Biomaterial Surfaces & Interfaces

Room Naupaka Salon 6-7 - Session BI-TuE

35 Years of NESAC/BIO II

Moderator: Sally L. McArthur, Swinburne University of Technology, Australia

5:40pm **BI-TuE-1 History of Biomaterials and the Founding of NESAC/BIO**, ***Buddy D. Ratner***, University of Washington **INVITED**

The history of biomaterials and the founding of NESAC/BIO is a big order for one abstract. The history of biomaterials can trace application back to the Neolithic period. But, more relevant to AVS, let's look at the early history relevant to considerations of the surfaces of biomaterials. Early pioneers in biosurface/biointerface studies include Agnes Pockels, Irving Langmuir, Katharine Blodgett, Henry Bull (proteins at interfaces), Leo Vroman, Robert Baier, Joe Andrade and Allan Hoffman. We also have pioneers in technique and instrumentation including William Zisman (contact angles), Kai Seigbahn, Alfred Benninghoven, Gabor Somorjai, Dave Clark, Ron Thomas, Michael Kelly, Chuck Bryson, Leroy Scharpen, Gerd Binnig and Heinrich Rohrer. In the late 1970's I became aware of the power of some of the newer methods for surface characterization for studying biomaterials, particularly electron spectroscopy for chemical analysis (ESCA). Inspired by the pioneers who were demonstrating the importance of surfaces for biology and powered by ESCA and a collaboration with Kelly and Scharpen of HP Corporation, I performed early studies that almost immediately began offering important insights into the biointerface. I came to realize that all biomaterials scientists should embrace biosurface studies, but most did not have access to the instrumentation and training in the use of the instrumentation. I learned about NIH National Resource Centers as a mechanism to provide services to the community and to advance my own studies. I applied to the NIH for such a center. After a failed application, on my second try, we were funded. That led to the formation of the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO). Bringing on board Dave Castner and later Lara Gamble strengthened the intellectual and instrumental base of NESAC/BIO. Thirty five years later, it is with great pleasure and pride to look back on NESAC/BIO's successes and service to the biointerface community.

6:20pm **BI-TuE-3 The Evolution of Biomedical Surface Analysis at NESAC/BIO**, ***David Castner***, University of Washington, USA **INVITED**

Biomedical surface analysis has undergone significant and numerous advances in the past decades in terms of improved instrumentation, introduction of new techniques, development of sophisticated data analysis methods, and the increasing complexity of samples analyzed. Comprehensive analysis of surfaces and surface immobilized biomolecules (peptides, proteins, DNA, etc.) with modern surface analysis instrumentation provides an unprecedented level of detail about the immobilization process and the structure of the immobilized biomolecules. Results from x-ray photoelectron spectroscopy (XPS or ESCA), time-of-flight secondary ion mass spectrometry (ToF-SIMS), near edge x-ray absorption fine structure (NEXAFS), surface plasmon resonance (SPR) and quartz-crystal microbalance with dissipation (QCM-D) biosensing, atomic force microscopy, and sum frequency generation (SFG) vibrational spectroscopy provide important information about the surface structure and composition of complex biomedical materials, as well as the attachment, orientation, conformation, etc. of biomolecules to those materials. However, even with the advances that have been achieved with these powerful surface analysis techniques, there still remain many significant challenges for biomedical surface analysis. These include characterizing the surface chemistry and structure of nanoparticles, determining the atomic level structure of proteins bound to surfaces, 3D imaging of cells and tissue sections, and maintaining biomolecules and materials in a biological relevant state when using ultra-high vacuum based analysis techniques. This talk will discuss the development of surface analysis tools at the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO). Also discussed will be the role of well-defined standards to develop new biomedical surface analysis methods for characterizing more complex, biological relevant samples.

