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.

Biomaterial Surfaces & Interfaces

Room Naupaka Salon 6-7 - Session BI-TuM

Bioimaging and Bionanotechnology

Moderator: Lara Gamble, University of Washington

8:00am **BI-TuM1 Exosomes and Extracellular Vesicles: Small Particles with a Big Impact**, ***Renee Goreham,*** Victoria University of Wellington, New Zealand **INVITED**

Extracellular vesicles (EVs), such as exosomes are membrane-bound vesicles released by most living cells and play a vital role in cell function and cell-cell communication. EVs have shown massive potential as biomarkers for a wide range of diseases and are found in most bodily fluids, including blood, saliva, breastmilk and urine. Detection and measuring cell specific EVs in complex solutions can lead to more sensitive detection of diseases, such as cancer. We have synthesised water soluble InP/ZnS (core/shell) quantum dots using optimised ligand exchange methods. Subsequently, the water-dispersible quantum dots were conjugated to EV-specific antibodies or aptamers. The quantum dot-antibody conjugates and their EV binding, were characterised using a suite of techniques to confirm the size, morphology and surface chemistry. The use of non-cadmium-based quantum dots implies that these conjugates would be more viable for use in a clinical setting. The same strategy has also been applied to bacteria cells (i.e. A*cinetobacter baumannii*) and bacteria derived EVs. Combined with custom designed platforms for surface plasmons resonance or spectroscopy detection, we aim to develop novel methods for EV detection.

9:00am **BI-TuM4 The Role of Lipid Surfaces in Molecular Mechanism of Alzheimer's Disease**, *E. Drolle, M. Robinson, B.Y. Lee, C. Filice, S. Turnbull, N. Mei,* ***Zoya Leonenko,*** University of Waterloo, Canada

Alzheimer's disease (AD) is a neurodegenerative disease characterized by dementia and memory loss for which no cure or prevention is available. Amyloid toxicity is a result of the non-specific interaction of toxic amyloid oligomers with the surface of plasma membrane. We studied amyloid aggregation and interaction of amyloid beta (1-42) peptide with lipid model membranes using atomic force microscopy (AFM), Kelvin probe force microscopy (KPFM) and surface Plasmon resonance (SPR). Using AFM-based atomic force spectroscopy (AFS) we measured the binging forces between two single amyloid peptide molecules. Using AFM imaging we showed that amyloid binding and aggregation are affected by charge and polarity of the surfaces (we studied chemically modified inorganic surfaces, phospholipid monolayers and bilayers (membranes)). Furthermore, we demonstrated that lipid membrane surfaces play an active role in amyloid binding and toxicity and thus in molecular mechanism of AD: changes in membrane composition and properties increase amyloid binding to the membrane and membrane damage. Effect of lipid composition, the presence of cholesterol and melatonin are discussed. We discovered that membrane cholesterol creates nanoscale electrostatic domains which induce preferential binding of amyloid peptide, while membrane melatonin reduces amyloid-membrane interactions, protecting the membrane from amyloid attack. These findings contribute to better understanding molecular mechanisms of Alzheimer's disease and aid to the developments of novel strategies for cure and prevention of AD.

References

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3. M.Robinson, B.Y.Lee, Z.Leonenko, Drugs and Drug Delivery Systems Targeting Amyloid-β in Alzheimer's Disease. *AIMS Molecular Science,* 2015, 2(3)*:* 332-358.

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9:20am **BI-TuM5 An PEEM and Imaging XPS study of Neutrophil Extracellular Traps Caputuring Nanoparticles**, *A. Skallberg, K. Bunnfors, C. Brommesson,* ***Kajsa Uvdal,*** Linköping University, Sweden

Photoelectron Emission Microscopy (PEEM) and Imaging X-ray Photoelectron Spectroscopy (XPS) have the potential to deliver element specific imaging useful for biomedical visualization. This may increase the understanding of biological processes on the cellular level, contributing with element specific information and data on topographical morphology combined. The technique is based on chemical composition, chemical states and work-function shifts.

This is hereby demonstrated by combined PEEM and Imaging XPS investigation of neutrophils and their activation processes. Neutrophils are vital components in the human defense system, with the fundamental role to fight invading pathogens. Neutrophils are also able to release nuclear DNA done by formation of extracellular web-like structures called neutrophil extracellular traps (NETs) to capture and occasionally kill intruding microbes.

Here we reportneutrophils externally triggered by in this case nanoparticle (NPs). The neutrophils and NETs formation are imagined in presence of NPs and we report elemental composition of single-cells and structure of NETs. Active cellular uptake of nanoparticles is imaged both before and after NETs release. Element specific imaging of this novel capability for mass transport. This shows the potential for element specific bio-related cell studies on surfaces and nanoparticle tracking on the cellular level.

9:40am **BI-TuM6 Chemical Imaging of Aggressive Basal Cell Carcinoma using ToF-SIMS**, *M. Munem, K. Dimovska Nilsson,* University of Gothenburg, Göteborg, Sweden*; O. Zaar, N. Neittaanmäki, J. Paoli,* Sahlgrenska University Hospital, Gothenburg*;* ***John Fletcher,*** University of Gothenburg, Göteborg, Sweden

Time-of-fight secondary ion mass spectrometry (ToF-SIMS) is starting to be of increasing value to clinicians and has been used on different tissue samples to successfully identify and localise chemical components to various areas of the tissue and answer disease related questions [1]. Compared to other methods, the main advantage of ToF-SIMS is the label free detection of a large number of different molecules within one experiment on the same tissue section. ToF-SIMS is successfully used for analysing lipids behaviour in biological samples like breast cancer tissue [2]. Basal cell carcinoma (BCC) is one of the most increasing cancers worldwide and it is the most common malignancy in white people. Although the mortality is low as BCC rarely metastasises, this malignancy causes considerable morbidity and places a huge burden on healthcare services worldwide. Furthermore, people who have this condition are at high risk of developing further BCC and other malignancies [3].

Samples were collected from patients with BCC, by Mohs surgery. The tissue was sectioned for ToF-SIMS analysis and H&E staining of consecutive tissue slices was performed. ToF-SIMS was performed using an Ionoptika J105 instrument using a 40 keV $(CO_2)_{6000}^+$ ion beam. The analysis provided detailed chemical information about the individual lipid species and the spatial distribution of these within the tissue. It was possible to observe differences between the layers of the skin as well as between healthy and cancerous tissue (see figure). ToF-SIMS data was correlated with H&E stained images to understand and confirm, from which structures or regions of the tissue that the individual signals originated.

10:20am **BI-TuM8 Combining the Benefits of GCIB-ToF-SIMS, MALDI-FTICR-MS and LC-MS/MS for Location specific Lipid Identification in Planarian Flatworm Tissue Sections**, ***Tina Angerer,*** University of Washington, USA*; D. Velickovic, J.E. Kyle, C. Nicora, C. Anderton,* Pacific Northwest National Laboratory, USA*; D.J. Graham, L.J. Gamble,* University of Washington, USA

Phagocata gracilis are planarian, non-parasitic flatworms. Planarians are best known for their fascinating regenerative abilities, requiring a complex interplay of a wide range of molecules. The regeneration process and the molecules involved are still poorly understood. Most notably there is a lack of lipid and fatty acid data, a diverse group of molecules fulfilling numerous functions such as energy storage and cell signaling.

To gain a better understanding of the lipidomic landscape in planarians we analyzed positive and negative ions from longitudinal sections of *P. gracialis* with MALDI-FTICR-MS and ToF-SIMS along with homogenized whole worm extracts with LC-MS/MS.

Imaging MALDI-FTICR-MS (15T, Bruker Solarix) provides location specific (50 µm/pixel), ultra-high mass resolution (R≈250,000 @m/z=400) and ultra-high accuracy (<1ppm) lipid data capable of distinguishing intact lipid

species of similar exact mass and showing their distribution in the tissue. The drawbacks for this technique are that the spatial resolution is too low to clearly identify features within the worm and its low fragmentation rate. While beneficial for molecular peak intensities, the lack of fragments does not allow for specific lipid assignments with structural information (e.g. lipid headgroup and both fatty acid chains identified).

