

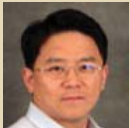
NHLBI **Inter-PEN Quarterly
Newsletter**



EILEEN A. CLER

PEN members in attendance at the 4th Annual Inter-PEN Conference, at Massachusetts General Hospital, in Boston, Massachusetts, on October 16-17, 2009.

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Program of Excellence in Nanotechnology

The final quarter of the original PEN grants

The next quarter will be the final quarter of the current PEN Program, which has supported four centers via a grant mechanism.

The final newsletter that is to be published May 1, 2010, will highlight the most significant accomplishments made by the four PENs. The PEN Programs are expected to change substantially with the awarding of new PEN centers over the next five years, due to shifts in research *foci* and an emphasis on clinical translational work, with support via a contract mechanism.



Please see the back cover of this issue for a collection of images documenting our annual Inter-PEN Meetings from 2006-2009, at each of the four hosting PEN locations.

PEN



Karen L. Wooley, Ph.D.
Principal Investigator
W. T. Doherty-Welch Chair
Professor of Chemistry
Texas A&M University

EMORY UNIVERSITY, GEORGIA TECH

Nanotechnology: Detection & Analysis of Plaque Formation



Transplanting cells that replenish blood vessels can also restore nerve function in an animal model of diabetic neuropathy

Jin-Ok Jeong, Mee-Ohk Kim, Hyongbum Kim, Min-Young Lee, Sung-Whan Kim, Masaaki Ii, Jung-uek Lee, Jiyoung Lee, Yong Jin Choi, Hyun-Jai Cho, Namho Lee, Marcy Silver, Andrea Wecker, Dong-Wook Kim, Young-sup Yoon
gang.bao@bme.gatech.edu

About 60 percent of people with diabetes have some form of neuropathy, damage to the peripheral nerves that can cause a loss of sensation in hands, arms, feet or legs. The damage, caused by high blood sugar, occurs gradually and in advanced cases can lead to amputation. Scientists have connected the damage to problems with peripheral nerves' blood supply.

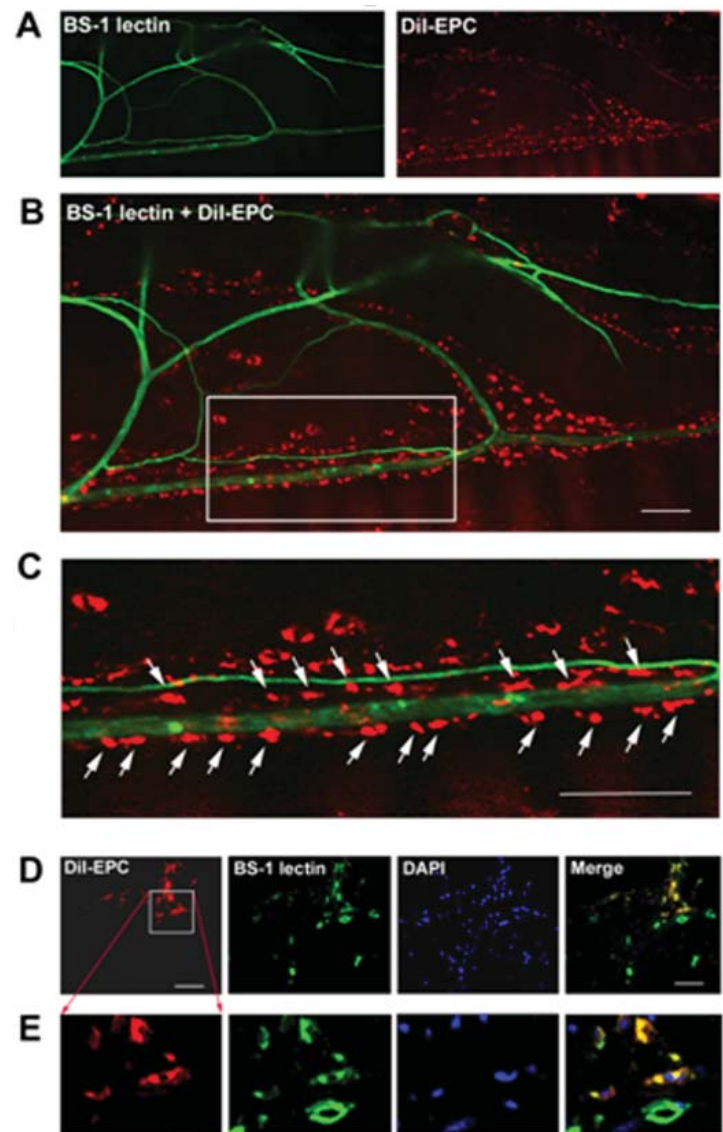
Cultured cells from the bone marrow can promote the regrowth of both blood vessels and the protective lining of nerves in the limbs of diabetic animals, a team led by Young-sup Yoon, M.D., Ph.D., associate professor of medicine (cardiology) at Emory University School of Medicine, found.

