This quarter marks the beginning of our third volume of the NHLBI Inter-PEN Quarterly Newsletter. We've introduced a new segment that highlights recent recognition for members on each of the four PENs. We are calling this new segment "Awards", and asking each PEN to submit significant accomplishments or awards received any time within the past six to twelve months.

Some examples of these awards are an induction as a fellow, a young investigator award or an annual award for a particular organization. Additionally, if you have a list of the criteria necessary to attain the award or any comments from those people making the selection for the award that explain why a PEN Participant was chosen, please include that information along with the name of the award.

Please forward these awards to Eileen A. Cler at eacler@wustl.edu.
New Approaches for Molecular MRI based on Iron Oxide Nanoparticles

Xiaoping Hu
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Superparamagnetic iron-oxide nanoparticles (SPIOs) have become widely used contrast agents for molecular imaging with MRI. However, there are potential limitations. Associated with the Emory-Georgia Tech PEN, our work focused on alleviating some of these limitations.

While iron-oxide particles are effective at dephasing the MR signal, leading to image signal loss, such signal losses can be difficult to distinguish from other signal voids and difficult to quantify, especially in environments where processes such as blood flow and susceptibility changes may also appear as regions of signal loss. While several techniques have been introduced to generate the so-called “positive” contrast from SPIOs, they are mostly based on macroscopic effects. We have developed techniques based on the microscopic changes in the magnetic field surrounding the nanoparticle. An iron-oxide nanoparticle produces a magnetic dipole field (1), leading to a nanoscale variation in the magnetic resonance frequency of protons. The first technique we have developed is based on off-resonance saturation (ORS), achieved by applying a saturation pulse, whose frequency corresponds to a shell surrounding the nanoparticle (2). Our results show that the saturation ratio increases with SPIO concentration (Fig. 1a).

In addition to depending on saturation offset, the ORS technique is also affected by how long each proton spends within the specified shell and how fast the saturated protons spread to other regions, making the ORS effect dependent on diffusion, a physiologically relevant parameter. Our second approach for probing the microscopic magnetic field of the nanoparticle is based on the use of an adiabatic preparation pulse. Adiabatic pulses are amplitude and phase modulated pulses where the magnetization follows the effective applied magnetic field provided the “adiabatic condition” is met (3). Near the nanoparticles, the adiabatic condition is violated because the resonance frequency of spins changes rapidly due to diffusion in the highly inhomogeneous magnetic field near the particles. Consequently, images acquired with and without the application of an adiabatic inversion can be subtracted (in magnitude) and normalized, producing a contrast that is linearly correlated with iron concentration (Fig. 1b). Additionally, imaging the nanoparticles in water and agar shows that the adiabatic technique is not very sensitive to magnetization transfer.

Exogenous SPIOs have been used as labels for cell tracking and conjugated with targeting domains for imaging specific molecular signatures. The use of exogenous particles in cell tracking can be limited by the dilution of the label due to cell growth and division over time, and exogenously generated targeting contrast can be limited by delivery and flexibility. Towards alleviating these limitations, our lab has established that...
In summary, we have developed alternative techniques for MRI contrast sensitive to magnetic nanoparticles and established a reporter whose expression leads to endogenous production of iron-oxide nanoparticles in mammalian cells. Our detection techniques are potentially more specific and quantitative. Our reporter could be used for MRI in a similar fashion as optical reporter genes for optical imaging.

References


About the Authors

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The Georgia Institute of Technology

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

Executive Committee Meetings
(Denis Buxton and Principal Investigators only)

**2009**
- March 6
- June 5
- September 4
- December 4

Program of Excellence in Nanotechnology
NATIONAL HEART LUNG & BLOOD INSTITUTE
Simplified syntheses of complex multifunctional nanomaterials

Jason R. McCarthy and Ralph Weissleder
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The synthesis of multifunctional nanoagents, or those bearing multiple targeting, imaging, and therapeutic ligands, or combinations thereof, is facilitated by the large surface area to volume ratio of nanoparticles, as well as well established conjugation methodologies. Typically, modifications of the nanoparticle surface are accomplished sequentially, resulting in non-stoichiometric conjugation, and varying ligand ratios. This can become problematic when fixed ratios are required, especially when non-chromophoric ligands are utilized, as their numbers can not be readily quantified. In order to circumvent this, Garanger et al. have developed multifunctional single-attachment-point (MSAP) reagents. These reagents, based upon small peptide scaffolds, are capable of conjugating 2-3 ligands in predefined ratios.

The bifunctional MSAPs, employing a Lys-Cys motif, were synthesized on solid support using traditional Fmoc chemistries. In one example, the N-terminus was reacted with a partially protected DTPA derivative (diethylenetriamine-N,N,N′,N′′-tetra-tert-butyl acetate-N′-acetic acid) prior to cleavage from the resin, which occurred concomitant with side chain Boc-deprotection. The amine of the lysine side chain was then free to react with the activated (succinimidyl) ester of the 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD) fluorophore. Lastly, the free thiol of the cysteine was reacted with a heterobifunctional crosslinking reagent (maleimidobutyryloxysuccinimide ester) to yield the amine-reactive activated ester.