6:40pm **BI-TuE-4 Future Directions and Challenges in Biomedical Surface Analysis**, ***Lara Gamble***, University of Washington **INVITED**

The NESAC/BIO center has been running as very successful NIH NIBIB funded P41 center since 1983. We are making plans for the future growth and expansion of the center resources and expertise. Our work and advances in ToF-SIMS imaging and analysis have prompted a lot of interest among biomedical academic as well as the clinical research community. As a result, we have developed many new tools and capabilities to improve sub-cellular resolution ToF-SIMS analysis of cells and tissues. In the future, the focus of the center will be to provide multimodal information in three dimensions addressing key questions to biomedical issues. While we have proposed instrumentation and research towards this end in the current proposal, we will also be building collaborations nationally with leading groups that have interests in cell and tissue 3D chemical analysis on a vertical and lateral scale that will take advantage of the resolution our NESACBIO surface characterization tools.

7:00pm **BI-TuE-5 Characterizing Protein Fiber Structures and their Interactions in Biological Environments with Vibrational Sum-frequency Scattering Spectroscopy**, ***Patrik Johansson***, *D Castner*, University of Washington

Biological processes are typically regulated by interactions at the interface of 3D structures, such as the membrane of cells or protein fiber surfaces. Collagen (the most common protein in mammals) forms large fibers that are responsible for the structural integrity of tissues. The structure, organization and interactions of these fibers are furthermore important for the survival, communication, migration, and proliferation of cells.

Investigating protein fiber interactions is challenging, particularly under biological conditions where the fibers exist in a 3D aqueous environment. Many techniques cannot interrogate interfaces buried in the bulk of a solvent and therefore require 2D surface models, while others need extensive purification and sample preparation. These approaches may not capture all characteristics of the fiber structures and their interactions in the real sample. However, vibrational sum-frequency scattering (SFS) spectroscopy, with inherent contrast for local molecular ordering, can be utilized towards these important goals.

As a first demonstration, we applied SFS to protein fibers in aqueous environments, self-assembled from collagen type I. We detected signals from the amide I band and the N-H stretching vibrations, both of which are related to the specific protein backbone structure. Signals from the C-H stretching and bending vibrations were also identified, which are associated with the side-chains in the fibers. The angular scattering patterns for the backbone (amide I) and side-chain (C-H stretches and bends) signals are different, making the spectra dependent on the angle of detection. While the backbone signals are dominant in the phase-matched direction, the side-chain signals remain high also at large scattering angles. Distinctions in the organizational symmetry and the relative fiber surface contribution to the overall signal are hypothesized as reasons for this observation.

Finally, we are investigating how changes to the environment (pH, surfactants, etc.) affect spectra and scattering patterns for the SFS signals. This can yield new insights to the structure and dynamics of collagen fibers in biological settings and guide decellularization protocols in regenerative medicine. The relevance of such investigations is enhanced by the fact that detection of vibrations from the surrounding molecules is a direct observation of their interactions with the collagen fiber surface, which thus can be correlated with the fiber structure. The relative orientations for the detected groups may also be obtained via vibrational SFS polarization analysis, for a deeper understanding of biomolecular interactions in biological processes.

7:40pm **BI-TuE-7 Albumin and Fibrinogen Adsorption on New Fluorinated Polyurethanes as an Indication of Blood-compatibility**, ***Le Zhen***, University of Washington, USA; *M Mecwan, S Zhang, F Simonovsky, B Ratner*, University of Washington

Clotting is a major complication for blood contacting biomaterials intended to sustain normal blood flow (biomaterials used in vascular grafts, stents, artificial hearts, etc.). FDA-approved devices for use in the blood stream suffer from thrombotic complications and can be blood reactive even after years of implantation. Thus, highly blood-compatible biomaterials have been long-sought after but not yet achieved. Polyurethanes, with their readily tunable chemical and mechanical properties, represent one of the most widely used classes of biomaterials. We synthesized fluorinated polyurethane materials via a one-step, solvent-free, catalyst-free reaction. The ratio of CF_3/CF_2 can be tuned by varying the composition of the monomers. Electron spectroscopy for chemical analysis (ESCA) and attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) were used to confirm the success of the reaction. ^{125}I labeled albumin and fibrinogen are used in a competitive format to quantitatively study the adsorption of both proteins on the fluorinated polyurethanes. The retention of both proteins was quantified after elution with a sodium dodecyl sulfate (SDS) solution. Since fibrinogen is implicated in surface-

