

### Mount Sinai/MIT/BWH/Columbia/NYU PEN



### First Annual Intra-PEN Meeting for new PEN "Translational Nanomedical Therapies for Cardiac and Vascular Diseases"

Mount Sinai School of Medicine

#### New York City, New York

#### May 26, 2011

#### Getting started

By Eileen A. Cler, B.S.

ahi A. Fayad, Ph.D., and Robert S. Langer, Sc.D., hosted their first Annual Joint PEN External Advisory Committee (EAC), Internal Advisory Committee (IAC) and Executive Steering Committee (EAC) Meeting on May 26, 2011, at the Mount Sinai School of Medicine in New York. At this meeting they reviewed their PEN's tracking to milestones and scientific progress accomplished since the beginning of the Program of Excellence in Nanotechnology contract in August 2010. Denis Buxton, Ph.D., our National Heart Lung and Blood Institute (NHLBI) Program Official, and Narasimhan Danthi, Ph.D., another Program Director at the NHLBI/NIH were in attendance along with about 40 others, ranging from co-project directors, investigators, collaborators and peers, to students, postdocs and other staff.

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**Research Review** 

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#### **Farewell from our Program Coordinator**

A fter five years as the Program Coordinator for the PENs, I have accepted a new position as a Project Manager of Information Systems for the Neuroinformatics Research Group here in the Department of Radiology at Washington University School of Medicine. My last day on the PEN will be July 16, 2011, at our Annual Intra-PEN Meeting. With my Bachelor's degree in Management Information Systems, and my twelve years of information technology experience as a mainframe COBOL programmer and a Lotus Notes Systems Engineer dating back to my post-college days, I have wanted for some time to return to an "IT environment". I look forward to "talking shop" again with (software) application developers and implementing formal project management methodologies.

This *NHLBI Inter-PEN Quarterly Newsletter* has been a "labor of love" for me, and I will miss the photography and this newfound graphic design skill, as well as the personal contact and camaraderie with so many of you while I took your portraits. It's hard to believe that this issue is the 14<sup>th</sup> edition. As the editor and producer of this newsletter, I want to thank Amy Tang (Georgia Tech/Emory) and Jason McCarthy (Massachusetts General Hospital/Harvard Medical School) for their dedication in delivering the many inputs for each issue, and their commitment to meeting the publication deadlines. I would like to invite everyone to visit two websites that are now available. The first is the new Administrative Center PEN (ACPEN) website, www.nhlbi-pen.net. This website, which went "Live" on June 29, introduces the general public to our new ACPEN Community, and has a secure section for PEN Participants. The website meets the new NHLBI design standards for web development. These federal government requirements mandate the color scheme, structure, and navigation of the website, with the intent of providing a uniform "look and feel" throughout all NHLBI and NIH websites. The second website, which I designed and built, went "Live" on June 17. It is a replacement for the previous Washington University-hosted PEN's website, and may be found at www.nhlbi-pen.info.

The agenda for the upcoming 5<sup>th</sup> Annual Inter-PEN Meeting on November 4-5, 2011, will be distributed in the coming weeks. For more information, see the back cover of this newsletter.

Eileen A. Cler

Eileen A. Cler, B.S. Program Coordinator for the NHLBI-PEN Contracts <u>clere@mir.wustl.edu</u>

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#### GEORGIA TECH, EMORY, & UC-DAVIS Center for Translational Cardiovascular Nanomedicine



#### Image-guided pharmacokinetics in cardiovascular disease

Katherine W. Ferrara, Jai W. Seo, M. Karen Gagnon, Lisa Mahakian, Hua Zhang, Juliana Hamzah and Erkki Ruoslahti laboratory gang.bao@bme.gatech.edu

argeted nanoimaging and delivery requires stable circulation but also frequently requires tissue penetration to reach the target of interest. Our laboratory has focused on the development of imaging strategies to assess the circulation, stability and extravasation of targeted nanocarriers. Imaging assays are applied to measure the concentration of radionuclide-labeled particles in real-time and formulations have been optimized for specific molecular targeting, stealth, plasma stability and activation. We have focused on labeling the nanoparticle shell for PET imaging and the core with a magnetic resonance (MR) agent or a fluorophore and find that this combination facilitates the visualization of stability and activation. In addition, we apply high resolution MRI to assess the intra-tissue distribution of targeted particles.

Several approaches have been developed and compared for the incorporation of a radiolabel. First, we developed a conjugation scheme for the attachment of a fluorine-18 label to a lipid molecule prior to its incorporation in a nanoparticle (Fig. 1A) (1, 2). The radiolabel remains attached until the lipid is metabolized; at this point the fatty acid tails are separated from the radiolabeled head group. This approach facilitates the vi-

sualization of the circulating nanoparticles and detection of lipid metabolism as the radioactivity accumulates within the bladder. When incorporated in a long-circulating particle and imaged with a small bore PET scanner, high resolution images of vascular structure can be produced (2). This approach was applied in (1) to track the accumulation of CRPPR-conjugated liposomes within the heart after systemic administration. We found that  $\sim 40\%$  ID/g of the injected liposomes accumulated within 1-2 minutes and remained bound (Fig. 1B,C). Recently, we found that the liposomal cargo (Fig. 2, red fluorescence) is initially co-located with the vasculature (Fig. 2, green lectin) and is transported through the endothelium over several hours. We have applied the combination of optical and PET imaging techniques to create an image-guided pharmacokinetic model (3) and are in the process of extending this model for intracellular uptake and transport.

An alternative method for directly labeling peptides using solid phase synthesis couples the peptide to a 4-[18F]fluorobenzoic acid ([18F]FBA) radio-tracer and was used to track peptides in the recent paper published in the Proceedings of the National Academy of Sciences (4). Here, we demonstrated the accumulation of LyP-1conjugated iron-oxide nanoworms (Fig. 3A) and the radiolabeled LyP-1 peptide (Fig. 3B) within atherosclerotic plaque in regions containing p32-expressing macrophages. Accumulation of the nanoworms was greater when conjugated to LyP-1, as compared with control (no target) and CREKA-conjugated nanoworms (Fig. 3C).