The trifunctional MSAP was synthesized from the tetrapeptide Lys-Lys-βAla-Cys, making full use of the variety of lysine side chain protecting groups. The peptide (Lys(ivDde)-Lys(Boc)-βAla-Cys(Trt)) was synthesized on solid support, and was initially reacted with the partially protected DTPA, as described above for the bifunctional analog. After selective removal of the ivDde protecting group, the resulting free amine was reacted with fluorescein isothiocyanate (FITC) to fluorescently label the peptide. The peptide was then cleaved from the resin and deprotected, and reacted with an activated ester of a 5 kDa methoxypolyethylene glycol (mPEG). The utility of this trifunctional MSAP was illustrated by conjugating them to gold nanoparticles.

Figure 1: Strategies to obtain multifunctional probes and the design of multifunctional single-attachment-point reagents (MSAPs). (a) A multifunctional probe can be obtained when a substrate is reacted with two chemically reactive functional groups in sequence. F1 = functional group 1; RG1 = reactive group 1. (b) A substrate can be reacted with an MSAP reagent to obtain a multifunctional probe in a single step. (c) A bifunctional MSAP scaffold consists of a Lys-Cys dipeptide to which two functional groups (F1 and F2) are attached. (d) A trifunctional MSAP scaffold consists of a Lys-Lys-βAla-Cys tetra-peptide to which three functional groups (F1, F2 and F3) are attached. A “probe” consists of a “substrate” modified by one or more “functional groups”.

The trifunctional MSAP was synthesized from the tetrapeptide Lys-Lys-βAla-Cys, making full use of the variety of lysine side chain protecting groups. The peptide (Lys(ivDde)-Lys(Boc)-βAla-Cys(Trt)) was synthesized on solid support, and was initially reacted with the partially protected DTPA, as described above for the bifunctional analog. After selective removal of the ivDde protecting group, the

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MSAP was bound to the gold surface, yielding 410 MSAPs per nanoparticle, as determined by the absorption of the fluorescein. The presence of the mPEG also brought about increased stability and decreased aggregation of the gold colloid.

While it may seem that any number of functional groups may be attached to the MSAP scaffolds, the authors found that this was not necessarily the case. Reagents that could cause crosslinking, such as DTPA dihydride could not be used. As well, a large number of near-infrared fluorescent dyes were not amenable to MSAP syntheses in which the dyes were conjugated prior to resin cleavage. Aside from these drawbacks, MSAPs may prove to be efficient reagents for the functionalization of a number of nanomaterials with controlled ligand ratios.

References

Hui Xie’s, Ph.D., journey from northwestern China to the Jeffrey W. Smith Lab in La Jolla, California, began with a stop in the Department of Chemistry at the University of Texas Dallas. While studying in the lab of Ray Baughman, who was recently selected to National Academy of Engineering, saw the possibilities of nanotechnology applied to his own interests.

Hui focused on biotechnology while getting his masters degree. His Ph.D. thesis discussed using bio-material to make carbon nano-tubes for use in medical applications.

The PEN grant at the Smith Lab offered him an opportunity to combine those two fields of study. Currently, he is focused on learning how to create bio-compatible nano-particles of a prescribed shape, size and composition. Each of these characteristics can have an impact on the efficacy of the nanoparticles.

“We are trying to tailor the size, shape, and composition to meet the particular drug delivery specs,” Hui says.

Ultimately, they want to build templates for future use in drug delivery applications.

To learn more ...
Visit the website for The Burnham Institute for Medical Research at:  http://www.burnham.org/
Emerging Breakthroughs

Dr. Niren Murthy and his group has developed a new method for \textit{in vivo} imaging of reactive oxygen species (ROS) using hydro-cyanines, which are a new family of fluorescent probes for superoxide and the hydroxyl radical. They found that the hydro-cyanines have nanomolar sensitivity to hydroxyl radical, high stability, and tunable emission wavelengths that extend into the near IR range, and can detect superoxide and the hydroxyl radical in cell culture and \textit{in vivo}.

We have discovered a novel method of making multi-constituent nanoparticles so that multiple drugs can be simultaneously delivered to the same targeted region.

Researchers at the Center for Systems Biology at the MGH have developed a technique for the facile labeling of nanoparticles with 18F for use in PET/CT imaging. This high yielding functionalization is facilitated by the Huisgen 1,3-dipolar cycloaddition or “click” reaction.

The cationic shell crosslinked nanoparticle (cSCK) platform, developed as a unique and general delivery system for genetic material by Ke Zhang of the Wooley laboratory, in collaboration, Huafeng Fang, and Gang Shen of the Taylor laboratory have proven increasingly interesting, as they can serve as modifiers for various other materials, showing an ability to effect cell transduction even for negatively-charged cylindrical nanostructures.
AWARDS
Recognition (honors, appointments, awards) received by members of the four PENs

By Michael C. Purdy
Washington University School of Medicine

The Society for Nuclear Medicine (SNM) has created an annual award named for Michael J. Welch, Ph.D., professor of radiology, of developmental biology and of chemistry at the School of Medicine.

