earlier this month the NHLBI Director, Dr. Betsy Nabel, announced the release of the NHLBI Strategic Plan: Shaping the Future of Research. The Strategic Plan is designed to guide NHLBI’s scientific directions over the next five to ten years, and adopts a cross-cutting approach that revolves around three goals – “form to function”, “function to causes”, and “causes to cures”. Each goal contains a number of challenges, and the plan also outlines strategies to reach these goals. Particularly notable for the PEN program is Goal 2.1.b: Apply discoveries in nanotechnology to the development of new diagnostic and therapeutic strategies. The goal stresses the importance of multidisciplinary collaborations in bringing nanotechnology into the clinical arena. The Strategic Plan, along with more information on how the plan was developed, can be found at http://apps.nhlbi.nih.gov/strategicplan/.
The PEN at Emory University and Georgia Tech has been developing novel approaches for both diagnosis and targeted therapy of disease. Among the 13 member laboratories of the PEN, the Murthy lab at Georgia Tech is a relatively young lab focusing on chemical synthesis, characterization and validation of new contrast agents and drug delivery vehicles. Here we showcase some of the projects in the Murthy lab, aiming to stimulate inter-PEN collaborations.

Polyketal: A new biomaterial for drug delivery. The polyketsals are a new class of biomaterials developed to enhance the treatment of inflammatory diseases. The polyketsals have several attractive properties for drug delivery, such as excellent biocompatibility, acid sensitivity and ease of synthesis (1-3).

Shown below in Figure 1 are the chemical structure, synthesis and applications of a polyketal termed poly(cyclohexane-1,4-diyl acetonedimethylene ketal) (PCADK). PCADK is designed to hydrolyze, after phagocytosis by macrophages, in the acidic environment of the phagosome and enhance the intracellular delivery of phagocytosed therapeutics. Other key attributes of PCADK for drug delivery are its well characterized degradation products and straightforward synthesis. PCADK hydrolyzes into 1,4-cyclohexanediol, a compound used in food packaging, and acetone, a compound on the FDA GRAS list. PCADK was synthesized using the acetal exchange reaction between 1,4-cyclohexanediol and 2,2-dimethoxypropane, and can be synthesized on a multigram scale in one step. The hydrolysis kinetics of the ketal linkages in PCADK have been measured, by $^1$H-NMR, and were determined to be pH-sensitive, having a half-life of 24.1 days at pH 4.5 and over 4 years at pH 7.4. We are currently using PCADK to deliver a wide variety of anti-inflammatory compounds, one example using the protein, superoxide dismutase (SOD), which scavenges reactive oxygen species, is shown below in Figure 1.

Figure 1. PCADK, a new polymer for drug delivery. (A) Synthesis of PCADK from 1,4-cyclohexanediol (CDM) and 2,2-dimethoxypropane (DMP), and acid hydrolysis of PCADK into CDM and acetone. (B) Formulation of SOD loaded microparticles by w/o/w double emulsion. Microparticles degrade after phagocytosis, in the acidic environment of the phagosome.
Polyketals for the treatment of inflammatory lung diseases. Inflammatory lung diseases, such as COPD, acute lung injury and lung fibrosis, cause millions of deaths each year and effective treatments are greatly needed. Polymeric microparticles have tremendous potential for enhancing the delivery of therapeutics to the lung. Drug loaded micron sized polymeric particles can be directly delivered to lung macrophages via inhalation, resulting in targeting of therapeutics to macrophages, via a non-invasive route. Porous microparticles, 20-30 microns in size, with a low density, can also be inhaled and deliver therapeutics to non-macrophage cells types. However, despite their potential, polymeric microparticles are not being used clinically for the treatment of inflammatory diseases. This is partially because existing microparticles used for lung delivery are based on polyesters, which are problematic for the treatment of their inflammatory lung diseases because of their acidic degradation products. We are currently using the polyketals as a drug delivery vehicle for the treatment of acute lung injury. Shown in Figure 2 is the general goal of this project.

