Biomaterial Interfaces Division
Room: 12 - Session BI+NS-WeM

Biomaterials and Nanomaterials Fabrication & In Honor of Dave Castner's 65th Birthday: Multitechnique Bio-Surface Characterization I

Moderator: Caitlin Howell, University of Maine

8:00am **BI+NS-WeM1 Plasma-Enhanced Chemical Vapor Deposition of an Antibacterial Coating from an Essential Oil-Derived Precursor**, ***Michelle Mann***, *E.R. Fisher*, Colorado State University

Polymeric constructs, such as filtration membranes for water treatment and nanocomposite scaffolds for wound healing and drug release, are often chosen for their ideal bulk properties (e.g., porosity, mechanical strength, and chemical inertness). Challenges faced when using such materials in aqueous settings, however, include their hydrophobicity and propensity for bacterial attachment, leading to biofilm formation and degradation of material performance. Modifying the surface of the constructs while simultaneously maintaining the bulk properties offers both the possibility of addressing these limitations and the potential for creating new advanced materials. Plasma processing is a valuable tool often used to improve material wettability and deposit antifouling coatings. Here, plasma-enhanced chemical vapor deposition is used to deposit films from eucalyptol (1,8-cineole), an antibacterial precursor derived from tea tree oil. Although the antibacterial properties of eucalyptol are supported by numerous clinical trials, it is unknown to what extent the monomer structure and biocidal capabilities are maintained in plasma polymerized films. Thus, we have explored the properties of the eucalyptol-based films as a function of plasma parameters (e.g., power, pressure). Surface analyses (contact angle goniometry, X-ray photoelectron spectroscopy, scanning electron microscopy, and optical profilometry) reveal film wettability directly correlates to precursor pressure, with water contact angles ranging from ~50° to 85°. To further improve wettability of these materials, they were subjected to H_2O (v) plasma modification, an approach that has been successful in past studies to improve polymer biocompatibility. After plasma treatment, wettability increased, with water contact angles of ~20-35°, and the films exhibited a significant enhancement in oxygen content (40-150%), while remaining stable in aqueous solutions. Attachment and biofilm formation assays allowed for assessment of bacterial interactions at 1 and 5 days after exposure, respectively, with gram-negative *E. coli* and gram-positive *S. aureus*. Using microscopy techniques, we observed attachment and growth are substantially diminished for as-deposited and H_2O (v) plasma treated films. Moreover, performance data (i.e., flux of coated ultrafiltration membranes) are presented. Surface analysis and performance testing results, combined with information about gas phase excited state species observed using optical emission spectroscopy, guide our development of additional antibacterial essential oil-based films for 2D and 3D constructs used in environmental and biomedical applications.

8:20am **BI+NS-WeM2 Transition Metal Nanoparticles and Quantum Dots with Tunable Electronic Properties by Bacterial Precipitation: Synthesis and Applications**, *K.E. Marusak, Y. Feng, E. Ngaboyamahina, Y. Cao, J.T. Glass, L. You,* ***Stefan Zauscher***, Duke University

We present a new method for the fabrication of semiconducting, transition metal nanoparticles (NPs) with tunable bandgap and useful photoelectric properties, through bacterial precipitation. *Escherichia coli* bacteria have been genetically engineered, by overexpression of a cysteine desulfhydrase gene, to precipitate transition metal NPs from solution, here more specifically, cadmium sulfide (CdS). Transmission electron microscopy (TEM), X-ray diffraction (XRD), and X-ray photoelectron spectroscopy (XPS) revealed that the bacterially precipitated NPs are agglomerates of mostly quantum dots (QDs), with a diameter of 4-5 nm, in a carbon-rich matrix. We discovered that the precipitation conditions of the bacteria can be tuned to produce NPs with bandgaps that range from quantum-confined to bulk CdS. Furthermore, we determined their photoelectrochemical (PEC) properties and their energy band structure by electrochemical measurements. In addition, by taking advantage of the organic matrix, which is residual from the biosynthesis process, we fabricated a prototype photocharged capacitor electrode by incorporating the bacterially precipitated CdS with a reduced graphene oxide (RGO) sheet. Our results show that bacterially precipitated CdS NPs are potentially useful components for PEC devices with applications for energy conversion and storage.

References:

1) Katherine E. Marusak, Yaying Feng, Cooper F. Eben, Stephen T. Payne, Yangxiaolu Cao, Lingchong You, Zauscher, S. “Cadmium sulphide quantum dots with tunable electronic properties by bacterial precipitation,” RSC Advances, **2016**,6, 76158-76166.