Despite a continuous increase in the incidence of diabetes and diabetic neuropathy, current treatments have yet to effectively treat diabetic neuropathy. Our group reported that experimental diabetic neuropathy is characterized by reduced microcirculation in peripheral nerves due to the destruction of the vasa nervorum, and thus administration of angiogenic factors such as vascular endothelial growth factors (VEGFs), and sonic Hedgehog (SHh) could restore neural function by augmenting angiogenesis. Also, deficiency of neurotrophic factors is regarded as one of the most plausible mechanisms underlying diabetic neuropathy. Alterations of nerve growth factor (NGF), ciliary neurotrophic factor, and brain-derived neurotrophic factor have been reported. However, in clinical trials, single neurotrophic cytokines turned out to be ineffective for treating diabetic neuropathy. Recently, many classical angiogenic factors were shown to possess neurotrophic activities and vice versa. VEGF, insulin-like growth factor (IGF)-1 and neurotrophins are some of the representative factors having these dual effects. Since diabetic neuropathy lacks both angiogenic and neurotrophic factors, using a therapeutic agent which has dual angio-neurotrophic activities may prove more beneficial for treating diabetic neuropathy.

Bone marrow is thought to contain endothelial progenitor cells (EPCs), which can divide into endothelial cells, forming a "patch" for damaged blood vessels.

EPCs contain high amount of angiogenic and neurotrophic cytokines that these effects may benefit to recover damaged peripheral nerve function and integrity.

Yoon's team cultured bone marrow cells in a way designed to enrich them for



Localization of transplanted EPCs along the vasa nervorum and trans-differentiation of EPCs into endothelial cells in nerves. A, B, C Whole mounted images of a sciatic nerve demonstrated that engrafted EPCs (red) were preferentially localized along the course of the vasa nervorum (green). Bars, 50 μ m in B and C. D, E, Cross sectional images of a diabetic nerve 4 wks after injection with EPCs revealed that a portion of the engrafted EPCs was colocalized with BS-1 lectin-positive endothelial cells suggestive of transdifferentiation of EPCs into endothelial cells. Bars, 50 μ m (D), 10 μ m (E).



“Alterations of nerve growth factor (NGF), ciliary neurotrophic factor, and brain-derived neurotrophic factor have been reported. However, in clinical trials, single neurotrophic cytokines turned out to be ineffective for treating diabetic neuropathy. Recently, many classical angiogenic factors were shown to possess neurotrophic activities and vice versa.”

EPCs and injected them next to the sciatic nerves of diabetic mice. The sciatic nerve is a large nerve that runs from the back to the rear leg. The mice were made diabetic by giving them streptozocin, a drug that poisons insulin-producing cells in the pancreas.

Endothelial progenitor cells—known to restore blood vessels—can halt neuropathy and restore nerve function in an animal model of diabetic neuropathy. The team found that over several weeks, nerve signal speed and sensitivity to temperature were restored to normal in diabetic mice injected with the bone marrow cells.

The researchers found that the restoration of nerve function occurred despite the fact that only a portion of the bone marrow endothelial progenitor cells actually became endothelial cells. These cells secrete molecules that stimulate the growth of both endothelial cells and Schwann cells, which protect and insulate peripheral nerves. The researchers also found that the cells appeared to “home” in on peripheral nerves, an attribute that makes their eventual application in humans even more promising.

Bone marrow-derived EPCs have also been used in studies of heart muscle repair after heart attack. However, most previous studies indicate that they disappear from the heart muscle after a few weeks.

“We were surprised to find that in this specific environment, they engraft and survive longer than in other tissues,” Yoon says. “These cells appear to home to peripheral nerves.”

Reference:

1. J.O. Jeong *et al.* “Dual angiogenic and neurotrophic effects of bone marrow-derived endothelial progenitor cells on diabetic neuropathy.” *Circulation*, **2009**, *119*: 699-708.

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About the Authors

Sungwhan Kim, Ph.D.

Postdoctoral Fellow
Tufts University
Emory University



Hyongbum Kim, M.D., Ph.D.

Postdoctoral Fellow
Tufts University
Emory University



Min-Young Lee, M.S.

Graduate Technician
Emory University



Young-sup Yoon, M.D., Ph.D.

Senior Investigator
Formerly of Tufts University
Director of Stem Cell Biology
Division of Cardiology
Department of Medicine
Emory University
School of Medicine



(not pictured)

Jin-Ok Jeong, M.D., Ph.D.

Postdoctoral Fellow, Tufts University

Mee-Ohk Kim, M.D., Ph.D.

Research Associate, Tufts University & Emory University

Masaaki Ii, M.D., Ph.D.

Collaborating Investigator, Tufts University

Jung-uek Lee, M.S.

Graduate Technician, Tufts University

JiYoon Lee, M.S.

Research Specialist, Tufts University & Emory University

Yong Jin Choi, M.S.

Research Specialist, Tufts University & Emory University

Hyun Jai Cho, M.D., Ph.D.

Postdoctoral Fellow, Tufts University

Namho Lee, M.D., Ph.D.

Postdoctoral Fellow, Tufts University

Marcy Silver, B.S.

Technician, Tufts University

Andrea Wecker, B.S.

Technician, Tufts University

Dong Wook Kim, Ph.D.

