Biomaterial Surfaces & Interfaces
Room Milo - Session BI-MoM

Buddy Ratner's 70th Birthday Session
Moderator: Lara Gamble, University of Washington, USA

8:00am **BI-MoM1 SIMS Surface Science from SAMs to 6S Scaffolds***, **Daniel Graham**, L.J. Gamble,* University of Washington, USA

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) generates chemically rich, complex data that can encode information about surface composition, molecular conformation, orientation and more. However, due to the complexity and magnitude of the data, it is imperative that research projects be carefully planned out before the researchers attempt to extract this information using ToF-SIMS. Professor Ratner understood this challenge and recognized that, after utilizing a well planned research strategy, applying multivariate analysis methods (MVA) could aid to better understand ToF-SIMS data and use it more efficiently. This idea, generated more than 20 years ago, led me on a journey exploring the complexities of ToF-SIMS through the use of self-assembled monolayers (SAMs) that has continued into exploring complex organic systems such as cells, tissues and tissue engineered scaffolds. In this presentation I will highlight the work pioneered by the ideas of Buddy Ratner that helped start the MVA revolution in SIMS analysis and has led to the development of methods that help to more efficiently process and better understand secondary ion mass spectrometry data. Examples will be shown from controlled experiments with SAMs, cells, tissues and tissue engineering scaffolds. Work with SAMs helped demonstrate that combining MVA with SIMS and well controlled substrates could help us better understand the SIMS fragmentation process and discover new information encoded in the relative intensities of the peaks. This set the foundation for processing more complex systems and using MVA and SIMS to characterize the chemical differences in more complex systems. This has led to one of our current projects involving characterizing tissue engineering scaffolds with cells in 2D and 3D.

8:20am **BI-MoM2 Genetic Level Programming of Molecular Assembly of Intrinsically Disordered Proteins***, **Gabriel López**,* University of New Mexico, NSF Research Triangle Materials Research Science and Engineering Center, Duke University, USA **INVITED**

A number of dynamic, protein-rich intracellular structures containing phase separated, unstructured proteins comprising low-complexity amino acid sequences have recently been shown to serve a variety of important cellular functions, including signaling, compartmentalization and stabilization. The understanding of these structures, and the ability to synthesize models of them, has been limited. This talk will present simple methods for programming diverse assemblies comprised of a series of elastin-like polypeptides, model intrinsically disordered proteins possessing sequences of low-complexity. By encoding the stimulus-induced aqueous phase behavior of proteins at the amino acid sequence level, we demonstrate the reversible formation of a variety of protein-rich structures, ranging from uniform nano-, meso-, and micro-scale puncta (small, distinct particulates) to multilayered, orthogonally-phase-separated, multicomponent microgranules. We further show how such nanoscale assemblies (i) can be stabilized by controlled biomineralization, (ii) can be used for simple bioassays for diagnostic or drug discovery applications, or (iii) can be used as building blocks for the hierarchical formation of micellar hydrogels with surprising mechanical properties and potential use in controlled delivery of nanoparticles for drug delivery applications. The talk is dedicated to Prof. Buddy Ratner, a mentor and friend of mine and of my collaborator, Prof Ashutosh Chilkoti, on the occasion of Buddy's birthday this year.

9:00am **BI-MoM4 Surface Activation of the VWF A1 Domain: The Relationship between Platelet Activity and Absorbed A1 Structure***, H. Tronic, E. Thomas, **David Castner**,* University of Washington, USA

When a material is placed in a biological environment, the surface of the material acts as the interface between that material and the biological environment. Upon contacting blood, plasma proteins attach to these surfaces and mediate platelet adhesion and activation and thrombosis. A key protein in this process is the clotting protein von Willebrand Factor (VWF) which binds to platelet receptor glycoprotein 1bα (GPIbα) when VWF is activated by chemicals, high shear stress, or immobilization onto surfaces. Activation of VWF by surface immobilization is an important problem in the failure of cardiovascular implants, but is poorly understood. Here we investigate whether some or all surfaces can activate VWF at least in part by affecting the orientation or conformation of the immobilized GPIbα-binding A1 domain of VWF. Platelets translocate rapidly on A1 adsorbed onto PS surfaces, and demonstrate shear-enhanced adhesion in that they detach at low rather than high shear stress. In contrast, platelets translocate more slowly on A1 adsorbed onto TCPS surfaces and are nearly stationary on A1 adsorbed onto glass surfaces, and demonstrate shear-inhibited adhesion in that they detach at high but not low shear stress. Both X-ray photoelectron spectroscopy and conformation independent antibodies reported comparable A1 amounts on all surfaces. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) suggested differences in orientation on the three surfaces, but none that could explain the biological data. Instead, ToF-SIMS determined Cys exposure and conformation-sensitive antibody binding suggest that A1 retains its native conformation when adsorbed onto PS surfaces, while TCPS surfaces and especially glass surfaces stabilized an alternative activated conformation of A1 that likely resembles the activated form of A1 that is also stabilized by disease-causing mutations. Regardless of the specific structure of the activated forms of A1, these studies demonstrate that it is not enough to determine the amount of various proteins that bind to different biomaterials placed in contact with the blood; instead, it is necessary to understand how different surfaces control the conformation of the many blood proteins that are capable of undergoing activating conformational changes. In particular, these studies demonstrate that the A1 domain of VWF has fundamentally different biological activity when adsorbed onto different surfaces. This is important when interpreting or designing *in vitro* experiments with surface-adsorbed A1 domain, and is also of likely relevance for blood-contacting biomaterials.

9:20am **BI-MoM5 Why do Bacteria Stick to Some Surfaces and Not Others? Characterisation of the Behaviour of Motile Bacteria at and above the Surface of Materials***, **Morgan Alexander**,* University of Nottingham, UK, United Kingdom of Great Britain and Northern Ireland

Antimicrobial resistance has been recognised as a pressing problem by the WHO, the UK government review [Jim O'Neill 2014] predicting a financial impact equal to cancer by 2050 and most recently a unanimous declaration by the UN General Assembly. Infections associated with medial devices are a significant contribution to this challenge. Hook et al. used high throughput screening to discover a new class of polymer with resistance to biofilm formation correlating with the chemistry of the uppermost nanometer of the material. [Nature Biotechnology 2012] Whilst a device using this material is progressing to regulatory approval for use in man, we are exploring the mechanism by which these work to enable us to develop improved devices.

Microorganisms cannot be approximated to inert objects since they possess surface responsive appendages such as flagella, which enable them to swim, pili that confer twitching motility and fimbriae that mediate surface attachment in response to surfaces. These 'devices' are in turn coupled to sophisticated signal transduction mechanisms that facilitate integration of multiple local environmental parameters at both single cell and population levels. Many of these sensory systems are postulated to contribute to surface sensing. As an example of the complexity of these processes, the opportunistic pathogen Pseudomonas aeruginosa has over 60 two-component sensor kinase response regulator systems involved in environmental adaptation.

We believe that bacterial decision-making is key to determining whether a surface is colonised or not. I will present the early results from our optical microscopy investigations of how individual bacterial cells respond to surfaces. We have developed a novel microscope that collects temporal 3D information on cell position using both holography and remote scanning microscopy. Surface tracking can be simultaneously achieved using DIC, TIRF and TIR microscopy. This allows us to track not only the motion of single cells at the surface, but also their approach to and behaviour after contact with the surface. We will combine these findings with our understanding of the surface chemistry-attachment relationships for certain subsets of materials and attachment regimes with in situ chemical analysis to build a complete description of this complex biointerface and the response of bacteria to it. This information is crucial in determining how bacteria behave with respect to defined surfaces and has important implications for the prevention of device centred infections and the development of the next generation of biofilm resistant surfaces.