Imaging hydrogen peroxide in vivo. Hydrogen peroxide has emerged as one of the central signaling molecules of inflammatory diseases. There is therefore great medical and scientific interest in developing technologies that can image the production of hydrogen peroxide in vivo, because of its potential to act as a diagnostic marker and also to better understand its physiologic role. At present technologies do not exist that can image hydrogen peroxide in vivo and information about the in vivo biological function of hydrogen peroxide can only be obtained indirectly from transgenic mouse experiments or from ex vivo tissue culture studies. This lack of imaging technology has limited the biological understanding of hydrogen peroxide and also the development of medical diagnostics.

We are currently developing a new family of nanoparticles that are designed to image hydrogen peroxide in vivo. These nanoparticles are designed to be suitable for deep tissue imaging of hydrogen peroxide and the detection of hydrogen peroxide in atherosclerotic plaques.

Figure 2: Delivery of SOD polyketals for the treatment of lung inflammation

1. Administration of SOD-loaded microspheres via inhalation
2. Accumulation of microspheres to lung tissue
3. Macrophages mediated release of SOD

References

Acknowledgment. Research in the Murthy laboratory is supported in part by the NHLBI Program of Excellence in Nanotechnology to Emory University (U01 HL80711-01).
Samuel Achilefu, Ph.D., one of the ten Senior Investigators under the direction of Principal Investigator, Karen L. Wooley, Ph.D., is part of the “Integrated Nanosystems for Diagnosis and Therapy” PEN. Achilefu is a professor of radiology and director of the Optical Radiology Laboratory (ORL) at Mallinckrodt Institute of Radiology, at Washington University School of Medicine in Saint Louis, Missouri.

Achilefu was featured in the Mallinckrodt Institute of Radiology, Focal Spot magazine, Winter 2006/2007, in an article titled “Advances in Optical Imaging” by Candace O’Connor. Some highlights from the article are outlined here.

* * * * *

Imaging assists both in finding a “suspicious mass” and in determining whether the chosen method of treatment is producing the desired results. Achilefu’s hope is that “by using optical methods we will be able to see, within hours of therapy being given, what is happening to the patient’s body”.

Achilefu’s work is supported by the National Institutes of Health, under a $7.5 million grant to study new molecularly targeted probes and strategies to make these highly sensitive evaluations. Research at the ORL focuses on the following projects:

1.) Innovative use of fluorescene sensitivity and lifetime imaging  
2.) Development of optical brain function imaging for neonates  
3.) Collaboration with interventional radiologists  
4.) Assessment of breast cancer biomarkers in collaboration with MIR faculty in the breast imaging section.

“In general, imaging takes place along a wide spectrum of light: from short-frequency radiation (conventional X rays and radionuclear imaging, for example), all the way to very long frequency waves (MR). Optical imaging occurs in a narrow window directly in the middle: the visible to infrared spectrum of light, in the 400-nanometer to 1600-nanometer range.”
“Because different tissues scatter light differently,” says Achilefu, “we can use that information to discriminate between diseased and normal tissue by the pattern of light scattering.”

“The light that researchers shine into the tissue does not emerge all at once, but at minutely different times - measured in nanoseconds - depending on the path the photons take after hitting the target tissue and scattering.”

For more information about Sam Achilefu and a full copy of the article visit the MIR website at www.mir.wustl.edu.

**JOB OPPORTUNITIES**

Within the four PENs

**Center for Molecular Imaging Research**

**Position:** Postdoctoral Fellows

**Research Area:** Chemistry

**Labs:** Dr. McCarthy and Dr. Hilderbrand

**Description:** The Center for Molecular Imaging Research (http://cmir.mgh.harvard.edu) at the Massachusetts General Hospital and Harvard Medical School is recruiting post-doctoral fellows for research in the synthesis and development of nanoparticulate delivery systems for the imaging and therapy of numerous disease states. We are expanding our chemistry facilities and are looking for scientists with experience in polymer and/or organic chemistry who will be interested in the development of biologically applicable nanoagents. CMIR is a diverse facility; therefore, we offer excellent training opportunities in a collaborative research environment including biology, chemistry, and imaging disciplines.

**Requirements:** The candidate (PhD or equivalent) should have experience in polymer and/or organic chemistry, familiarity with peptides, and an understanding of nanotechnology.

