Biomaterial Surfaces & Interfaces
Room Naupaka Salon 6-7 - Session BI-MoM

35 Years of NESAC/BIO I

Moderator: David Castner, University of Washington

8:00am **BI-MoM1 Adventures in Biointerface Engineering Inspired by NESACBio – Combining and Integrating Techniques to Gain Insight into Biointerfaces (and Most Instruments Wins!)**, ***Sally L. McArthur,*** Swinburne Institute of Technology, Australia **INVITED**

Control and the ability to elicit specific responses from a biological system lies at the heart of most bioengineering. We want to immobilize proteins on biosensors but ask them to as sensitive as they are in solution or in the body, stimulate cells to assemble into tissues, reconstructing our bodily functions. We want methods that prevent bacteria forming biofilms and better still we would like them to stop bacteria attaching to surfaces full stop. But biology is soft and normally has lots of water associated with it, so how and why would you want to use vacuum based techniques to create coatings or characterise these systems?

This talk will explore how in my group and our collaborators, have tackled the challenges associated with interfacing vacuum deposited plasma polymers with water, proteins, lipids and cells to create a wide number of model systems and devices. At the same time, we have developed methods for chemically characterising these systems in vacuum, integrating XPS and ToF-SIMS with a range of other surface analytical and biological tools to gain insight into the materials we create and their interactions with biological systems.

8:40am **BI-MoM3 ToF-SIMS Label Free Chemical Imaging of Surface Modifications in Materials with Extreme Topography**, ***Michael Taylor,*** *D.J. Graham, L.J. Gamble,* University of Washington

ToF-SIMS is uniquely suited towards high spatial resolution imaging of surface modifications in materials with structure. While imaging 2D "flat" surfaces is relatively simple, working with three-dimensional (3D) "rough" surfaces is challenging due to the topography. This topography interferes with the ability to extract spatio-chemical differences in the sample and results in image shadowing and decreased mass resolution. In 3D depth profiling, topography can cause differential etching as the sputtering beam can impact the surface at different heights and angles. In many cases the combination of these factors prevents acquiring high quality imaging data since features associated with surface chemistry can be obscured. Multivariate image analysis methods have been used to assess the impact of topography on ToF-SIMS data, and AFM has been applied for topographical correction. However, the former method only assesses the impact, while the latter is time consuming and does not mitigate the effects of surface topography entirely. We propose an alternate methodology for imaging topographic samples with ToF-SIMS, demonstrating that through a simple polymer embedding methodology, topographic materials, ill-suited for ToF-SIMS analysis can be transformed into surfaces where topographical effects are minimized and high spatial resolution label free imaging of chemical modifications can be performed in topographic materials[1]. Imaging surface modifications in the pores of biopolymer scaffolds will be presented, using a highly focused Bi_3^+ LMIG beam for analysis in 2D, and the addition of an Ar_{1500}^+ sputter beam for depth profiling the pore in 3D.

FC (fluorocarbon) modification of PCL pores will be imaged, showing FC film deposits in the scaffold pore, and its distribution can be imaged in 3D. Water plasma hydrolyzed PCL scaffolds, modified with bovine serum albumin (BSA) through EDC/NHS bioconjugation chemistry can similarly be imaged, unlocking label free imaging of protein fragments at the scaffold/pore interface. We will also show that lipid imaging is possible in this challenging material class, extracting information in 2 and 3D from the pores of lipid modified poly(2-hydroxyethyl methacrylate)-co-methacrylic acid scaffolds.

1 M. J. Taylor, H. Aitchison, M. J. Hawker, M. N. Mann, E. R. Fisher, D. J. Graham and L. J. Gamble, *Biointerphases*, 2018, **13**, 03B415.