Collaborating Investigator, Yonsei University, Korea

BI, BWH, MGH, HARVARD, MIT

Translational Program of Excellence in Nanotechnology



Binding Affinity and Kinetic Analysis of Targeted Small Molecule-Modified Nanoparticles¹

By Jason McCarthy and Ralph Weissleder
weissleder@helix.mgh.harvard.edu
jason_mccarthy@hms.harvard.edu

The targeting of nanomaterials to sites of interest is a non-trivial matter. In order to facilitate this, researchers have utilized a multitude of affinity ligands, including antibodies, aptamers, and peptides. One of the main advantages of nanomaterials is that they allow for the multivalent display of targeting ligands, which can serve to increase the affinity of the nanoagent for its target. One class of targeting ligands that is just beginning to find utility are non-natural product small molecules. These species allow for a larger portion of the chemical space to be utilized for targeting, while simultaneously enabling the synthesis and screening of libraries of small molecule modified nanoparticles. The main question that must be answered is how to best characterize the binding of the resulting functionalized nanoparticles with its target.

degree of functionalization. For each ligand, low (3-5 small molecules per particle) and high-loading (13-18 small molecules per particle) species were synthesized for comparison. Importantly, initial investigation focused upon the loading density of the FKBP12-GST (glutathione S-transferase) fusion protein immobilized on the SPR sensor chip. Interestingly, with loadings typically utilized SPR experiments, the small-molecule modified nanoparticles did not show appreciable dissociation, even after extended periods of time (12 min). The authors therefore decreased the protein loading to lower densities (1 protein per 648 nm²), which closer mimics physiologically relevant receptor densities.

“These species allow for a larger portion of the chemical space to be utilized for targeting, while simultaneously enabling the synthesis and screening of libraries of small molecule modified nanoparticles. The main question that must be answered is how to best characterize the binding of the resulting functionalized nanoparticles with its target.”

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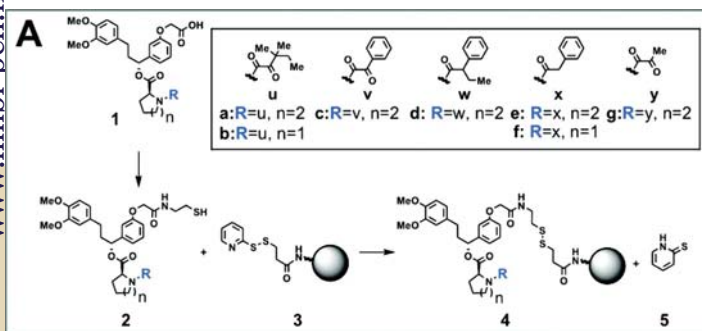


Figure 1. Conjugation of a series of synthetic derivatives of FK506 (1) by sulfhydryl exchange.

To this end, Tassa *et al.* have investigated the use of surface plasmon resonance (SPR) to study the affinity and binding kinetics of small molecule modified nanoparticles.¹ In order to facilitate direct comparisons, the authors investigated a series of structurally related ligands for the same protein, FKBP12, with dissociation constants (K_D) over a 4500-fold range. In these experiments the small molecules were conjugated to a dextranated iron oxide nanoparticle, CLIO, via sulfhydryl exchange. The initially N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) modified particles release pyridine-2-thione upon reaction with the thiol-modified small molecules, which can be monitored spectrophotometrically, and allows for the determination of the

For each small molecule and their respective conjugates, the association rate k_a , the dissociation rate k_d , and the dissociation constant K_D (k_d/k_a) were calculated. Interestingly, all conjugates demonstrated significant decreases in k_d , whereas changes in k_a varied greatly. As is demonstrated in Figure 2, the multivalent attachment of small molecule ligands to the surface of the nanoscaffold results approximately the same K_D , yet they display markedly different kinetics. The authors subsequently examined the multivalent effect by looking at the enhancement factor β , which is equivalent to the ratio of the K_D for the functionalized nanoparticle to that of the free ligand, which resulted in β values from 4 to 9500. For example, exceedingly weak

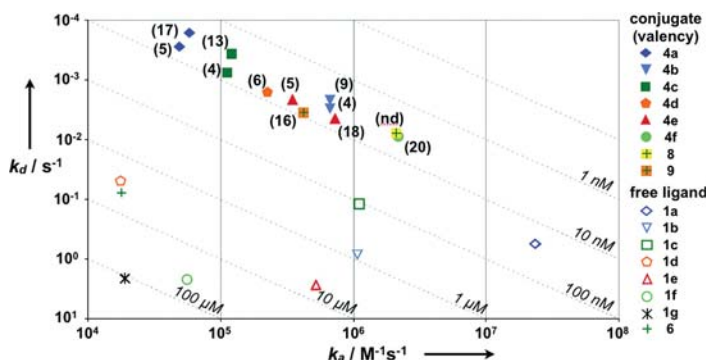
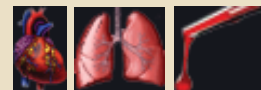


Figure 2. Rate maps summarizing binding affinity and kinetics. Different combinations of k_a and k_d that result in the same K_D are indicated by dashed lines. Data for free ligands are depicted by open symbols; for each ligand, the corresponding nanoparticles are depicted by a solid symbol of the same shape, with conjugation valency listed in parentheses next to the solid symbol. Valency of 8(nd) was not determined.

ligand 1f displayed a $39 \mu\text{M}$ K_D as a free ligand, which increased to 4.15 nM upon conjugation to CLIO (20 ligands per particle).

The authors conclude that this approach is general and can be applied to rapidly screen nanomaterials, as it does not require the development of cell-based assays for each target. Thus, its systematic implementation may enable the guidance of nanomaterial design.

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About the Authors

Carlos Tassa, Ph.D.

Research Scientist
Center for Systems Biology
Massachusetts General Hospital
Harvard Medical School



Jason R. McCarthy, Ph.D.

Senior Investigator
Center for Systems Biology
Massachusetts General Hospital
Harvard Medical School



Ralph Weissleder, M.D., Ph.D.

