sacchetti



Mahmoud Elsabahy



Gyuseong Heo



Ang Li



Lily Yun Lin



Danielle Policarpio



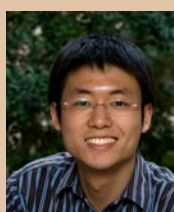
Sandani Samarajewa



Ritu Shrestha



Fuwu Zhang



Shiyi Zhang



Jiong Zou

Not pictured: Ang Li, Young Lim, and Judy White

University of California - Berkeley



Jean Frechet



Peter Wich

Not pictured: Paul Kierstead and Cezar Ramiro

Meet Our PEN (Cont'd)

University of California – Santa Barbara



Craig Hawker



Roey Amir



Nathaniel
Lynd



Eric Pressly

University of Texas Southwestern Medical Center



Carolyn
Cannon



Parth Shah

Not pictured: Justin Smolen and Kangmee Woo

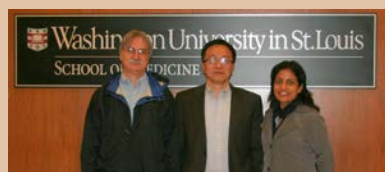
Seminar Series

October 11, 2011 -

Adah Almutairi, Ph.D.

Assistant Professor, Pharmaceutical Sciences
University of California, San Diego

“The art of falling apart & coming together:
Exploiting nanomaterial properties for medicine”



November 3, 2011 –

Gang Bao, Ph.D.

Distinguished Professor and
Robert A. Milton Chair in
Biomedical Engineering
Georgia Institute of Technology

“Engineering molecular imaging probes for disease studies”

December 12, 2011 –

Kwang-Jin Kim, Ph.D.

Professor of Medicine
University of Southern California –
Keck School of Medicine

“*Nanomaterial Interactions with and Trafficking
Across Lung Alveolar Epithelium*”

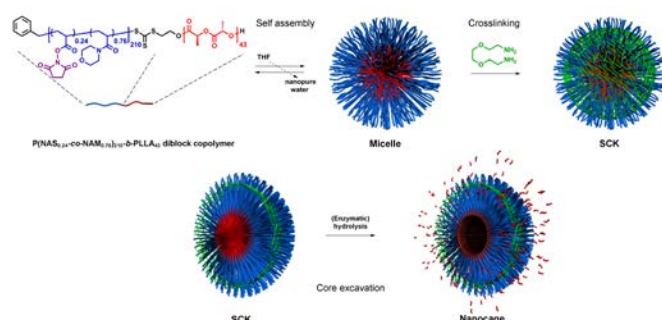


Degradability of Poly(Lactic Acid)-based Nanoparticles Toward Controlled Release of Therapeutics and Biological Clearance

Sandani Samarajeewa, Ritu Shrestha, Yali Li, and Karen L. Wooley

There has been a growing interest in employing degradable materials for applications in biomedical settings, due to their reduced toxicity and ability to clear through biological systems. Therapeutics can be loaded into polymeric nanoconstructs and the release of these encapsulated guest molecules can then be triggered by external stimuli such as changes in pH, light and temperature. However, for rapid and effective clinical translation, it is imperative that these nanostructures are comprised of biocompatible materials, which can package therapeutics, gate their release and provide efficient delivery. Complex polymeric nanostructures with core-shell morphologies that constitute degradable polyesters in the core domain have shown great potential as vehicles for delivery of active therapeutics.

As an extension to the previous studies focused on incorporating chemical and hydrolytic degradability into nanostructures, this current research integrates degradable poly(lactic acid) (PLA), and specifically investigates the hydrolytic degradation behaviors of PLA within the core regions of micelles and shell crosslinked knedel-like (SCK) nanoparticles, under acid and enzyme catalysis.¹ As the enzymatic cleavage of the PLA core requires the enzyme to be accessible to the core domain, this work also provides information on the permeability of the crosslinked shell.



Scheme 1. Preparation of SCK nanoparticles by self assembly of amphiphilic diblock copolymer followed by crosslinking, and production of a nanocage-like structure from selective hydrolysis of the PLA core of the SCK template.

Core-degradable SCK nanoparticles were constructed by the supramolecular assembly of a novel amphiphilic diblock copolymer poly(N-(acryloyloxy)succinimide-*copolymer*-N-acryloylmorpholine)-*block*-poly(L-lactic acid) (P(NAS-*co*-NAM)-*b*-PLLA), followed by crosslinking within the shell of the nanoparticles by the addition of a diamino crosslinkers. Sequential ring opening polymerization (ROP) and reversible addition-fragmentation chain transfer (RAFT) polymerization were employed to obtain the initial homopolymer and subsequent diblock co-polymer. Scheme 1 illustrates the

preparation of SCK nanoparticles from the polymer precursor and the enzymatic degradation process of the SCKs.