Imaging GCIB-ToF-SIMS (J105, Ionoptika) generates high mass accuracy (< 5ppm), cell/organ-specific data (3 µm/pixel) consisting of intact lipids, lipid fragments and fatty acids. The moderate mass resolution (R≈10,000@m/z=700) is sufficient to resolve most lipid species. Mass peaks consisting of more than one species are indicated by broad and/or asymmetric peaks with poor mass accuracy. However co-localizing fragments can add confidence for the assignment of overlapping species, provide us with structural information and allow for unambiguous identification of resolved peaks.

LC-MS/MS (Thermo Velos Pro Orbitrap) separates different lipid species prior to fragmentation so, in contrast to SIMS, the observed lipid fragments are guaranteed to stem from the analyzed species. The drawback with this technique is that it provides no location specific information. Similar to SIMS, lipids with similar mass are not separated leading to mixed fragments in the MS/MS data. Comparing LC-MS/MS to SIMS data shows that the same lipid fragment species are present in both spectra.

This work demonstrates that only by correlating all 3 techniques can we get highly accurate, high mass, high spatial resolution, structural and location specific lipid information. Together this data provides detailed information about all major structures and organs within planarians.

10:40am **BI-TuM9 Hybrid SIMS: A New SIMS Instrument for High Resolution Organic Imaging with Highest Mass-resolving Power and MS/MS***, **Nathan Havercroft**,* ION-TOF USA, Inc.*; A. Pirkl,* IONTOF GmbH, Germany*; D. Scurr, N. Starr,* University of Nottingham*; R. Moellers, H. Arlinghaus, E. Niehuis,* IONTOF GmbH, Germany

Introduction

Secondary ion mass spectrometry (SIMS) offers the possibility to acquire chemical information from submicron regions on inorganic and organic samples. This capability has been especially intriguing for researchers with life science applications. In recent years, the vision to image and unambiguously identify molecules on a sub-cellular level has been driving instrumental and application development. While new ion sources expanded the usability of SIMS instruments for biological applications, SIMS analyzers lacked the required mass resolution, mass accuracy and MS/MS capabilities required for the thorough investigation of these materials.

Methods

To specifically address the imaging requirements in the life science field a powerful new Hybrid SIMS instrument [1] was developed in a research project by IONTOF and Thermo Fisher Scientific™, following Prof. Ian S. Gilmore's original idea, in close cooperation with the National Centre of Excellence in Mass Spectrometry Imaging (NiCE-MSI), GlaxoSmithKline, and the School of Pharmacy of the University of Nottingham. The instrument combines an Orbitrap™-based Thermo Scientific™ Q Exactive™ HF mass analyzer with a high-end ToF-SIMS system (IONTOF GmbH). The instrument provides highest mass resolution (> 240,000) and highest mass accuracy (< 1 ppm) with high lateral resolution cluster SIMS imaging.

Preliminary data

Secondary ions, generated by primary ion bombardment from liquid metal ion clusters or large gas clusters can be analyzed in either of the mass analyzers. Fast switching between the mass analyzers is achieved by pulsing of a hemispherical electrode. This even allows combined measurement modes using the TOF for very fast imaging and the Orbitrap mass analyzer during intermediate sputtering cycles for generation of spectra with high mass resolution and mass accuracy from the same sample area in a single experiment.

First application data including high resolution SIMS spectrometry, MS/MS analyses, high resolution imaging of tissues and depth profiles of biological samples with this new instrument will be presented. For example, single beam depth profiling data were collected, from porcine skin samples, that clearly exhibited different molecular ion signals for different skin layers. This method potentially allows one to measure the permeation of skin for various compounds, e.g. drug molecules.

[1] The 3D OrbiSIMS – Label-Free Metabolic Imaging with Sub-cellular Lateral Resolution and High Mass Resolving Power, Passarelli et al., Nature Methods, 2017, 14(12):1175-1183, DOI 10.1038/nmeth.4504

11:00am **BI-TuM10 Latest Developments in Cluster Beam Technology for ToF SIMS: Towards Greater Spatial Resolution, Improved Ion Yields, and Faster Etch Rates!***, **Paul Blenkinsopp**,* Ionoptika Ltd, UK

The emergence of Gas Cluster Ion Beams (GCIB) for SIMS has significantly extended the available mass range of the technique, and in so doing has also widened the scope of its applications. The low-damage nature of GCIB sputtering greatly improves yields of higher mass molecular species, however spatial resolution remains a challenge. Of particular interest is the ability to detect and image intact bio-molecules in tissue and cells at spatial resolutions below 1 micron. Here, we present on the latest advancements in gas cluster beam technology for ToF SIMS, demonstrating significant progress towards these goals.

We report on the results of our latest innovation – a 70kV Gas Cluster Ion Beam, the GCIB SM. Most current GCIB sources operate at energies between 10 and 40kV, however there are several theoretical benefits to extending the energy range beyond this, including improved focusing and greater secondary ion yields. With the GCIB SM, we demonstrate both a reduction in spot size – improving the resolving power by a factor of 3 – as well as an increase in total current – which has benefits for both speed of analysis, as well as for greater depth profiling capabilities.

We also present on methods to increase ion yields of high-mass species by utilizing alternative source gases to argon. We demonstrate that by choosing an appropriate source gas, yield enhancements greater than 10 times can be achieved. As analysis volumes decrease with greater resolving power, techniques such as this are expected to play a vital role in obtaining the highest quality imaging SIMS data. These latest developments are now enabling SIMS imaging of species such as lipids and gangliosides in tissue at resolutions greater than 2 microns.

11:20am **BI-TuM11 SIMS with Higher Resolution and Higher Signal: 40keV Water Cluster Primary Ion Beam and Prospective Orbital Ion Trapping***, J. Hood, **Peter Cumpson**, I. Fletcher,* Newcastle University, UK*; S. Sheraz,* Ionoptika Ltd, UK

Increasing the secondary ion yield from organic and biological molecules has been a key pursuit in the development of secondary ion mass spectrometry (SIMS) since the inception of the technique, with novel primary ion sources a promising avenue of research. The development of a water cluster primary ion beam has offered improvement in this regard, with ion yield enhancements of the order of 100 to 1000 times observed for beams with water cluster size 7,000, relative to argon cluster beams of size 2,000 [1] [2].

We demonstrate that exploiting larger cluster sizes, in excess of $(H_2O)_{10,000}^+$, with higher beam energy of 40 keV, offers further enhancement of the secondary ion yield, including for large fragments.

To complement the increased secondary ion yield of higher mass fragments, higher mass resolution is desirable. One way to achieve this is through the coupling of a high resolution Fourier transform mass spectrometer (FT-MS) to a SIMS instrument. One form of such hybrid instrumentation utilizes an orbital trapping mass analyser [3] [4], which we have designed and fabricated for our J105 SIMS instrument [3]. However, as with ion cyclotron resonance (FT-ICR MS) techniques, orbital trapping analysers operate at a much slower repetition rate than time-of-flight (ToF) variants, with acquisition dwell times per pixel of the order of 100ms to several seconds, as opposed to as little as 10µs for modern ToFSIMS instruments such as the Ionoptika J105[5].

In FT-MS the field which governs ion motion can potentially be manipulated by applying different voltages to the component electrodes, a process known in ICR-MS as Stored Waveform Inverse Fourier Transform (SWIFT)[6]. The time-domain excitation waveform is formed from the inverse Fourier transform of the appropriate frequency-domain excitation spectrum, which is chosen to excite the resonance frequencies of selected ions. The application of a SWIFT signal to the orbital ion trap improves the speed of acquisition, making high mass resolution SIMS practical.

The combination of a water cluster primary ion beam with high mass resolution orbital ion trapping offers considerable potential for analyzing the molecular chemistry in organic and biological systems.