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Figure 2: CRPPR-conjugated liposomes filled with Alexa555 accumulate on the cardiac vasculature with transmission of the cargo to the surrounding myocardium over a period of minutes to hours. Vascular lectin (Green) demonstrates the morphology of the vasculature, scale bar = 10µm. A. Free Alexa555 (red) was cleared within 30 minutes after systemic injection. B. Control liposomes (without the CRPPR conjugation as in Figure 1a) filled with Alexa555 are rarely observed. C. When the ligand is buried within the PEG brush layer, the Alexa555 signal level is low. D. With the CRPPR ligand exposed, the Alexa555 signal level increases coincident with the vasculature and within the surrounding myocardium.



**Figure 3: Targeting of LyP-1 to atherosclerotic lesions.** A. Nanoworms (NW) containing iron oxide particles and the [18F]FBA conjugated peptide (B) are systemically injected and imaging with MRI or PET is then conducted. C. The heart, aortic arch, descending aorta and spine were excised and T2\*-weighted MRI scans were acquired with a 7-Tesla MR imager (Bruker Biospin) using a FLASH sequence with flip angle of 30°, TR/TE of 1000/15 ms, 512 × 512 acquisition, 290  $\mu$ m slice thickness and 2.9 by 2.9 cm regions of interest extending over the heart, aortic arch and descending aorta. The resulting T2\* signal from the untreated mouse tissues was then compared with tissues excised after the injection of CREKA and LyP-1-conjugated NW and demonstrated the enhanced penetration of LyP-1 NW within the plaque.



Previous reports have indicated that intra-plaque macrophage uptake of iron oxide particles is limited by the ability of the particles to traverse through the arterial endothelial wall. Here, we found that a nanoparticle that is approximately 80 nm long and 30 nm wide was effectively carried into the interior of the plaques by LyP-1 despite its large size (**Fig. 3C**). LyP-1 homing co-localized with lymphatic markers (podoplanin and LyVE-1), in addition to plaque macrophages. The lymphatic localization and the absence of blood vessels in the plaque intima suggest that LyP-1 may enter plaque intima *via* the lymphatics.

In summary, we have developed a suite of methods to track nanoparticles and demonstrated their preferential accumulation within the heart and within atherosclerotic plaques. These techniques are now being applied in guiding therapeutic delivery.

#### References

- Zhang, H.; Kusunose, J.; Kheirolomoom, A.; Seo, J.W.; Qi, J.; Watson, K.; Lindfors, H.; Ruoslahti, E.; Sutcliffe, J.L.; Ferrara, K.W. "Dynamic imaging of arginine-rich heart-targeted vehicles in a mouse model", *Biomaterials*, **2008**, *29*, 1976-88.
- Marik, J.; Tartis, M. S.; Zhang, H.; Fung, J. Y.; Kheirolomoom, A.; Sutcliffe, J. L.; Ferrara, K. W., "Long-circulating liposomes radiolabeled with [F-18]fluorodipalmitin ([F-18]FDP)", *Nuclear Medicine and Biology*, 2007, *34*, 165-171.
- Qin, S.; Seo, J.W.; Zhang, H.; Qi, J.; Curry, F.-R. E.; Ferrara, K. W. "An Imaging-Driven Model for Liposomal Stability and Circulation", *Molecular Pharmaceutics*, 2009, *7*, 12-21.
- 4. Hamzah, J.; Kotamraju, V. R.; Seo, J.W.; Agemy, L.; Fogal, V.; Mahakian, L.M.; Peters, D.; Roth, L.; Gagnon, M.K.J.; Ferrara, K.W.; Ruoslahti, E. "Specific penetration and accumulation of a homing peptide within atherosclerotic plaques of apolipoprotein E-deficient mice", *Proceedings of the National Academy of Sciences*, 2011, 108, 7154-7159.



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

Visit our website at <u>www.nhlbi-pen.net</u> to read about:

- Aims of each PEN Contract (2010-2015)
- Research slides from past Inter-PEN meetings
- Past newsletter issues
- Seminar Series
- Initial PEN Grant information (2005-2010)



### SEMINARS

July 14, 2011 A collaboration among PENs



Omid Farokhzad, M.A., M.D., Co-Project Director on the MSSM/MIT/ BWH/Columbia/NYU PEN, will give a presentation on "Medical Nanoengineering: A Bench to Bedside " at the Washington University School of Medicine, in St. Louis, MO.

#### June 23, 2011 Invited Speaker

Angelique Louie, Ph.D., Associate Professor and Vice Chair for the Department of Biomedical Engineering at the University of California, Davis, gave a presentation on "Multimodal Probes for Molecular Imaging" at the Washington University School of Medicine, in St. Louis, MO. Forty people attended the meeting locally, while faculty, students and postdocs from TAMU, UCB, UCSB and UTSW connected via Cisco WebEx

# conferencing software.

**Did you know?** 

#### **ACPEN Website** Available soon

The development of the website is complete and the new Administrative Center (ACPEN) website will "Go Live" soon. The URL will be emailed to all PEN Participants in the coming weeks.



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#### In collaboration with ...