9:40am **BI-MoM6 Antibody Microarrays for Point-of-Care Detection from a Single Drop of Blood***, **Ashutosh Chilkoti**,* Duke University, USA

I will discuss a point-of-care diagnostic that we have developed, in which all reagents are printed and stored on a "non-fouling"—protein and cell resistant—polymer brush. The D4 assay, involves four sequential events:

(1) Dispense (droplet of blood); (2) Dissolve (printed reagents on chip); (3) Diffuse (across surface); and (4) Detect (binding event). The D4 POCT antibody (Ab) consists of microarrays printed on the polymer polymer brush yields quantitative results, with picomolar sensitivity within 30 minutes. All reagents are inkjet-printed and stored on D4 POCT cassettes, which do not require refrigeration. Upon direct application of fingerstick blood onto a cassette, analyte capture and detection occur automatically, generating a quantifiable fluorescence signal obtained by placing the cassette in a small device that magnetically attaches to a smart phone, which images and analyzes microarrays via on-board App. Examples of quantitative dose-response from whole blood will be presented. The D4 assay can be used for the diagnosis of all markers for which antibody pairs are available with a speed and sensitivity that is as good or better than commercially available point-of-care tests and is far simpler, cheaper more rugged, and does not require a cold-chain.

10:20am **BI-MoM8 Plasmas, Proteins and Other Things Buddy has Inspired me to Play with in Vacuum Chambers**, ***Sally L. McArthur***, Swinburne University, 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 behave as they would 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.

Biomaterial Surfaces & Interfaces
Room Milo - Session BI-MoE

Soft Surface & Biofunctional Coatings

Moderator: Duncan McGillivray, University of Auckland, New Zealand

6:00pm **BI-MoE2 Functionalisation of Polymeric Biomaterials by Graft Copolymerisation**, ***Lisbeth Grondahl,*** The University of Queensland, Australia **INVITED**

The surface of a material is the first contact with the cellular environment upon inoculation (in vitro) or implantation (in vivo) and as such these surfaces must, at minimum, possess properties amenable to cell adhesion. However, many polymers used as biomaterials lack functional moieties and the overall hydrophobic nature of the polymers encourage non-specific protein adsorption and make them less than ideal for controlled protein attachment and hence directed cell attachment and expansion.

One method of changing the surface properties of polymeric biomaterials is graft copolymerisation of functional polymers thereby providing functional groups and/or reduced hydrophobicity to the material. Work will be presented on graft copolymerisation of both the biodegradable polymer polycaprolactone (PCL), an FDA approved aliphatic semicrystalline polyester studied extensively for tissue engineering, and the biostable polymer expanded polytetrafluoroethylene (ePTFE), used for example in facial reconstruction. The ability to modify the interior of scaffolds and membranes as well as introducing various functional groups (eg. phosphate, carboxylate and amine) will be illustrated. Recent studies on creation of dual functional materials will show how the approach taken (one-pot or consecutive grafting) affects the material properties.

Protein adsorption studies using albumin, lactoferrin and lysozyme illustrate that the surface density of carboxylate groups does not correlate to the amount of adsorbed protein rather a correlation to the degree of grafting was observed indicating penetration of the proteins into the grafted layers. Furthermore, the topology of the graft copolymer is shown to be more important than the functional group in regards to the outcome of in vitro mineralisation when comparing a series of phosphate and carboxylate functionalised surfaces.

6:40pm **BI-MoE4 Blood Compatibility Evaluation of Fluorinated Bioresorbable Polylactic Acid For Coronary Artery Stents**, ***Razieh Khalifehzadeh,*** *B.D. Ratner,* University of Washington, USA

Bioresorbable stents are an emerging, novel treatment for improving long-term stent outcomes. Rigid metallic stents are associated with failure over time partly due to their permanent presence in vessel walls inhibiting physiologic vasomotion and stimulating neointimal hyperplasia. Among various polymers, poly(lactic acid) (PLA) has been extensively used for making bioresorbable stents. PLA undergoes degradation through hydrolysis of ester bonds. The degradation products are lactic acid and oligomers that later metabolize into CO_2 and water.

Despite the various long-term advantages of bioresorbable stents, their reported thrombosis is higher than metallic stents. In an attempt to improve blood compatibility of PLA, we used radio frequency glow discharge (RFGD) to modify the surface of this polymer with perfluoro compound. Fluoropolymers have been shown to lower thrombogenicity and platelet reactivity, and are extensively used in blood contacting materials.

Here, we have developed a process to coat the surface of PLA with perfluoro compound. Electron spectroscopy for chemical analysis (ESCA) was used to analyze the surface composition of these polymers. In addition, contact angle measurements, cell cytotoxicity, and degradation profile were evaluated.

Finally, we will assess the blood compatibility of these modified surfaces by using radiolabeled blood plasma proteins (albumin and fibrinogen) adsorbed onto their surface. The adsorption of plasma proteins is the central event in the biofouling of blood-contacting surfaces, which occurs immediately upon exposure of blood to biomaterial. We hypothesiz that tight binding of adsorbed albumin on fluoropolymers accounts for its success in blood-contacting applications.

7:00pm **BI-MoE5 Multifunctional Bionanotubular Implant Surfaces**, ***Tolou Shokuhfar,*** University of Illinois at Chicago, USA

Bionanotubular surfaces offer exciting progress toward the design of multifunctional medical implants. To bring this to reality, we have synthesized and optimized the mechanical, physical, biocompatibility, and interfacial properties of titania nanotube surfaces using in-situ TEM, SEM, FIB, FTIR, and WCA measurements. We have observed that the fabrication of bionanotubular titania surfaces with elastic modulus close to actual bone promotes osteoblast growth and prevents stress shielding. In addition, bionanotubular titania surfaces could be considered a suitable alternative route for the development of drug-eluting and antimicrobial implants due to the fact that these nanostructures are not an added coating but rather are rooted in the implants and will not delaminate from the surface. Such drug-eluting implants can prevent unnecessary side effects caused by oral administration of drugs, increase drug efficiency, and prevent infection related implant complications and failures.

7:40pm **BI-MoE7 NAP-XPS and EnviroESCA – Surface Analysis Entering New Fields of Applications: XPS from Liquids and Solid-Liquid Interfaces**, ***Thomas Schulmeyer,*** SPECS Surface Nano Analysis, Inc.

For decades XPS has been the well-accepted standard method for non-destructive chemical analysis of solid surfaces. To fulfill this task, existing XPS tools combine reliable quantitative chemical analysis with comfortable sample handling concepts integrated into fully automated compact designs.

Recently however, it has been possible to develop XPS systems that can work far beyond the standard of high or ultrahigh vacuum conditions. Near Ambient Pressure (NAP) XPS has become a rapidly growing field in research, inspiring many scientist to transfer the method to completely new fields of application. By crossing the pressure gap, new insights in complicated materials systems have become possible using either synchrotron radiation or laboratory X-ray monochromators as excitation sources under NAP conditions.

Based on this experience, SPECS Surface Nano Analysis GmbH has developed two lines of products: a portfolio of research instruments with various setups optimized for different applications of NAP-XPS, and EnviroESCA. Both of these revolutionary tools realize the long existing dream in many analytical laboratories: reproducible chemical surface analysis under any environmental condition. EnviroESCA™ allows for different applications, such as extremely fast solid surface analysis of degassing (but also non-degassing) samples, XPS analysis of liquids or liquid-solid interfaces, chemical analysis of biological samples, materials and device analysis under working conditions (in situ/in operando studies of catalysts, electrochemical devices etc.).