References

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[2] S Sheraz Nee Rabbani et al. *Analytical Chemistry* 87(4), 2015, 2367–2374

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[6] RB Cody et al. *Rapid Communications in Mass Spectrometry* 1(6), 1987, 99–102

11:40am **BI-TuM12 In-Situ TEM Studies of Biomineralization***, **Tolou Shokuhfar**, R. Shahbazian-Yassar,* University of Illinois at Chicago

This talk will provide an overview of the PIs' efforts to understand the dynamics of biomineralization via in-situ transmission electron microscopy. First we demonstrate how to utilize graphene sheets to build a liquid-cell nanoreactor that fits the chamber of high-resolution TEM. Graphene is impermeable to liquids such as aqueous solutions and therefore can be used to seal liquid solutions from leaking to the high vacuum of TEM environment. In addition, the excellent electrical conductivity of graphene and its ability to scavenge the radicals produced by the interaction of electron beam and liquid solutions provide an excellent platform to perform imaging of biological or hydrated specimens. We then demonstrate our success to observe the biomineralization of calcium oxalate crystals that are the primary constituent of kidney stones. We show that the addition of citrate and other molecular inhibitors can affect the crystallization pathway of these minerals. In addition, we will showcase example of biomineralization of iron oxide core in ferritin proteins and demonstrate the ability to monitor the biomineralization of these crystals using graphene liquid cells in TEM. We will show that the ratio of L and H subunits in the ferritin protein shells can affect the nucleation and growth of iron oxide cores. We also will present our latest results on the biomineralization of magnetosomes in magnetotactic bacteria grown in iron-rich media using in situ GLC-TEM studies.

Biomaterial Surfaces & Interfaces

Room Naupaka Salon 1-3 - Session BI-TuP

Biomaterial Interfaces Poster Session

Moderator: David Castner, University of Washington

BI-TuP1 Inhibiting Upstream Motility of *Pseudomonas Aeruginosa via* Nanopillared Surface Structuring, ***Rachel Rosenzweig***, *V.K. Ly, K. Perinbam, A. Siryaporn, A.F. Yee,* University of California, Irvine

Bacteria often populate environments where fluid flow is present such as the lungs of mammals, vasculatures of plants, industrial transportation fuel lines, and medical devices. *Pseudomonas aeruginosa* is an opportunistic biofilm forming bacterium that exhibits the ability to twitch upstream when surface attached. The upstream movement is facilitated by the retraction and extension of their type iv pili mechanosensor ATPase motors, pilT and pilU, when encountering shear stress. Such motility modalities of *P. aeruginosa* lead to bacterial surface adherence, colonization, and infectious biofilm formation. Here, upstream motility inhibition and surface detachment of *P. aeruginosa* were accomplished on polymeric biomaterial structures with arrays of nanopillared geometries.

Nanopillared surface structures were fabricated using thermal nanoimprint lithography on a synthetic polymer, poly(methyl methacrylate) (PMMA), commonly used in medical devices. The arrays of nanopillars range in periodicities from 200nm, 300nm, 500nm to 600 nm. Upstream motility direction, displacement, velocity, and detachment of wild-type *P. aeruginosa* expressing GFP were monitored in microfluidic flow channels with flat or nanopillared bottom surfaces and quantified using fluorescence microscopy. The cell motility inhibition and detachment under shear stress were observed to have a nanopillar surface area dependence most likely due to decrease in surface mechanosensing capabilities of the type iv pili. This bacteria-nanostructured surface interface phenomenon allows us to tailor surfaces with specific nanopillared geometries for structurally controlling cell motility and detachment under fluid flow. The disruption of surface attached biofilm forming bacterial upstream movement is crucial in preventing harmful infection from contaminated medical devices such as catheters and has broad application in industrial fuel line dependent transportation.

BI-TuP2 Effect of Preheating Treatments on Interfacial Reaction between Dental Porcelain and Low Magnetic Susceptibility Zr–14Nb Alloy, ***Atsushi Takaichi***, Tokyo Medical and Dental University, Japan*; Y. Kajima,* Tohoku University, Japan*; H. Doi, T. Hanawa, N. Wakabayashi,* Tokyo Medical and Dental University, Japan

[Objective]

In this study, we focus on using the Zr-14Nb alloy for porcelain-fused-to-metal (PFM) restoration in dental prosthetics, owing to their good mechanical properties and biocompatibility, as well as the low magnetic susceptibility. The interface between the alloy and the porcelain is of critical value in ensuring the long-term integrity of the PFM restoration, thus we investigated the changes at the ceramo-metal interface induced by preheating treatments.

[Methods]

Cylindrical cast specimens of the Zr–14Nb alloy were prepared. After sandblasting with Al_2O_3, the Zr–14Nb samples were subjected to a preheat treatment at 700 °C for 5, 10, or 20 min and those without treatment were taken as control samples. Dental porcelain was veneered on them; then, their bond strength (MPa) was evaluated by performing shear bond tests (n = 8/group) and the results were analyzed using ANOVA and Tukey's tests (p = 0.05). The surface characteristics of the preheated Zr–14Nb specimens were evaluated by scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS), laser microscope, and X-ray diffractometry (XRD). The elemental distribution on the interface between the Zr–14Nb alloy and the porcelain was determined by SEM-EDS. MR images were obtained using 3.0 T MR scanners (MAGNETOM Spectra 3T), and artifacts volume from the specimens were quantified by constructing 3D image.

[Results and Discussion]

The samples subjected to the heat treatment for 5 min showed the highest mean bond strength (43.7±5.9 MPa). On the preheated sample groups, white oxide layers, which were predominantly composed of monoclinic zirconia, were formed, exhibiting a greater roughness than control samples; besides, on the interface of the metal–ceramics, a greater diffusion range of Nb was observed than that found on the control samples, which could contribute to increase the bond strength between porcelain and Zr-Nb alloy. On the other hand, the bond strengths of the samples subjected to 20 min preheating treatment were the lowest (33.6±3.2 MPa), which may be ascribed to the formation of a brittle thick oxide layer under excessive heat treatment.

[Conclusion]

The suitable preheat treatment performed on the Zr–14Nb substrates contributed to the increase in the surface roughness and the diffusion of Nb, which enhanced the micro-retention and chemical bonding and improved the bond strength of Zr–14Nb and porcelain. The Zr-14Nb alloy is a promising candidate for fixed dental prosthesis, as long as the appropriate treatment conditions are adopted.

BI-TuP3 Surface Characteristics and Corrosion Behavior of CoCrMo Alloys Fabricated by Selective Laser Melting after Various Heat Treatments, ***Yuka Kajima***, Tohoku University, Japan*; A. Takaichi, T. Oishi, N. Kittikundecha, Y. Tsutsumi,* Tokyo Medical and Dental University, Japan*; N. Nomura,* Tohoku University, Japan*; N. Wakabayashi, T. Hanawa,* Tokyo Medical and Dental University, Japan*; A. Kawasaki,* Tohoku University, Japan

[Objective] Selective laser melting (SLM) has attracted significant attention as an advanced method for fabricating biomedical devices. SLM-manufactured parts easily accumulate large amounts of residual stress due to rapid heating and cooling. Thus, they require a post-fabrication heat treatment to relieve the residual stress. However, the heating process inevitably changes the microstructure of the alloys, which may affect their corrosion behavior. The objective of this study was to assess the morphological characteristics and corrosion properties of SLMed CoCrMo alloys following various heat treatments.

[Methods] Block specimens were prepared using an SLM machine equipped with a fiber laser (EOSINT M280) and commercially available CoCrMo alloy powders (MP1). Specimens were heated to 750 or 1150 °C and held at this temperature for 1 or 6 h in a furnace in an Ar atmosphere. Specific section cuts of XY and YZ planes were used for analyzing microstructures and corrosion resistance. Microstructures were investigated via scanning electron microscopy (SEM), field-emission transmission electron microscopy (FE-TEM), field-emission electron probe microanalysis (FE-EPMA), electron backscattered diffraction (EBSD), and X-ray diffraction (XRD). Additionally, anodic polarization was performed with a potentiostat (HABF-501A) with a function generator (HB-111).

[Results and Discussion] The SEM images showed that fine precipitates were formed within the grains and at the grain boundaries in the specimens heated to 750 °C. On the other hand, after heating to 1150 °C, coarse precipitates, identified as $M_{23}C_6$ by TEM and EPMA analysis, grew along the grain boundaries. Both γ and ε phases formed in all heat-treated specimens, and the volume fraction of the ε phase decreased with increasing heat-treatment temperature and time. In the samples heated to 750 °C, the microstructures exhibit the epitaxial growth of columnar grains with a < 001 > fiber texture along the build direction as well as the as-built state. In samples heated to 1150 °C, defect-free equiaxed grains with random orientations were found, indicating that recrystallization occurred. Considering the anodic polarization curves, the heat treatment process did not greatly affect the corrosion resistance of the SLMed specimens; resistances of all heated samples were comparable to traditional cast samples, with those heated to 750 °C exhibiting the highest corrosion potential. The enhanced corrosion resistance of SLMed CoCrMo alloys provides further support for their use in medical applications.