induced clotting and albumin is a benign protein, we hypothesize that materials which tightly bind albumin and have reduced binding to fibrinogen will be less platelet activating and more blood compatible. Compared to non-fluorinated polyurethane and PTFE, the fluorinated polyurethane showed the highest albumin binding and retention. The albumin/fibrinogen ratio of the fluorinated polyurethane is higher than the non-fluorinated polyurethane and comparable to PTFE. These results show promise in blood-compatibility. We will further examine the albumin and fibrinogen adsorption to fluorinated polyurethanes with varying CF_3/CF_2 ratios and correlate composition to adsorption properties. The candidates optimized for high albumin binding and low fibrinogen binding from these experiments will be subjected to human platelet interaction studies to further test albumin hypotheses aimed at achieving enhanced blood-compatibility.

8:00pm **BI-TuE-8 Disclosing the Aggregation Mechanism and Orientation of Self-assembled Cysteine-modified Oligopeptides through Low Energy Dual Beam Depth Profiling Experiments**, ***Luca Tortora,*** *S De Rosa,* National Institute of Nuclear Physics Roma Tre, Italy*; M Dettin,* University of Padua, Italy*; V Secchi, C Battocchio, G Iucci,* Roma Tre University, Italy

The use of short peptide-modified planar gold surfaces or gold nanoparticles is extensively reported in the literature regarding nanoscience and nanotechnology [1]. The mechanism by which these small biomolecules interact to form a film is a crucial information when a solid surface must be functionalized. At the same time, it must be taken into account that the final result in terms of chemical, topological, and functional features is strongly influenced by the orientation of the active layer. Here, a self-assembling peptide (SAP) with a Cys as a terminal residue was used to modify a planar gold surface. The SAP-Cys self-assembled monolayer (SAM) was obtained by o/n incubation of Au surfaces with 1mM SAP-Cys aqueous solution. The presence of alternating positively and negatively charged amino acids (H-Cys-Ala-Glu-Ala-Glu-Ala-Lys-Ala-Lys-Ala-Glu-Ala-Glu-Ala-Lys-Ala-Lys-OH) should guarantee the anchorage of the SAP to the metal surface preserving at the same time the ability of the SAP to self-assemble in antiparallel β-sheet structures. In recent studies [2], XPS analysis has allowed to estimate the film thickness as 4.45 nm and confirm the presence of sulfur atoms of Cys covalently bonded to the metal surface. In addition, an estimate of the mean angle between the peptide bond axis and the substrate surface of about 60° has been calculated by taking advantage of AD-NEXAFS investigations. In this work, we aim to obtain a more detailed understanding of the aggregation mechanism and orientation of SAP-Cys onto the gold surface through ToF-SIMS imaging and depth profiling experiments. Preliminary results obtained in static conditions showed the presence of SH negative ion signal coming from the top surface of the SAM, confirming the self-assembling of the SAP in antiparallel β-sheet structure. The signal intensities of the amino acid fragment ions were used to calculate the following ratios: Ala/Cys, Glu/Cys, Lys/Cys, and AuS/Au. In particular, AuS/Au peak intensity ratio values suggest a gold surface coverage percentage ranging from 8% to 12%. Low values of coverage could be strictly correlated with a strong presence of inorganic ions such as K, Na, spread over the gold substrate, as revealed by ToF-SIMS imaging. Finally, the SAP-Cys film was successfully profiled recording SH^- and S^- ion signal intensity variations during low energy dual beam depth profiling experiments.