Welch, who specializes in the synthesis of new radioactive chemicals for medical imaging, is head of the Radiochemistry Laboratory Institute at the Mallinckrodt Institute of Radiology and a member of the Senior Leadership Committee of the Siteman Cancer Center. Over the course of more than 40 years at the School of Medicine, he has developed imaging agents for use in a wide variety of medical contexts. He focuses his research on agents that can help researchers better understand the connections between diabetes and heart disease. The Michael J. Welch Award, created by the Radiopharmaceutical Sciences Council (RPSC) of the SNM, will be given annually for outstanding work in the field. It includes a $1,000 honorarium. RPSC president Robert H. Mach, Ph.D., professor of radiology, of cell biology and physiology, and of biochemistry and molecular biophysics at the School of Medicine, said that the RPSC had been debating for several years about whom to name the award for.

“We had a difficult time deciding on the most appropriate honoree until we detailed all the criteria the honoree needed to meet,” he said. “Then it became apparent to everyone that Michael J. Welch was our most deserving member, the one researcher who unreservedly met all the criteria.”

For more information, click here: http://record.wustl.edu/news/page/normal/12283.html

Michael J. Welch, Ph.D. - The 2nd Century Award at Washington University School of Medicine recognizes individuals whose long-term commitment and participation truly have made a difference, enabling the School of Medicine to enter its second century with strength and confidence. A tradition that began in 1991. 2008 Award Recipients: Charles F. Knight, Jane E. Phillips-Conroy, Ph.D. and Michael J. Welch, Ph.D.

Carolyn J. Anderson, Ph.D., received the Society of Nuclear Medicine Presidential Distinguished Educator Award at the Society of Nuclear Medicine annual meeting in June, 2008.

Pamela K. Woodard, M.D., will be inducted as a Fellow of the American College of Radiology at the ACR Annual Meeting and Leadership Conference; May 2-6, 2009, Washington, DC.

Karen L. Wooley, Ph.D., has been awarded an American Competitiveness and Innovation (ACI) Fellowship by the Division of Materials Research of the National Science Foundation for her transformative research on shell-crosslinked nanoparticles and her exceptional contributions on broader impacts, particularly in K-12 education. The ACI Fellows receive a monetary supplement to their current grants, and have those grants extended for two additional years “for Special Creativity”. The objective of such extensions is to offer an enhanced capability to attack adventurous, “high-risk” opportunities in the same general research area, but not necessarily covered by the original/current proposal.

Craig J. Hawker, Ph.D.*, Professor of Chemistry, Biochemistry and Materials and Director of the Materials Research Laboratory at the University of California, Santa Barbara, has been awarded the DSM Performance Materials Award 2008 in recognition of his exceptional contributions to the advancement of the materials sciences. Professor Put of DSM said: ‘A real paradigm shift took place in synthetic chemistry in the past fifteen years which resulted in the building up of well defined large functional systems mimicking biological systems. This was made possible by a combination of very well controlled chemistry, non covalent interactions and biotechnological approaches. Professor Hawker played a vital role in this development, both by opening up new synthetic pathways and by looking into possible applications in microelectronics and biomedical.’

UCB, UCSB, WUSTL

Michael J. Welch, Ph.D. (Center) at the October 10-11, 2008, Inter-PEN meeting in Atlanta, Georgia, with fellow WUSTL Senior Investigators, Carolyn J. Anderson, Ph.D., and Dana R. Abendschein, Ph.D.
Harvard, MGH, MIT, BWH

The Radiological Society of North America (RSNA) Board of Directors and the Research & Education Foundation Board of Trustees present the 2008 Outstanding Researcher Award to Ralph Weissleder, M.D., Ph.D.

Emory, Georgia Tech

Xiaoping Hu, Ph.D.*, was named both a Fellow of Institute of Electrical and Electronics Engineers (IEEE) and also a Fellow of American Institute for Medical Biological Engineering (AIMBE).

Niren Murthy, Ph.D., was awarded the 2009 Society for Biomaterials Young Investigator Award. This prestigious award is specifically given "to recognize an individual who has demonstrated outstanding achievements in the field of biomaterials research within ten years following his/her terminal degree or formal training".

Burnham, Scripps, UCSB

Samir Mitragotri, Ph.D.*, of the University of California at Santa Barbara was given the Controlled Release Society Young Investigator Award.

Erkki Ruoslahti, M.D., Ph.D.*, of the University of California at Santa Barbara received the DoD Breast Cancer Program Innovator Award this past year.

Joseph Zasadzinski, Ph.D., was named a Fellow of the American Physical Society, Division of Biological Physics for 2009.

Matthew Tirrell, Ph.D., of the University of California at Santa Barbara was elected to the Indian National Academy of Engineering.