Real-time monitoring of the enzymatic degradation was accomplished by observing the methyl protons of oligo (lactic acid) (OLA) and lactic acid (LA) ranging from 1.64 ppm to 1.44 ppm in deuterated buffer (Figure 1-a), in comparison to an external chloroform reference. As shown in Figure 1-b, kinetic analyses by ¹H NMR spectroscopy showed less than 20% lactic acid released from enzymatically-catalyzed hydrolysis of PLA in bulk, whereas *ca.* 70 % of the core degraded within 48 h for block copolymer micelles, with only a slight reduction to *ca.* 50 % for the shell crosslinked derivatives.

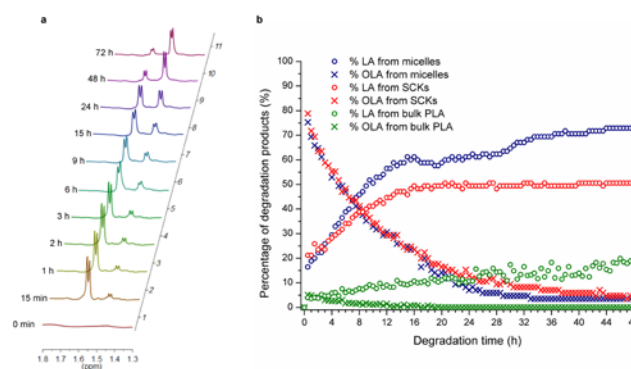


Figure 1. (a) Real-time ¹H NMR spectra of the OLA (1.64-1.56 ppm) and LA (1.48-1.44 ppm) methyl protons for micelles monitored over 3 d following addition of the enzyme, (b) percent degradation products vs. degradation time plot from integration of OLA or LA peaks from micelles, SCKs and bulk PLA monitored over 48 h of enzyme exposure.

Additionally, as illustrated in Figure 2, Atomic Force Microscopy (AFM) images revealed the conversion of SCK nanoparticles to hollowed nanocages (which appear as donut-like structures after collapse onto the mica substrate) upon hydrolytic degradation of the PLA core for 3 months, with a reduction of the particle heights (from 3 ± 1 nm to <1 nm, before and after hydrolytic core degradation, respectively) and a substantial increase in the average particle width (from 38 ± 10 nm to 88 ± 12 nm, before and after hydrolytic core degradation, respectively).

Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) studies provided insight into the dimensional changes in the hydrodynamic diameters and average core diameters of the nanoparticles upon core excavation, respectively. It was observed that upon hydrolysis of the PLA segment, the resulting hydrophilic nanocage-like structures underwent swelling, due to the diffusion of water into the core region, increasing progressively as the hydrophobic components were eliminated.

In summary, we have reported fundamental advances in the synthetic methodologies for the preparation of hydrolytically-degradable, functionalizable nanoparticles, together with rigorous characterization of their degradation properties. Specifically, we have demonstrated enzyme-triggered selective excavation of the polyester-based core of block copolymer micelle assemblies and their shell crosslinked nanoparticle analogs. Loading of active therapeutics into the PLA based SCKs and the release kinetics of the guest molecules as a function of core degradation are being evaluated, and will be reported in the near future.

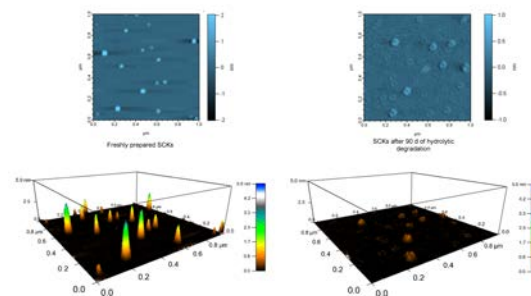


Figure 2. Tapping-mode AFM images of freshly-prepared SCKs and hydrolytically-degraded SCKs, showing collapse of the nanocage-like structures on the mica substrate upon core excavation.

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Hot Topic

This quarter's Hot Topic for Washington University comes from Pamela Woodard's laboratory:

Dosimetry Results for Targeted PET Atherosclerotic Imaging Agent Favorable

In preparation for pre-IND meetings, we performed dosimetry assessment for the 25% ^{64}Cu -CANF-comb atherosclerotic PET imaging agent. This radiopharmaceutical targets atrial natriuretic peptide receptor C (C-ANF).

The radiation dose was assessed in 6 groups of 5 mice each, by injecting $10\mu\text{Ci}$ of the 25% ^{64}Cu -CANF-comb into each mouse. Animals were kept in metabolic cages for excretion measurement. Groups were sacrificed sequentially and assessed at 1, 2, 6, 12 and 48 hrs. post injection. Organ residence times were calculated from the time integral of biodistribution data.

Results showed that ^{64}Cu -DOTA-CANF is principally excreted through the feces, with critical organ dose to the bone surface at 0.307 rad/mCi and heart at 0.245 rad/mCi. The effective dose was 93 mrem/mCi. As extrapolated to human subjects, with a 5 rad limit to any organ, our results indicate a permissible maximum of 16.3 mCi injected, well above the 10 mCi necessary for adequate image quality in human subjects.