2) Yaying Feng, Edgard Ngaboyamahina, Katherine E. Marusak, Yangxiaolu Cao, Lingchong You, Jeffrey T. Glass, and Stefan Zauscher, "Hybrid (Organic/Inorganic) Electrodes from Bacterially Precipitated CdS for PEC/Storage Applications," The Journal of Physical Chemistry C **2017***121* (7), 3734-3743

8:40am **BI+NS-WeM3 Plasma Surface Modification of 2D and 3D Constructs: Creating and Evaluating New Materials for Biomedical Applications**, ***Ellen Fisher***, Colorado State University **INVITED**

Plasma processing represents a powerful approach to modification of 2D and 3D substrates with an array of chemistries appropriate for use in biomedical applications. It is an attractive methodology because of its versatility, low waste, and scalability. The three major plasma surface modification classifications are deposition (film formation, polymerization), etching (removal of material) and functional group implantation (covalent bonding of chemical functional groups), which collectively provide a large landscape for creating materials with specific properties tailored for particular applications. Combining a range of spectroscopic techniques, materials characterization tools, and basic biological interaction studies provides a platform for deeper insight into these underlying mechanisms than just one approach alone. Yet, this can also lead to a range of obstacles, especially when seeking to apply traditional diagnostic methodologies to new systems and materials. For example, data on the surface chemistry of 3D constructs coated with thin films having a range of film chemistries (via utilization of allylamine/allylalcohol mixed gas plasmas) combined with an understanding of the gas-phase chemistry in these systems and cell-surfaces interaction studies highlights key properties required to tune the surface chemistries that can promote or deter cell viability and proliferation. Thus, this presentation will highlight a few key examples, including inherent challenges, where such a unified, comprehensive approach has been fruitful for 2D and 3D materials intended for use as antimicrobial materials, in separations, and for tissue engineering applications.

9:20am **BI+NS-WeM5 The Ins and Outs of Functionalized Natural Materials for Applications in Drug Delivery and Separations**, ***Norma Alcantar***, *R. Toomey, Z. Veisi,* University of South Florida, *A. Cardenas-Valencia, M. Cardenas,* SRI International, *R. Falahat,* Moffitt Cancer Center, *T. Peng, F. Guo,* University of South Florida **INVITED**

In the last decade, numerous natural materials have been investigated as platforms in functionalized surfaces. In our case, we have studied the structure and properties of two natural materials, chitosan from crustaceous shells and cactus mucilage from cactus plants. Those two natural materials have been used as building blocks in drug delivery systems, and as flocculants or adsorbent materials to remove contaminants from water. In the drug delivery systems, the natural material is used as surface membranes capable to respond to external stimuli. Our team has discovered that chitosan has a specific bond with the MUC1 enzyme found in epithelial-type cancers, which can enhance its specificity towards cancer cells when used in drug delivery systems. The results of our research have also shown that depending on the biophysical conditions surrounding the natural materials, their response to hydrophilic and hydrophobic interactions to separate organic and inorganic contaminants are controlled by their structure, which can then be finely tuned to enhance their performance. The use of natural materials for functional applications is an area of study that could lead to discoveries in microfluidic devices, health applications, cosmetics, coatings and paintings, and water purification systems.

11:00am **BI+NS-WeM10 Combinatorial Material Chemistry-Topography Screening: The ChemoTopo Chip**, ***Britta Koch****, University of Nottingham, UK, *A. Vasilevich, N. Beijer, J. de Boer,* Maastricht University, The Netherlands, *M.R. Alexander,* The University of Nottingham, UK

The interaction of cells with their culture substrate is critical to their fate, having a profound impact on cell response and viability. However, complex cell-cell as well as cell-matrix interactions in native tissue make it challenging to emulate *in vivo* cell behavior in the lab. The design of man-made, biomimetic cell environments hold great potential for biomedical applications like tissue engineering, disease modeling and drug screening.

*** BID Early Career Researchers Award**

Therefore, suitable biomaterials are sought that can interface with cells and provide adequate physical, chemical and biological characteristics to elicit the desired cell response in a well-defined *in vitro* environment.