The Four PENs and their Principal Investigators

Nanotechnology: Detection & Analysis of Plaque Formation

Emory University
Georgia Institute of Technology

P.I. - Gang Bao, Ph.D.
Director, Emory-GT, Program of Excellence in Nanotechnology
CoE Distinguished Professor
The Wallace H. Coulter Department of Biomedical Engineering
Georgia Institute of Technology
Emory University

Nanotherapy for Vulnerable Plaque

The Burnham Institute
University of California Santa Barbara
The Scripps Institute

P.I. - Jeffrey W. Smith, Ph.D.
Professor
Director, Center on Proteolytic Pathways
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Translational Program of Excellence in Nanotechnology

Harvard University
Massachusetts General Hospital
Massachusetts Institute of Technology
Brigham and Women's Hospital

P.I. - Ralph Weissleder, M.D., Ph.D.
Director, Center for Molecular Imaging Research
Professor of Radiology, Harvard Medical School
Massachusetts General Hospital

Integrated Nanosystems for Diagnosis and Therapy

Washington University in Saint Louis
University of California Santa Barbara
University of California Berkeley

P.I. - Karen L. Wooley, Ph.D.
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

*Except where noted
Synthetic nanoparticles for noninvasive imaging of angiogenesis

Adah Almutairia, Raffaella Rossinb, Monica Shokeenb, Aviv Hagoolyb, Ashwin Ananthc, Dana R. Abendscheinc, Carolyn J. Andersonb, Michael J. Welchb, and Jean M. J. Fréchet1
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A biodegradable positron emitting dendritic nanoprobe targeted at $\alpha\beta_3$ integrin, a biological marker known to modulate angiogenesis, was developed for the noninvasive imaging of angiogenesis. The nanoprobe has a modular multivalent core-shell architecture consisting of a biodegradable heterobifunctional dendritic core chemoselectively functionalized with heterobifunctional polyethylene oxide (PEO) chains that form a protective shell, which imparts biological stealth and dictates the pharmacokinetics. Each of the eight branches of the dendritic core was functionalized for labeling with radiohalogens. Placement of radioactive moieties at the core was designed to prevent in vivo dehalogenation, a potential problem for radiohalogens in imaging and therapy. Targeting peptides of cyclic arginine-glycine-aspartic acid (RGD) motifs were installed at the terminal ends of the PEO chains, to enhance their accessibility to $\alpha\beta_3$ integrin receptors. This nanoscale design enabled a fifty-fold enhancement of the binding affinity to $\alpha\beta_3$ integrin receptors with respect to the monovalent RGD peptide alone, from 10.40 nM to 0.18 nM IC50. Cell based assays of the 125I-labeled dendritic nanoprobes using $\alpha\beta_3$ positive cells showed a six-fold increase in $\alpha\beta_3$ receptor mediated endocytosis of the targeted nanoprobe compared to the non-targeted nanoprobe, while $\alpha\beta_3$ negative cells showed no enhancement of cell uptake over time. In vivo biodistribution studies of 76Br-labeled dendritic nanoprobes showed excellent bioavailability for the targeted and non-targeted nanoprobes. In vivo studies in a murine hindlimb ischemia model for angiogenesis revealed high specific accumulation of 76Br-labeled dendritic nanoprobes targeted at $\alpha\beta_3$ integrins in angiogenic muscles, allowing highly selective imaging of this critically important process.

The nanoprobe reported here has increased selectivity for cells that express a specific integrin receptor, $\alpha\beta_3$, which serves as a biological marker for angiogenesis. These adhesive receptors are critical for the proliferation, survival and function of new blood vessels. Figure 1 shows representative microPET/CT slices of two mice injected with either the non-targeted (A) or targeted (B) dendritic nanoprobe at 24 h post injection. In both animals, the coronal slices show diffuse radioactivity in the abdominal area, due to urinary (Figure 1B) and hepatobiliary excretion. Remarkably, transaxial and sagittal slices obtained from the mouse injected with RGD-conjugated dendritic nanoprobe show accumulation of radioactivity in the entire thickness of the ischemic muscle, compared to the non-ischemic limb (Figure 1B). In contrast, no difference between the two limbs is visible in the sagittal slices from the mouse injected with non targeted dendritic nanoprobe (Figure 1A).

Angiogenesis, or the formation of new blood vessels, plays an important role in many human diseases such as weakening of the heart muscle (cardiomyopathy) or thickening and hardening of the arteries (atherosclerosis). Nanotechnology has the potential to revolutionize the diagnosis and treatment of these disorders.

The image- and biodistribution-derived ratios of probe accumulation (ratio of ischemic to non-ischemic limb, R/L) for $\alpha\beta_3$-targeted vs. non targeted dendritic nanoprobe in proximal and distal hindlimbs are depicted in Figure 2.

The R/L image ratio was markedly higher in mice that received the targeted dendritic nanoprobe compared to those injected with the non-targeted dendritic nanoprobe particularly in the area distal to the excised segment of femoral artery (Figure 2A).
These findings suggest specific interactions between the targeted nanoprobe and $\alpha_\beta_3$ integrins in angiogenic capillaries. In contrast to previous studies, our image analysis showed larger ratios for targeted compared with non-targeted nanoprobes. When the skeletal muscles were excised and the tissue radioactivity measured in a well counter, we found similar results to those obtained by micro PET (Figure 2B) and a strong linear correlation between the methods in all the mice tested ($R^2 = 0.84$). This further substantiates the use of small animal PET imaging to non-invasively quantify the presence of biological targets, such as those related to angiogenesis, in vivo. Additionally, we observed a slight increase in the ischemic/non-ischemic R/L ratio with the non-targeted nanoprobe in the distal hindlimb. This may reflect some non-specific retention of the non-targeted nanoprobe, perhaps due to interstitial activity associated with changes in vascular permeability or residual blood activity within the hypervascular ischemic hindlimb at 7 days post ischemic injury. Importantly, muscle sections from the ischemic hindlimb showed increased staining for $\alpha_\beta_3$ receptors compared with the non-ischemic hindlimb (Figure 2C).