Principal Investigator
Director, Center for Systems Biology
Massachusetts General Hospital
Professor of Radiology and Systems Biology
Harvard Medical School



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
Director, Program of Excellence in Nanotechnology
Burnham Institute for Medical Research



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 Systems Biology
Massachusetts General Hospital
Professor of Radiology and Systems Biology
Harvard Medical School



Integrated Nanosystems for Diagnosis and Therapy

Texas A&M University
Washington University in Saint Louis
University of California Santa Barbara
University of California Berkeley
University of Texas, Southwestern Medical Center at Dallas

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

W. T. Doherty-Welch Chair
Professor of Chemistry
Texas A&M University



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AWARDS

Recognition (honors, appointments, awards) received by members of the four PENs

By Eileen A. Cler, B.S.

WUSTL, TAMU, UCB, UCSB, UTSW



Jasmine N. Hunt, Ph.D. Candidate, of Craig J. Hawker's laboratory at the University of California at Santa Barbara, received a National Science Foundation Integrative Graduate Education and Research Traineeship (IGERT) fellowship.



Ke Zhang, Ph.D., of Karen L. Wooley's laboratory has accepted a postdoc position at Northwestern University, Department of Chemistry, in the laboratory of Chad A. Mirkin.



Guorong Sun, Ph.D., of Karen L. Wooley's laboratory, has accepted a postdoctoral fellow position at Washington University in the Department of Biomedical Engineering, in the laboratory of Professor Younan Xia.



Zicheng Li, Ph.D., of Karen L. Wooley's laboratory, has accepted a postdoctoral fellow position at University of Texas at Austin, in the Department of Chemistry, in the laboratory of Professor Christopher W. Bielawski.

Kyle S. McCommis, B.S., a Research Technician in Pamela K. Woodard's laboratory at the Washington University School of Medicine, won 2nd place in the American Heart Association, Cardiovascular Radiology and Intervention (CVRI) Young Investigator's award.



Ashley L. Fiamengo, Ph.D. Candidate (not pictured), of Carolyn J. Anderson's laboratory, has accepted a postdoctoral researcher position at the University of Pennsylvania in the Department of Chemistry, in the laboratory of Ivan J. Dmochowski.

Emory, Georgia Tech



Allison M. Dennis, Ph.D., of Gang Bao's laboratory, has accepted a postdoc position at the Los Alamos National Laboratories (LANL) in Los Alamos, New Mexico.



D. May Wang, Ph.D., Two bioinformatics tools (caCORRECT and omniBiomarker) we developed at Georgia Tech has received silver level compatibility certification from National Cancer Institute (NCI) cancer Biomedical Informatics Grid (caBIG). This work has been cited by 20+ news media around the country.



Mark M. Goodman, Ph.D., Professor of Radiology, received the "Emory Office of Technology Transfer Innovation of the year 2008" award.

Harvard, MGH, MIT, BWH



Peter Panizzi, Ph.D., of Dr. Matthias Nahrendorf's laboratory has received a Young Investigators Award from the XXII Congress of the International Society on Thrombosis and Haemostasis for his recent work entitled "Atherosclerosis-associated Ly-6Chi Blood Monocytosis Impairs Wound Healing in Mice with Myocardial Infarction." This work will appear in an upcoming issue of the *Journal of the American College of Cardiology*. Co-authors include Drs. Nahrendorf, Pittet, Swirski, Libby and Weissleder.



Late breaking news in the field of Nanotechnology

Exciting new ideas and/or research in the field of nanotechnology on unpublished data

By Eileen A. Cler, B.S.

Emerging Breakthroughs



Gang Bao

Dr. Charles D. Searles' group is working on the use of molecular beacons and other nucleic acid probes to detect mRNA and miRNA in RNA isolates and in live cells. We are examining various modifications that will significantly enhance the sensitivity of our probes but will maintain their specificity and overall simplicity of use. These modifications include creation of nucleic acid-iron nanoprobe and biotinylated molecular beacons. We think that these new approaches will facilitate the development of *in vitro* assays for detection of changes in gene expression that are diagnostic of atherosclerotic disease progression.



Charles D. Searles



Ralph Weissleder

Dr. Scott A. Hildebrand and his group have furthered the utility of their oxazine-based hypochlorous acid activated nanoparticles by chemically modifying them to shift their absorption and emission spectra further into the near infrared, and also enabling their therapeutic potential.



Scott A. Hildebrand



Karen L. Wooley

A collaborative team of researchers including Drs. Wooley, Youngs (University of Akron) and Cannon (UTSW) have demonstrated *in vivo* activity of once-daily, nebulized SCK nanoparticles core-loaded with silver carbene complexes and shell loaded with silver cations in a mouse model of *Pseudomonas aeruginosa* pneumonia. The nebulized silver-loaded SCK nanoparticles proved as efficacious as twice-daily nebulized tobramycin for inhalation and significantly more efficacious than 500 fold higher doses of free silver carbene delivered twice-daily. Further development of these unique nanoparticle systems for treatment of lung infectious diseases is underway.