Juliana Humzah, Ph.D. Postdoctoral Research Fellow Sanford-Burnham Institute







### MGH, BWH, BI, HARVARD, MIT

Translational Program of Excellence in Nanotechnology

#### Probing Intracellular Biomarkers and Mediators of Cell Activation Using Nanosensors and Bioorthogonal Chemistry<sup>1</sup>

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raditional methods utilized to assess the spatial and temporal status of signal transduction networks in size-limited clinical samples, such as Western blotting, flow cytometry, and immunohistochemistry suffer from prohibitively large required cell numbers, low throughput, and lack multiplexing capabilities. Nanomaterial-based methodologies, on the other hand, allow for several advantages, including increased signal sensitivity, stability, and the capability for multiplexing that are afforded by the materials unique physical properties. Detection platforms based upon a 2-step bioorthogonal nanoparticle detection (BOND-2) have been reported for the coupling of nanoparticles to affinity molecules pretargeted to the cell surface.<sup>2</sup> This system utilized an inverse-electron demand Diels Alder reaction between a strained dienophile such as norbornene of transcyclooctene (TCO) and a tetrazine, a reaction which is irreversible and extremely fast. In their recent manuscript, Haun et al. significantly improve on their initial work by optimizing their system for the detection of intracellular protein biomarkers.1

Initially, the authors attempted to optimize the fixation and permeabilization of cells for the subcellular delivery of nanoparticles while limiting background signal. Cells were treated in suspension, similar to clinical samples, such as fine needle aspirates or other fluid samples. A number of methodologies were identified from the literature, which were prescreened, resulting in the ultimate use of a mixture of formaldehyde and the detergent saponin for initial fixation and permeabilization, followed by further saponin treatment and mechanical disruption via freeze-thaw treatment, as illustrated in figure 1c, which was utilized in subsequent experiments. BOND-2 was next optimized, as compared to direct immunoconjugation, using cytokeratin (CK)as a target protein. Initially, TCO conjugation to an anti-CK antibody was optimized, resulting in ~10 TCO per antibody (Figure 1d). When the BOND-2 methodology was compared to the direct conjugate, the authors found that BOND-2 resulted in an order of magnitude higher signal.

The authors then utilized this methodology to investigate whether nanoparticle binding could be correlated with target expres-

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sion. To accomplish this, the authors employed panels of cell lines varying in expression of CK and a nuclear protein, Ki-67. The CK panel included negative (U118), low (SK- OV-3), moderate



(HeLa), and high (SK-BR-3 and PANC-1) expressing cell lines. The Ki-67 panel included cell populations exhibiting low (SK-OV-3 and SK-BR-3), moderate (A549 and HT-29), and high (PANC-1) percentages of actively growing cells. Dr. Haun and his colleagues found that the nanoparticle signals for both markers, as examined by flow cytometry, correlated closely with expression levels determined by fluorescent antibody staining and Western blot. This methodology was further utilized to profile intracellular proteins using diagnostic magnetic resonance (DMR), a technology that was discussed previously in this newsletter (Dec 2008). As is shown in figure 2, eight different cell lines were profiled for eight intracellular biomarkers relevant to cancer screening and therapeutic monitoring using 1000 cells per experiment in 1 µL sample volume. As is shown in figure 2b, there was an excellent correlation between magnetic resonance signal and expression levels. This methodology was further utilized to measure drug efficacy. When A431 (highly sensitive due to EGFR amplification), NCI-H1650 (moderately sensitive due to exon 19 deletion of EGFR),



**Figure 1. Targeting nanoparticles to intracellular markers using bioorthogonal chemistry coupling.** (a) Semipermeabilization of intact cells allows nanoparticle targeting to a variety of intracellular biomarkers as wells as indicators of cell growth, activation, and survival. (b) BOND targeting scheme using a TCO-modified antibody followed by Tz nanoparticle to amplify nanoparticle binding. (c) Investigation of various cell treatments to optimize secondary permeabilization following fixation. (d) Increasing the TCO valency on the anti-CK antibody increased MFNP signal. (e) Binding isotherms obtained for targeting CK using BOND-2 and a direct MFNP immuno-conjugate prepared using the TCO/Tz chemistry (BOND-1).



Figure 2. Profiling scant tumor cell populations for key biomarkers of cancer using DMR. (a) Detection of eight biomarkers in eight different cell lines using MFNP based on nuclear magnetic resonance signal. (b) Magnetic measurements showed excellent correlation with marker expression levels determined independently by antibody staining. (d) Confocal images demonstrating varying cellular localizations and signal intensities obtained for each specific marker (top) and controls (bottom). PANC-1 cells were used for CK, vimentin, p-ERK, p-S6RP, Ki-67, and p53 images. A431 cells were used for both EGFR cases.

and A549 (not sensitive due to KRAS mutation) cells were treated with gefitinib, an EGFR inhibitor or rapamycin, an mTOR inhibitor, a dosedependent inhibition of p-S6RP, a shared downstream target, could accurately be quantitated.

Overall, the work by Haun *et al.* demonstrates that methodologies can be developed to improve nanoparticle targeting to cytoplasmic or nuclear proteins, allowing for increased sensitivity in their detection. These results also show broad applicability and remarkable reproducibility across a platform of different intracellular biomarkers of diagnostic interest, thereby increasing the number and type of biomarker targets available to nanomaterials for sensitive molecular detection and comprehensive profiling, while also enabling real-time monitoring of therapeutic treatment efficacy.

#### References

 Haun, J.B.; Devaraj, N.K.; Marinelli, B.S.; Lee, H.; Weissleder, R. "Probing intracellular biomarkers and mediators of cell activation using nanosensors and bioorthogonal chemistry", ACS Nano, 2011, 5, 3204-3213. Haun; J.B., Devaraj; N.K., Hilderbrand; S.A., Lee; H., Weissleder; R. "Bioorthogonal chemistry amplifies nanoparticle binding and enhances the sensitivity of cell detection." *Nat Nanotechnol*, **2010**, ePub.



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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



**Ritu Shrestha**, **Ph.D. Candidate**, of Texas A&M University received a Student Poster Award at the 2nd Annual Biomaterials Day at Texas A&M University, Organized by the Society for Biomaterials on May 16, 2011.



Stephanie Florez, Undergraduate, of Texas A&M University won first place at the TAMU 14th Annual Student Research Week Competition.



Karen L. Wooley, Ph.D. of Texas A&M University received the Distinguished Professor award, effective September 1, 2011.



**Eileen A. Cler, B.S., accepted a position** as the Project Manager of Information Systems in the Neuroinformatics Research Group at Washington University School of Medicine, effective July 18, 2011.