**Conclusion**

Non-invasive molecular imaging sets forth not only to probe the molecular abnormalities that are the basis of disease rather than the end result, but to directly image non-invasively the effects of therapy in patients. Recently the effects of size, architecture, and topology of multivalent carriers, in addition to the density of ligands presented, have emerged as decisive factors in their ability to perform as molecular imaging agents. A novel design and a facile synthesis have provided us with a radiohalogen nanoprobe...
A PET nanoprobe showed a 50-fold enhanced binding affinity to \( \alpha_\beta_3 \) integrin receptors, known to modulate angiogenesis, with respect to the monovalent RGD peptide alone. Additionally we found a six-fold increase in \( \alpha_\beta_3 \) receptor mediated endocytosis of the targeted nanoprobe compared to the non-targeted nanoprobe. In vivo pharmacokinetic studies showed excellent bioavailability for the targeted and non-targeted nanoprobes. In vivo studies in a murine hindlimb ischemia model for angiogenesis revealed high specific accumulation of \( ^{80} \)Br-labeled dendritic nanoprobes targeted at \( \alpha_\beta_3 \) integrins in angiogenic muscles, allowing highly selective imaging of this critically important process. The modular nature of this nanoprobe allows tuning its blood circulation time, through changes in dendritic branching and PEO length, to suit a variety of radiohalogens used in both imaging and therapy. Thus we envision this nanoprobe could potentially have implications in both therapy and imaging.

"A novel design and facile synthesis have provided us with a radiohalogen nanoprobe capable of non-invasively imaging angiogenesis. This targeted PET nanoprobe showed a fifty-fold enhanced binding affinity to \( \alpha_\beta_3 \) integrin receptors, known to modulate angiogenesis."
Targeting micelles to plaques

Erkki Ruoslahti
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This project is a collaborative effort between two laboratories participating in the PEN program. The laboratory of Matthew Tirrell specializes in making and studying micelles. The advantages of micelles as nanoparticles are that they self-assemble from simple amphiphilic compounds and that the self-assembly process makes it easy to incorporate multiple functions into a single micelle. My laboratory develops peptides that recognize specific markers in the endothelium of diseased tissues, including cancer and atherosclerotic plaques. We (David Peters, with help from Ramana Kotamraju, Priya Karmali, Kunal Gujraty) combined forces with the Tirrell laboratory (Mark Kastantin), and the result was a multifunctional micelle for the detection of atherosclerotic plaques and for delivering a therapeutic agent to the plaques.

The targeting is based on the subtle clotting that occurs on the luminal surface of atherosclerotic plaques, which presents a novel target for nanoparticle-based diagnostics and therapeutics. We had previously identified 3 different peptides that recognize clotted plasma proteins in tumor vasculature and tumor stroma (Pilch et. Al., PNAS 103, 2800, 2006; Simberg et al., PNAS 104, 932, 2007). We surmised that there might be enough occult clotting on the surface of atherosclerotic plaques for these peptides to bind to the plaques. This prediction turned out to be accurate, and we chose one of them, a 5-amino acid peptide (CREKA, cysteine-arginine-glutamic acid-lysine-alanine) for the development of multifunctional, modular micelles that contain in the same particle the CREKA targeting element, a fluorophor for imaging purposes and, when desired, a drug component.

We used atherosclerotic plaques in ApoE null mice fed a high fat diet as the target to test the CREKA micelles. Intravenously injected CREKA micelles bound to the surface of the plaque and were readily detected by fluorescent imaging of isolated aortic tree and microscopy of tissue sections. In contrast, there was no detectable binding to normal looking endothelium of the same vessels, or of vessels in normal mice, and non-targeted micelles did not bind to the plaques. Other controls also confirmed the specificity of the CREKA micelle binding. Of potential importance, the CREKA micelles appeared to concentrate at the shoulders of the plaque, a location where plaques are prone to rupture. We also incorporated an anticoagulant drug, hirulog, into the CREKA-targeted micelles and showed that the targeted micelles delivered an increased concentration of the drug to plaques when compared to untargeted micelles.

In tumors, iron oxide particles coated with the CREKA peptide caused additional clotting that occludes some tumor vessels. Although this effect was not seen in the liver, which non-specifically accumulates a high concentration of injected nanoparticles, the possibility that the CREKA micelles might increase the clotting over the plaques was of concern. However, the CREKA micelles showed no clotting activity in tumors, and their homing to the plaques was not inhibited by heparin treatment of the mice, indicating that the CREKA micelles do not have procoagulant activity and that they target pre-existing clotted material in the plaques.

Our results show that the modularity inherent to our micellar nanoparticle platform allows multiple functions to be built into a nanoparticle allowing selective delivery a diagnostic imaging dye and a therapeutic compound to atherosclerotic plaques in vivo. The targeting should allow lowering of the doses of drugs, such as the anticoagulant, resulting in reduced side effects in normal tissues. We are in the process of publishing these results.
Meet our PEN

Harvard University
Massachusetts General Hospital
Massachusetts Institute of Technology
Brigham and Women’s Hospital

Principal Investigator
Ralph Weissleder

Program Official
Denis Buxton

Our Students, Postdocs and Staff Scientists

Scott A. Hilderbrand, Ph.D.
Fangwei Shao, Ph.D.