Carolyn L. Cannon



Wiley J. Youngs

WUSTL, TAMU, UCB, UCSB, UTSW

Integrated Nanosystems for Diagnosis and Therapy



Shell crosslinked nanoparticles carrying silver antimicrobials as therapeutics

Yali Li, Khadijah Hindi, Kristin M. Watts, Jane B. Taylor, Ke Zhang, Zicheng Li, Yun Lin, David A. Hunstad, Carolyn L. Cannon, Wiley J. Youngs, and Karen L. Wooley
Carolyn.Cannon@UTSouthwestern.edu, wooley@mail.chem.tamu.edu

Silver compounds are widely used as antimicrobial agents, especially in the treatment of wounds and burns. Silver cation (Ag^+) is highly toxic, or described as “oligodynamic”, against a broad spectrum of microorganisms, probably because of its inhibition of certain oxidative enzymes, protein denaturation, or interference with DNA replication. Unlike traditional antibiotics, Ag^+ is of low toxicity to human tissues and has elicited only rare instances of bacterial silver resistance.¹ A variety of silver-based antimicrobials has been synthesized and evaluated. Over the past decade, an array of silver *N*-heterocyclic carbene (NHC) complexes, which exhibit improved stability to light and aqueous solution, have been synthesized and investigated by Youngs and Cannon as potential antimicrobial agents and have shown very promising results with both *in vitro* and *in vivo* studies in a variety of bacteria including BSL3 organisms.^{2,3} Small molecule antibiotics also have a major problem, however, that of rapid clearance from the human body and, in the case of silver, reaction with sulfur containing proteins and chloride in the bloodstream. Therefore, there remains a need for packaging and protection for therapeutic delivery of Ag^+ or silver carbene complexes (SCCs).

"A variety of silver-based antimicrobials has been synthesized and evaluated. Over the past decade, an array of silver *N*-heterocyclic carbene (NHC) complexes, which exhibit improved stability to light and aqueous solution, have been synthesized and investigated by Youngs and Cannon as potential antimicrobial agents and have shown very promising results with both *in vitro* and *in vivo* studies in a variety of bacteria including BSL3 organisms.^{2,3}"

In this study, we developed shell crosslinked knedel-like (SCK) nanoparticles,⁴ as an antimicrobial device, designed to encapsulate and protect

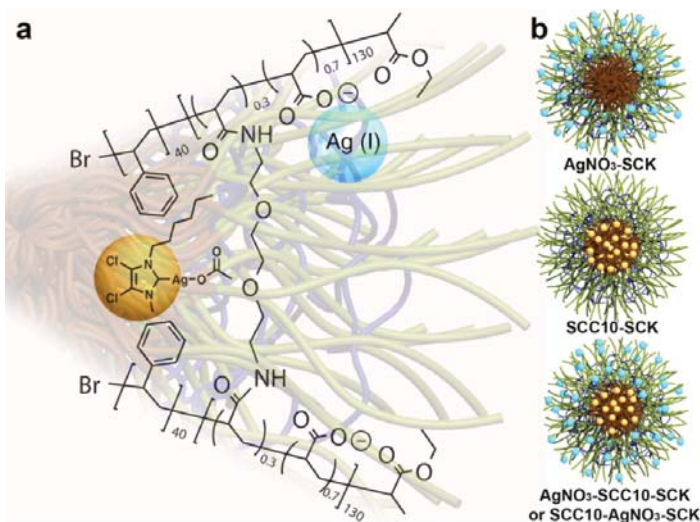


Figure 1. Schematic representations of (a) SCC10 (yellow ball) incorporated into the core and Ag^+ (blue ball) from AgNO_3 chelated into the shell of an SCK prepared from PAA_{130} - b - PS_{40} ; and (b) SCK-Ag complexes

Ag^+ SCCs, or the two agents coincidentally, and evaluate the relative efficacy of each system. The SCKs were constructed by the supramolecular assembly of amphiphilic block copolymers, poly(acrylic acid)-*b*-polystyrene (PAA -*b*- PS), into micelles, followed by covalent crosslinking throughout the shell layer to afford discrete nanostructures having a hydrophobic core domain and a hydrophilic shell region. Four procedures were then followed for loading of the SCKs with silver: (1) Ag^+ was incorporated from AgNO_3 into the hydrophilic PAA shell region (AgNO_3 -SCK); (2) 1-hexyl-3-methyl-4,5-dichloro-imidazole-2-ylidene silver (I) acetate (SCC10, which undergoes decomposition in the presence of saline solution to release active Ag^+) was loaded into the hydrophobic PS core domain and/or the core-shell interface (SCC10-SCK); (3) and (4) both methods were applied in opposite order of addition (AgNO_3 -SCC10-SCK or SCC10- AgNO_3 -SCK) (Figure 1). In all cases, free silver was eliminated using a centrifugal filter device (100 kDa MWCO), and the filtrates were examined by UV-visible spectroscopy to confirm removal of free silver. The resulting silver-bearing nanoparticles were characterized and their antimicrobial activities against common Gram-negative pathogenic bacteria were evaluated *in vitro*.