BI-TuP4 Analysis of Drug Coated Polymer Stents Studied by XPS and Ar_n^+ Sputter Profiling, ***David Surman***, Kratos Analytical Inc.*; J. Counsell,* Kratos Analytical Ltd., UK

Cardiovascular interventional therapy with stents has emerged as one of the most effective treatment methods for coronary heart disease, however, thrombosis and hyperplasia are the usual pathological responses to the implantation of foreign devices into the body. Originally stents were made of steel although these have now been superseded by polymer based materials. Recent developments have introduced a new range of stents made from bio-resorbable polymers, however problems such as thrombosis and hyperplasia still remain. To suppress this immune response and that of overgrowth and subsequent restenosis anti-inflammatory drugs are now loaded onto the surface of stent implants.

In this presentation we investigate the surface of drug loaded polymer stents using X-ray photoelectron spectroscopy (XPS) and sputter depth profiling using Ar_n^+ clusters. The stents studied are made of polylactic acid (PLA) dosed with an anti-inflammatory drug with a molecular structure of $C_{51}H_xNO_{13}$. XPS yields quantitative information regarding drug distribution

which is shown to be higher on the abluminal (outer) than the luminal (inner) surfaces of the stent. Combining Argon cluster sputtering with XPS enables the distribution of the drug into the stent structure to also characterised.

Conventional methods to study the effects of aging and drug mobility in stents involve immersion in a buffer solution for varying periods of time. Subsequent analysis of the solution with HPLC determines the extent of drug dissolution from the stent. Although this approach is accurate in determining the amount of drug dissolved, it is still unknown how much drug remains and how it is distributed. This is addressed in this study where the drug distribution for stents immersed in PBS buffer solution for 1-3 months was determined by Ar_n^+ cluster depth profiling of the stents themselves. These results were used to determine the effects on simulated ageing and the propensity for the drug to migrate into the solution with time.

BI-TuP5 Anchored Protease-Activatable Polymersomes for Molecular Diagnostics of Cancer Cells, ***Jong-Woo Lim,*** Yonsei University, Republic of Korea*; H.-O. Kim,* Korea University, Republic of Korea*; J. Choi,* Yonsei University, Republic of Korea*; H. Lee,* Korea Basic Science Institue, Republic of Korea*; H.Y. Son, J. Kim, G. Park, H. Chun,* Yonsei University, Republic of Korea*; D. Song,* Korea University, Republic of Korea*; Y.-M. Huh, S. Haam,* Yonsei University, Republic of Korea

Real-time quantitative and qualitative analyses of metastasis-associated proteases are critical for precise diagnosis and novel therapeutic treatment of advanced cancers. However, conventional methods based on DNA, peptides, and proteins require sophisticated chemistry and additional processes to expose detection moieties, and they lack elements of temporal control, which limit their applicability. We designed unique protease-activatable polymersomes (PeptiSomes) for high sensitivity, in situ quantitative analysis of activating membrane-type 1 matrix metalloproteinases (MT1-MMP, MMP14). To do this, we first synthesized an amphiphilic block polymer–peptide and a copolypeptide based on mPEG -b-pLeu and MT1-peptide-b-pLeu, respectively. Amphiphilic self-assembled PeptiSomes in water were capable of disassembling and releasing the encapsulated self-quenched fluorescence dye (calcein) via enzymatic activation by MT1-MMP. Our PeptiSome system may potentially prevent the initiation and progression of cancer metastasis. Furthermore, the PeptiSome approach described here is likely to facilitate the development of rapid protease assay techniques and further extend the role of proteases as metastasis indicators and therapeutic targets.

BI-TuP6 Study on Meta-material Structure in Oil Repellent Bile Duct Stent, ***Tomoki Nishino,*** Ritsumeikan University, Japan*; H. Tanigawa,* The Research Organization of Science and Technology, Japan*; A. Sekiguchi,* Litho Tech Japan Corporation, Japan*; K. Aikawa,* Saitama Medical University, Japan

The bile duct is a tubular pathway that drains the bile made in the liver into the duodenum. In bile duct cancer, biliary atresia or the like, the bile duct narrows, the flow of bile is inhibited and it becomes difficult for the duodenum to flow from gall bladder to the duodenum. For this reason, bile flows back to the liver, causing a disorder that causes jaundice. Natural progression will result in liver failure, possibly leading to death. As a treatment method, in order to open the obstruction of the bile duct, the endoscope is used to indwell the stent in the bile duct to ensure discharge of the bile into the duodenum. By placing the stent with a metal tube or a resin tube having a mesh-like structure, a path through which bile normally flows is secured.

However, since bile is a viscous liquid containing oil, it is known that the tube clogs by adhesion or gelling in the bile duct stent. Currently, no effective technology has been studied for this problem. When the tube is clogged, there is only a countermeasure therapy to replace the biliary stent by reoperation. If it is possible to reduce clogging of the stent, it is possible to reduce the number of reoperation, which not only reduces the burden on the patient but also leads to a reduction in the burden on the medical field including doctors.

This study is a technology development to reduce clogging of the biliary stent. We introduce a biliary stent combining metamaterial technology that realizes oil repellency and antifouling property which is not realized on the surface of the material prepared. When considering the oil repellency function for oil containing fluid such as bile, we considered the snail shell structure with nano hydrophilic effect to be effective. On the shell surface of the snail, there is a concavo-convex structure of 200 nm to 400 nm, and it is running a dirt by making a thin water film.

The film having a metamaterial structure was produced by a semiconductor fine processing technique. When the produced film was evaluated for oil repellency, it was confirmed that good oil repellency in water was obtained. Therefore, in order to evaluate a bile duct stent with a metamaterial structure, bile ducts were placed in pigs for 7 days. The inner surface of the usual bile duct tube resulted in bile sticking and a lot of contamination, but the bile duct tube of the meta-material structure was a result that the bile was repelled and there was no stain. We present that bile duct with oil repellency and antifouling property is effective as new metamaterial technology.

BI-TuP7 The Blood Cell-nanoparticle Interface: Functional Cellular Responses, Mechanisms of Interaction and Signaling pathways, *C. Brommesson, N. Abrikossova, P. Eriksson, Z. Hu, K. Uvdal,* ***Andreas Skallberg,*** Linköping University, Sweden

The use of nanomaterials in biomedical applications create a large need for studies elucidating potential harmful effects of the materials in living systems. Nanomaterials specifically aimed for *in vivo* applications are sure to encounter all components of blood, directly or indirectly, and increased knowledge of underlying mechanisms at the blood cell-nano interface will be valuable in the further development of these materials.

We have specifically investigated blood platelet and neutrophil granulocyte interaction with several types on nanoparticles. These blood cells are rapidly responding, and potent cells involved the immune response and following inflammatory processes. Using well-defined and characterized NPs we have focused on clarifying the induced functional cellular responses, interaction mechanisms and involved signaling pathways. Our results demonstrate uptake of polymeric (Pdots) and Cerium/Gadolinium based NPs in neutrophils and show that both active and passive uptake processes contribute to the internalization of these particles. In connection, cellular mechanisms underlying our previously described antioxidative properties of cerium containing NPs[1] are further investigated and identified. Nanoparticle induced platelet aggregation and release of inflammatory mediators is also shown herein to be valuable for evaluating NP- blood compatibility.