#### MGH, BWH, Broad, Harvard, MIT



Ralph Weissleder, M.D., Ph.D., has been awarded the Gold Medal of the European Society of Radiology in recognition of his exceptional contributions to science, research and the development of medical imaging.

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-- Transitions --

Jessica L. Cohen, Ph.D., accepted a position as Postdoctoral Research Associate in Professor Michael Czech's laboratory at the University of Massachusetts Medical School.



Karen M. Kharasch, B.S., accepted a position as the Executive Director of Business Affairs for the Siteman Cancer Center at Washington University School of Medicine on May 1, 2011.



**Carolyn J. Anderson**, **Ph.D.**, accepted a position as the Director of Molecular Imaging and Professor of Radiology at the University of Pittsburgh, effective May 6, 2011. Her lab will be developing molecular imaging probes for cancer and other diseases.

#### Mt. Sinai, MIT, BWH, Columbia, NYU



Kiyotake Ishikawa, M.D., was recently promoted to Instructor in medicine at Mount Sinai School of Medicine.

#### Emory, Georgia Tech, UCD



Gang Bao, Ph.D., of The Georgia Institute of Technology, has been named the Director of the Center for Pediatric Nanomedicine at Children's Healthcare of Atlanta, Georgia.

#### PEN

If someone from your PEN has received an Award that you'd like to recognize, please forward the information to **Kari Alca** at <u>alcak@mir.wustl.edu</u>



### Meet our PEN

**Mount Sinai School Of Medicine Massachusetts Institute of Technology Brigham and Women's Hospital Columbia University New York University** 

Zahi A. Fayad Principal Investigator

Robert S. Langer Co-Principal Investigator

### Directors, Co-Directors and Investigators Translational Nanomedical Therapies for Cardiac and Vascular Diseases

By Eileen A. Cler, B.S.

### Our Aims (abridged)

NanoCarD: Nanomedicines for Cardiovascular Disease

This contract aims to establish a unique multidisciplinary Program of Excellence in Nanotechnology (PEN) by

integrating the cardiovascular medicine and imaging expertise of highly productive NHLBI-funded investigators at

Mount Sinai School of Medicine, New York University, and Columbia University, with the cutting-edge biomolecular and nanomedical engineering expertise of world-renowned pioneers at Massachusetts Institute of Technology and Brigham and Women's Hospital. The overarching long-term goal of this PEN contract is to establish an innovative research and training program focused on developing translational nanomedical tools for the imaging-facilitated

Cardiovascular disease is the leading cause of morbidity and mortality in developed nations with an enormous

societal and economic burden that costs millions of lives and over \$360 billion per year in the US alone. Since the development of novel treatment strategies for atherosclerosis has stagnated in recent years, and the conventional treatment modalities to reduce heart failure (HF) mortality are only slowly progressing, there exists an urgent need to explore new and potent therapeutic approaches. The exploitation of nanotechnology in cardiovascular disease

diagnosis and minimally-invasive treatment of vascular and cardiac diseases.





Omid C Farokhzad



Zahi A. Fayad



Edward A. Fisher



(1) We will investigate minimally invasive nanotechnology-based approaches to induce genetically driven regeneration of infarcted myocardium.

In the current PEN contract we propose to develop sophisticated translational nanoparticle technologies for

(2) We will develop novel nanotherapeutic approaches for defective inflammation resolution in atherosclerosis. (3) We will develop a theranostic nanoparticle platform with surface activation properties to efficiently and specifically target macrophages in atherosclerotic plaques.

For more information, visit the ACPEN website at: http://www.nhlbi-pen.net/centers/mssm-mit.html



Robert S. Langer



Willem Mulder



Martin A. Schwarz



Ira Tabas

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has been largely unexplored, but may have unprecedented benefits in both preventing atherosclerosis progression and treating irreparable clinical events like myocardial infarction. Nanotherapies have the potential to help increase the efficacy of anti-atherosclerotic drugs and novel regenerative therapies, with significantly reduced adverse side

effects.





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WUSTL, TAMU, UCB, UCSB , & UTSW

Integrated Nanosystems for Diagnosis and Therapy



#### Evaluation of Multivalent, Functional Polymeric Nanoparticles for Imaging Applications<sup>1</sup>

Monica Shokeen, Eric D. Pressly, Aviv Hagooly, Alexander Zheleznyak, Nicholas Ramos, Ashley L. Fiamengo, Michael J. Welch, Craig J. Hawker, and Carolyn J. Anderson shokeenm@mir.wustl.edu

he goal of this study was to develop a synthetic strategy for accurate loading of targeting moieties and diagnostic units onto a polymeric nanoparticle scaffold and to exploit this high level of control to understand and evaluate the competing effects of ligand loading on nanoparticle binding affinity and uptake in cellulo compared to the effect on their pharmacokinetic properties. Here we demonstrated that the modular and tunable nature of the synthetic approach to these multifunctional comb nanoparticle (CNP) carriers allows for the design of systems with increased specific integrin binding and cellular uptake, optimal blood retention, and RES response based on an intermediate loading of targeting peptides. Of the many molecular targets available,  $\alpha_{v}\beta_{a}$ , a well-studied type of integrin upregulated in tumor angiogenesis, metastasis, inflammation, certain cardiovascular abnormalities, and bone resorption, was selected as a model system for evaluation. To synthesize agents capable of detecting  $\alpha_{\mu}\beta_{\nu}$ , small peptides containing the amino acid sequence Arg-Gly-Asp (RGD), which bind to  $\alpha_{\nu}\beta_{\alpha}$  with high affinity, were linked to the polymeric backbone of nanoparticles at various concentrations. Notably, the novel modular and tunable synthetic approach ensures accurate control over conjugation of RGD peptides to the backbone. Finally, this series of RGD-comb nanoparticles were radiolabeled with  $^{64}\text{Cu}$  (  $T_{_{1/2}}$  = 12.7 h,  $\beta^+$  = 17.86%), a positron emitter commonly used in positron emission tomography (PET), via the DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) chelator for evaluations in cellulo and in vivo.