In this presentation, the various basic designs showcasing their different applications are introduced. The results primarily show how measurements can be taken from liquids or solid/liquid interfaces, and the essential bits of information that can be derived from these measurements. The applications range from measurements on static and dynamic liquids, biological samples and fuel cells to in-operando measurements on electrodes under potential in static and dynamic electrochemical cells. For the first time, systematic XPS analysis from liquids is possible and evidenced. Thus, realistic opportunities as well as experimental challenges in liquid-XPS analysis will be discussed and summarized.

8:00pm **BI-MoE8 Wearable Microprojection Array Skin Patches for Sampling Biomarkers from the Skin**, ***Jacob Coffey,*** University of Queensland, Australia*; S. Corrie,* Monash University, Australia*; M. Kendall,* University of Queensland, Australia

Microprojection array (MPA) skin patches capture circulating blood biomarkers from the skin as a needle-free alternative to traditional blood sampling. The skin, due to its abundance of superficial capillary vessels offers an alternative route to access circulating biomarkers with minimal invasiveness for more frequent monitoring. This diagnostic potential has been largely unrealised due to the lack of convenient methods to sample biomarkers from the skin. To address this challenge we surface modified MPAs with an anti-fouling polymer (poly(ethylene glycol)) and capture probes that selectively bind circulating disease markers. These MPAs are engineered to penetrate only the upper layers of the skin and selectively bind circulating disease markers, thus avoiding bulk fluid sampling. Accessing the biomarker of interest, however, from the complex milieu of the tissue environment remains a key challenge critical to enable high MPA detection sensitivity.

We report MPAs rapidly sample dengue, malaria, and IgG (antigen-specific) disease markers in animal models. We then characterise the effect of MPA design (length, density, array size) on biomarker capture, which increases with the penetrated surface/tissue contact area of MPAs. Investigating the effect of MPA application and projection design on blood protein extravasation from skin vasculature showed MPA insertion induces blood protein extravasation, which may play a key role in accessing circulating biomarkers *in vivo*. MPAs with improved design rapidly, reliably and

reproducibly sample antigen-specific IgG for immunoassays in 30 sec – currently the best reported sampling time and diagnostic sensitivity using microprojections, making them highly suitable for rapid diagnostic tests.

We also developed 'wearable' MPAs for longer implantation times (24 h) which improves the sampling of low concentration biomarkers up to 6-fold. However, a significant decrease in the functionality of the capture surface was observed during implantation,with an approx 60% decrease in biomarker capture and corresponding increase in non-specific background signal. This suggests significant degradation or fouling of the capture surface *in vivo*. A key remaining challenge is to identify the causes of this functionality loss and to develop stable surfaces for long term *in vivo* sampling. An inflammatory response was also observed in the tisue surrounding the MPA, which may contribute to this surface degredation. Preliminary studies with zwitterionic antifouling polymer coatings (polysulfobetainemethacrylate) show improvemed biomarker capture over shorter sampling times (<10 min), which may offer promise to improve long term sampling.

Biomaterial Surfaces & Interfaces
Room Milo - Session BI-TuM

Bioimaging & Bionanotechnology
Moderator: Sally L. McArthur, Swinburne University, Australia

8:00am **BI-TuM1 Gas Cluster Ion Beams for Improved Biological Imaging with ToF-SIMS**, ***John Fletcher,*** University of Gothenburg, Göteborg, Sweden

The quest for improved molecular signal levels in imaging secondary ion mass spectrometry has been a long one. As the nature of the primary ion beam used to sputter the surface has a direct influence on the species detected there has been a long history of research in this area. The latest ion beam technology of SIMS is based on gas cluster ions, introduced by Matsuo and co-workers. These beams are routinely used for sample etching and cleaning in SIMS and XPS instruments but are not normally employed as analysis beams in SIMS instruments due to challenges associated with fast pulsing, focusing and low analyte ionisation efficiencies.

The use of a higher energy (40 keV) gas cluster ion beam (GCIB) on a J105 ToF-SIMS instrument (Ionoptika Ltd), where fast beam pulsing is not required for good spectral quality, offers great benefits for biological analysis.

Comparison of signal levels on rodent brain between the 40 keV GCIB and equivalent energy C_{60} shows a 30-50× increase in secondary ion yield for intact lipids while spot sizes of approximately 2 µm have been achieved.

The application of this system for the imaging of animal and human tissue samples in cardiovascular and breast cancer research will be presented. Lipid changes following surgically induced infarction in mouse hearts have been imaged with distinct differences detected between the infarcted and healthy regions of the tissue along with specific lipid signals associated with the interface between the 2 regions. Breast cancer biopsy tissue has been imaged and the lipid distributions studied in the tumour and surrounding stroma. Changes in the abundance of lipids arising from modification of dietary fatty acids versus de novo synthesised lipids shed new light onto lipogenesis processes in the tumour microenvironment.

8:20am **BI-TuM2 ToF-SIMS Imaging for Nano-Bio Applications**, ***Tae G. Lee,*** Korea Research Instutue of Standards and Science (KRISS), Republic of Korea **INVITED**

Although the collisional cascade process in ToF-SIMS is unable to produce secondary ions with a molecular weight of over m/z 2,000 without the use of noble metals or special matrixes, time-of-flight secondary ion mass spectrometry (TOF-SIMS) imaging is a powerful technique for producing chemical images of small biomolecules (ex. metabolites, lipids, peptides) “as received” because of its high molecular specificity, high surface sensitivity, and submicron spatial resolution. For large biomolecules, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has been used for molecular identification of proteins in the discovery of disease-related biomarkers as a key platform technique in proteomics.

For this talk, I will show that the label-free ToF-SIMS imaging technique can be a platform technology for characterization of organic-conjugated nanoparticles, disease diagnosis and drug screening. In addition, I will discuss the potential capability of Ar-cluster SIMS to study omics, particularly proteomics and lipidomics for brain studies.

9:00am **BI-TuM4 Label-Free Imaging of Biological Tissue with Micron Spatial and 240k Mass Resolution using a New Sims Hybrid Mass Analyzer**, ***Nathan Havercroft,*** ION-TOF USA, Inc.*; A. Pirkl, R. Moellers, H. Arlinghaus, F. Kollmer, E. Niehuis,* ION-TOF GmbH, Germany*; A. Makarov, S. Horning,* Thermo Fisher Scientific, Germany*; M.K. Passarelli, R. Havelund, P. Rakowska, A. Race, A.G. Shard,* National Physical Laboratory, UK*; A. West, P.S. Marshall, C.F. Newman,* GlaxoSmithKline, UK*; M.R. Alexander,* University of Nottingham, UK*; C.T. Dollery,* GlaxoSmithKline, UK*; I.S. Gilmore,* National Physical Laboratory, UK