Significance – what does this mean for us as we head into an eIND? *The human radiation dose as extrapolated from this data is low at radiopharmaceutical doses well above that required for adequate image quality.*

Washington University PEN: Nanotechnology Outreach for Middle School Students

In November the WU PEN Skills Development Core, in conjunction with the Washington University Institute for School Partnership organized a nanotechnology in-reach event for middle school students. The focus was to expand the reach of the WU-centered PEN to the general community and raise awareness about the latest innovations in nanotechnology. Built upon our previous outreach efforts through the St. Louis Science Center, and St. Louis area Elementary schools, this year we extended our scope to include Middle School students. The previous curriculum was reinforced to include a more didactic approach to presenting the experiments and challenged the students to write up their own experimental plans and reports. Forty students, recruited from both Hixson Middle School and Brittany Woods Middle School, came to the Washington University campus to be introduced to and carry nanoscience experiments at the university chemistry laboratories.

Chemistry Experiments

Students were organized into groups of three and given a lab report to fill out as a group. The students were required to fill out the hypothesis and procedure portions of their write ups before the start of each experiment. They recorded their observations while doing the experiments and developed their own conclusion.

Testing Stain-Resistant Nano-fabrics Experiment: The goal was to show how nanotechnology is present in their everyday life. The goal of this experiment was to show how nanotechnology can have a significant effects on basic properties of materials, such as the ability to resist stains. A few drops of water, ketchup, grape juice, coffee, mustard and tumeric were deposited on 12 pieces of fabric (regular and Nano-Tex). The students were required to determine the fabric coated with nanoparticles and its effect in making stain resistant materials.

Ultraviolet (UV) Radiation Detection Experiment: The goal was to qualitatively grade the amount of UV light transmission. Students used UV beads that contain particles that change color when exposed to UV lamp. The students assessed the color change of the beads through a transparent surface coated with sunscreen with different protection factors (High and Low SPF numbers). The students ranked unknown creams with regards to UV blocking efficiency.

Dynamic Light Scattering (DLS) Experiment: In this activity, the students were required to observe the effect of particle size and solubility of five solutions (water, salt, milk, polystyrene (PS)-polyacrylic acid (PAA) di-block polymer and micelle (nanoparticle)) based on the reflected and scattered light from a laser.

For many of the students, this was their first visit to campus. The students were remarkably enthusiastic about the science activities. The most common complaint was that it didn't last long enough! In the survey sheets that the students filled out at the end of the activity, one student even replied next time, "I would want to stay overnight, I loved it."

This year, there was active volunteer participation from Washington University in Saint Louis SPIE Student Chapter. The skills development team would like to thank our gracious volunteers for all their help!!!



Drs. Culver, Shokeen and Lapi gave introductory lectures on nanoscience and the length scales.



← A nanoscience in-reach day, brought students from both Hixson and Brittany Woods Middle Schools into the campus of Washington University in St. Louis. For many students, this was their first visit to Washington University.

Here the students are playing a paper folding game that illustrates the exponential effects of the power's of 2. →



← Students working on a dynamic light scattering experiment. Polymer compounds were combined to create micelle structures. The changes in size and the formation of the micelles were monitored using dynamic light scattering.

Rather than give the students a recipe, the general idea of the experiments were discussed with the students. The groups of 3-4 for students then wrote their own experimental plans and drew their own conclusions. In this experiment, they worked to distinguish which of the creams was most effective at blocking the UV irradiation from a Lamp. →



Would you like to learn more about the four PEN contracts?

Visit our website at www.nhlbi_pen.net to read about:

- Aims of each PEN contract (2010-2015)
- Publications
- Past newsletter issues

Principal Investigators

Gang Bao, Ph.D.
The Georgia Institute of Technology
Emory University
University of California, Davis

Zahi A. Fayad, Ph.D.
Mount Sinai School of Medicine

Robert S. Langer, Sc.D.
Massachusetts Institute of Technology
.....
Brigham and Women's Hospital
Columbia University
New York University

Ralph Weissleder, M.D., Ph.D.
Brigham and Women's Hospital
Massachusetts General Hospital
Broad Institute
Harvard Medical School
Massachusetts Institute of Technology

Michael J. Welch, Ph.D.
Washington University School of Medicine

Karen L. Wooley, Ph.D.
Texas A&M University
.....
University of California, Berkeley
University of California, Santa Barbara
University of Texas, Southwestern

Principal Investigator of the PEN Administrative Center

Robert J. Gropler, M.D.
Principal Investigator of
Administrative Center
Washington University School of Medicine

Inter-PEN Administration

Kari E. Alca
Project Manager for NHLBI- PEN
Washington University School of Medicine

Terry L. Sharp
PEN Finance Manager
Washington University School of Medicine

Program Official

Denis Buxton, Ph.D.
National Heart, Lung, and Blood Institute