Biomaterial Interfaces
Room: 317 - Session BI+AS+NS-MoA

Bio/Nano Interfaces
Moderator: Patrick Koelsch, University of Washington

2:00pm **BI+AS+NS-MoA1 Controlling Bio/Nano Interface Response using Metal Oxide Atomic Layer Deposition: Zinc Oxide ALD Modifies how Human Lung Fibroblasts respond *In Vitro* to Multiwall Carbon Nanotubes**, ***Erinn Dandley***, *A. Taylor, G.N. Parsons, J. Bonner*, North Carolina State University

Carbon nanotubes have been reported to cause pulmonary fibrosis in mice after inhalation exposure. When inhaled, multiwall carbon nanotubes (MWCNTs) activate macrophage inflammasomes and interleukin (IL)-1β release, key cellular components of the innate immune response. Macrophages are the first line of defense that engulf and remove inhaled MWCNTs from the lungs. Macrophages are also a source of secreted osteopontin (OPN), which promotes tissue matrix remodeling and fibrosis. These responses may be triggered by the unique aspect ratio, aggregation or surface chemistry of MWCNTs. In previous studies, we explored atomic layer deposition (ALD) as a means to modify the surface functionality of MWCNTs and studied how the surface coating affected the toxic response of THP-1 cells, a widely used human monocyte/macrophage cell line, and primary peripheral blood monocytes (PBMCs) obtained from normal human donors. Compared to uncoated MWCNTs, we found that nanotubes with Al_2O_3 nanocoatings showed enhanced IL-1β secretion and decreased OPN production in THP-1 cells and PBMCs, indicating that the coating enhances the innate immune response and decreases pro-fibrotic activity.

In this study we examined the effect of ALD ZnO coatings on the fibrogenic response in human lung fibroblast (HLFs) using mRNA expression and secretion of transforming growth factor (TGF)-b 1 and CXCL10, mediators that promote and deter fibrosis respectively. We find that the ALD ZnO layer thickness can be controlled down to ~5nm, and the thickness scaled directly with the number of ALD cycles, as observed by TEM. Thicker coatings inhibited MWCNT aggregation, and sonication allowed us to induce fiber fragmentation. In this way the ALD coating allowed us to independently adjust surface termination, fiber aggregation, and fiber aspect ratio, providing us a unique tool to examine how each of these factors influences cellular response. Initial results show that the ZnO coating significantly increased TGF-β1 mRNA expression and stimulated a larger pro-fibrogenic response in HLFs compared to uncoated MWCNTs. Control experiments using ZnO nanoparticles also showed potent induction of TGF-β1 mRNA in HLFs. Also, the response tends to correlate with extent of dispersion, and is nearly independent of MWCNT aspect ratio. These experiments show that nanoscale surface functionalization of nanoscale materials may help us gain better understanding of the mechanisms associated with toxicology of nanomaterials, and expand knowledge of biological response at nano/bio interfaces.

2:20pm **BI+AS+NS-MoA2 Mechanically Optimized Fe (III) Doped Silica Nanoshells as a Contrast Agent for Ultrasound Imaging and HIFU Therapy**, ***James Wang***, *A. Liberman, R. Viveros, C. Barback, S.L. Blair, Z. Wu, R. Mattrey, W. Trogler, A.C. Kummel*, University of California at San Diego

Ultrasound (US) is a common medical imaging modality due to its flexibility, low-cost and therapeutic potential. 500 nm silica nanoshells were synthesized as a contrast agent to improve US imaging signal for better diagnostic performance. Iron (III) was included into the silica network to enhance the biodegradability of the silica nanoshells. Previously, ferric iron was shown to facilitate silica nanoshell biodegradation due to its strong binding affinity with serum transferrin proteins. The removal of iron from the silica network by serum proteins fragments the nanoshells enabling effective biodegradation for *in vivo* applications. The silica nanoshells are filled with perfluorocarbon (PFC) vapor which expands and shatters the nanoshells during US irradiation. A mechanically weaker silica nanoshell increases US signal at lower power. A range of alkoxysilanes with selected R-groups such as long chain hydrocarbons, fluorinated carbon chains, fluorinated phenyl groups and vinyl groups were employed along with tetramethyl orthosilicate and iron (III) ethoxide in a modified sol-gel synthesis to create structural defects that alter the mechanical properties of the nanoshells. Monodispersed 500 nm polystyrene beads were used as a soft template during the reaction. The silica nanoparticles were calcined at 550 C to remove the polystyrene core and form hollow nanoshells. SEM and TEM showed that 500 nm silica nanoshells with different microstructures were synthesized incorporating alkoxysilanes with different R-groups. Formulations with higher concentrations of alkoxysilanes with large R-groups such as long chain hydrocarbons resulted in stronger *in vitro* contrast enhanced ultrasound (CEUS) signals due to the increase of structural voids that resulted in weaker shell strength. CEUS experiments demonstrated that mechanically weaker silica nanoshells exhibited longer signal life time and required a lower mechanical index (MI) for imaging. The high intensity focused ultrasound (HIFU) properties of the modified silica nanoshells were tested for potential therapeutic applications. Mechanically weaker silica nanoshells were shown *in vitro* to require a lower HIFU power to fracture which is consistent with safer HIFU therapy. By synthesizing strength tunable silica nanoshells as US contrast agents, it is possible to improve diagnostic US imaging performance in order to detect smaller tissue structures or early stage tumors. Additionally, mechanically weaker silica nanoshells may also increase the efficiency of HIFU enabling HIFU at lower US power and/or higher speed.