Further information and several other positions can be found at http://cmir.mgh.harvard.edu/

Please send your application to (please specify position): Serena Laft, sdlaft@PARTNERS.ORG

CMIR, MGH, 149 13th Street, Room 5407; Charlestown, MA 02129-2060.
All-electronic sensing: Developing tools for a non-optical world

By Andrew N. Cleland, Jeffrey W. Smith (jsmith@burnham.org)

Biomedical sensing relies heavily on optically-based tools, with the pervasive use of molecular fluorophores, self-assembled quantum dots, and fluorescent beads to identify, concentrate, and isolate specific cell types or target molecules. There has been little progress made in developing analogous tools based on electronic labeling, detected or read out using electronic sensing, in part due to the success the optical approaches have enjoyed. However, there likely is a need for a parallel, all-electronic approach to some of these applications, due to the potential for extremely low-cost, disposable electronic analogues, with equal or higher throughput capability in screening or other applications.

The Cleland nanostructures group has been developing a suite of tools aimed at addressing this area. Our approach is based on the use of radiofrequency electronic signaling and detection, which affords large bandwidth (fast readout), with the possibility of multiplexing, using the broad spectrum that can thereby be addressed. Electronic signals at frequencies above 1 MHz have the advantage of being easily coupled through the ionic double layer formed at the electrode-aqueous interface, bypassing the problematic electrochemical reactions that occur with low-frequency signals.

Sensing is achieved using reflectometry, where a weak radiofrequency signal is reflected from a sensor, typically a lithographed, thin-film coplanar waveguide, defined on a glass substrate. When balanced, we can detect very small changes in the electrical impedance in the vicinity of the waveguide, with sensitivities of 10 parts per million. We have implemented these sensors for whole-cell sensing, and more recently for molecular sensing.

We first used this reflectometer to demonstrate a radiofrequency version of a Coulter counter, where particles pass...
ing in an elastomeric microfluidic channel, over the micron-scale gold electrodes defined on an underlying gold substrate, could be detected with very high sensitivity at very high particle count rates (in excess of 100,000 per second). A schematic of this device is shown in Figure 1.

We then developed an electronic labeling scheme, that should allow the identification and sorting of cells based on surface protein expression. The labeling uses micron-scale epoxy bars, with embedded metal stripes, which can be read out using the same reflectometry method used for the Coulter counter. The metal stripes digitally encode a unique bar identifier; the present version, with 10 stripe positions, can encode 1024 unique codes. Doubling to 20 stripes will enable more than a million identifiers. The bars can be functionalized using antibodies specific to certain cell types, and when adhering to a cell, identify that cell type. After identification, sorting and purification should then be possible.

More recently, we have focused on the development of molecular-scale versions of these devices. We have replaced the planar, lithographically defined waveguide with a vertical electrode stack, with a self-aligned vertical etch defining the stack edge. The metal electrodes in this format are spaced by distances of 10-100 nm, and are sensitive to molecular changes in the immediate vicinity, or eventually in the space between, the stacked metal electrodes. Our goal is to coat the electrode surfaces with specific antibodies or receptor nucleotides, and electronically detect specific binding of target molecules to the functionalized surfaces, using our highly sensitive reflectometric approach. A schematic of the proposed approach is shown in Figure 3. The goal is to develop sensors that can specifically identify and detect molecules related to vulnerable plaque, either present in plaques formed on artery walls, or in suspension in blood.