[1] *P. Eriksson el al. Cerium oxide nanoparticles with antioxidant capabilities and gadolinium integration for MRI contrast enhancement. Scientific Reports 2018; 8:6999*

BI-TuP8 Developing a pH Responsive Hydrogel as an Alternative for Colonoscopy Preparation, ***Phuong Nguyen,*** University of New Mexico*; S. Mounho,* University of Texas at Austin, USA*; D. Cuylear, H. Canavan,* University of New Mexico

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States. The most reliable screening method of CRC is a colonoscopy which requires a 4-Liter poly(ethylene glycol) electrolyte lavage solution (PEG-ELS) for preparation. ~40% of patients are non-compliant to their colonoscopy schedules, with many patients who abstain reporting refusal due to significant discomfort associated with this preparation. Furthermore, there are distinct gender differences in the tolerance of PEG-ELS in male and female populations. We hypothesize the differences in clinic are a result of cytotoxicity effects of PEG. PEG is approved by the FDA for use in medical devices, and has been recognized for many years as a biocompatible/bioinert polymer but few studies have truly studied the short-term and long-term effects of high concentrations of PEG on multiple cell lines. We have developed a pH responsive hydrogel to control the release of PEG – reducing adverse effects associated with colonoscopy preparations. The hydrogels have been characterized using NMR, and XPS to ensure chemical identity, rheometry to assess the stiffness/robustness of the hydrogels in varying environments, and SEM and other techniques to confirm uniformity of size. Biocompatibility testing of exposure to increasing PEG concentrations over a period of 3 hours, 6 hours, 12 hours, 24 hours, and 48 hours shows PEG is biocompatible to intestinal human cell lines in short intervals and low concentrations. Furthermore, at low concentrations PEG increases cell growth and viability as seen in previous studies. At higher concentrations, however, PEG is cytotoxic to cells. Although it would be difficult to get to toxic levels of PEG in the body in a single dose, current uses of PEG in which large, non-uniform, quantities are ingested in a short time frame should be re-evaluated due to possible adverse cumulative effects due to the cytotoxicity effects seen *in vitro.* Further directions of this work will evaluate the pH responsiveness of our hydrogel formulation to deliver PEG *in vitro* and *in vivo,* and assessment of the cellular response to the hydrogels using mammalian cells specific to the gastrointestinal system of humans, as well as imaging analysis to envision their penetration.

BI-TuP9 Atmospheric Pressure Mass Spectrometric Imaging of Live Tissue Specimen using Electrospray assisted CW Laser Desorption and Ionization Source, ***Jae Young Kim,*** Daegu Gyeongbuk Institute of Science & Technology*; S.Y. Lee, M.H. Shin,* Daegu Gyeongbuk Institute of Science & Technology, Korea*; D.W. Moon,* Daegu Gyeongbuk Institute of Science & Technology, Republic of Korea

Although atmospheric pressure mass spectrometry (AP-MS) is a promising analytic technique for biological samples because of its ambient analytic process and no or minimal sample pretreatment, still many challenges must be overcome to acquire MS data from them. Because mass spectrometry is a quantitative technique for the measurement of the masses of the charged molecules that comprise a sample of material, small but actual parts should be severed from the sample. Thus, desorption and ionization methods for small spot sampling to the biological sample are the most important technique to minimize the sample damage and to obtain high resolution MS imaging. Currently many ambient desorption/ionization sources except lasers are working in ionized gaseous state or spray state of charged droplet, reducing the size of source devices is limited.

We use electrospray ionization source and visible continuous wave (CW) lasers as AP-sampling/ionization sources and develop/combine these electrospray and lasers for the purpose of mass spectrometry applications. The energetic light generated by CW lasers focuses on a very small spot of the sample through the objective lens. At same time, the electrospray device forms the charged droplet particles on the sample. Regarding desorption and ionization procedures in open air, CW lasers mainly desorb actual parts from the sample and electrospays mainly ionize these small substances.

An inverted optical microscope was used as a sampling stage and the additional pumping system including ion transfer tubing, chamber, and dry pump known as air flow assisted ion transfer equipment was installed with the MS inlet. Therefore an AP-MS system is developed and preliminary MS data are achieved with a help of gold nanoparticles. With an addition of the two-dimensional programmed scanning stage to this ambient MS system, many bio-molecular mass spectrometric imaging were obtained from the mouse hippocampal tissues. High spatial resolution MS imaging with a pixel size of four micrometer can be secured at a sample moving velocity of 30 µm /s. MS imaging of bio-molecules including monoacylglycerols, cholesterols, fragments of sphingolipids and glycerophospholipids has been obtained from mouse hippocampal tissues.

BI-TuP10 Improvement of Cell Imaging by Graphene Encapsulation in ToF-SIMS Method, ***Sun Young Lee,*** Daegu Gyeongbuk Institute of Science & Technology, Korea*; H.J. Lim, J.Y. Kim,* Daegu Gyeongbuk Institute of Science & Technology*; D.W. Moon,* Daegu Gyeongbuk Institute of Science & Technology, Republic of Korea

For last decades, ToF-SIMS has been in use in bio sample imaging and has resulted in some important bio issue discoveries. SIMS analysis requires ultrahigh vacuum environment and analysis in atmospheric not allowed. Its ultra-high vacuum based operation necessitates proper sample preparations ranging from simple ones like washing and drying to relatively sophisticated as frozen hydration.[1] Therefore, any of them can neither represent the biologically native state nor preserve membrane integrity. So we have developed a new sample preparation using wet cells covered by single layer graphene for hydrated and native state bio sample.

Graphene acts as a gas impermeable honeycomb mesh on cells preventing solution evaporation and keeping cells wet even under ultra-high vacuum. Besides, the high electrical conductivity of graphene compensates the charging effects during analysis. ToF-SIMS images in this study were obtained using ToF-SIMS 5 (ION-TOF GmbH) equipped with liquid metal ion gun (LMIG) [2,3] and analyzed both positive and negative ion modes and about 1000 specimen-related spectra were obtained from A549 lung carcinoma cell. Thus, we obtain various lipid SIMS images from cell membrane through a graphene layer without cracking.

Major limitations of applying mass spectrometry imaging techniques such as SIMS to biomedical researches are due to harsh bio-sample preparation including freezing, matrix addition and drying. The possibility that secondary molecular ions can be sputtered through a single layer graphene from wet cells will open innovative applications of mass spectrometry imaging of wet cells to various biomedical research areas.

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BI-TuP11 Behavior of Shewanella Oneidensis MR-1 in a Sulfur and Zinc-Rich Medium and its Applications for Biosensing and Biomaterials, ***James Rees,*** *S. Sawyer, Y. Gorby,* Rensselaer Polytechnic Institute

An increasing focus of current microbiology research is the fact that some strains of bacteria, called dissimilatory metal-reducing bacteria (DMRB), are capable of utilizing certain metallic ions as terminal electron acceptors in their metabolic processes. One strain in particular, Shewanella oneidensis MR-1, can reduce ions of iron, lead, arsenic, and uranium, among others. Under anaerobic conditions it has also been shown to reduce sulfur compounds, nitrates, and chromates. The cultivation of DMRB under controlled conditions therefore has significant implications for the low-energy, room-temperature synthesis of metal sulfide and/or metal oxide semiconductors. Furthermore, Shewanella and other DMRB can form biofilms that interact electronically with solid-phase minerals in their environment. For this reason there exists a potential to grow DMRB directly into porous substrates in order to create biosensors that are capable of producing electrical signals that provide information about metal ion concentration in water as well as a range of other water quality variables.

I will highlight my recent work exploring the behavior of Shewanella oneidensis MR-1, in a medium rich in both zinc and thiosulfate ions. I have grown Shewanella bacteria in a three-electrode system and used a potentiostat to hold the system at a fixed DC voltage during cultivation while also measuring current output. After completing the cultivation step, I have used cyclic voltammetry and electrochemical impedance spectroscopy to characterize the DC and AC current-voltage dynamics of the system, which can reveal the reduction-oxidation activity of key bacterial proteins. In the second experiment, I have grown Shewanella bacteria under both aerobic and anaerobic conditions in media rich in zinc and thiosulfate ions and used scanning electron microscopy and energy dispersive microscopy to characterize the minerals that precipitate within the batches. I compare results from a minimal medium containing only the zinc and thiosulfate sources to a more traditional Shewanella medium containing various vitamins, minerals and other nutrients to support growth. I also compare the inoculated batches to sterile control batches containing no bacteria in order to infer the effect that the bacteria have on mineralization in their environment. Finally, I use confocal microscopy to explore the fluorescence behavior of the precipitates generated in both inoculated and sterile batches.

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

35 Years of NESAC/BIO II

Moderator: Sally L. McArthur, Swinburne Institute of Technology

5:40pm **BI-TuE1 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-TuE3 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-TuE4 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-TuE5 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-TuE7 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.D. 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-TuE8 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.

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8:20pm **BI-TuE9 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-TuE10 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.

Biomaterial Surfaces & Interfaces
Room Naupaka Salon 6-7 - Session BI-WeM
Soft Surfaces and Biofunctional Coatings

Moderator: Tobias Weidner, Aarhus University

8:40am **BI-WeM3 Surface Micropatterning Techniques for Reconstituting Functional Neuronal Networks in Culture**, ***Hideaki Yamamoto***, *A. Hirano-Iwata,* Tohoku University, Japan **INVITED**

Nerve cells in culture take irreplaceable roles in molecular and cellular neuroscience. However, the fact that neurons form random connections, which are substantially different from the actual brain, has limited the wide application of cell culture in systems-level studies.