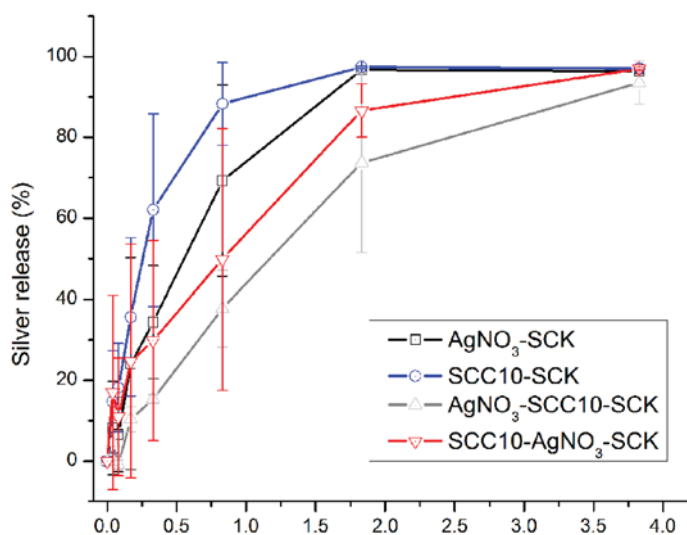
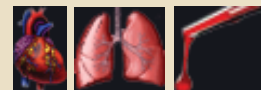


Figure 2. Release profiles of silver from silver-bearing nanoparticles at 37 °C in 5 mM PBS at pH 7.4 (Duplicate)

Release of silver from the SCK nanoparticles was assessed by monitoring the decrease over time of the concentration of silver in dialysis cassettes, performed at 37 °C in 5 mM PBS at pH 7.4 and analyzed by ICP-MS. Each loading protocol gave *ca.* 50% release of silver within *ca.* 1 day and *ca.* 80 % release within 2 days, obtaining a plateau with full silver release by *ca.* 4 days. Moreover, the stability of these Ag-SCK complexes over many hours in PBS is a distinct advantage, relative to simple silver salt solutions.

The antimicrobial activities of the silver-loaded nanoconstructs against representative strains of *E. coli* (strain UTI89; MIC [SCC10] = 2 µg/mL) and *P. aeruginosa* (strain PAM57-15; MIC [SCC10] = 1 µg/mL), common Gram-negative pathogenic bacteria were measured. Defined suspensions of these strains in Mueller-Hinton broth were treated in 96-well plates with the silver-bearing SCKs, equalized for [Ag] by the ICP-MS data. Bacterial growth was measured by optical density (650 nm) in a microplate spectrophotometer 6 h after treatment. SCKs without loaded silver had no antimicrobial activity (data not shown). Independent of the silver-loading method, decrements in growth of *E. coli* UTI89 were observed at [Ag] of 1 µg/mL, and growth was completely inhibited at [Ag] of 2 µg/mL (Figure 3a). For *P. aeruginosa* PAM57-15, decrements in growth were observed at [Ag] of 2-4 µg/mL and growth was completely inhibited at [Ag] of 8 µg/mL (Figure 3b). Activity of the silver-bearing SCKs was generally inferior to that of naked AgNO₃ by ≤ 1 two-fold dilution in inhibition of bacterial growth, suggesting that the SCKs provide availability of silver for antimicrobial action.

These silver-loaded SCK nanoparticle delivery systems exhibited antimicrobial activities, which were nearly comparable to

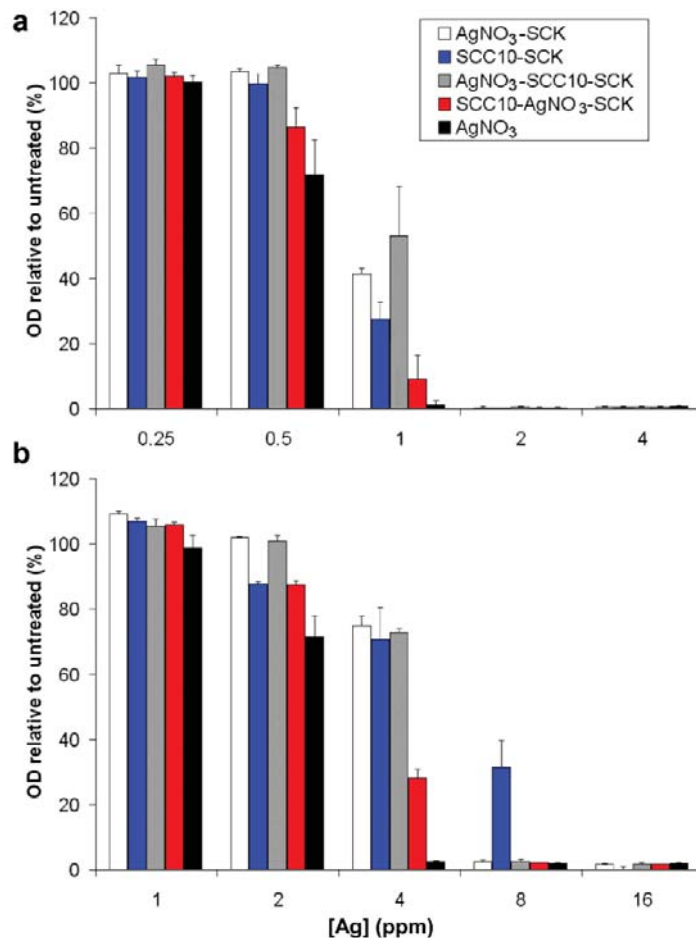


Figure 3. Inhibition of growth of *E. coli* strain UTI89 (a) and *P. aeruginosa* strain PAM57-15 (b) by silver-bearing nanoparticles and naked AgNO₃. Relative optical density (650 nm) after 6 h is shown for each construct at the indicated silver concentrations.

AgNO₃. There appeared to be no advantage to the use of the silver carbene compounds *vs.* loading with silver cations directly. The sustained release over a period of hours suggests that these nanoparticle delivery systems may be beneficial in the treatment of microbial infections *in vivo*. Packaging in the nanoparticle framework is expected to provide for *in vivo* stability. Furthermore, they can be functionalized, which may permit control over biodistribution, tissue-selective targeting and *in vivo* clearance. We are currently investigating their potential in the treatment of pulmonary and urinary tract infections.