References
Clinical evidence suggests that coronary atherosclerosis and aortic valve stenosis share similar risk factors. Thus, it is surprising that the molecular markers of the progression of valvular heart disease have received little attention. Recently, the overexpression of matrix metalloproteinases (MMP-1, -2, -9, and -13), cysteine proteases (Cathepsins S and K), adhesion molecules, embryonic myosin, and interleukin-1β were observed in activated valvular cells. The biological processes that occur due to this upregulation result in pathologies ranging from mild valve thickening to severe calcification with impaired leaflet motion or aortic valve stenosis, which can only be treated by valve replacement. It is therefore important to detect early changes in valvular cell functions and identify targets for the prevention of aortic valve stenosis. To this end, a team led by Dr. Elena Aikawa utilized a combination of protease activatable near-infrared fluorescence (NIRF) imaging agents sensitive to gelatinases and cathepsins, as well as a series of targeted or untargeted magnetofluorescent nanoparticles (MFNP) that recognize vascular cell adhesion molecule-1 (VCAM-1) and macrophages, respectively, to probe the environment of aortic valves of hypercholesteremic apolipoprotein E-deficient (apoE-/-) mice. As compared to wild type mice, whose leaflets are composed predominantly of quiescent fibroblast-like cells, apoE-/- mice exhibit thickened leaflets with macrophage-rich subendothelial lesions. When probed by the VCAM-1 targeted MFNP, endothelial cell activation was evidenced by both magnetic resonance imaging (MRI) and fluorescence microscopy. When treated with the untargeted MFNP, areas of the aortic valve in apoE-/- mice which were rich in immunoreactive macrophages demonstrated a fourfold higher NIRF signal than corresponding saline treated wild type mice. In order to...
assess the detrimental aspects of macrophage localization within the valves, hypercholesteremic mice were injected with Prosense 680, which is activated mainly by cathepsin B, or Gelsense 680, which is activated by MMP-2 and -9 (Figure 1). Histopathological analysis of the resulting valves colocalized the NIRF signal from the activatable probes and macrophages (as ascertained by Mac3 staining). The presence of macrophages, and thus enzymes which are known to contribute to collagen and elastin degradation, brings about vascular and valvular remodeling, and deleterious structural changes. In order to detect osteogenic activity within the inflamed aortic valves, a cohort of mice were coinjected with Osteosense 750, and the macrophage specific MFNP labeled with VivoTag 680. Ex vivo imaging revealed that the NIRF signal in the 750 nm and 680 nm channels did not colocalize (Figure 2). In fact, osteoblastic activity was localized to activated myofibroblasts. In total, this study revealed the underlying molecular mechanisms involved in the thickening and calcification of aortic valves, which are similar to those observed in atherosclerosis. It also demonstrated that a number of multimodal fluorescence imaging agents can be used to visualize changes in cellular function due to calcific aortic valve disease in vivo.

References

Labeling of Polymer Nanostructures for Medical Imaging

By Jinqi Xu, Guorong Sun, Raffaella Rossin, Aviv Hagooly, Zicheng Li, Ken-ichi Fukukawa, Benjamin W. Messmore, Dennis A. Moore, Craig J. Hawker, Michael J. Welch* and Karen L. Wooley*

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Nanoscale architectures with well-defined structures and diverse properties have exhibited great promise for biomedical applications.1 Many efforts have been devoted to incorporate various functionalities within such nanoobjects and investigate the interactions between synthetic nanomaterials and biological systems in order to achieve drug delivery to specific target sites while detecting and monitoring the biological activities in vivo.1 Obviously, it is crucial to develop and employ an efficient strategy to functionalize nanoobjects. For instance, shell crosslinked nanoparticles (SCKs),2 developed from polymeric micelles (by performing chemical crosslinking in the shell domain) to provide robust nanostructures that overcome critical micelle concentration (CMC) restrictions,3 have been functionalized with surface-accessible, biologically-active ligands through various methodologies.4 One of the versatile and straightforward pathways is the direct conjugation of ligands onto pre-established nanoparticles.4 The coupling chemistries utilized were effective in many cases, but they could be unpredictable, especially when the system was complex. Therefore, transformation of nanoobjects into functional materials required a better understanding on the coupling chemistry and interactions between targeting ligands and nanoobjects. Based upon multidisciplinary collaborations among our NHLBI-PEN (HL080729), we were able to study the conjugation of SCKs (derived from amphiphilic diblock copolymers, poly(acrylic acid)-b-polystyrene) with two types of DOTA (1,4,7,10-tetraazacyclododecane-N,N’,N”,N’’-tetraacetic acid) derivatives, DOTAamine and DOTAllysine, via amidation chemistry (Scheme 1).5 DOTA has been used widely to chelate copper-64 (^64Cu) and other radiometals for applications in both positron emission tomography (PET) and radiotherapy.6 Notably, radiolabeling of DOTA-SCK transforms these nanoparticles into in vivo imaging agents, but the process also provides a quantitative measurement to determine the yield of conjugation chemistries and better understand the fundamental chemistry of nanoscale systems. Our studies5 present an excellent example of how “simple” amidation chemistry could
be complicated as the reaction is aimed to functionalize nanoobjects, and of how such complications may be addressed. We have found that: (1) the coupling yield of the DOTAamine to the SCKs was much higher than that of DOTAlysine, suggesting that the existence of a lengthy, inert spacer on the designed functionalities is essential to minimize both steric and electrostatic hindrances (Figure 1) and improve the reaction efficiency; (2) the overall structure and composition of the SCKs and the degree of crosslinking in the shell regions of SCKs could also affect the coupling chemistry with DOTAamine. These results provided many new insights into the design and functionalization of nanoparticles. Further efforts are devoted toward enhancing the radiolabeling yields, thereby improving the sensitivity of such conjugates and allowing decreases in the number of labeled nanoparticles required to produce in vivo detection.