2:40pm **BI+AS+NS-MoA3 Synthesis, Functionalization, and Biological Imaging with Quantum Dots**, ***Preston Snee***, University of Illinois at Chicago **INVITED**

Semiconductor quantum dots (QDs, or nanocrystals), are very bright chromophores that possess unlimited potentials in alternative energy generation and for biological sensing and imaging applications. Our group has made advances in the synthesis QDs to produce 100% efficient emitters; furthermore, we can dope the semiconductor with guest ions to alter the bandgap. We recently invented a method to dope each quantum dot with an exact number of guest ions, a feat that was previously considered impossible. As very bright fluorophores, quantum dots are ideal for biological imaging and sensing. Our first contribution in this regard was to develop methods of chemical and biological functionalization of water-soluble quantum dots as many existing methods either quenched the QDs or had very low reaction yields. We have circumvented these problems by synthesizing polymers which serve as QD functionalization reagents; the polymer – QD activated intermediate has increased stability and allows us to conjugate chemical and biological vectors to the nanocrystals with ~100% reaction yields. We use these methods to functionalize QDs with organic fluorophores that can report on the local chemical and biological environment. We have synthesized several ratiometric, or "self-calibrating" sensors, for pH, toxic metals, DNA, and proteins. In our recent work on protein sensing, we have developed an all optical method for sensing un-labeled proteins with a better detection limit than any currently existing technology. We have also circumvented the well-known problem of cytocellular delivery of quantum dots into live, adherent cells.

3:40pm **BI+AS+NS-MoA6 Easynanofab: Fast, Simple, Combinatorial Routes to Reusable Plasmonically Active Gold Nanostructures Over Macroscopic Areas**, *A. Tsargorodska, O. El Zubir,* ***Graham Leggett***, University of Sheffield, UK

Plasmonic effects associated with gold nanocrystals have attracted widespread interest for the interrogation of biological molecules. Existing approaches to fabrication of plasmonic nanostructures fall into two categories: high precision methods such as electron beam lithography that rely on complex, specialised infrastructure; and simple, low-cost methods such as colloidal lithography that offer limited capacity. Here, we describe a fast, simple method for the fabrication of re-usable, robust gold nanostructures over macroscopic (cm^2) areas that provides enormous scope to control nanostructure morphology and dimensions, and which also uses only simple apparatus and requires no access to a clean-room. We have assembled a combinatorial library of over 200 different samples consisting of highly crystalline gold nanostructures that exhibit varying morphologies, dimensions and periodicities but yield intense plasmon bands. These structures enable the rapid identification of optimum substrates for the detection and analysis of biological targets, and provide a platform for exploring the relationship between particle morphology and optical properties. Self-assembled monolayers (SAMs) of alkylthiolates on chromium-primed polycrystalline gold films are patterned using a Lloyd's mirror interferometer and etched using mercaptoethylamine in ethanol in a rapid process. The use of a Cr adhesion layer facilitates the cleaning of specimens by immersion in piranha solution, enabling their repeated re-use without significant change in their absorbance spectra over two years. Annealing yields structures with a uniformly high degree of crystallinity that exhibit strong plasmon bands. Because of the ease with which nanoparticle morphology may be controlled using interferometric lithography (IL), it provides a convenient means to investigate the correlation between structural parameters (particle dimensions, spacing) and optical responses. The shift in the position of the plasmon band after site-specific attachment of histidine-tagged green fluorescent protein (His-GFP) and after adsorption of chlorophyll and bacteriochlorophyll was measured

for a range of nanostructured films, enabling the rapid identification of structures that yielded the largest shifts. Strong resonant coupling was observed when light-harvesting membrane protein complexes from plants and bacteria were coupled to gold nanostructure arrays, yielding absorbance spectra that were very different from those of the clean gold nanostructures. This approach offers a simple route to the production of durable, reusable, macroscopic arrays of gold nanostructures with precisely controllable morphologies.