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is an established, highly sensitive analytical technique for mass spectrometry (MS) imaging applications with a lateral resolution below 100 nm. Application of this technique for the localization of drugs and their metabolites in drug-doped cells could be used to find regions in which a pharmaceutical compound accumulates. This information would be extremely helpful for selection of possible drug candidates in pre-clinical studies, thereby reducing the development costs for new pharmaceutical products. Furthermore, surveying biologically relevant molecules, such as lipids, in tissue can give valuable information on the molecular fundamentals of diseases and the effects of treatments. However, in complex biological samples identification of unknown compounds can be hampered by mass interferences and a high number of possible assignments for a single mass peak. In order to overcome these limitations, the 3D nanoSIMS project [1] is developing a revolutionary new SIMS instrument that combines the high lateral resolution and speed associated with TOF-SIMS (TOF.SIMS 5, ION-TOF GmbH, Muenster, Germany) with the high mass resolution and high mass accuracy of an orbital trapping mass analyzer (QExactive™ HF [2], Thermo Fisher Scientific™, Bremen, Germany). The instrument is equipped with a newly developed gas cluster ion beam column which provides the capability to image with a lateral resolution down to the micron level with minimum sub-surface damage. In this contribution we will report about results obtained from different biological application areas such as tissue imaging, lipidomics, and single cell analysis. From coronal mouse brain tissue slices, we fully separate the (3'-sulfo)Gal-Cer(d18:1/24:1(2-OH)) and (3'-sulfo)Gal-Cer(d18:1/25:0) sulfatides, which reveals a difference in spatial distribution. In the low mass region, mass resolving powers of >400,000 are achieved allowing clear separation of the low abundance metabolite dopamine from other peaks, which has not been possible before.

Analyzing NR8383 cells, we show the ability to image the drug amiodarone with sub-cellular resolution and show that the mass spectra are not affected by sample topography.

Furthermore the MS/MS capability of the QExactive Instrument is used to confirm proposed assignments on tissue and from single cells.

[1] The 3D nanoSIMS project, http://www.npl.co.uk/news/3d-nanosims-label-free-molecular-imaging

[2] Scheltema, et al. Mol Cell Proteomics (2014).

9:20am **BI-TuM5 Advancing our Understanding of Tumor Biology with Imaging Time-Of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS)**, ***Lara Gamble,*** *D.J. Graham, B. Bluestein,* University of Washington, USA

Imaging time-of-flight secondary ion mass spectrometry (ToF-SIMS) can provide images of tissues with chemical and molecular specificity. These chemically specific images can improve our understanding of biological processes. Our current research has utilized this technique to map the chemical changes in the composition and distribution of metabolic related molecules within a tumor and the tumor microenvironment in order to study tumor development and progression in inducible and regressible mouse pancreatic β-cell neoplasia. Using ToF-SIMS, the chemistry of tumor microenvironments and lipid metabolomics relationship to cancer and tissue can be visualized on a cellular and sub-cellular level. Samples mounted on ITO substrates can be analyzed with ToF-SIMS and directly correlated with immunohistochemical and/or H&E images taken on the same sample. Imaging principal component analysis (PCA) is used to identify chemical regions that correlate with the tumor and the surrounding tumor environment. Preliminary results using PCA analysis of ToF-SIMS image data easily separate the tumor chemistry from the surrounding tissue within the first principal component. Differences in chemistry between the tumor and surrounding tissue suggest a preferential uptake of fatty acids 18:3, 18:2 within the tumor. The data shows an absence of Mg^+ within the islet tumor and small, higher signal regions on the periphery of the tumor that correlate with increased CN^-, CNO^-, $C_7H_{10}O^+$, and Fe^+ ToF-SIMS peaks. This work demonstrates the high resolution capability of ToF-SIMS as the data clearly reveals intratumor chemical heterogeneity as localized high intensity regions for specific chemical signatures. It also highlights the utility of acquiring ToF-SIMS images and traditional H&E images on the same sample.

9:40am **BI-TuM6 Multi-stain Live Confocal Microscopy of *Balanus Amphitrite* Provides New Insight on Interfacial Processes**, ***Kenan Fears,*** *C. So,* US Naval Research Laboratory, USA*; B. Orihuela, D. Rittschof,* Duke University, USA*; K. Wahl,* US Naval Research Laboratory, USA

The adhesion of hard foulers (e.g., barnacles and tubeworms) has plagued the maritime community for as long as mankind has been setting sail. Since the biological processes responsible for adhesion occur at buried interfaces, elucidating the mechanisms by which foulers adhere is

challenging. Through the use of multiple fluorescent probes, peptides, and antibodies, we have been able to discern an unprecedented level of detail about biological processes that occur at the interface between acorn barnacles (Balanus Amphitrite) and the underlying substratum during the barnacle growth cycle. Barnacles secrete a lipidaceous substance around the outside of their shell, prior to expansion that dislodges microorganisms and biofilms to present a cleaned surface. During molting, epithelia cells build a new interfacial cuticular layer, which becomes autofluorescent as it is sclerotized, above the existing cuticle whose degradation coincides with the exuviation of the main body's cuticle. Rather than being directly secreted onto the substrate, nanostructured barnacle cement accumulates in the space in between the new and old cuticle. As the barnacle expands, the cuticular layers are stretched and pulled around the outside of the side plate. The strain causes the old cuticle to randomly tear, allowing the new cuticle to deposit cement into the interface as it is dragged across the substrate. Furthermore, antibody staining allowed us to spatially and temporally identify where different cement proteins are presenet. These results illustrate that the methodologies we have developed to break down and analyze barnacle cement collection are yielding a more accurate representation of the proteins at the buried interface, and providing insight on their roles which will lead to improved strategies to both combat and mimic barnacle adhesives.

10:20am **BI-TuM8 Nanodiamonds for Targeted Biolabeling**, ***Olga Shimoni***, *K. Bray, L. Cheung, I. Aharonovich,* University of Technology Sydney, Australia

In the last decade, nanodiamonds (NDs) have attracted much interest from the biological community as the ultimate agent for biomedical applications, such as biomarkers, drug and gene delivery and biocatalysts, owing to their chemical inertness, biocompatibility, prolonged photostability and negligible toxicity. Their fluorescence emanates from point defects (centres) that possess an unprecedented photostability and exhibit visible emission at room temperature. One of the most well-known centres is nitrogen-vacancy (NV) defects, mostly because of its potential use in quantum computer and nanomagnetometry applications. Here, we will demonstrate the utilisation of commercially available NDs with NV centres and in-house fabricated NDs with silicon-vacancy (SiV) defects for application in bio-imaging. We will discuss their optical properties, luminescence differences and opportunities for fluorescence enhancement. An additional advantage of using NDs in bio-imaging is that they are purely made of carbon, and carbon can be readily modified with functional groups to attach biomolecules using standard organic chemistry procedures. Therefore, we demonstrate surface functionalisation of NDs to achieve selective intracellular targeting. In summary, our results bring new advancement in fabrication and utilisation of NDs as targeted biomarkers.

10:40am **BI-TuM9 Encapsulation of Selected Natural Novel Nutraceutical Biomolecules**, ***Selim Kermasha***, McGill University, Canada; *S. Karboune,* McGill Universit, Canada

Although there is an increasing interest in the development of novel natural nutraceuticals, it is important to ensure their preservation and delivery by their encapsulation, a technology that is gaining popularity in the food industry. The encapsulation of selected nutraceuticals, including enzymatically-synthesized phenolic lipids and self-assembly polymers of proteins and polysaccharides, was carried out. Our research group succeeded in the production of novel biomolecules of high nutritional value and antioxidant capacity, phenolic lipids (PLs), by a biotechnological process involving the esterification of selected phenolic acid models and endogenous edible oils. The encapsulation of PLs was carried out by the development of a process to yield gelatin-gum Arabic multinuclear microcapsules, via complex coacervation. The overall experimental findings indicated that the microencapsulation of PLs was effective for preventing their oxidation and hence by maintaining their antioxidant potential. On the other hand, the self-assembly of selected proteins (patatin and lysozyme)/polysaccharides (galactan, gum Arabic and xanthan gum)-based nanoparticles was investigated. The results indicated that the nanoparticles have spherical shape and their sizes were dependant on the pH and the molar ratio of protein to polysaccharide. Through the zeta (ζ) potential measurements, the formation mechanism of amphoteric patatin/xanthan gum and lysozyme/galactan nanoparticles was illustrated.