References
Reflections on the American Chemical Society National Meeting, Spring 2007 and the Arthur M. Sackler Colloquia by the National Academy of Sciences

By Monica Shokeen,
Carolyn Anderson
(andersoncj@wustl.edu)

A s part of my postdoctoral training under the direction of Prof. Carolyn J. Anderson, I got an opportunity to attend the 233rd American Chemical Society National Meeting & Exposition in Chicago, March 25-29. Without any doubt, the ACS National meetings are a pure delight for chemists! It’s a great venue where all levels of chemists ranging from budding undergraduates to graduate students to postdocs to PI’s come together to present their work and learn about new science from their peers. This year’s spring meeting was held in the windy and wonderful city of Chicago with over 9,400 papers presented in oral and poster sessions. Several PEN group members also took upon the opportunity and presented their work under various divisions (list available on the PEN website at http://www.nhlbi-pen.info/Events/ACS%20-%20March%2025-29%20%202007).

Representation from the Skills Development Component:

One of the chief goals of the skills development component is to develop interdisciplinary courses that bring together students and experts from diverse fields. One such initiative was the “Special Topics in Organic Chemistry-Nanomedicine” course that was offered in the Fall of 2006 through the Department of Chemistry (Course Master: Prof. Karen L. Wooley). The course was a success by all means with enrollment from over 20 students and lectures contributed by experts from PEN (details covered in the first newsletter, Vol 1 (1), Feb 2007). As new such courses are being designed for the future, it is imperative that we share our story with other educators and get their perspective and suggestions. With that goal in mind, an abstract was submitted for oral presentation in the Division of Chemical Information with the title “Taking the Graduate Classroom Teaching a Step Further”. During the 30 minute presentation, the methodology, success, and challenges of the course were presented. The talk was well received and some good questions and suggestions were put forward. The big take home message from this and related talks in the Division of Chemical Education and Information was the use of smart multimedia technology for dissemination of knowledge at all levels. There was also a dedicated section on “Nanotechnology across the curriculum” which covered the innovative methodologies that educators are developing for incorporating...
nanotechnology into standard curriculum.

The ACS experience was augmented by participation in another nanotechnology specific conference: “Arthur M. Sackler Colloquia” organized by The National Academy of Sciences, Washington D.C. from April 10-11, 2007. The theme of the meeting was “Nanomaterials in Biology and Medicine: Promises and Perils.” As apparent from the title, the colloquium was highly appropriate for PEN members as it covered the cutting edge nano-science done at premier research institutions among other related issues. There were four major sessions: (1) New technologies to create nanomaterials; (2) Society and ethical concerns of nanotechnology in Biology; (3) Functional nanomaterials in biology; and (4) Frontiers of nanotechnology. There was also a poster session with 24 participants including participation from Washington University in Saint Louis. The speakers ranged from the academic labs of Princeton University, Northwestern University, Harvard University, Massachusetts Institute of Technology, Oxford University, Rice University etc. to industrial labs of Nanorex, Inc. BioMEMS,IMEC, Belgium etc. Although all the talks were phenomenal, the 7th Annual Sackler Lecture given by George Whitesides (Harvard University) stood out as it covered the whole spectrum of nanotechnology with references to quantum mechanics, cell biology, information technology, new materials, and energy issues. Interestingly, Whitesides quoted PET imaging as the imaging modality of the future, and a great beneficiary of nanotechnology as it can image molecular processes efficiently in real time unlike the traditionally used modalities. All these points came together in drawing the big picture of nanotechnology. The social, economical, and ethical impact of nanotechnology was also put forth for public discussion.