#### Synthesis of Comb Copolymers (Scheme 1).

Using the binding of RGD to the integrin  $\alpha_{\nu}\beta_{3}$  as a prototypical system, well-defined amphiphilic graft copolymers and associated comb nanoparticles (CNPs) having a controlled number of RGD peptide targeting moieties were prepared. The modular approach used in this study is based on four important building blocks: (a) poly(ethylene glycol) (PEG) as a hydrophilic, protein-resistant unit; (b) methyl methacrylate as a hydrophobic backbone which controls self-assembly; (c) 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) as a chelator for imaging with the positron emitter <sup>64</sup>Cu (T1/2 = 12.7 h,  $\beta$ + = 17.86%); and (d) GRGDS as a linear targeting peptide.

By controlling the relative hydrophobic/hydrophilic balance of the copolymers through the ratio of methyl methacylate and PEG units, assembly into CNPs was observed and nanoparticles with approximately the same size (~22 nm), irrespective of the level of incorporation of the RGD units obtained (**Table 1**).



Scheme 1. Synthesis of functionalized comb copolymers by RAFT polymerization and their assembly into CNPs.

Polymer	M <sub>n</sub> (GPC)	PDI (GPC)	Mn (NMR)	CNP	CNP Diameter (nm)	RGDs per CNP
0% RGD	120 kDa	1.3	140 kDa	CNP-0%	20.4 nm	0
5% RGD	125 kDa	1.3	141 kDa	CNP-5%	22.0 nm	7
10% RGD	130 kDa	1.3	141 kDa	CNP-10%	21.8 nm	14
20% RGD	120 kDa	1.3	142 kDa	CNP-20%	22.9 nm	28
50% RGD	110 kDa	1.3	145 kDa	CNP-50%	20.9 nm	70

Table 1. Structural features of RGD-functionalized comb copolymers.



### In Vitro Evaluation of $\alpha_v \beta_3$ Targeted CNPs by Isolated Integrin Binding Assays

In evaluating the biological activity of RGD-functionalized CNPs toward  $\alpha_{\nu}\beta_{3}$ , the effect of multivalency on integrin binding affinity was assessed by performing isolated integrin binding assays. CNPs with variable levels of RGD attachment were evaluated for their binding affinity and specificity toward the integrins  $\alpha_{\nu}\beta_{3}$ ,  $\alpha_{\nu}\beta_{5}$ , and  $\alpha_{\mu b}\beta_{3}$  in heterologous competitive binding experiments with biotinylated vitronectin ( $\alpha_{\nu}\beta_{3}$ ,  $\alpha_{\nu}\beta_{5}$ ) and fibronectin ( $\alpha_{\mu b}\beta_{3}$ ) as the competitive natural ligand, respectively. Nonlinear regression was used to fit the binding curves and calculate inhibitory concentration values of 50% (IC<sub>50</sub>), with the  $\alpha_{\nu}\beta_{3}$  binding kinetics following a classic sigmoid path that could be fit with a nonlinear regression curve (**Figure 1**). The results demonstrate a *ca.* 75-fold increase in  $\alpha_{\nu}\beta_{3}$  binding between the 5% RGD–CNPs (IC<sub>50</sub> = 78.46 nM)



 $\alpha_{v}\beta_{3}$  obtained by competition with biotinylated vitronectin.

and 50% RGD–CNPs (IC<sub>50</sub> = 1.08 nM), which is in contrast to the number of RGD targeting ligands where the increase is only a factor of 10. The 10 and 20% RGD–CNPs had intermediate IC<sub>50</sub> values of 12.48 and 5.21 nM, respectively, with the significant finding being the nonlinear nature of the relationship between the IC<sub>50</sub> and the number of peptides (**Table 2**). The results indicate that

increasing the number of targeting peptides per CNP translates to increased binding affinity to the integrin, with the nontargeted control CNP showing no appreciable binding to  $\alpha_{\nu}\beta_{3}$  (**Table 2**). The RGD–CNPs were also screened against the integrins  $\alpha_{\nu}\beta_{5}$  and  $\alpha_{11b}\beta_{3}$ , found on macrophages and blood platelets, respectively. The binding to these two integrins was tested to evaluate the specificity of the multivalent CNPs toward the integrin  $\alpha_{\nu}\beta_{3}$ . Significantly, all four CNP constructs demonstrated no specific binding toward the integrins  $\alpha_{\nu}\beta_{5}$  and  $\alpha_{11b}\beta_{3}$ , with IC<sub>50</sub> values of >1000 nM.

#### Internalization Studies.

The cellular uptake trend of the <sup>64</sup>Cu-labeled multivalent CNPs was clearly demonstrated by assays performed with the  $\alpha_{v}\beta_{3}$ -positive U87MG cells. Significantly, the confocal images showed internalization of the 20% RGD–CNPs within 1 h of incubation with the cells **(Figure 2)**. The confocal images combined with the cellular studies using radioactive assays illustrate that high cellular uptake and internalization by receptor-mediated endocytosis can be achieved and manipulated through tuning of the nanoparticle structure.



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Figure 2. Confocal microscopy slides (red channel image) showing the internalization of 20% targeted CNPs in U87MG glioblastoma cells. The cells were incubated with respective nanoparticle solutions (143 nM) for 1 h at 37 °C. (A) Image showing accumulation of 20% RGD-comb nanoparticles into the cells. (B) Image showing no accumulation of 0% RGD-comb nanoparticles into the cells.