11:00am **BI-TuM10 Sequential Drug Release by pH/redox Dual Responsive Non-covalent Polymer Gatekeepers in Hollow Mesoporous Silica Nanoparticle**, ***Ja-Hyoung Ryu***, Ulsan National Institute of Science and Technology, Republic of Korea

Nanoscopic delivery vehicles capable of encapsulating drug molecules and releasing them in response to

external stimuli are of great interest due to implications in therapeutic applications. Sequential drug delivery with dual

stimulus responsive nanotherapeutics is highly desirable for disease specific treatment in cancer therapy with

minimized adverse effects. In addition to this, on-demand therapy received considerable attention among the

treatment techniques. Herein, we present the design of robust, new and simple pH dependent charge conversional

non-covalent polymer gatekeepers technique by preparing the hydrophilic and hydrophobic drug loading at high

capacity and improved encapsulation stability in hollow mesoporous container for target specific cellular uptake for

cancer treatment. The di-isopropyl methacrylate functionalized monomer facilitates the fast cellular uptake at acidic

environment of cancer cells and allows the on-demand release of hydrophillic drug at acidic pH of endosomes upon

protonation. Pyridine disulfide facilitates the strong encapsulation of loaded cargo upon crosslinking by thiol-disulfide

exchange and releases the cargo upon exposure with increased intracellular glutathione concentration. The codelivery

of the multi-drugs in single carrier enables a synergistic chemotherapeutic effect. Based on this new design, a

wide range of sequential and synergistic therapy can be achieved to satisfy varied clinical requirements.

11:20am **BI-TuM11 Development Of Biometric Identification Technique Of High Reliability Based On Atomic Force Microscopy**, ***Vlad Ageev***, Biomaging and Bionanaotechology, Russia

In this paper a method of biometric identification with high reliability based on measuring the elastic properties of the skin of a human finger while scanning his finger prints is presented. This method is shown to allow with a high degree of veracity to distinguish the skin from the inorganic materials used to create the fingerprint. It is found that the elasticity of the skin varies at 15% with increasing interval between the cut and measurement of the skin from 5 to 30 minutes. The elasticity of the skin also depends on the age of the person and is 60,2 ± 4,2 and 42,4 ± 2,6 kPa to 20 and 40 years, respectively. These dependencies can be used for creating additional levels of protection of biometric identification method and preventing such methods of its compromетation as the use of moulds and pre-made cuts of skin. The results can be used in the development of biometric identification systems with a high level of protection that verifies either the fingerprint pattern of skin of human finger or its elasticity.

Nanotechnology; biometrics; biometric identification; skin; elasticity; atomic force microscopy

Biomaterial Surfaces & Interfaces
Room Mauka - Session BI-TuP
Biomaterial Surfaces & Interfaces Poster Session

BI-TuP1 A Real Time Observation of Nano/Bio Interface of Protein Nanoparticles Using Graphene Liquid Cell ELectron Microscopy, ***Tolou Shokuhfar,*** University of Illinois at Chicago, USA

Investigation of crystalline structure and chemistry of biomaterials including biopolymers, bacteria cultures and proteins has always been a great interest for both materials and biomedical communities. The difficulties associated with the imaging of biological structures can be listed as follows: 1) If the imaging is conventional in Scanning / Transmission Electron Microscope (S/TEM), all liquid phase will be evaporated in the vacuum environment when inserted into the microscope, otherwise room temperature fixation, dehydration, infiltration, fixation, embedding and staining should be applied, but in this case, the sample will go through several chemical and thermal processes which involves loss of liquid phase and artificial contrast improvement through preferential staining so that the sample will not be in its native state. 2) If cryogenic imaging is utilized, the sample will be frozen, so all these steps mentioned in 1 will be eliminated. By lowering diffusion rates at liquid nitrogen temperatures, the electron beam induced sample damage will be less, which is good for imaging, but the other energy activated processes, which again depend on the electron beam-sample interaction, are not possible for these frozen samples to occur. 3) In order to see the dynamic processes, *in situ* fluid cell holders can be used. Even though electron beam related dynamic processes can be visualized with these holders, the sample thickness will be too much including the two silicon nitride windows and the liquid sandwiched in between the windows. 4) To characterize the chemistry and crystal structure knowledge at the native state with the highest resolution, the material needs to be thin and should be sealed/sandwiched in between two single layers of graphene sheets forming graphene liquid cell (GLC), so that the Selected Area Electron Diffraction (SAED) and Electron Energy Loss Spectroscopy (EELS) studies can be carried out in the Cs corrected S/TEMs with the highest resolution available in terms of the crystal structure and chemistry, respectively. We have shown even individual iron ions can be detected in the liquid state when released from the ferritin structures encapsulated in GLC and which proves the necessity of using GLC for the achievement of this sort of resolution [1-2].

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[2] The authors acknowledge funding from the National Science Foundation- CAREER award- Grant No- DMR- 1350734.

BI-TuP2 Combined Effect of Antimicrobial Coatings, Gamma Radiation and Negative Air Ionization with Ozone on *Listeria Innocua*, *Escherichia coli* and mesophilic bacteria on ready-to-eat cauliflower florets, ***Monique Lacroix,*** INRS-Institut Armand-Frappier, Canada

The objective of this study was to evaluate the effect of an antimicrobial bioactive edible coating on the microbiological quality of ready-to-eat cauliflowers. Combined treatments using antimicrobial coating in combination with a low γ-radiation dose or negative air ionization (NAI) with ozone on the microbiological quality of ready-to-eat cauliflowers was also evaluated. The antimicrobial coating was based on a microemulsion of citrus and lemongrass extract in mixture. The microemulsion was also mixed with a polymeric formulation based on maltodextrin and methylcellulose.

Results showed that each treatment alone was effective on *Listeria innocua*, *Escherichia coli* and mesophilic bacteria.

The antimicrobial coating was able to reduce by 2 Log CFU/gr, the level of *E. coli* and *L. innocua* and by 1.5 log CFU/gr, the level of total mesophilic bacteria. The bioactive coating acts also in synergy with γ-radiation, inducing no bacterial growth of *L. innocua* and *E. coli*, as well as a control of the growth of mesophilic bacteria during 7 days of storage. However, the use of NAI + ozone did not act in synergy with the antimicrobial coating to reduce the level of the pathogens under study. However, storage of coated vegetables under NAI + ozone atmosphere would be a good technique to reduce and control bacterial growth during storage to prevent cross-contamination.

BI-TuP3 Antibacterial Nano-film Fabrication for Ophthalmic Application, ***Minwook Chang,*** Dongguk university, Republic of Korea; *J. Hong,* Chungang university, Republic of Korea

Super-hydrophilic coatings have been extensively studied because of their diverse applications, especially for anti-bacteria films. Anti-bacterial coatings in biomedical devices need to be durable and bio-compatible, but super-hydrophilic films are commonly very fragile due to a porous structure, which is essential for super-hydrophilic functionality, chemical contamination, and thermal stability. To overcome these drawbacks of antibacterial coatings, we introduced polymeric silsesquioxane into the nano-coating, resulting in superior thermal stability and matrix structure based on siloxane groups. Layer-by-layer assembly was used as a multilayer fabrication method to exquisitely control morphology, thickness, and functionality of the nano-coating, and fabricate suitable structures for super-hydrophilic films through simple dipping and washing steps. Antimicrobial and nanoindentation tests were carried out to demonstrate the successful enhancement in the antibacterial and mechanical properties of the nano-coatings.