Hakho Lee, Ph.D.
Tae-Jong Yoon, Ph.D.

Peter Libby, M.D.
Thomas Christen, M.D., Ph.D.
Koichi Shimizu, M.D., Ph.D.
Change on the Horizon: A chronological perspective of the Washington University-based PEN Skills Development Component

By Monica Shokeen and Kenya T. Powell
Photos by Eileen A. Cler and Kenya T. Powell

Looking Back:
Skills Development from 2005-2008

In 2005, The Washington University (WU)-centered Skills Development Component (SDC)† was formed under the leadership of Principal Investigator Professor Karen L. Wooley and the direction of component leader Professor Carolyn J. Anderson. Overall, the goals of the SDC were to (1) train PEN personnel in the field of nanomedicine, (2) create nanotechnology-based outreach programs, and (3) promote awareness of the WU-based PEN among the general and scientific communities (Figure 1). In the fall of 2006, two postdoctoral associates joined the SDC, Drs. Monica Shokeen and Kenya T. Powell, and with Wooley and Anderson, set about the task of addressing the SDC’s global objectives.

COURSE DESIGN

The WU-centered PEN project is intended as Integrated Nanosystems for Diagnosis and Therapy, in which novel, nanoparticle constructs are evaluated in vitro and in vivo by using a variety of characterization tools, including sensitive imaging. As a result, the courses were devised to provide a careful examination of the syntheses, formation, characterization, and performance of inorganic, organic, and polymeric nanosystems, incorporating the principles of their design and applications, and their implications in the biological, medical, and imaging sciences. The initial effort, Special Topics in Organic Chemistry: Nanomedicine (CHEM555), led by Wooley as course master, was a remote learning, technology-intensive pilot course developed to fulfill the goal of PEN personnel training, but also to promote consciousness of the many advances in and advantages of nanotechnology among science and non-science students.

The successful employment of CHEM555 depended heavily on the construction of an interactive learning classroom built by the unification of three essential components: a cyberinfrastructure, learner-centered practices, and course content derived from current advances in nanomedicine. Web-based resources Telesis, WebCT™, and RealVNC™ were combined with teleconferencing, allowing students enrolled at three different universities to experience lecture in real time and to access and retrieve course-related materials easily and independently. Furthermore, students were encouraged to be active participants in and equally responsible for their learning gains. For example, students were given the assignment of designing a novel nanoprobe based upon the concepts covered throughout the course or to present findings on recent literature related to nanomedical fields.

To further enhance the interactive nature of the course, top experts in nanomedicine gave instruction based upon their most current results in their specific areas of expertise, a strategy that took advantage of the existing pool of renowned chemistry and imaging faculty in the PEN. Gang Bao (Georgia-Tech, PEN), John-Stephen A. Taylor (WU, PEN), Dana R. Abendschein (WU, PEN), Samuel I. Achilefu (WU, PEN), Michael J. Welch (WU, PEN), and others participated in this effort.

The SDC is a part of the National Heart Lung and Blood Institute (NHLBI) of the National Institutes of Health’s (NIH) Program of Excellence in Nanotechnology (PEN).
**MENTORING**

A considerable portion of the SDC’s efforts are devoted to training students to explore and master the latest advances in nanomedicine using inventive course designs, but also by way of real, hands-on experiences. Over the course of the last three years, this specialized, one-on-one training has taken place for several undergraduate students in the academic laboratories of PEN senior investigators (Figure 2). These mentorships have resulted in the formulation of new protocols for ascertaining the efficiency of synthetic nanoparticles in complex biological systems, a Master’s thesis based upon the in vitro evaluation of dendritic nanoparticles for biomedical applications, and presentations at interdepartmental conferences and seminars.

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**OUTREACH**

In the fall of 2006, a pilot outreach program addressed the SDC’s aim to extend the reach of the WU-centered PEN into the general community and further raise awareness of the latest innovations in nanotechnology. Collaborating with educators from Ritenour Middle School in Saint Louis, MO, the SDC designed a hands-on, student-centered, after-school program entitled “The Cutting Edge of Nanotechnology: The interplay between robotics and polymer chemistry”. In addition to teaching middle school students the basics of robotics programming, introductory laboratory techniques, and the fundamentals of polymer chemistry and nanotechnology, the nine-week course was careful to compliment the Missouri standard curriculum.

Following the success of the SDC’s first kinesthetic outreach program, similar collaborations were subsequently formed. In most cases, the ensuing events were built on elementary or common concepts of nanotechnology, encompassing topics such as atomic force microscopy, the chemistry of fireworks, and dynamic light scattering. The SDC deliberately varied its outreach initiatives, partnering with different community and campus educators and teaching students of various age groups, in order to service as wide a spectrum of students and teachers, at as many academic levels as possible, increasing the range and scope of the WU-centered PEN’s dissemination efforts (Table 2).

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**Table 1:** Highlight of the Special Topics Courses designed by the Skills Development Team from 2006-2008.