CNPs	# of Peptides per CNP	IC <sub>50</sub> (nM)	α <sub>v</sub> β₃	IC <sub>50</sub> (nM)	$\alpha_{v}\beta_{5}$	IC <sub>50</sub> (nM)	α <sub>IIb</sub> β <sub>3</sub>
5%	7	78.46		> 1,000		> 1,00	D
10%	14	12.48		> 1,000		> 1,00	D
20%	28	5.21		> 1,000		> 1,00	D
50%	70	1.08		> 1,000		> 1,00	D
0% (Non-targeted) Control	0	> 1,000		> 1,000		> 1,00	D

Table 2. Integrin binding (IC<sub>50</sub>) results from the RGD-CNP series from an isolated integrin binding assay in competition with vitronectin ( $\alpha_{\mu\beta_3}$  and  $\alpha_{\nu}\beta_{\gamma}$ ) and fibronectin ( $\alpha_{\mu\beta_3}$ ).

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#### **Biodistribution Studies.**

The blood retention profiles for the RGD-CNP series are shown in Figure 3. The 10, 20, and 50% RGD-CNPs demonstrate relatively fast blood clearance to 1 h followed by stabilization of blood activity to 48 h. In contrast, the 5% RGD-CNPs exhibit a similar blood retention profile as the control particle without RGD, with the blood retention remaining high out to 48 h. These results show that, for the GRGDS ligand, low loa dings have minimal effect on blood retention while higher drasticallv loadings decrease blood retention. There was no significant accumulation in the lungs. as expected from non-aggregating nanoparticles.



Figure 3. Blood (top), liver (middle), and spleen (bottom) retention profiles for the <sup>64</sup>Cu-labeled CNPs in Sprague- Dawley rats. Data are expressed as percent injected dose (% ID) per organ±SD.

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**Conclusions.** The tunable and modular synthesis of functionalized comb copolymers has enabled facile development of a library of multivalent nanoparticles incorporating diagnostic <sup>64</sup>Cu-DOTA units with varying amounts of surface-accessible RGD peptides for  $\alpha_{\nu}\beta_{3}$  targeting. By controlling the level of targeting moieties, while maintaining similar nanoparticle sizes, fundamental structure/bioperformance relationships were developed and the effects of functional group density on pharmacological profiles and biological behavior explored. The in cellulo results indicate an upper limit of RGD loading for effective cellular uptake in  $\alpha_{\nu}\beta_{3}$ -positive U87MG glioblastoma cells, while the *in vivo* results show a balance between the level of targeting ligand and biologistribution.

#### References

1. Shokeen, M.; Pressly, E. D.; Hagooly, A.; Zheleznyak, A.; Ramos, N.; Fiamengo, A.L.; Welch, M.J.; Hawker, C. J.; Anderson, C. J. "Tunable Synthesis and Pharmacokinetic Evaluation of Copper-64-labeled  $\alpha_{\gamma}\beta_{3}$  Targeted Polymeric Nanoparticles", *ACS Nano*, **2011**, 5(*2*), 738-47. Available online at DOI: 10.1021/nn102278w.



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### Hot Upcoming Topics

### 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.

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### **Emerging Breakthroughs**



Gang Bao

**Dr. Thomas Barker's** lab has recently been exploring the discovery and development of unique imaging and therapeutic targeting elements that recognize nascent fibrin protofibrils, but not the soluble circulating precursor fibrinogen. Through sequential screening and quantitation of binding via surface Plasmon resonance, these targeting agents show extremely high specificity to fibrin polymer. These agents have the potential to guide imaging probes and nanoparticle delivery systems directly to microthrombi and vulnerable plaques throughout the body enabling early detection and treatment of these cardiovascular pathologies. We are currently exploring these agents in dual imaging modalities coupling PET and optical probes.



Thomas Barker



Ralph Weissleder

**Dr.** Hakho Lee's lab at the Center for Systems Biology at the Massachusetts General Hospital has recently made significant advances in the detection of Mycobacterium tuberculosis. Using a bioorthogonal conjugation approach and highly magnetic nanoprobes, they were able to demonstrate increased sensitivity and lower bacterial detection thresholds, as determined using their diagnostic magnetic resonance (DMR) system.



Hakho Lee



Robert S. Langer

Stem Cell Factor (SCF) Gene Transfer Promotes Cardiac Repair After Myocardial Infarction *via* In Situ Recruitment and Expansion of c-kit+ Cells. The effects of c-kit ligand/SCF gene transfer are being tested in a rat myocardial infarction model and a swine model of myocardial infarction to determine whether SCF mediates cardiac regeneration through c-kit+ progenitors expansion and myocyte proliferation. Nanoparticle enabled SCF gene transfer in mesenchymal stem cells will be evaluated through pericardial transfer in large animal models of heart failure.



Roger Hajjar



Michael J. Welch



Karen L. Wooley

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**Dr. James C. Sacchettini** and his group has performed high throughput screening (HTS) using his diversity library of chemically dissimilar drug-like compounds to look for novel antimicrobials active against Mycobacterium tuberculosis. His group has performed similar screens to find compounds active against Pseudomonas aeruginosa. Numerous "hits" have been identified, some of which are active against both microorganisms. Assays to determine the minimal inhibitory concentration (MIC) of the "hits" against each bacteria are underway. The most active compounds will be loaded into biodegradeable nanoparticles for aerosol delivery to the lung.



Carolyn L. Cannon



James C. Sacchettini

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### MSSM, MIT, BWH, COLUMBIA, NYU

Translational Nanomedical Therapies for Cardiac and Vascular Diseases



#### **Development of a Pre-clinical Model of** Ischemic Cardiomyopathy in Swine

Kiyotake Ishikawa, Roger J. Hajjar kiyotake.ishikawa@exchange.mssm.edu, roger.hajjar@exchange.mssm.edu, zahi.fayad@mssm.edu, rlanger@mit.edu

he development of novel therapies for patients with severe ischemic heart failure has accelerated in recent years and novel targets have emerged at the cellular and molecular levels. Many potential novel therapies such as nanotechnology, gene therapy, and stem cell therapy are gaining popularity. Pre-clinical models of ischemic cardiomyopathy that closely mimic the clinical condition are important to develop in order to test the efficacy and safety of these new strategies of treatment.