Surface modification combined with microfabrication has a high potential to circumvent this limitation of cell culture technology in neuroscience [1-2]. By patterning biomolecules that scaffolds cellular growth, a glass coverslip can be functionalized so that growth of primary neurons can be controlled extrinsically, at the level of both individual cells [3-5] and cell populations [6]. Taking advantage of the cell micropatterning technology, we reconstitute functional neuronal networks of rat cortical neurons and investigate how meso-scale connectivity among neurons determines network dynamics. We focus on the modular organization of brain networks, characterized by the presence of densely-connected subsystems, i.e., modules, that are weakly interacting with each other [7]. Analysis of spontaneous neural activity by fluorescence calcium imaging shows that an atypical dynamics of the cultured networks, characterized by a bursting activity that is highly-synchronized across the whole network, is suppressed by the induction of modular organization in the networks. Increasing the degree of modularization causes the networks to generate activity patterns that are spatiotemporally more complex. Our results demonstrate that surface micropatterning expands the cell culture system as a unique tool to model and study the structure-function relationships in living neuronal networks.

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9:20am **BI-WeM5 Inhibiting Bacterial and Fungal Growth *via* Biomimetic Nanopillared Surface Structuring**, ***Rachel Rosenzweig***, *V.K. Ly, K. Perinbam, M. Marshall, E. Pearlman, A. Siryaporn, A.F. Yee,* University of California, Irvine

Bacterial and fungal contamination occur in our everyday lives from food spoiling, oral disease, appliance clogging, and industrial naval and aviation fuel-line dependent transportation. More perilously, human pathogenic bacteria and fungi often contaminate medical device surfaces leading to 1.7 million annual nosocomial infections in the US alone. Such infections result in 99,000 annual deaths and $20 billion in healthcare costs. Current solutions that are declining in efficacy due to antimicrobial resistance (AMR) include chemical antimicrobials applied topically to or impregnated onto devices and implants. The rise of AMR has created an urgent need for alternative strategies. In this work, the physical antimicrobial effects of nanoimprinted polymer surface structures inspired by insect wing nanotopography are investigated.

Natural nanopillared surface structures found on dragon fly and cicada wings have been found to cause bacterial cell lysis, yet their possible effect has not been studied when applied to eukaryotic filamentous fungi. In this work, AMR prokaryotic bacteria, *Pseudomonas aeruginosa*, and clinical isolates of AMR eukaryotic filamentous fungi, *Aspergillus fumigatus* and *Fusarium oxysporum*, were cultured on flat and engineered biomimetic nanopillared surfaces on a material often used in medical devices, *viz*., poly(methyl methacrylate). Surfaces of nanopillared arrays with varying periodicities of 200nm, 300nm, 500nm, and 600nm were fabricated using nanoimprint lithography. Notably, this surface structuring technique is a low-cost and scalable lithographic method translatable to flat and curved surfaces. Cell growth and survival were measured using fluorescence microscopy of GFP tagged bacteria and fungi with propidium iodide DNA stain to indicate compromised cell membranes. The cell-nanosurface interface was further analyzed with scanning electron microscopy. A decrease in *P. aeruginosa, A. fumigatus,* and *F. oxysporum* cell growth and an increase in cell death were observed on the biomimetic nanopillared surfaces compared to the flat. This work presents the first demonstration of a scalable, nanostructured, antimicrobial surface against both drug resistant prokaryotic bacteria and eukaryotic fungi. This biofunctional coating can be applied to a broad range of applications in healthcare, industrial transportation, and environmental conservation.

9:40am **BI-WeM6 Chemo-enzymatic Pathways for Sustainable Terpene-based Polymeric Materials**, ***Arne Stamm***, *L. Fogelström, P.-O. Syren, E. Malmström,* KTH Royal Institute of Technology, Sweden

Polymers play an essential role in everyday life as materials in automotive, packaging and electronics and as compounds in medicine. Nevertheless, the finite supply of fossil fuels leads to an increased need for development of more sustainable materials from renewable sources. Renewable natural products from forestry, especially hemicellulose and terpenes, offers a highly versatile platform for green building blocks. By using Nature's own biofunctionalizations, enzymes can be used as green catalysts for the valorization of abundant terpenes from pine-wood extractives. Enzymatic biotransformations enable mild processes for "activating" inert molecular building blocks in a highly controlled manner to afford renewable monomers. By combining *in vitro* synthetic biology and polymer chemistry, we have generated a novel class of bio based polymers, star ting from a naturally abundant terpene found in wood. Specifically, the terpene sobrerol, which can be achieved both enzymatically and by traditional organic chemistry, represented a promising starting compound for the preparation of such bio based monomers. Sobrerol consists of a multiple substituted cyclohexene unit, containing secondary and tertiary hydroxyl functionalities. The functionalities of sobrerol enable certain chemical modifications, whereas the cyclic structure provides hardness in subsequent polymeric products. Especially, the stereoselective methacrylation of the secondary hydroxyl group constituted a suitable monomer for radical polymerization. We were able to demonstrate that the enzymatic functionalization under benign conditions showed superior properties concerning yield, stereo selectivity and workup procedures of the methacrylated sobrerol (SobMa). Further, SobMA could be polymerized using both traditional and enzymatic procedures enabling a completely green route from a natural abundant product to a highly versatile polymer. Due to the remaining functional groups in the side chain, polySobMA provides a variety of possibilities for post-functionalization reactions and crosslinking. Polymeric films were obtained by crosslinking reactions using either the ene-, or the hydroxyl functionality of the sobrerol unit and their properties evaluated. Thus, the unaffected second functionality could be used for a broad range of further modifications to produce tailor-made polymer films targeting different fields of application. In conclusion we were able to present that the use of enzymatic or chemo-enzymatic processes is an ideal approach to convert terpenes into highly versatile polymeric coating materials.

10:20am **BI-WeM8 Chemical Surface Modification of Carbon Nanostructures Towards Biological Applications**, ***Mildred Quintana***, Universidad Autónoma de San Luis Potosí, México **INVITED**

The unique combination of properties of carbon nanostructures, such as high specific surface area, chemical stability, mechanical strength, flexibility, high electrical and thermal conductivity, and tunable band gap and shape, make them ideal materials for the development of a number of bio-applications including biosensors [1], photodynamic therapy agents [2], and active surfaces for cellular growth [3]. However, for applicability several problems arise, including scalability, dispersibility, stability, and reproducibility. Several authors have proposed chemical functionalization as a feasible solution to render carbon nanostructures dispersible in many solvents, comprising water, and readily for its integration in hybrids materials [4]. Furthermore, by performing chemical organic reactions on carbon nanostructures, it is possible to exactly adjust the interfacial properties to increase biocompatibility [5] or to prompt lipid membrane translocation [6]. In this work, I will describe our recent efforts on the chemical functionalization of carbon nanotubes and graphene towards the development of SERS biosensors, photodynamic therapy agents and active surfaces for cellular growth. The importance of the tailored design of the chemical surface of the nanostructure for the desired application will be extensively discussed.

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11:00am **BI-WeM10** ***Roles of Anodic Oxide Layer on the Improvement of Cellular Response of Titanium Implant*** *,* ***Naofumi Ohtsu****, T. Kuji, M. Hirano,* Kitami Institute of Technology, Japan

Anodic treatment of titanium (Ti) has been used to improve its biocompatibility. The process leads to the formation of TiO_2 layer and the layer growth can be controlled by varying the processing voltage. Concomitantly, the surface roughness increases accompanying with the layer growth. Some researchers have believed that the enhanced biocompatibility through anodization is derived from the chemical property of TiO_2 itself, whereas other groups have insisted that the roughness increase relates with the biocompatibility. To obtain the valuable clue regarding this argument, in the present study, we prepared a TiO_2 layer with different roughness through the anodization in H_3PO_4 electrolyte with various voltages ranging from 5 to 500 V and thermal oxidation at 723 K in air. Thereafter, surface roughness and cellular response were compared to discuss the dominant property contributing the enhancement.