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About the Authors

Yali Li, Ph.D.

Former Ph.D. Candidate - Wooley Laboratory
Department of Chemistry
Washington University in St. Louis



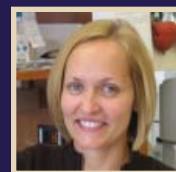
Khadijah Hindi, Ph.D.

Former Postdoctoral Associate
Cannon Laboratory
Washington University School of Medicine
Currently a Postdoctoral Fellow
Henke Laboratory
Department of Medicine
Harvard Medical School
Brigham and Women's Hospital



Kristin M. Watts, B.S.

Research Technician in the Hunstad Laboratory
Department of Pediatrics
Department of Molecular Microbiology
Washington University School of Medicine



Ke Zhang, Ph.D.

Former Ph.D. Candidate - Wooley Laboratory
Department of Chemistry
Washington University in St. Louis
Currently a Postdoctoral Fellow
in the laboratory of Chad A. Mirkin
Northwestern University



Zicheng Li, Ph.D.

Former Ph.D. Candidate - Wooley Laboratory
Department of Chemistry
Washington University in St. Louis
Currently a Postdoctoral Fellow
in the laboratory of Christopher Bielawski
University of Texas at Austin



Yun Lin

Ph.D. Candidate in the Wooley Laboratory
Department of Chemistry
Texas A&M University



Authors - continued

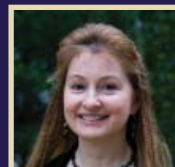
David A. Hunstad, M.D.

Assistant Professor
Department of Pediatrics
Department of Molecular Biology
Washington University School of Medicine



Carolyn L. Cannon, M.D., Ph.D.

Former Associate Professor
Washington University School of Medicine
Currently Associate Professor
Department of Pediatrics
Divisions of Respiratory Medicine,
Pulmonary and Vascular Biology
University of Texas
Southwestern Medical Center



Wiley J. Youngs, Ph.D.

Distinguished Professor
Department of Chemistry
Center for Silver Therapeutics Research
University of Akron



Karen L. Wooley, Ph.D.

Principal Investigator
W. T. Doherty-Welch Chair in Chemistry
Department of Chemistry
Texas A&M University

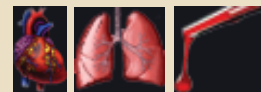


The *in vitro* efficacy of the shell crosslinked nanoparticles carrying silver antimicrobials has been demonstrated *in vivo*.

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- Research slides from past Inter-PEN meetings
- Past newsletter issues
- Joint Seminar Series with the Siteman Center of Cancer Nanotechnology Excellence



PEN SPOTLIGHT ON:

Yuefei Shen, Ph.D. Candidate at Washington University



Yuefei Shen is a third-year Ph.D. candidate in Professor John-Stephen A. Taylor's group in the Department of Chemistry, at Washington University in St. Louis. She received her Bachelor of Science degree in 2007 at Nanjing University in China.

Yuefei Shen has been working in the Acute Lung Injury (ALI) group led by Steven L. Brody, since she joined the PEN team. The goal of her study is to develop antisense mRNA expression imaging agents for inducible nitric oxide synthase (iNOS) as specific markers for diagnosis of ALI. In her study, antisense binding sites on iNOS mRNA were mapped by a reverse transcriptase random oligonucleotide library (RT-ROL) method which was developed in John-Stephen A. Taylor's group. PNAs (peptide nucleic acids) complementary to the antisense binding sites were found to have good binding affinities for iNOS mRNA by a quantitative Dynabead antisense binding assay. Tissue culture experiments indicated that some PNAs identified by RT-ROL method had better anti iNOS activities than those previously reported.

Additionally, she found that cSCKs developed by Ke Zhang of Karen L. Wooley's group in the Department of Chemistry, at Texas A&M University, were very efficient in delivering anti-iNOS PNA-ODN hybrids into cells. She is now collaborating with Ritu Shrestha of Karen L. Wooley's group to construct and evaluate PET imaging agents by conjugating PNAs with SCKs. NP-PNA conjugates showing good *in vitro* properties will be evaluated as potential iNOS PET imaging agents *in vivo* by the Acute Vascular Injury (AVI) group, led by Michael J. Welch, in the Department of Radiology at Washington University School of Medicine.



Collaborators . . .



Michael J. Welch



Steven L. Brody



Karen L. Wooley



Ke Zhang



Ritu Shrestha

NP-PNA conjugates showing good *in vitro* properties will be evaluated as potential iNOS PET imaging agents *in vivo* by the Acute Vascular Injury (AVI) group, led by Michael J. Welch, in the Department of Radiology at Washington University School of Medicine.

PEN

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Upcoming Executive Committee Meetings

(Denis Buxton and Principal Investigators only)

Final Meeting - March 12



Program of Excellence in Nanotechnology (PEN) Executive Committee.
(from left to right): Gang Bao (Emory/Georgia Tech), Karen L. Wooley (WUSTL/TAMU/UCB/UCSB/UTSW), Ralph Weissleder (Broad/BWH/Harvard/MGH/MIT), Jeffrey W. Smith (Burnham/Scripps/UCSB) and Denis Buxton (NHLBI)

About the Authors

Yuefei Shen, Ph.D. Candidate

in the John-Stephen A. Taylor Laboratory
Department of Chemistry
Washington University in Saint Louis
yshen@artsci.wustl.edu



John-Stephen A. Taylor, Ph.D.