BI-TuP4 Micro/Nano-Bioactive Structure o f Titanium Implant Surface, ***Zhoucheng Wang,*** Xiamen University, China

Multi-level micro/nano-structure on titanium surface was constructed by Al_2O_3 sandblasting, acid etching in H_2SO_4 and HCl solution, and then anodizing in HF solution at a constant potential. The biological activities of different treated titanium samples were observed in vitro experiment . Scanning electron microscopy (SEM), [app:ds:Energy] [app:ds:Disperse] [app:ds:Spectroscopy] ([app:ds:EDS]) and X-ray diffraction (XRD) were employed to characterize the morphology, composition and crystalline phase of the different treated titanium samples. The results showed the multi-level micro/nano-composite structure in which approximately consisting of 10~20 μm diameter hollow by sandblast, 5~8 μm diameter pit by acid etching and 10 nm diameter nanotexture by anodizing. The results of vitro experiments showed that the multi-level micro/nano-composite structure had better biological activity than the control group. After modification and heating treatment of the surface, the multi-level micro/nano-composite structure sample showed [app:ds:excellent] hydrophilic property and biological activity.

BI-TuP6 Assembly of Proteins and Oriented Purple Membrane on Functionalized Carbon Nanomembranes, ***Natalie Frese,*** Bielefeld University, Germany; *D. Rhinow,* Max Planck Institute of Biophysics, Germany; *A. Turchanin,* Friedrich Schiller University, Germany; *N. Hampp,* Philipps University, Germany; *A. Gölzhäuser,* Bielefeld University, Germany

This presentation is about hybrid structures comprising carbon nanomembrane (CNM) as a functional substrate and oriented assembled purple membranes (PMs). CNMs are monomolecular cross-linked layers of aromatic amphiphilic molecules with lateral dimensions of several square centimeters and a thickness of about 1 nm. PM from Halobacterium salinarum is a membrane consisting of bacteriorhodopsin (BR), which is a light-driven proton pump, and lipids.

CNM has already been successfully tested as a substrate for electron cryo-microscopy of PM. To realize the oriented assembly of PM patches on CNM, we used a PM mutant, which has histidine (HIS) tags selectively on one side of the membrane and a nitroliotriacetic acid (NTA) terminated NBPT-CNM. The functionalized CNM has also been tested with different HIS-tagged proteins.

BI-TuP7 Detection Fecal Occult Blood for Early Colorectal Cancer by TOF-SIMS, *C.C. Yu,* Vanung University, Taiwan, Republic of China; *W.J. Lin, S.M. Wang,* Central Police University, Taiwan, Republic of China; ***Fu-Der Mai,*** Taipei Medical University, Taiwan, Republic of China

Due to a dramatic change in both the lifestyle and eating habits, a steady increase in risk of gastrointestinal diseases every year. Early detections and treatments can reduce the mortality caused by gastrointestinal diseases. Fecal occult blood (FOB) is a small amount of blood in the stool which is invisible to human eye. If blood is detected in FOB, the cause may be gastrointestinal disorders, such as colorectal polyps, colorectal cancer and, etc. Fecal occult blood test (FOBT) is a rapid and non-invasiveness method for early diagnostics about gastrointestinal diseases. In this study, we present an alternative method for FOBT other than the traditional methods by using the time-of-flight secondary ion mass spectrometry (TOF-SIMS). TOF-SIMS can analyzes the molecular composition of the sample surface with a simple pre-treatment or even without any pre-treatment. Furthermore this method can avoid the potential pitfalls found in the traditional FOBT, such as the false positive results due to the interfere of

food and drugs in the chemical method and the false negative responses due to the hemoglobin degradation during the storage in the immunization method. Preliminary results shown that there are significant differences at m / z = 86 and m / z = 184 for the stool samples with FOB. Currently we test different pre-treatments to achieve consistency and accuracy of the experimental results.

BI-TuP8 Preparation of Water-Based Cationic Polyurethane Dispersion for Antibacterial Applications, *G.H. Wu,* ***Cheng-Tien Hsieh,*** National Taiwan University, Taiwan, Republic of China*; S. Hsu,* National Taiwan University,Taiwan, Republic of China

Waterborne biodegradable cationic polyurethane (WCPU) nanoparticle dispersion was synthesized by reaction of polycaprolactone, isophorone diisocyanate, and N-methyldiethanolamine under 75 °C and vigorous stirring under acidic condition. The nanoparticles dispersed in the aqueous medium were uniform with an average size of ~80 nm and a zeta potential of ~60 mV. The WCPU nanoparticle dispersion may be cast into films. The contact angle of the films was ~67°and the zeta potential was ~16 mV. The WCPU nanoparticles demonstrated excellent antibacterial activity against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) (100% inhibition with a contact time of 3 h). Meanwhile, the antibacterial ratio of WCPU films to E. coli and S. aureus reached 100% after 24 h of contact. Moreover, The WCPU nanoparticles could be used as a transfection reagent without significant toxicity for concentrations less than 1000 mg/mL and showed the ability to condensate plasmid DNA. The transfection efficiency for HEK293T cells was ~60% after 48 h of transfection. Moreover, the WCPU dispersion can be made into microspheres conveniently. The WCPU dispersion prepared in the study has potential antibacterial and biomedical applications.

BI-TuP9 Hydrophilic Itaconic Acid Based Material Coating on Silicone Implants Surface for Inhibition of Capsular Contracture, *M. Birajdar,* Chung Ang University*; YB. Choy,* Seoul National University, Korea*; S. Lee,* CHA*;* ***Hansoo Park,*** Chung Ang University

The capsular contracture has been a serious problem for silicone implants, leading to surgical removal or surgical replacement. Recently, the silicone implants were modified with textured surface and coated with biomimetic materials to overcome this complication. In this work, we modified the surface of silicone implants with highly hydrophilic itaconic acid (IA) based materials having antibacterial properties and investigated their effect on inhibition of capsular contracture. Various formulation including IA alone, Oligo IA, IA-gelatin polymers, gelatin particles containing IA were tested. The results confirmed the coating of silicone surface with thin layers of IA based materials with concentration dependent manner. It was also found that the contact angle was decreased in all groups, confirming the increase in hydrophilicity. In vitro cell adhesion and protein adsorption were also tested on the modified silicone and compared to the unmodified silicone. These surface modification of IA based materials could be used as a potential alternative to inhibit the formation of capsular contracture.

BI-TuP10 Photoacoustic Microscopic Imaging with Bone Targeted Near Infrared Fluorophore, ***TaeJoong Eom***, *H.D. Lee,* Gwangju Institute of Science and Technology (GIST), Republic of Korea

In order to obtain 3D bone structure images, we should regard x-ray dose for CT imaging or ultrasound image confusing from other organs. By using a bone-specific near infrared (NIR) fluorophore functionalized by phosphonate groups, we demonstrate photoacoustic microscopy (PAM) system for in-vivo bone imaging with out regarding x-ray dose. The NIR light has certain key advantages for biomedical imaging, including relatively ultra low tissue absorption with red blood cell, reduced scatter, and minimal autofluorescence. Especially, the phosphonated NIR fluorophore targets bone tissue with high specificity and this property is inherent to the chemical structure of the fluorophore. Since the heptamethine indocyanine structure has absorption/emission spectrum at ~780/ ~800 nm and the quantum yield of fluorophore is around 10-20 %, the NIR fluorophore can be applied for PA imaging system. The most of surplus optical energy is transferred to the thermal energy which can generate acoustic signal. For the NIR pulse excitation, we applied a high power pulsed laser, which has output power of 600 mW, pulse duration of 1 ns, and repetition rate of 10 kHz at the center wavelength of 780 nm. The NIR fluorophores were administered intravenously into nude mouse to obtain in-vivo PA bone imaging. The foot and tail bone structures of mouse image were successfully obtained by scanning a PAM system with a single ultrasound transducer.