The surface image of the anodized substrates, observed by SEM, revealed that the surface roughness increased with increasing the voltage. To investigate the cellular response, MC3T3-E1 cells, an osteoblast-like cell line, were seeded on the sample surface and cultivated for 72 h, after which the numbers of the attached cells were counted. The numbers of the cells on the anodized surfaces were larger than those on an untreated and the thermally oxidized surfaces, whereas the difference depending on the processing voltage was hardly observed. Ii was conjectured that the enhanced biocompatibility is due to the anodized TiO_2 itself, of which surface property is different with that of TiO_2 prepared by thermal oxidation.

11:20am **BI-WeM11 (Electro)Chemically Synthesis et Characterization of New Coating having N-Halamine Groups giving them Regenerative Antibacterial Properties***,* ***Vincent Humblot****, N. Nazi,* LRS - CNRS Sorbonne Université, France*; C. Debiemme-Chouvy,* LISE - CNRS Sorbonne Université, France

In the presence of moisture, surfaces are an ideal support for the development of biofilms containing bacteria that can be pathogenic. This poses a real public health problem, economic or even environmental in view of the use of biocides to fight against this phenomenon. The first step in the formation of a biofilm is the adsorption of molecules, especially proteins, followed by the colonization of surfaces by bacteria.

The goal of this study is the development of **new regenerative antimicrobial coatings**, **containing haloamine (or N-halamine) functions(> N-Cl or> N-Br)** that have oxidative properties due to the degree of oxidation +I [(a,b)]. N-halamines are broad-spectrum biocidal groups; due to their mode of action, i.e. oxidation, bacteria should not develop resistance, unlike after repeated use of antiobiotics. The protection of surfaces with N-halamine compounds requires the immobilization of amine, amide or imine functions that will be transformed into haloamine either during synthesis or by post-treatment in the presence of NaOCl or NaOBr.

In this study, we will present a new approach of gold surfaces functionalization with the use of a biopolymer: **polydopamine**. The synthesis of the polymer has been implemented with two original approaches: a chemical and an electrochemical synthesis. We will present a comparative study of both chemical and electrochemical polymerisation and functionalization of gold surfaces characterized by means **of PM-RAIRS, XPS and (E)-QCM surfaces techniques**. The control of the polymer thickness shows a clear dependence of the antibacterial response with the degree of chlorination or bromination. Finally, the simple regeneration of the biocidal surfaces will be presented together with the biocidal activity upon re-use of the surfaces.

(a) Antimicrobial *N*-halamine polymers and coatings: A review of their synthesis, characterization and applications. F. Hui, C. Debiemme-Chouvy, *Biomacromolecules* **2013**, 14, 585-601. (b) N-halamine coating formed via the electroreduction of *in situ* generated diazonium cations: toward antimicrobial surfaces. S. Gao, H. Cachet, C. Debiemme-Chouvy. *Surf. Interface Anal*. **2016**, 48, 630-635.

11:40am **BI-WeM12 Effect of Salts on Friction of Zwitterionic Polymer Brush: Molecular Dynamics Simulation***,* ***Shuichi Uehara****, Z. Liu, N. Miyazaki, Y. Ootani, N. Ozawa, M. Kubo,* Tohoku University, Japan

In recent years, concentrated polymer brush (CPB), which is constructed by grafting polymers onto a substrate at high density, has been developed [1]. Especially, zwitterionic CPB produces ultra-low-friction surface and has biocompatibility in aqueous environment. Thus, zwitterionic CPB has attracted much attention for application to a low friction material as artificial joints. Recently, experiment showed the friction force of zwitterionic CPB decreases with increasing ionic strength for salts [2]. However, the details of this mechanism are still unknown because the in-situ observation is difficult. For enhancing the performance of zwitterionic CPB as a low friction material in biological applications, it is important to understand the effect of salt existing in biological environment. Thus, computational simulation is required.

In the present study, we performed molecular dynamics friction simulation between CPB and Au tip to elucidate the effect of salts on friction force of zwitterionic CPB. In the CPB model, 9 zwitterionic polymer chains of 10 monomers were grafted to a silicon (111) substrate (area, 5.75nm×5.98nm) via covalent bonds. For comparison, we prepared two systems: with salts (80 KCl) and without salts. Both system of CPB solvated in 6000 water molecules.

At a low load (up to 10 MPa), zwitterionic CPB with salt showed lower friction force than system without salts. This result is qualitatively consistent with experimental data [2]. The mean square displacement of water in the system with salts is lower than that in system without salts. This result suggests that waters in the system with salts have higher viscosity. Whereas, we find that zwitterionic chain with salts is harder to move in the sliding direction than system without salts. Therefore, we revealed that the binding of salts to polymer chain made polymer chains hard to collapse in spite of increasing viscosity of waters. Thus, zwitterionic CPB with salt reduced contact area between Au tip and polymer chains. Our previous study showed that the reduction of contact area of CPB in friction interface leads to low friction [3]. On the other hand, at a high load (20 MPa), the friction force of zwitterionic CPB with salts and system without salts were comparable. This is because salts desorb from inner layer of zwitterionic CPB due to the severe load. Therefore, to enhance performance of zwitterionic CPB as a low friction material, it is necessary to design of CPB so as to hold salts which make CPB hard to collapse in the severe load.

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

Biomolecule/Material Interactions and Medical Applications

Moderator: Buddy D. Ratner, University of Washington

5:40pm **BI-WeE1 Engineered Biointerfaces – Organisation and Functionalisation of Proteins at Surfaces***, **Jenny Malmstrom,*** University of Auckland, New Zealand **INVITED**

In my research group, we are interested in the interface between materials and biological systems – such as proteins and cells. Structured or organised surfaces with nanoscale features are important in a range of fields ranging from energy and computing to controlling cellular adhesion or differentiation. The precise organisation of proteins at surfaces is one route to creating such engineered interfaces. Proteins exist with an enormous structural and chemical versatility and lend themselves well to be functionalized with different moieties. The ability to rationally engineer proteins enables the use of proteins as carefully designed nanometer sized building blocks.

I will present work from our group focussed on using protein-protein interactions to build up higher order protein structures, and our efforts to organize and functionalise these structures. Proteins like Lsmα and peroxiredoxin self-assemble into robust doughnuts whose pore size can be tuned specifically to encapsulate metal complexes or nanoparticles and then assemble further into stacks to create magnetic, electrical or optical nanorods. We are harnessing this potential to create functional arrays of these self-assembling protein rings. We have explored ways of arranging these protein rings, for example through templating using a self-assembling block copolymer, or through specific binding to a patterned surface. Furthermore, the protein core has been used to template the synthesis of small (~4 nm) iron oxide nanoparticles. Throughout all of this work, imaging is an important characterisation tool and I will show how we use AFM (including magnetic force microscopy) and other techniques to understand our systems.

Building on this work, I will also present how we are developing some of these thin block copolymer films as biointerfaces, with the aim to control both protein and cellular interactions at the interface.

6:20pm **BI-WeE3 Tunable Thermal Transport and Reversible Thermal Conductivity Switching in Topologically Networked Bio-Inspired Materials***, J. Tomko,* University of Virginia*; A. Pena-Francesh, H. Jun,* Pennsylvania State University*; M. Tyagi,* National Institute of Standards and Technology*; B. Allen, M. Demirel,* Pennsylvania State University*; **Patrick Hopkins**,* University of Virginia

The dynamic control of thermal transport properties in solids must contend with the fact that phonons are inherently broadband. Thus, efforts to create reversible thermal conductivity switches have resulted in only modest on/off ratios, since only a relatively narrow portion of the phononic spectrum is impacted. Here, we report on the ability to modulate the thermal conductivity of topologically networked materials by nearly a factor of four following hydration, through manipulation of the displacement amplitude of atomic vibrations. By varying the network topology, or crosslinked structure, of squid ring teeth-based bio-polymers through tandem-repetition of DNA sequences, we show that this thermal switching ratio can be directly programmed. This on/off ratio in thermal conductivity switching is over a factor of three larger than the current state-of-the-art thermal switch, offering the possibility of engineering thermally conductive biological materials with dynamic responsivity to heat. More details of this work can be found in the recently published paper, Tomko, J.A., Pena-Francesch, A., Jung, H., Tyagi, M., Allen, B.D., Demirel, M.C., Hopkins, P.E., "Tunable Thermal Transport and Reversible Thermal Conductivity Switching in Topologically-Networked Bio-Inspired Materials," *Nature Nanotechnology* **DOI: 10.1038/s41565-018-0227-7**.