Senior Investigator
Department of Chemistry
Washington University in Saint Louis



Photography by Eileen A. Cler, B.S.

Meet our PEN

WINTER 2010



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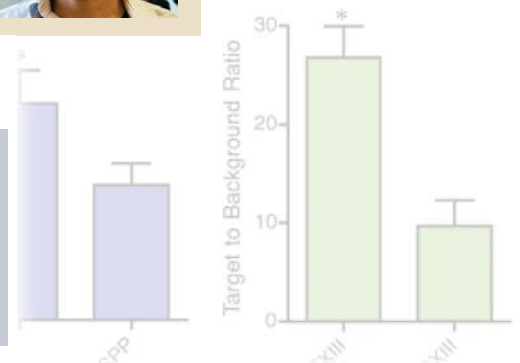
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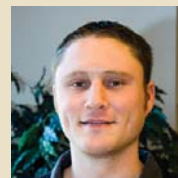


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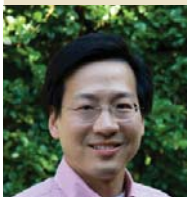
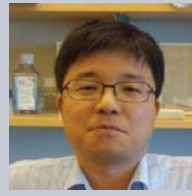




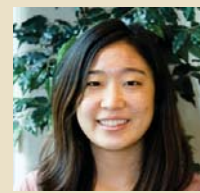
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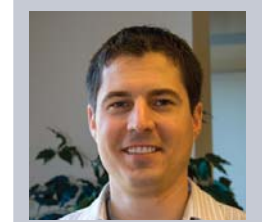
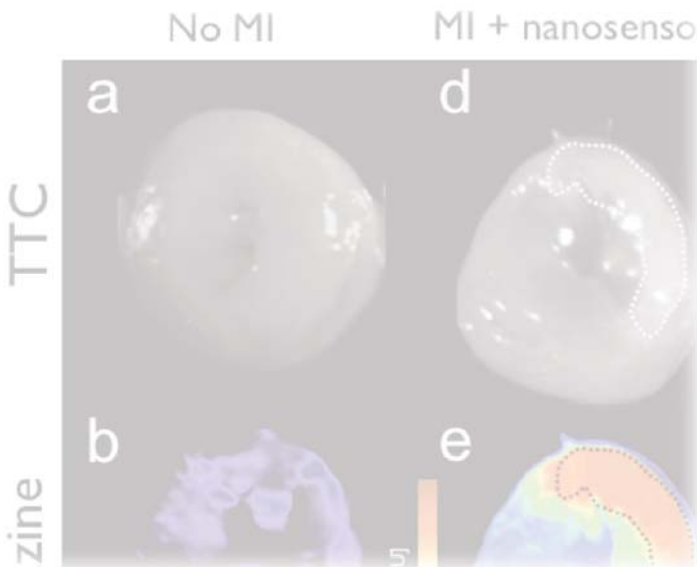
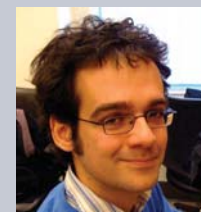
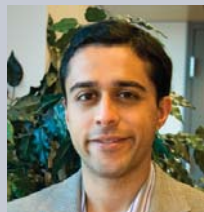
Matthias Nahrendorf
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 Sohini Bose*
 Carlos Tossam
 Erica Ueda



Ralph Weissleder
 Neal K. Devaraj
 Jered B. Haun
 Edmund Keliher
 Thomas Reiner*
 Greg M. Thurber



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About the Author

Eileen A. Cler, B.S.
 Photography* and Graphic Design
 Program Coordinator for the PENS
 Department of Chemistry
 Washington University in Saint Louis



Remembering the Inter-PEN Meetings

Research presentations, poster sessions, workshops and a symposium



2006

2007

NHLBI Inter-PEN Quarterly

<http://www.nhlbi-pen.net/default.php?page=news>

Program Official

Denis B. Buxton

National Heart, Lung, and Blood Institute
Email: buxtond@nhlbi.nih.gov
<http://www.nhlbi.nih.gov>

Principal Investigators

Gang Bao

Emory University
Georgia Institute of Technology
Email: gang.bao@bme.gatech.edu
<http://pen.bme.gatech.edu/>

Jeffrey W. Smith

The Burnham Institute
The Scripps Research Institute
University of California, Santa Barbara
Email: jsmith@burnham.org
<http://www.pennvp.org>

Ralph Weissleder

Harvard University
Massachusetts General Hospital
Massachusetts Institute of Technology
Brigham and Women's Hospital
Email: rweissleder@partners.org
<http://csb.mgh.harvard.edu/>

Karen L. Wooley

Texas A&M University
Email: wooley@mail.chem.tamu.edu
www.nhlbi-pen.info

Production Designer, Editor, Photographer

Eileen A. Cler

Web Designer & Developer, Graphic Designer
Program Coordinator for NHLBI-PEN Grants
Washington University in Saint Louis
Email: eacler@wustl.edu

Engineering Science

Chemistry

2008

2009

Renewal

Good luck to everyone in the Renewal. We expect the research awards to be announced **any day now**. The Administrative Core selection will be announced sometime in **February**.



Eileen A. Cler, B.S.

Program Coordinator
for the PEN Grants
Photography & design
(314) 935-7482
eacler@wustl.edu