<table>
<thead>
<tr>
<th>Course Title</th>
<th>Course Objective</th>
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<tbody>
<tr>
<td>Fall 2006: Special Topics in Organic Chemistry Nanomedicine</td>
<td>Fundamental Topics, Rational Design, and Applications</td>
</tr>
<tr>
<td>Fall 2007 and 2008 Current Topics in Nanomedicine</td>
<td>Seminar Style Course on Current Nanomedicine Topics: “Biomedical applications of nanotechnology”</td>
</tr>
<tr>
<td>Spring 2007 and 2008 Contrast Agents for Biological Imaging (CABI)</td>
<td>Principles of chemistry and biology for designing contrast agents in imaging applications such as nuclear medicine, magnetic resonance imaging (MRI) and optical imaging</td>
</tr>
<tr>
<td>Fall 2007 and 2008 Principles and Applications of Biological Imaging (PABI)</td>
<td>Introduce the interdisciplinary nature of the imaging sciences and conduct a comprehensive survey of the array of interrelated topics that define biological imaging</td>
</tr>
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**Table 2:** A sampling of the outreach and educational programs developed and implemented by the SDC Team from 2006-2008.

<table>
<thead>
<tr>
<th>Program/Objective</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>Fall 2007: Homer G. Phillips. Focus on Basic Research (One credit-hour class offered by the College of Arts &amp; Sciences; designed for freshmen in a research-based career track)</td>
<td>Fostered discussion, critical thought, and personal and professional growth; highly evaluated (12 students)</td>
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<tr>
<td>April 2008: Take Our Daughters and Sons to Work Day: Chemistry of Fireworks (Designed to illustrate the chemical reactions responsible for producing fireworks colors)</td>
<td>Students learned history, chemistry of fireworks; did flame tests (~40 students)</td>
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<tr>
<td>May 2008: Annual Wilson School Event: “How to See Invisible Things” A DLS Experiment for 2nd Grade Students (A Dynamic Light Scattering (DLS) micromodule was used to illustrate how scientists utilize instruments for characterization)</td>
<td>Students learned fundamentals of nanotechnology; made and “saw” nanoparticles in the laboratory (~20 students)</td>
</tr>
<tr>
<td>September 2008: ACS Younger Chemists Committee (STL): Careers in Chemistry Night (Presentations by a diverse selection of chemistry professionals; question and answer session; networking reception)</td>
<td>New memberships into ACS and YCC; new community, academic, and industry partnerships (~100 attendees)</td>
</tr>
</tbody>
</table>
I’m junior at Washington University in St. Louis. I am majoring in Chemistry with a minor in Jazz Studies and African and African-American Studies. I am originally from Middleton, WI, and would like to go to Medical School after graduating from Washington University. As a sophomore I worked with Monica Shokeen on competitive plate binding assays on difference nanoparticles. I learned a lot about different types of nanoparticles and their use for imaging and treatment. I will continue my work screening different nanoparticles this semester. While working in the lab, I learned many valuable lab techniques and enjoyed working with everyone.

I am currently a senior at the University of Southern California. I am majoring in Biological Sciences and have two minors: Theatre and Psychology and Law. I have been accepted into a program for Teach for America, so after I graduate, I plan to teach high school biology in Baltimore, Maryland, for two years. I have also applied for a Fulbright grant to study Aboriginal diabetes and cardiovascular disease in Australia. After either or both of those programs, I plan to attend medical school. During my two summers at Washington University, I have not only learned about various imaging techniques but also how those techniques can be applied to various diseases (specifically, metastatic breast cancer). I also did a lot of work with nanoparticles and learned how they can be used as targeting devices for imaging and treatment.

I am currently a senior at Washington University in St. Louis where I am majoring in Biology and Anthropology. After graduating in May, I look forward to a relaxing back home in Fort Wayne, Indiana, before I head off to medical school (not yet decided where) next Fall. My time in the lab has been very valuable in that it has given me a concrete framework in which I was able to further explore the concepts and ideas that were merely introduced in my coursework. Additionally, the lab has provided me with a very accommodating learning environment where my mentors are always available for questions and support. As such, my time here has been an integral and enjoyable component of my undergraduate experience.

Figure 2: Undergraduate students Jessica Wilson (top), Tyler Mains (middle), and Nikko Ramos (bottom) detail their experience receiving one-on-one training by PEN faculty and SDC members.

WORKSHOPS & SEMINARS

As part of the Skills initiative aimed at promoting research on the cutting-edge of nanomedicine, the WU-PEN hosted a 1.5 day symposium in collaboration with the National Cancer Institute (NCI) entitled “Nanotechnology & the Life Sciences Workshop” in March of 2007. The workshop consisted of oral and poster sessions presented by faculty, students, and clinicians who gathered to address the current state of scientific research in the nanotechnologies.

Additionally, monthly seminars were organized and attended by students, faculty, and PEN personnel featuring prominent scientists in the area of nanomedicine. Among those who gave Skills-sponsored seminars in 2008 were Professor George W. Gokel (University of Missouri, Saint Louis, UMSL); Chancellor Thomas F. George (UMSL); Professor Renat R. Letfullin (Rose-Hulman Institute of Technology); Professor Sangeeta N. Bhatia, MD (Massachusetts Institute of Technology); Professor Kenneth N. Raymond (University of California, Berkeley); Professor Maulik R. Shah (Saint Louis University); Dr. Robert A. Beardsley (President and CEO Kereos, Inc.). These diverse presentations provided a platform that allowed an inter-disciplinary group of scientists and students to hold discussions, engage in networking, and initiate new and exciting collaborations, among others. Moreover, the workshop and seminars served to raise awareness and promote interest of the promises of nanotechnology among the general and medical communities.