References

1. D.Compagnone et al., *Biosens. Bioelectron.*, 2013, 42, 15, 618-625.

2. V. Secchi et al., *J. Phys. Chem. C*2018, 122, 6236−6239.

8:20pm **BI-TuE-9 Multimolecular Omics in Single Frozen-hydrated Cells using High-resolution Gas Cluster Ion Beam Secondary Ion Mass Spectrometry Imaging (GCIB-SIMS)**, ***Hua Tian,*** *N Winograd,* Pennsylvania State University

The cross-talk between molecular network is central to signaling pathways that mediate cellular functionalities of all aspects. To understand the molecular mechanisms, it is necessary to unfold a complete spectrum of molecular species (e.g., lipids, metabolites and proteins) in parallel. Previously, independent molecular extraction is conducted to identify each classes using ensembles of cells. This poses a major limitation to study interconnection between lipid metabolism/protein based signaling from a global perspective and overlooks cell heterogeneity. Moreover, the spatial distributions, a vital piece for understanding biological processes is lost. It is a great technique challenge to detect all biomolecules in single cells near their nature state, and currently there is no method to directly detect metabolites in situ because of their rapid and dynamic nature and impossibility of amplifying.

The development of high resolution CCIB-SIMS in our lab has positioned us to image multiple biomolecules in cryofixed cells in a single run. The approach takes advantage of three aspects of GCIB-SIMS - low chemical damage, high yield of intact biomolecules, and the possibility of sub-micron lateral resolution. In this work, we utilize a DC beam buncher-ToF SIMS instrument to achieve high lateral resolution. Moreover, this configuration simplifies depth profiling since erosion and spectral acquisition are performed with a single beam.

To illustrate this instrumental protocol, single HeLa cells expressing purine de novo biosysthesis (PDNB) are imaged in 3D using a novel 70 keV $(CO_2)_{14000}^+$ beam with a spot size of 1 µm. Purine de novo biosynthesis (PDNB) is essential for supporting cellular proliferation, survival and metabolic adaptation under varying nutritional environment. Using isotope tracer experiments, the stable PDNB intermediates are localized as distinct isolated punctate within cellular boundary. The simultaneous imaging of enzyme to catalyze the pathway is also developed to show the interactions of enzyme and protein. The approach provides a complete chemical picture of single cells at near original physiological and morphological state, opening the opportunities for single cell omics and heterogeneity studies using SIMS.

8:40pm **BI-TuE-10 Pretty Gross: Surface Analysis Illustrating How Beauty Tools Aren't Only Biocompatible for the Human Face**, *P Nguyen, V Mitchell, J Romero-Kotovsky, B Mattheson, L Ista,* ***Heather Canavan,*** University of New Mexico

Tools such as "beauty blender" sponges have become a multi billion dollar product in the cosmetic industry. Introduced in 2007 as reusable utensils for the reliable application of liquid foundation, these applicators have become the largest growing area of the cosmetic industry. Current sales in the USA alone equate to $445B USD/yr in 2017, and are expected to climb to $805/yr by 2023. Although the manufacturers recommend that their sponges be cleaned prior to each use, and have a limited lifetime, many users are relatively complacent about the hygiene of their utensils. In this work, we evaluate how the surface properties of the various makeup blending sponges on the market correlate with their utility and propensity to harbor unwanted bacteria and other microbes. Using traditional surface analysis tools such as X-ray photoelectron spectroscopy, atomic force microscopy, Fourier transform infrared spectroscopy, and scanning electron microscopy, the surface chemistry, porosity, tensile and Young's modulus of the dominant sponges currently sold on the market were evaluated. In addition, the relative hospitality of the sponges to culture bacteria such as *E. coli, Staphlococcus aureus, and Propionibacteriumacnes* were evaluated using confocal microscopy, dilution colony cell counts, and XTT analyses. Preliminary results indicate that these sponges, which are primarily poly(urethane)-based, are capable of forming colonies of these bacteria, as well as other microbial such as fungi, within days if not hours.

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