To sum up, these two conferences were both fun and a great learning experience and contributed immensely to the intellectual tool box of laboratory research and teaching. Also, getting to know the similar research done in nanomedicine in other leading institutions gives a refreshing perspective on our own work and is an excellent training tool!

Acknowledgements

Special Thanks to Prof. Carolyn J. Anderson and Prof. Karen L. Wooley for providing the opportunity to attend the meetings.

The Four PENs and their Principal Investigators

**Nanotechnology: Detection & Analysis of Plaque Formation**

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**Georgia Institute of Technology**

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- CoE Distinguished Professor
- The Wallace H. Coulter Department of Biomedical Engineering
- Georgia Institute of Technology
- Emory University

**Nanotherapy for Vulnerable Plaque**

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**University of California - Santa Barbara**

**The Scripps Institute**

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**Translational Program of Excellence in Nanotechnology**

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**Massachusetts General Hospital**

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**Integrated Nanosystems for Diagnosis and Therapy**

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- James S. McDonnell Distinguished University Professor in Arts & Sciences
- Professor, School of Arts & Sciences, Department of Chemistry
- Professor, School of Medicine, Department of Radiology
- Faculty member in the Center for Materials Innovation
SECOND ANNUAL
Nanotechnology and the Life Sciences
St. Louis, Missouri
March 30-31, 2007

GANG BAO AND KAREN L. WOOLEY ARE CO-AUTHORING A “CONFERENCE SCENE” ARTICLE FOR THE JOURNAL, NANOMEDICINE, ON THE SECOND ANNUAL NANOTECHNOLOGY & THE LIFE SCIENCES SYMPOSIUM.

COMING SOON, ...

Poster Awards

Left: Second Place went to Peter Jones (Programmer) and Dennis Thomas, Ph.D., from WUSTL. Peter developed the logical model for representing the nanoparticle and tumor data. Dennis developed the controlled vocabulary and ontology for data about nanotechnology applications in cancer diagnostics and therapeutics, collected and curated the data, and designed the poster.

First Place was awarded to Soubir Basak of WUSTL (not pictured).
Event Organizers: (from left to right) Angela Benassi, Siteman Cancer Center; Lynn Coulter, C-TRAIN, Kay Duchek, Continuing Medical Education; Eileen Cler, Program of Excellence in Nanotechnology, Department of Chemistry.
Principal Investigator, Jeffrey W. Smith, of The Burnham Institute for Medical Research, University of California in Santa Barbara, will host the upcoming Second Annual Inter-PEN Meeting.

We have selected the Best Western South Coast Inn - Santa Barbara-Goleta, CA as the preferred hotel for the Second Annual Inter-PEN Meeting. A complimentary airport shuttle is included in the guest room rate ($135.00 single/double + 10% tax, for a total of $148.50). A shuttle service to and from the University of California, Santa Barbara will be arranged by UCSB.

**Hotel Information:**

Best Western South Coast Inn -
Santa Barbara/Goleta
5620 Calle Real
Goleta, California, 93117-2319
United States
Tel: (805) 967 - 3200
Fax: (805) 683 - 4466
http://www.bwsci.com

For questions or information regarding the Inter-PEN Meeting, please contact either Eileen Cler (WUSTL) at eacler@wustl.edu or James Lee (UCSB) at jameslee@burnham.org. You can also visit the Inter-PEN website at www.nhlbi-pen.net for more details.

Inter-PEN presentation slides from October 2006, as well as prior versions of the Inter-PEN NHLBI-Quarterly Newsletter are available on the Inter-PEN website at www.nhlbi-pen.net.