Recognition

“In the area of education and dissemination (SKILLS), the Washington University PEN is outstanding. The development, delivery, and favorable reception of several new courses were very impressive, as were the outreach activities. The EAB lauds the Washington University PEN for developing novel tools for training young scientists in the imaging sciences and for raising awareness of the young public for the nature and benefit of the science of nanoparticles. The EAB further suspects that these outstanding efforts are unequalled within the PEN program nationally and likely within any other major federally supported research program.”

EAB (External Advisory Board), NHLBI (NIH) Program of Excellence in Nanotechnology, July 24, 2008.

Looking Ahead:

Exciting new advances in Skills Development

Dr. Shokeen plays an integral role in projects that are significant to both AVI (Acute Vascular Injury) component, as well as SDC of the WU-centered PEN program. During her postdoctoral career, she has introduced the use of nanotechnology to the Radiological Department at Mallinckrodt Institute of Radiology (MIR), developing methods to evaluate novel, molecularly targeted nanoparticles in cell-based assays. Her efforts have led to a co-authorship on a recent paper in PNAS.

The SDC gratefully acknowledges WU-PEN Program Coordinator and Photographer, Eileen A. Cler; IT Specialist, James Kozlowski; and Media Coordinator, Christopher Sherman for their dedication and service.
Currently, Dr. Shokeen is engaged in work that will further highlight the significance of in vitro screening methods in understanding the structure-activity relationships in biologically significant nanoparticles. As a member of SDC, Dr. Shokeen is developing new methodologies for incorporating the latest technological innovations and teaching modalities into the modern classroom, including the use of video-conferencing in place of teleconferencing to better accommodate remote learners, web resources with more interactive user interfaces and features to promote a more enriched and engaging experience for students, and empirically-derived, active learning strategies to maximize student learning gains. Furthermore, she continues to assist in designing special topics courses related to PEN interests, taking a leadership role in curriculum development, lecturing, writing exams, and coordinating course logistics. As an extension of similar efforts, Dr. Shokeen is also deeply involved in the Imaging Sciences Pathway program at Washington University School of Medicine.

Her long-term goal is to further her efforts in both research and teaching in nanotechnology and the imaging sciences with lasting endurance, tapping into nurturing opportunities that would improvise these efforts. Specifically, she seeks to continually enrich her research career via the development of new ways of investigating nanoparticles for biological and imaging applications, one-on-one laboratory mentoring, nanotechnology-based course creation, and exciting K-12 outreach. Dr. Shokeen is entertaining several prominent research-based teaching professorships that would allow her to exploit the unique and expansive skill set she has acquired as a result of her work in the SDC.

Dr. Powell was pleased to accept an appointment as Science Education Researcher at Washington University. Extending from the Science Outreach Office directed by Asst. Dean Victoria L., the appointment involves the formation of science outreach programs and courses emphasizing cutting-edge research. Furthermore, it is heavily dependent upon partnerships with WU faculty, students, and community educators and is resultant from her work in the SDC of the WU-centered PEN program. The appointment is designed to be both inclusive and adaptive, with the acquisition of supplemental grant support catalyzing its evolution over time.

Dr. Powell’s research now encompasses the use of ethnographic, qualitative evaluations of the classroom phenomena effecting student achievement. Specifically, she is designing an observation matrix that allows classroom participants to diagnose, monitor, and mediate the unfavorable, oft times silent, interpersonal overtures that can hinder maximized learning gains.

In addition to developing a science outreach laboratory and conducting research, she’s excited to assist the Science Outreach Office in integrating the Department of Chemistry into WU’s outreach infrastructure, providing advice, direction, and/or leadership in the outreach component(s) of federally funded grants, and creating training opportunities for chemistry students wishing to incorporate science education into their undergraduate or graduate studies.

The accomplishments of the WU SDC have demonstrated a pathway by which the tenets of innovation, creativity, and engagement can be converted into pioneering and independent careers (Figure 3). More importantly, these undertakings present a clear picture of the inherent value of skills development, including its transformative power to positively affect the lives of individuals, programs, institutions, communities, and nations, and its prospects as a long-lived and vital component of the future of STEM education. We hope our outreach activities will inspire other PENs to expand their efforts in these important and rewarding endeavors.

Figure 3: Drs. Shokeen (left) and Powell (right) celebrate their new positions, the changes taking place in STEM education, and the re-energized directions of the WU-PEN Skills Development Component.

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4th ANNUAL INTER-PEN MEETING
Massachusetts General Hospital, Boston, Massachusetts October 16-17, 2009

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October 16-17, 2009

4th Annual Inter-PEN Meeting

Massachusetts General Hospital
Center for Systems Biology
185 Cambridge Street, Simches Research Bldg.
3rd floor Conference Center
Boston, Massachusetts 02114

Hosted by Ralph Weissleder

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