In recent years, microarray technology in combination with high-throughput surface characterization methods has proven to be a valuable tool for the cost-efficient and rapid screening of large libraries of biomaterial candidates. However, until now screening has been performed either on planar samples, focusing on optimizing sample chemistry rather than topography [1] or on topography with no chemical variation [2]. Here we propose a novel platform that augments the chemical screening approach with deterministic control of the topography. This new platform called the 'ChemoTopo Chip' allows the systematic investigation of combinatorial effects of well-defined surface chemistry and topography and moves closer to recapitulating the range of 3D cues at play *in vivo* within an *in vitro* screen. The first results on the identification of hit combinations supporting mesenchymal stem cell growth are presented and future steps aiming at enhancing our global understanding of the context-dependent cell response are outlined. The ChemoTopo Chip platform contributes to the discovery of novel substrates with the potential to ultimately translate these into biomedical applications. Also, the gathering of data allows to develop surface structure-property relationships from which understanding can be generated to support rational design of the *in vitro* cell environment.

[1]. Y. Mei, K. Saha, S. R. Bogatyrev, J. Yang, A. L. Hook, Z. I. Kalcioglu, S.-W. Cho, M. Mitalipova, N. Pyzocha, F. Rojas, K. J. Van Vliet, M. C. Davies, M. R. Alexander, R. Langer, R. Jaenisch, D. G. Anderson. *Nature Materials* 9, 768-778 (2010).

[2]. H. V . Unadkat, M. Hulsman, K. Cornelissen, B. J. Papenburg, R. K. Truckenmüller, A. E. Carpenter, M. Wessling, G. F. Post, M. Netz, M. J. T. Reinders, D. Stamatialis, C. A. Bitterswijk, J. de Boer. *PNAS* 108, 16565-16570 (2011).

11:20am **BI+NS-WeM11 Combining Surface Analytical and Computational Techniques to Investigate Orientation Effects of Immobilized Proteins**, ***Elisa Harrison***, *G. Interlandi, D.G. Castner*, University of Washington, Seattle

Controlling how proteins are immobilized (e.g. controlling their orientation and conformation) is essential for developing and optimizing the performance of *in vitro* binding protein devices, such as enzyme-linked immunosorbent assays. The objective of this work is to develop new methodologies to study proteins and complex mixtures of proteins immobilized onto surfaces.

The focus of this study was to control and characterize the orientation of protein G B1, an IgG antibody-binding domain of protein G, on well-defined surfaces as well as measure the effect of protein G B1 orientation on IgG antibody binding using a variety of surface analytical and computational techniques. The goal was to immobilize protein G B1 into well-ordered films with different orientations that control the accessibility of antibody binding sites.

The surface sensitivity of time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to distinguish between different proteins and their orientation by monitoring the changes in intensity of characteristic amino acid mass fragments. Amino acids with asymmetric distributions (Asn, Trp, Gly, Ala, and Thr) were used to calculate peak intensity ratios from ToF-SIMS data in the C- and N-terminus of protein G B1 to determine the orientation of five different cysteine mutants of protein G B1 covalently attached to a maleimide surface.

To study the effect of protein orientation on antibody binding, we formed multilayer protein films. Quartz crystal microbalance with dissipation monitoring (QCM-D) detected protein coverages of 69 - 130 ng/cm^2. QCM-D and X-ray photoelectron spectroscopy (XPS) analysis revealed that packing density along with orientation affected the antibody binding process. Spectra from ToF-SIMS using large Ar gas cluster ion sources distinguished between different proteins in multilayer protein systems.

Additionally, development of computational methods to study proteins on surfaces can complement surface analytical data. A Monte Carlo algorithm was developed to predict protein orientation on surfaces. Two distinct orientations of protein G B1 adsorbed onto a hydrophobic surface were found and characterized as two mutually exclusive sets of amino acids on the outermost β-sheets contacting the surface. This prediction was consistent with sum frequency generation (SFG) vibrational spectroscopy results. In fact, theoretical SFG spectra calculated from an equal combination of the two predicted orientations exhibited reasonable agreement with measured spectra of protein G B1 on polystyrene surfaces. This method has been expanded to predict protein G B1 orientations on more complex surfaces, such as self-assembled monolayers.

11:40am **BI+NS-WeM12 Characterizing the Tumor Microenvironment and Tumor Progression**, ***Blake Bluestein***, University of Washington, *F. Morrish, D. Hockenbery*, Fred Hutchinson Cancer Research Center, *L.J. Gamble*, University of Washington

Solid tumors are not simply masses of malignant cells but are a structurally complex system, composed of a myriad of cells. The interactions between malignant cells and non-transformed cells form the tumor microenvironment. The tumor microenvironment has been associated with regulating tumor cell growth, metastatic potential, and drug resistance. Here, a combination of techniques including imaging time-of-flight secondary ion mass spectrometry (ToF-SIMS), H&E staining, and second harmonic generation (SHG) microscopy are used to analyze pancreatic biopsies from a mouse model with Myc-dependent inducible pancreatic β-cell neoplasia to relate changes in the composition and distribution of metabolic related molecules with tumor development. Myc, one of the most frequently deregulated oncogenes in human cancers, contributes to tumorigenesis through various mechanisms, including the deregulation of cell proliferation and metabolic alterations.