4:00pm **BI+AS+NS-MoA7 Impacts of Nanoparticle Synthesis Route, Structure and Serum Proteins on the Dispersion and Dissolution of Ag Nanoparticles in Biological Media**, *P. Munusamy, J.N. Smith, C. Liu, C.-M. Wang,* Pacific Northwest National Laboratory, *S. Chen,* Imperial College London, UK, *M.H. Engelhard,* Pacific Northwest National Laboratory, *A.E. Porter, M.P. Ryan,* Imperial College London, UK, ***Donald Baer***, Pacific Northwest National Laboratory

The wide-spread use of silver nanoparticles in consumer products raises questions of environmental impact and toxicity. Because both silver particles, and silver ions formed by particle dissolution, may impact biological systems, it is important to understand the characteristics of silver nanoparticles as they are made and their stability and dissolution in the medium relevant to environmental and toxicological studies. Silver nanoparticles produced by different synthesis routes can have significantly varying physical and chemical characteristics. In this talk we will summarize the characterization and dissolution stability of three types of silver nanoparticles (20 nm particles synthesized with and without gold core (~7 nm) and 110 nm particles with gold core) in cell culture media with serum proteins: FBS10%/RPMI, the culture media used at Pacific Northwest National Laboratory for *in-vitro* toxicity studies. These nanoparticles were synthesized and prepared for biological study in aqueous solution. They were examined *in situ* using dynamic light scattering, zeta potential measurements and optical adsorption and *ex situ* with x-ray photoelectron spectroscopy and transmission electron microscopy. For the dissolution studies, concentrations of particles examined were varied from 1 μg/ml to 50 μg/ml, consistent with the range of concentrations typically used during *in-vitro* studies. Silver particles with gold cores had smaller crystallite size and higher apparent solubility than three different batches of pure ~ 20 nm silver particles. A simple dissolution model was found to describe the time variation of particle size and amount of dissolved silver for particle loadings above 9 μg/ml. The effective solubility product obtained from fitting the data was higher for the 20 nm particles with the gold core in comparison to the pure silver or 110 nm particles. The dissolution of silver nanoparticles was also found to be enhanced by presence of serum proteins contained in fetal bovine serum (FBS). In addition, the protocol of dispersion in cell culture medium was found to influence particle agglomeration and the rate of dissolution. In these measurements focusing on a 24 hour time point, we found that the structure of the silver nanoparticles can have a significant impact on the concentration of dissolved silver in media and thus the dosimetry to which cells would be exposed during in vitro studies.

This work has been supported by the NIEHS under Center grant U19 ES019544. Portions of this work were performed using EMSL, a national scientific user facility sponsored by the US Department of Energy, Biological and Environmental Research and located at PNNL.

4:20pm **BI+AS+NS-MoA8 Analysis of Protein Coated Nanoparticles by X-ray Photoelectron Spectroscopy and Solution-Based Particle Size Techniques**, *C. Minelli*, ***Natalie Belsey***, *A.G. Shard*, National Physical Laboratory, UK

The attachment of proteins to nanoparticles' surface is of increasing interest in medicine for applications such as drug delivery and diagnostics. The unintentional acquisition of a protein corona from biological media is also important in determining the performance and potential toxicity of such particles. Understanding and refinement of the performance of nanoparticles of use in medical applications require accurate and quantitative characterisation of their protein interface. Our efforts are focussed upon developing measurement techniques to enable useful characterisation of this interface. In this study, three biomolecules of a range of sizes, shapes and mechanism of interaction with gold surfaces, i.e. 16 AA peptide, BSA and IgG, were adsorbed to gold nanoparticles (10, 20, 40, 60 and 80 nm) and the shell thickness was measured in solution using dynamic light scattering (DLS) and differential centrifuge sedimentation (DCS). UV-visible spectrophotometry was used to monitor localised surface plasmon resonance (LSPR) shifts of the nanoparticles due to the acquisition of the protein shell. Combination of this information with thickness measurements allowed for an estimation of the protein shell refractive index and average number of biomolecules at the nanoparticle surface. X-ray photoelectron spectroscopy (XPS) analysis of the same nanoparticles deposited on a PTFE substrate enabled determination of the nanoparticle shell chemical composition and dehydrated thickness, from which the number of molecules at the nanoparticle surface was also estimated. Parallel characterisation of the nanoparticles in their colloidal form and in vacuum provided consistent results and the combination of the techniques revealed farther insight into molecular adsorption at nanoparticles' interfaces. The complementarity of the approaches also allowed for validation of the methods, which is important for their application to a wide range of nanoparticle types. For example, DLS and LSPR analysis are not suitable for dealing with aggregated samples, but XPS is, while XPS measurements of organic nanoparticles are challenging and liquid based techniques may be preferred.