BI-TuP11 Histological Analysis of Bone Regeneration with Hydroxyapatite Isolated from Two Natural Sources (Gallus Domesticus and Sciaenops Ocellatus) in Bone Defects induced in Tibiae of Rabbits, *D.I. Balleza-Ovalle,* Universidad Autónoma de Tamaulipas, México*; H. Hernández-Cocoletzi,* Benemérita Universidad Autónoma de Puebla, México*;* ***J.H. Luna-Domínguez,*** *C.A. Luna-Lara, H. Tellez-Jimenez,* Universidad Autónoma de Tamaulipas, México*; E. Águila-Almanza,* Benemérita Universidad Autónoma de Puebla, México

In the field of dentistry, biomaterials that meet the requirements for optimal bone tissue formation have play a vital role for the treatment of bone reabsortion caused by periodontal disease, dental extractions or periapical lesions. Hydroxyapatite (HA) is a biocompatible nonabsorbable material chemically similar to the mineral component of bones and hard tissues, therefore, it can be used as a scaffold. HA can be isolated from natural sources by the thermal calcination method. The aim of this study is to assess the osteoconductive effect of hydroxyapatite isolated from two natural sources (Gallus domesticus and Sciaenops ocellatus) by histological analysis applied in bone defects induced in tibiae of rabbits over a period of four weeks. In this experimental study, five healthy adult male rabbits of New Zealand strain, weighing approximately 3.5 kilograms, which were kept in cages according to the Mexican Official Standard NOM 062 Z00-1999 for the use of laboratory animals were used. Four defects with a size of 4 mm in diameter and 6 mm of deepness were made in the proximal metaphyseal planodiáfiso of both tibias and HA was applied to the defect according to the grouping. After four weeks of the surgery, both tibias were recovered in blocks containing all the graft area for histological analysis. Results: In the hydroxyapatite groups the new bone growth involved an area of 78.53% (Sciaenops ocellatus) and 72.23% (Gallus domesticus). The control group involved 15.97%. Conclusion: After four weeks, HA groups shown to be osteoinductive agents and they allowed the growth of bone tissue to a higher growth rate and bone of higher quality than in the control group.

BI-TuP12 Cell based Tissue Engineering using Hydrogel Materials, ***Kangwon Lee,*** Seoul National University, Republic of Korea

Stem cell and growth-factor based therapies hold tremendous promise, but clinical effectiveness has been limited by transplanted cell death, efficacy of growth factors and limited control over cell fate *in vivo*. Macroporous biomaterials have been used in partially circumvent these problems by improving transplanted stem cell survival and controlling phenotype by providing molecular cues. In this presentation, we demonstrated strategies for bone repair. Mechanotransduction pathways have been harnessed to control stem cell behavior by manipulating the elasticity of both porous and non-porous materials. Here, we developed injectable, void-forming hydrogels in which critical biomaterial properties controlling stem cell behavior, including elasticity, could be decoupled from pore formation and cell confinement. Upon controlling of matrix elasticity, bone regeneration could be regulated and optimized. Next, CPC is promising for dental and craniofacial applications due to its ability to be injected or filled into complex-shaped bone defects and molded for esthetics, and its resorbability and replacement by new bone. The objective of this strategy was to investigate bone regeneration via novel macroporous CPC containing absorbable fibers, alginate hydrogel microbeads and growth factors in critical-sized cranial defects in rats. Macroporous CPC scaffolds containing porogen, fibers and microbeads with growth factors were investigated in rat cranial defects for the first time and had new bone up to 2-fold that of traditional CPC control at 4 weeks, and 3-fold that of traditional CPC at 24 weeks, and hence may be useful for dental, craniofacial

BI-TuP13 Collagen Fibrils Imaging in Air and in Liquid Using Atomic Force Microscope-Based Fast Nanomechanical Mode, *B. Kim,* ***Mina Hong,*** *G. Pascual, K. Lee,* Park Systems Corporation

Collagen is a protein that provides structure in various connective tissues in animals and can be found in ligaments, tendons, and skin. The characterization of collagen's mechanical properties at nanoscale can potentially reveal significant insights into the causes of macroscale phenomena such as the elasticity of skin and its degradation as we age. One tool that has been used to acquire nanoscale data of collagen is the atomic force microscope (AFM). Conventional AFM techniques based on force-volume spectroscopy have been used to analyze the topography and mechanical properties of collagen. However, these techniques are extremely time-consuming—acquisition of a quantifiable elasticity map can take hours to complete. A new AFM-based nanomechanical mode has been developed to address this drawback and can perform the same task significantly faster without sacrificing resolution. Our investigation revealed

that our sample collagen bundles had diameters ranging from 60 to 600 nm and an average elastic modulus of about 1.9 GPa, a value in agreement with other reported research. The total time taken to acquire this data was measured in minutes as opposed to hours.

Biomaterial Surfaces & Interfaces
Room Milo - Session BI-TuE

Medical Applications

Moderator: Michael Grunze, KIT, Germany

5:40pm **BI-TuE1 Challenges in Translating Surface Designs to Clinical Medical Device Applications**, ***David Grainger***, University of Utah, USA **INVITED**

Surface strategies for translational clinical performance for 1) medical devices with antimicrobial properties, and 2) nanomaterials in imaging and drug delivery are discussed.

1. Antimicrobial medical devices.[1-5] Increasing medical devices are used in clinical implants: in aging populations, diverse patient genetic profiles, ethnicity and health status, and increasing developing countries. Notably, infection related to implanted devices is a primary concern both for patient risk and healthcare cost reasons. Medical device surfaces and interfaces have long been a focus to produce diverse antimicrobial strategies, yet few translate to clinical use. A classic problem is lack of in vitro-in vivo correlation, validation or efficacy for surface methods and antimicrobial approach in vivo. A second issue is lack of commercial enthusiasm to take approaches forward thru regulatory pathways to clinical use. Improved methods are required to assess and validate new antimicrobial technologies that reduce implant-associated infections and risks in translation.

2. Nanomaterials exposure to the human physiome.[6-10] Human exposure to engineered nanotechnology is an increasing concern. Importantly, medical grade standards of purity and contamination validation and analysis are difficult for nanomaterials and not commonly followed in most in vivo studies. Since surface area is critical nanomaterials property, surface analysis is critical but rarely performed.[6] Much published data demonstrate that particles placed in blood circulation are rapidly filtered by the reticuloendothelial system (RES) comprising liver, spleen, lung, kidney, and marrow, performing blood scavenging. Particle removal is mostly independent of size or chemistry, and refractory to targeting strategies by coatings. Wide variations in circulating nanomaterials properties produce quite similar results in mammalian biodistributions and RES clearance (>90% RES filtration). Issues with connecting nanomaterials surface properties to complex biological interactions will be discussed.