Pancreatic tissues were harvested and frozen in optimal cutting temperature (OCT) at 6 days post Myc induction and without any Myc induction (control). Cryosections (4 µm thickness) were serially cut, with one used for H&E staining and SHG microscopy, and one for ToF-SIMS analysis. ToF-SIMS data was acquired using an IONTOF TOF.SIMS 5. Regions identified by analysis and principal components analysis (PCA) were cross-referenced against immunohistochemical, H&E, and SHG images to differentiate the tumor areas from the surrounding tissue.

PCA analysis of ToF-SIMS image data separate tumor from surrounding tissue and reveal the differences in chemistries between the two regions. The Myc-induced islet tumors exhibit a signal of C14:0, a likely product of *de novo* fatty acid synthesis within the islet tumor. Image data shows higher signal regions within the interior of the tumor. These regions exhibit an increased, localized signal of CN^-, CNO^-, Fe^+, and characteristic histidine fragments, $C_5H_8N_3^+$ and $C_6H_5N_2O^+$. SHG images showed that there were no organized structures in these higher signal regions and immunohistochemistry showed no signs of angiogenic processes, confirming that these areas are blood pools resulting from vascular hemorrhaging. Further metabolic analyses showed that when compared to control islets, Myc-induced tumor islets exhibited increased intensities of amino acids and phosphatidylcholine lipids (30:0, 32:1, 32:2), which are known to be related to tumor growth. Tissue surrounding the Myc islet tumors exhibited lower intensities of serine, glycine, and arginine when compared to the tissue surrounding the control islets, which suggests tumor uptake or an increased catabolism induced by the adjacent tumor.

12:00pm **BI+NS-WeM13 Observing the Molecular Mechanisms of Insect Adhesion by Sum Frequency Generation Spectroscopy**, *J.E. Fowler*, Oregon State University, *S.N. Gorb*, Kiel University, Germany, *T. Weidner*, Aarhus University, Denmark, ***Joe Baio***, Oregon State University

Many insects can walk on a range of natural surfaces through an adhesion process that combines an expansive array of hairy contacts on their feet, known as setae, and an adhesive fluid, forming contact between the setae and a substrate. Previous studies of this adhesion system have focused almost exclusively on the mechanical and kinematic aspects of adhesion, while ignoring the molecular interactions at the fluid – substrate interface. However, recent experiments illustrate that substrate chemistry does influence the adhesive forces produced by this fluid. Additionally, mass spectrometry results demonstrate that this adhesive fluid is a complex mixture containing both hydrophobic (*i.e.* fatty acids and lipids) and hydrophilic (*i.e.* sugars, alcohols, and carbohydrates) compounds. We hypothesize that the molecular structure at the adhesive fluid-substrate interface is dynamic, with different molecules within the fluid selectively organizing at the interface as a function of substrate hydrophobicity. In the work presented here we probe the molecular interactions between the adhesive fluid taken from lady bugs (*Cocinella septempunctata*) and three model substrates, polyethylene oxide, polystyrene and CaF_2 with vibrational sum frequency generation (SFG) spectroscopy and scanning electron microscopy (SEM). The observed water contact angles for the polyethylene oxide, polystyrene and CaF_2 substrates were 66^o, 92^o and 106^o, respectively. High-resolution SEM images of individual seta-fluid footprints on the surfaces indicate localized "water in oil" emulsion de-wetting with no sign of distinct patterning. SFG spectra collected, from the three substrates, at the C-H (2800-3100 cm^{-1}) contain peaks at 2850 cm^{-1} and 2870 cm^{-1}, characteristic of symmetric CH_2 and CH_3 stretches, respectively. The presence of these peaks suggests an ordered hydrocarbon monolayer at the interface. However, subtle changes in ordering of these molecular groups at the interface were observed across substrates by comparing the ratio of the intensities of observed vibrational modes related to the CH_2 and CH_3 modes. Across the three different substrates this ratio increased with surface hydrophobicity suggesting that the fluid-surface interactions adapt to different substrate chemistries.

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