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The porcine heart exhibits similar gross anatomic structure and important characteristics of the human heart, as well as, similar coronary artery anatomy without inherent collaterals. Surgical or percutaneous occlusion of coronary arteries which mimics acute infarct and their sequelae, or the implantation of plastic occluders to the coronary arteries which mimics hibernation, are the current models of ischemic heart disease. However, patients with ischemic cardiomyopathy are more likely to have non-transmural infarcts with viable myocardium. A study showed that 55% of ischemic cardiomyopathy patients with depressed cardiac function have viable myocardium (1). Thus, the current models do not offer a reasonable model of ischemic cardiomyopathy. Accordingly, establishment of an ideal chronic ischemia model of swine brings in a significant advantage in translational research, and eventually leads to future development of treatment in ischemic cardiac diseases. To this end, we developed a new swine model of chronic ischemia which presents total occlusion of the left anterior descending (LAD) coronary artery with rich collaterals.

To induce progressive and gradual coronary artery stenosis, a plastic occluder with a fixed diameter of 1.0 mm fitted with an 18 gauge copper wire was placed around the proximal LAD. A total of 39 pigs received this occluder. Overall mortality was 26% and approximately 80% of the pigs showed total occlusion at 1 month. A representative coronary angiogram before and after the total occlusion of the LAD is shown in Figure 1. The pigs with total occlusion exhibited a significantly decreased (+dP/dt) maximum throughout the study compared to that of the control pigs. Although stroke volume was comparable between the chronic ischemia and control pigs, ejection fraction was significantly impaired in the pigs with total occlu-



sion. At the same time, these pigs tend to have larger end diastolic volume and end systolic volume when

measured with an echocardiogram. Anterior wall motion was impaired in pigs with total occlusion, however, a dobutamine stress test demonstrated improved wall motion of the ischemic area. The coronary flow pattern in the ischemic area took the pattern of hibernating myocardium as described in previous studies (2). This data indicates that the myocardium in the ischemic area is in the stage of hibernating myocardium.

The post mortem examination exhibited approximately 10% of scar tissue with a patchy pattern predominantly at the endomyocardium. Masson's trichrome staining revealed an interlaced fibrosis



Figure 1. Representative coronary angiography of the pigs with occluder. Only stenosis shaped by the occluder was found at 2 weeks (A) without any collaterals from RCA (B). LAD showed total occlusion at the proximal at 1 month (C). Arrow indicates the occlusion site with copper wire placed on the LAD. Rich collateral from RCA was seen (D). LAD = left anterior descending coronary artery, RCA = right coronary artery.



**Figure 2. Histological assessment of cardiac fibrosis.** An increased fibrosis (blue area) was found predominantly toward the Endo by Masson's trichrome staining. There was only a minor change found in the inferior area. Ant = anterior, Inf = inferior, Epi = epimyocardium, Mid = midmyocardium, Endo = endomyocardium.

inside the normal myocardium, with more enhancement toward the endomyocardium **(Figure 2)**. In correspondence to the histological staining, western blot analysis revealed increased markers of both fibrosis and apoptosis in the anterior midmyocardium and endomyocardium.

This model has a number of advantages for pre-clinical studies targeting ischemic heart disease. Low mortality, cardiac dysfunction with non-transmural scar, ideal time frame with minimal increase in the body weight, and easily reproducible technique were demonstrated. The mixture of various stages of myocardium from normal to hibernating, to apoptotic, and to fibrotic myocardium, closely mimics the state of human chronic ischemic cardiomyopathy. Thus, our model would be very suitable to test therapies such as small molecule delivery, gene transfer, and stem cell therapy, targeting specifically ischemic cardiomyopathy. In summary, our model combines useful characteristics that are present in chronic ischemic cardiomyopathy in humans and it will be a useful experimental model to evaluate novel therapies.

#### References

- Auerbach, M.A.; Schoder, H.; Hoh, C.; Gambhir, S.S.; Yaghoubi, S.; Sayre, J.W.; Silverman, D.; Phelps, M.E.; Schelbert, H.R.; Czernin, J. "Prevalence of myocardial viability as detected by positron emission tomography in patients with ischemic cardiomyopathy", *Circulation*, **1999**, *99*, 2921-2926.
- 2. Fallavollita, J.A.; Malm, B.J.; Canty, J.M. Jr. "Hibernating myo-

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ed fibrosis (blue area) was found predominantly toward the ge found in the inferior area. Ant = anterior, Inf = inferior, Epi im. cardium retains metabolic and contractile reserve despite regional reductions in flow, function, and oxygen consumption at rest", *Circ Res*, **2003**, *92*, 48-55.



#### PEN

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## PEN Seed Grant Program at GT/Emory, Gang Bao, Ph.D.

Supported by NHLBI as a Program of Excellence in Nanotechnology, we have established the Center for Translational Cardiovascular Nanomedicine (CTCN) at the Georgia Institute of Technology, Emory University and the University of California, Davis. Our goals are to develop and apply nanotechnology and biomolecular engineering tools and approaches to address the compelling medical needs in the detection and treatment of atherosclerosis and the repair of damaged vasculature and heart tissue, and to train the next generation of leaders in cardiovascular nanomedicine.



To help achieve the goals of the CTCN, explore new ideas in cardiovascular nanomedicine, and facilitate the development of new collaborations between Georgia Tech and Emory University in cardiology and molecular imaging, with matching funds from Georgia Tech and Emory University, we have established a PEN seed grant program to expand our PEN both in terms of the number of investigators and the scope of the technology development. Four seed grants were awarded on November 1, 2010, with each having a budget of \$50,000 direct cost per year:

1. Temporal and spatial control of cardiac regeneration using biomaterials

Co-PIs: Michael Davis (Emory) and Andres Garcia (GT)

The goal of this project is to achieve better spatial and temporal control of post-infarct healing by combining bioartificial hydrogels and polyketal nanoparticles.