9:00am **BI-MoM4 NESAC/BIO IMPACT: Innovative Multivariate Programs Applied Carefully to ToF-SIMS**, ***Daniel Graham,*** *L.J. Gamble, D. Castner,* University of Washington

ToF-SIMS data is complicated. Even a single spectrum can contain hundreds if not thousands of peaks. Each peak corresponds to a unique element, fragment or molecule from the surface analyzed. The relative intensity of these peaks can encode information about the chemistry, structure and composition of the surface. With modern ToF-SIMS instrumentation it is straight forward to collect multiple spectra across multiple samples resulting in large, complex data sets. To further add to the scale of the data one can also produce 2D and 3D ToF-SIMS images which can consist of millions of spectra and fill gigabytes of storage space. Since 1992 NESAC/BIO has lead the way in developing innovative tools that enable digestion of this smorgasbord of ToF-SIMS data. This included some of the first papers published applying multivariate analysis (MVA) methods to ToF-SIMS data. This effort has lead to the creation of the NBToolbox which contains a set of advanced tools to process and display ToF-SIMS spectra and images. Though the ToF-SIMS community is relatively small, the NBToolbox has over 300 users across 39 countries on 6 continents. It is regularly used in research presented in publications and presentations around the world. In this presentation I will highlight the developments spearheaded through the years by NESAC/BIO in ToF-SIMS data processing from spectra to 3D imaging. Examples will be presented from the early beginnings of "simple" controlled systems to current work with complex tissue samples in 2D and 3D.

9:20am **BI-MoM5 Challenges to Nanoparticle Preparation and Analysis: An Unexpected Phase Transformation of Ceria Nanoparticles**, ***Donald Baer,*** Pacific Northwest National Laboratory*; S.V.N.T. Kuchibhatla,* Parisodhana Technologies Pvt. Ltd.*; A.S. Karakoti,* Ahmedabad University*; S. Seal,* University of Central Florida

Nanoparticles in a variety of forms continue to grow in importance for fundamental research, technological and medical applications, and environmental or toxicology studies. Physical and chemical attributes that lead to multiple types of particle instabilities complicate the ability to produce, appropriately characterize, and consistently deliver well-defined particles, frequently leading to inconsistencies, and conflicts in the published literature. In previous work examining 3-5 nm cerium oxide crystallites that had formed ~10 nm soft agglomerates in aqueous media we had observed chemical state changes (the ratio of Ce^{+3}/Ce^{+4}) and related optical absorption changes during particle formation and in response to environmental changes. The transformations have been further examined using micro-X-ray diffraction and Raman spectroscopy. We observed that in response to the environmental changes – adding H_2O_2 to the solution – these particles transformed from a ceria structure to an amorphous complex and returned to the crystalline phase upon solution aging. For comparison, 40 nm ceria nanoparticles were not observed to undergo this transformation and particles made up of crystallites of ~ 8 nm appeared to partially transform (or transform more slowly). We note that ceria nanoparticles of smaller size frequently have beneficial biological effects in comparison to the larger particles. The chemical state changes observed in ceria nanoparticles are usually assumed to be particle size dependent and to involve a change from cubic fluorite-type dioxide (CeO_2) to a hexagonal cerium sesquioxide (Ce_2O_3) with a continuous range of partially reduced CeO_{2-x} phases, where oxygen vacancies can be rapidly formed, arranged or eliminated. Our XRD and Raman data suggest that a much more complex transformation can occur for smaller ceria crystallites. Such changes were not readily identified by macroscopic *in situ* measurement such optical measurements or *ex situ* examination using TEM and XPS but were discovered by examination of ceria nanoparticles with molecularly and structurally sensitive methods with the particles in wet conditions (near *in situ*). Considering cerium oxide's useful abilities to scavenge radicals, control the oxygen environment and provide regenerative oxidation state switching, it appears that the ease of ceria nanoparticles to transform between Ce^{4+} and Ce^{3+} rich phases is facilitated by small size, but is not constrained to be a transformation between defected and non-defected ceria phases.