4:40pm **BI+AS+NS-MoA9 Development of Nanofibrous Meshes as Smart Dressings for Chronic Wound Care**, ***Martina Abrigo***, *P. Kingshott, S.L. McArthur*, Swinburne University of Technology, Australia

Diabetic, pressure, venous and arterial ulcers are a large social, economic and healthcare burden. These chronic non-healing wounds show delayed and incomplete healing processes exposing patients to high risk of infection. The design of wound dressings that combine the necessary morphological and physical requirements for wound healing with the value-added capability to address optimal cell responses and impair bacterial proliferation represents a major challenge in chronic wound care. Polymeric nanofibrous meshes fabricated through the electrospinning process are promising candidates as wound dressings due to their high surface area, micro-porosity and non-woven structure. In this study, the parameters of the electrospinning process (such as spinning rate and electric field intensity) were optimized to fabricate nanofibrous membrane in Polystyrene (M.W. 250.000). The morphological properties of the electrospun meshes were analysed by bright microscopy, three-dimensional optical profiler, Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). Electrospun materials have been used as scaffolds for tissue engineering for a number of years, but there is surprisingly little literature on the interactions of fibres with bacteria. In order to understand microbial infiltration and control in wound dressings, a number of microbiological assays (MTT, MTS and live/dead) were completed using *E. Coli*, *P. Aeruginosa*, *S. Aureus* in an effort to understand how the morphological and structural properties of the electrospun meshes influence bacterial attachment, proliferation and growth.

5:00pm **BI+AS+NS-MoA10 Electrophoretic Stretching of Tethered DNA in Nanoslits**, ***Jia-Wei Yeh***, *K. Szeto, H.G. Craighead*, Cornell University

We have investigated the field-extension of tethered DNA in nanoslits with slit heights ranging from 30 to 130 nm, and performed an analysis from an approximated modified worm-like chain (mWLC) field-extension relation. DNA molecules attached to microspheres were anchored at a micro-nanofluidic interface and the molecules electrophoretically extended. We demonstrated that both the DNA segmental correlation and equilibrium lengths increased as the slit height decreased. Furthermore, for extremely confined DNA where h≤30 nm, we observed reptation of the DNAs' contours within the nanoslit, a phenomena that may be induced by inhomogeneous surface charge distributions. This nano-confined system may have implications for single-molecule sensors on detecting and analyzing genetic, epigenetic markers, and related nanobiotechnological applications.

5:20pm **BI+AS+NS-MoA11 Measuring DNA Looping Pathways using Nanofluidic Manipulation**, *M. Roushan, Z. Azad, H. Wang,* ***Robert Riehn***, NC State University

DNA performs a carefully choreographed ballet during the cell cycle. The organization is driven by the specific binding of proteins to form tertiary DNA-protein-DNA complexes. The search process that precedes the formation must overcome the challenge of very low effective mobility of genomic-sized DNA pieces in the dense cellular environment.

In this paper we will discuss a group of nanofluidic device that force two DNA molecules to either slide past each other in parallel, or cross over each other at a steep angle. Nanochannel cross-sections are 100x100 nm^2, and are hundreds of microns long. Because DNA is elongated through confinement, loop with a length down to 2 kb can be directly observed in real time. Channels are made of fused silica, enabling single-molecule observation of both DNA and proteins. Because the effective concentration of DNA inside channels exceeds 1 mg/ml with the channel at the point of DNA-DNA contact, protein-mediated capture cross-sections are very high.

We will present analyses of different DNA-binding proteins that demonstrate that we can distinguish dense and sparse binding modes and the compensation of electrostatic DNA-DNA repulsion through protein binding. We further report the detection of long-lived tertiary complexes acting as a lock for looped DNA configurations, and the presence of very short-lived transient links. We further demonstrate a pathway for loop formation that is enhanced in nanochannel devices, and that may be

important in a cellular context. By using precision hydrodynamic flows, we are able to measure free energies of the search process.

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