**2. Visualization of Acute Atherothrombosis using a Nanoparticle Based Magnetic Resonance Contrast Agent** Co-Pls: John Oshinski (Emory), Hui Mao (Emory) and David Ku (GT)

The goal of this project is to develop a magnetic nanoparticle imaging probe which binds to activated platelets and would allow visualization of acute thrombosis inside of the MRI scanner in a realistic vessel model.

3. Novel molecular probes for detecting fibrin deposition in cardiovascular disease

Co-PIs: Tom Barker (GT) and Bob Taylor (Emory)

The goal of this project is to identify motifs that bind specifically to aberrant fibrin in cardiovascular disease using phage display screens for single chain variable antibody fragment (scFv).

4. Imaging Bacterial Infections by Targeting the Maltodexrin Transport Pathway

Co-PIs: Niren Murthy (GT) and Mark Goodman (Emory)

The goal of this project is to develop a new PET contrast agent that can detect early stage bacterial infections of lung and heart tissues.

Each seed grant project team is required to submit a final progress report after one year of funding, and the co-PIs of the projects are invited to our PEN monthly PI meetings. A seed grant may be renewed on a competitive basis for a second year.

Georgialnstitute







The "Spotlight" rotates between the PENs. If someone on your PEN has made a significant contribution to your PEN's success and you would like to recognize their work, please send a brief biography to Kari Alca at <u>alcak@mir.wustl.edu.</u>



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### LAUNCH OF WEBSITE ...

### NEW PEN ADMINISTRATIVE CENTER (ACPEN) WEBSITE

Robert J. Gropler, M.D., Washington University School of Medicine

The new Administrative Center website for the four PENs is hosted at the Washington University School of Medicine in the Department of Radiology.

In addition to presenting the research aims for the projects on each of the PEN contracts, the new website also contains archive links to the "Initial PEN" information. Some of the new features introduced are:

- A new "Publications" page, complete with a break down of the Manuscripts, Abstracts/Presentations and Patents by each PEN, plus a customized direct link to Pub Med Central by PEN.
- A new Skills Development page hosting information regarding Science Outreach events, Courses in nanotechnology, Seminars and Training of PEN students and postdocs.
- A new secure Member Pages section for PEN Participants contains information about the upcoming Inter-PEN meeting, the PEN Personnel Directory, the protocols and information about the Executive Committee meetings.

Today the website may be found at: <u>www.nhlbi-pen.net</u>. Ultimately, the website will be migrated to a new ".gov" URL, once the recent "freeze" on ".gov" domain names is lifted by the Obama Administration.



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Skills Development

Making a Difference

### **COMMUNITY OUTREACH**

"Nano Days" at the St. Louis Science Center Monica Shokeen shokeenm@mir.wustl.edu

The skills development core of the Washington University PEN organized a science outreach event at the Saint Louis Science Center on April 8th and 9th, 2011. The event was part of NanoDays 2011, which included educational programs and hands-on demonstrations about nanotechnology and its potential impact on the future. The two day event included participation by local Universities and companies displaying various uses of nanotechnology. This event was a great resource for the skills development core as it was an ideal way to reach out to the local community to describe the impact nanotechnology was creating in their day-today lives. The booths were set up such that there was effective communication with the demonstrators and visitors. The booths were attended by all age groups, ranging from kindergartners' to middle school students to adults



Tiffany Gustafson, a Postdoctoral Research Associate in Mikhail Y. Berezin's laboratory, discusses and demonstrates various nanotechnology applications.

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from various walks of life. The curiosity and enthusiasm of the audience was a testimony to the event's success!

The experiments that were covered by the Washington University School of Medicine PEN group were:

A. Investigation of the UV-blocking abilities of different nanoparticle containing sunscreen lotions.

**UV-beads** respond to UV irradiation by changing color, and were used to assess how much of the UV light passed through a transparent surface coated with the lotions with different protection factors.



Assistant Professor, Suzanne E. Lapi, uses an Ultra Violet light pen as a convenient method for applying a focused light source on the beads.

Afterwards, the children enjoyed the fun seeing the bright color change of the UV-beads in the sunshine outside.







#### B. Testing the stain-repelling ability of normal and nanotechnologyenhanced fabric.

A few drops of coffee, fruit punch, ketchup and mustard were deposited on two pieces of cloth, one made of plain cotton and one made with cotton coated with "nano-whiskers." The students observed whether or not staining occurred.

#### C. How does the car windshield treatment (Rain-x) works.

Beading of water droplets on the rain-x sprayed glass vs. non-treated side was demonstrated.

This year, there was active volunteer participation from Washington University School of Medicine staff members. including, Evelyn Madrid, Efrem Mebrahtu, Tiffany Gustafson, Maiko Kume, Curtis Carey, Eileen Cler.

The skills development team would like to thank our gracious volunteers for all of their help!!!



PEN

Evelyn Madrid and Monica Shokeen demonstrate the hydrophobic property of the fabric coated with "nano-whiskers".

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Washington University School of Medicine

5th Annual Inter-PEN Meeting

November 4-5, 2011 - Washington University School of Medicine

The 5<sup>th</sup> Annual Inter-PEN meeting on November 4-5, 2011, at Washington University School of Medicine. This will be the first Annual Inter-PEN Meeting for the new PEN Contracts. All PEN participants are welcome to attend. The agenda for the meeting will available soon.

The hotel is less than one block from the meeting location. Both the hotel and meeting location may be accessed by taking the Metrolink train from the airport to the Central West End station. The senior investigators will stay on the Executive Level (top three floors) of the Parkway Hotel, with the students and postdocs on the Lower Level.

NHLBI Inter-PEN Quarterly http://www.nhlbi-pen.net/newsletter.html

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#### The meeting will be held at:



Washington University School of Medicine Farrell Learning and Teaching Center Connor Auditorium 520 S. Euclid Avenue St. Louis, MO 63110

#### Hotel accommodations will be at:



Parkway Hotel Contact: Jaci Bolsega, Sales Manager (314) 256-7777 4550 Forest Park Avenue St. Louis, Missouri 63108 http://www.theparkwayhotel.com/

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