10:20am **BI-MoM8 Protein Catalysis of Minerals and Ice – A Molecular View**, ***Tobias Weidner,*** University of Arrhus, Denmark **INVITED**

Proteins can act as Nature's engineers at interfaces and manipulate both hard and soft tissue – they can shape biominerals, manipulate cell membranes and control water. Despite the apparent importance for chemists working in the fields of biomineralization, surface engineering and drug delivery the molecular mechanisms behind interfacial protein action have largely remained elusive. We use static and time resolved sum frequency generation spectroscopy combined with computer simulations to determine the structure and the mode of action by which these proteins interact with and manipulate interfaces. Here, I discuss our recent advances in the study of protein driven nucleation.

Taking clues from Nature we aim at understanding biomineralization processes at the molecular level to develop design rules for biogenic nanophase materials. Especially the high fidelity control of nanostructured

silica within diatoms has been the envy of material scientists for decades. Where diatoms can grow silica using proteins at cell interfaces under ambient conditions, we still need high pH and harsh conditions to structure silica. Despite the apparent importance for physicists and chemists working in the fields of biomineralization, surface engineering, drug delivery, or diagnostics, the molecular mechanisms behind interfacial silica protein action have remained largely elusive. Our goal is to probe the structure and structural dynamics of such active proteins – in action at the surface. As a first step we study the diatom silica peptide R5 when interacting with silica. We use methods based on theoretical and experimental sum frequency generation spectroscopy combined with computer simulations to determine the structure and the mode of action by which these proteins interact with and grow extended 2D silica interfaces.

A particularly fascinating example of protein driven nucleation and phase transitions are ice-nucleating proteins. These proteins are used by specific bacteria to attack plants and cause frost damage by growing ice crystals at temperatures that would otherwise not allow ice formation. A recent survey by the NASA found that biogenic ice nucleators in the troposphere may affect global precipitation patterns. We have followed the interaction of biogenic ice seeding proteins with surrounding water to gain a detailed picture of protein-driven ice nucleation.

11:00am **BI-MoM10 Multi-Functional Polyampholyte Hydrogels with Covalently Attached SIBLING Proteins for Bone Tissue Engineering**, ***Matthew Bernards***, *S.L. Haag, E.M. Mariner,* University of Idaho

In the ten years since leaving the NESAC/BIO, the Bernards group has focused on developing polyampholyte polymers for biomedical applications due to their unique multi-functional properties. Polyampholyte polymers resist nonspecific protein adsorption, while being able to covalently attach biomolecules. The physical properties (mechanical, degradation, etc.) of these polymers are also tunable by changing their underlying chemistry. Therefore, polyampholyte hydrogels represent a promising platform technology. In this presentation we will cover the development of these polymers for biomedical applications and present recent efforts to understand the degradation behavior of polyampholyte hydrogels as a function of chemistry, while also applying this platform technology for bone tissue engineering. Specifically a polyampholyte hydrogel composed of equimolar mixtures of [2-(acryloyloxy)ethyl] trimethylammonium chloride (TMA) and 2-carboxyethyl acrylate (CAA) is being pursued as a bone tissue scaffold. This hydrogel scaffold is being used as a delivery platform for individual members of the SIBLING (small integrin binding N-linked glycoprotein) family of proteins. SIBLING proteins are the primary non-collagenous proteins found in mineralized tissue and they all contain a cell binding RGD amino acid sequence, a collagen binding domain, and a hydroxyapatite binding domain. This family includes seven proteins or protein cleavage products. Following hydrogel synthesis, individual SIBLING proteins are conjugated to the hydrogel using EDC/NHS chemistry. The initial MC3T3-E1 osteoblast recruitment was investigated using 2-hour cell adhesion assays and the short-term response of the cells was investigated following 24 hours of culture. Hydrogels with conjugated osteopontin exhibited the highest cell recruitment after 2 hours, so polyampholyte hydrogels with conjugated OPN were also used in primary synoviocyte and primary bone marrow derived connective tissue progenitor cell studies. Characterizations with the primary cells include an evaluation of the initial stages of bone matrix production and cell differentiation. The results presented throughout this presentation demonstrate the promising potential for polyampholyte hydrogels in bone tissue engineering applications and beyond.

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