7:00pm **BI-WeE5 Design Principles and Potential Applications of Cyclic Peptide Polymer-based Nanomaterials***, **Kenan Fears,*** US Naval Research Laboratory, USA

We present a new class of bioinspired nanomaterials that are stabilized by a combination of covalent and hydrogen bonds. Prior work by others has shown that cyclic peptides can self-assemble to form supramolecular assemblies through backbone-backbone hydrogen bonding. To improve upon this molecular architecture, we develop a synthesis route to polymerize cyclic peptides and form a linear polymer chain that can transition between a rigid nanorod and a "soft" unfolded conformation. For a cyclic peptide polymer containing amine-terminated side chains on each ring, we demonstrate self-assembly can be triggered in aqueous solutions by varying the pH. We measure the elastic modulus of the rigid nanorods to be ca. 50 GPa, which is comparable to our molecular dynamics (MD) prediction (ca. 64 GPa). Our results highlight the uniqueness of our molecular architecture, namely their exemplary toughness (up to 3 GJ m^{-3}), in comparison to other cyclic peptide-based assemblies. Finally, we demonstrate the potential of these novel nanomaterials for biomedical applications, such as wound healing.

7:40pm **BI-WeE7 Metal Oxides and Bone Healing***, H. Nygren,* University of Gothenburg, Göteborg, Sweden*; C. Zhang,* Science for Life Laboratory, Stockholm, Sweden*; **Per Malmberg**,* Chalmers University of Technology, Sweden

Metal oxides are widely used in implant materials and trace metals are known to deeply influence bone healing. The present study was undertaken to elucidate the mechanisms of the effect of metal ions on bone healing, starting with analyses of the ability of different metal oxides to catalyze the formation of hydroxyapatite (HA) and ending with a global analysis of the transcriptome of bone tissue after implantation of metal ions.

Incubations of MnO and ZnO with cell culture medium followed by analysis with XPS, ToF-SIMS and SEM/EDX showed that these metal oxides are covered with a layer of HA within 12h. Implantation of MnO and ZnO in rat tibia stimulated the formation of callus bone. After 3w of healing of ZnO implants, the bone mineral contained high levels of Zn. This was considered a potential hazard and the use of ZnO was omitted from the study. Sham-operated tibia and bone implanted with MnO were taken to RNA-extraction and global analysis of differently expressed genes at the Science for Life Laboratory in Stockholm (head M. Uhlen). After 4 days of healing, the enrichment analysis showed upregulation of genes reflecting response to cytokines, cytokine regulation and cytokine production in the bones implanted with MnO, compared to sham. Furthermore, genes reflecting leukocyte migration, inflammation and celldeath were upregulated. Analysis of upregulated single genes shows reactions to hypoxia (RGS5), reactions to platelet Ca levels (LHFPL2), genes related to osteogenesis (FetuinB, RUFY4,NFkBIA) and osteoclast differentiation (CPMB6B). The data are still undergoing further analysis.

Manganese has been described as an essential trace metal for bone formation since the mid 1930´s when low levels of Mn in the feed was shown to cause skeletal defects in chicken, rats and rabbits. Mn has been suggested as a trace metal in bone cement based on its effect on biochemical markers of bone metabolism. Manganese is widely used in biomaterials, most extensively as a component of stainless steel.

8:00pm **BI-WeE8 Thin Films, Coatings and Surface Solutions for Medical Devices***, **Shahram Amini,*** Johnson Matthey Inc.

As medical device manufacturers are pressed to design ever-smaller devices with increasingly long service life, optimizing the performance and profile of each component becomes more crucial. During the past few decades, various medical devices, for instance cardiac rhythm management and neurostimulation devices, have been invented and used in clinical practice to achieve electrical stimulation. These devices function via artificial stimulation of living tissue through transfer of an external electrical signal to an implantable electro-conductive microelectrode across to the membrane of the neural cells or tissue. These electrodes and their surface properties have been a focus for innovation at the Center for Coatings and Surface Solutions (CCSS) to give the next-generation devices a competitive edge via advances in coatings technology that can enable electrodes with better charge exchange capacity, thereby improving accuracy and efficacy of treatment – while also extending the devices' battery life. In developing these electrodes, the substrate, its surface, and its interface with the electrolytic physiological environment all play important roles in the stimulation process. This presentation will focus on the process development and characterization of coatings that exhibit high electrochemically-active surface areas for implantable stimulation devices. In particular, effect of various electrode surface treatment technologies on microstructural characteristics will be discussed. The results presented in this work demonstrate an unprecedented approach that has facilitated discovery of many unique features in these coatings, and the effect of electrode surface on coating surface and sub-surface features.

8:20pm **BI-WeE9 Effects of Metal Implants on Bone Healing Analysed by Transcriptomics**, ***Håkan Nygren,*** University of Gothenburg, Göteborg, Sweden*; C. Zhang, M. Arif, M. Uhlen,* Science for Life Laboratory, Stockholm, Sweden

Bone fractures affect hundreds of millions people worldwide and are a leading cause of long-term pain and disability. Fractured bone normally heals *ad integrum* through a process undergoing characteristic stages of blood coagulation, inflammation, formation of soft and hard callus and, finally, remodeling to its original structure. In approximately 10% of femur-neck fractures, healing meets with failure, or delay. Common causes of failure to heal are critical size defects, infection, or mobility of the fracture parts. Internal stabilisation of fractures with metal implants is an efficient aid of fracture healing. Tissue engineering of bone healing is efficiently made by implanting metal species like Mg, Sr, Zn and Mn. These metals often have a capacity to catalyze formation of hydroxyapatite in bone tissue (Nygren, Pacsurf 2016) suggesting a possible common pathway for the well documented effect of trace metals on bone healing. In this study we analysed the transcriptomics of fracture healing with and without implanted Mg and Mn after 4 and 7 Days of healing, before mineralization and after completion of the callus bone.

Proteins coded by the most differentially expressed genes during normal fracture healing after 4 Days of healing where regulators of platelet degranulation, upregulators of TGF-beta, regulators of Beta-1 Integrin, IL10 receptor antagonist and ROBO proteins guiding cell movement in embryos.

Proteins most differentially expressed after 7 Days of healing were an enzyme hydrolysing lysine, inhibitors of inflammation, NFkappaB, microtubule associated scaffold protein, angogenic proteins and BMP-2 signalling proteins.

Venn diagrams comparing the up-regulated genes after healing with Mg and Mn after 4 Days of healing showed no overlap between the activated genes in these Groups. After 7 Days of healing, there was an 80% overlap between genes upregulated by Mg and Mn. The data suggest that pathways of bone healing at metal implants differs after 4 Days of healing, before the start of mineralisation, but are more congruent after 7 Days of healing when the callus bone is mineralised and remodelling starts.

8:40pm **BI-WeE10 Synthesis and Characterization of Reactively Sputtered Platinum Group Metal Oxides for Stimulating and Recording Applications**, *G.V. Taylor, N. Page, A. Marti, R. Paladines,* Rowan University*; A. Fones,* Johnson Matthey Inc., UK*; S.D. Tint,* Johnson Matthey Inc.*; H. Hamilton,* Johnson Matthey Inc., UK*; S. Amini,* Johnson Matthey Inc.*;* ***Jeffrey Hettinger,*** Rowan University

A range of materials have been examined as coatings over the past several decades to improve the performance of implantable devices used in neurostimulation and recording applications. Iridium oxide (IrO_2) has been widely investigated due to its biocompatibility and high charge storage capacity. Modification of the synthesis conditions, as one means of improving the coating performance, led to reports of surface platelets forming at high deposition pressures. This study complements earlier research by extending the range of deposition parameters for the IrO_2 system and investigates the ruthenium oxide (RuO_2) system under the same experimental conditions. The results show that the platelet microstructure in tetragonal IrO_2 is due to the formation of a specific orientation of crystallite. In contrast to previous reports that platelet formation coincided with a decrease in coating performance, it will be shown that the presence of platelets can improve the electrochemical performance of the coatings as measured by cyclic voltammetry in a phosphate buffered saline electrolyte. Furthermore, the platelet microstructure, and thereby the effective surface area, can be systematically controlled by adjusting deposition parameters, including temperature and oxygen partial pressure, used during the reactive sputtering. No such platelet formation has yet been observed in the RuO_2 system.

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