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6:20pm **BI-TuE3 Metal Oxides, Hydroxyapatite and Bone Healing**, ***Håkan Nygren***, University of Gothenburg, Göteborg, Sweden; *P. Malmberg, L. Ilver,* Chalmers University of Technology, Sweden

Magnesium alloy, Zinc and Manganese have been explored extensively for application as degradable metal implants, but there is still a lack of understanding of the biological response to the corrosion products and their influence on the local tissue repair process at the implantation site. The approach, in this study is to correlate the effect of corrosion products on human embryonic stem cells *in vitro* and on bone healing with and without stainless steel implants *in vivo* in rat tibia by preparing a paste of MgO or $MgCO_3$, ZnO and MnO. The metals and the formation of mineral were analysed by ToF-SIMS, EDX and XPS. Metal oxides, incubated in tissue culture medium (DMEM), induced the formation of hydroxyapatite (HA), covering the oxide grains. The apatite was low crystalline and carbonated. We found that when cultured human embryonic stem cells were presented with the HA-coated corrosion products they were able to maintain viability and proliferate over time. The presence of HA-coated metal corrosion products resulted in the up-regulation of hydroxyapatite formation by the stem cells *in vitro* and enhanced bone formation *in vivo*, preceded by the formation of hydroxyapatite in the tissue. The results of the present study suggest that metal corrosion products catalyse the formation of hydroxyapatite in the tissue, that the formation of apatitite is amplified by stem cells and that the hydroxyapatite is an active species in promoting osteogenesis.

7:00pm **BI-TuE5 Modulation of Macrophage Polarization Using Surface Immobilized Bioactive Molecules**, ***Alex Chen***, *B.D. Ratner,* University of Washington, USA

Introduction: The polarization of macrophages is highly influential in modulating the foreign body response. Macrophages characterized by the M2 (anti-inflammatory) phenotype are believed to reduce the formation of the foreign body capsule. It is hypothesized that surface immobilizing M2 promoting bioactive molecules will reduce the formation of the foreign body capsule by increasing M2 polarization as well as decreasing M1(inflammatory) polarization of macrophages. Collagen VI (col6) and α-1 acid glycoprotein (AGP) have been shown to induce M2 polarization of macrophages when introduced in solution. This work demonstrates the feasibility of modulating macrophage polarization via immobilization of col6 and AGP onto hydrogel coated surfaces.

Methods: 2-hydroxyethyl methacrylate (HEMA) was plasma deposited onto 10mm circular glass slides. HEMA coated glass slides were washed three times in p-dioxane and then surface activated by incubation with 100mM carbonyl diimidazole (CDI) in dioxane for 2.5 hours at 40°C. CDI activated glass slides were then incubated in either 440µg/mL AGP only or 250µg/mL AGP plus 62.5 µg/mL col6 solutions in pH 10.2 sodium carbonate/bicarbonate buffer for 24 hours at 40°C. Bone marrow derived macrophages (BMDMs) were cultured by harvesting marrow from the femurs of sacrificed mice, which was then dispersed and cultured in RPMI with macrophage colony stimulating factor for 7 days. BMDMs were then transferred to glass slides coated with immobilized bioactive molecules and cultured for 48 hours. CDI activated HEMA coated glass slides without immobilized bioactive molecules was used as a control. Macrophage polarization was assessed via ELISA measurements of tumor necrosis factor-α (TNF-α), a cytokine released by M1 macrophages, as well as RT-qPCR of arginase 1 (Arg1), an enzyme highly expressed by M2 macrophages. ELISA experiments involved the addition of 10uM lipopolysaccharide(LPS) to culture media in order to induce an M1 polarization of macrophages. RT-qPCR experiments did not involve the use of LPS and solely focused on the expression of Arg1.

Results and Conclusions: ELISA experiments showed a decrease of TNF-α expression in macrophages cultured on surfaces with immobilized AGP (~30%). RT-qPCR experiments showed an increase in Arg1 expression of macrophages cultured on surfaces with immobilized AGP (2.6x) or immobilized AGP + col6 (5.85x).

These experiments show the potential of using immobilized bioactive molecules to modulate the polarization of macrophages, which can potentially be used to reduce the foreign body response and foreign body capsule formation.

7:40pm **BI-TuE7 Activatable Molecular Nanoprobes for the Perception of Cancer Activity**, ***Seungjoo Haam***, Yonsei University, Korea, Republic of Korea **INVITED**

Stimuli responsive, i.e. activatable, nanomaterials are capable of providing their conformational or phase changes corresponding to specific environmental stimuli variations in biological systems including temperature, pH and reactive oxygen species (ROS). Further, specific biomolecules such as DNA, RNA and enzymes can represent the biological status, particularly for cancer activity allowing better understanding physiological and pathological processes. These stimuli variations would be small but they can trigger drastic changes in the structures of materials because they interact facilely with sub-nanometre-sized drugs or other nanometres-sized biomolecules. In particular, matrix metalloproteinases (MMPs) are highly attractive targets for molecular imaging because degrading and modifying the extracellular matrix by enzymatic activity is required for the invasive process of cancer cells. On the other hand, microRNAs (miRNAs), small, non-coding RNA molecules, play an important role as negative gene regulators and have been found to control various biological functions, such as cellular proliferation, differentiation, metastasis, and apoptosis. Emerging evidence suggests that miRNAs can also function as a diagnostic biomarker and a therapeutic target for a wide range of diseases, including human cancers, because miRNAs themselves can act as tumour suppressor genes or oncogenes. In this presentation, we describe the case examples of the development of activatable nanoprobes enabling precise recognition of the expression of specific enzyme (MT1-MMP) and miRNA34a which could provide deep perception of cancer activity, metastasis and invasion.

8:20pm **BI-TuE9 Thin Film Metallic Glass: A Novel Coating for Biomedical Applications**, *C. Yu, Y. Tanatsugu, C. Li, C. Lee,* ***Jinn P. Chu,*** National Taiwan University of Science and Technology, Taiwan, Republic of China*; M. Chen, S. Chang,* Mackay Memorial Hospital Tamsui Campus, Taiwan, Republic of China

Thin film metallic glasses (TFMGs) possess exceptional mechanical properties adopted from its bulk form such as high strength, large elastic limits, and excellent corrosion and wear resistances owing to their amorphous structure. In addition, the smooth surface, due to the grain boundary-free structure, and low surface free energy of TFMGs in certain compositions can be achieved and leads to the relatively high hydrophobicity and the low coefficient of friction.

In our studies, TFMG coatings are deposited using RF magnetron sputtering for various biomedical applications, including the property enhancements of dermatome blades and syringe needles, adhesion resistance of platelet, as well as the suppression of cancer cell attachments. The TFMG-coated dermatome blades show great enhancements in sharpness and durability, compared with those of the bare one. For the syringe needle, significant reductions in insertion and retraction forces for TFMG-coated needle are found due to the non-sticky property and relatively low coefficient of friction. For thrombosis reduction, less platelet aggregations are observed on the TFMG than that of on the bare glass in platelets adhesion test, suggesting TFMG-coated catheters is potentially useful to be placed into vessels for long periods of time with reduced numbers of the aggregation of blood platelets. For cancer cell attachment suppressions, TFMG exhibits the least cancer cell attachment among other control groups. Thus, anti-proliferation and anti-metastasis of medical tools can be achieved with TFMG coating.

8:40pm **BI-TuE10 Monitoring Human Physiological Signals Using Artificial Flexible Graphite Thin Films**, ***Takanari Saito****, Y. Kihara, J. Shirakashi,* Tokyo University of Agriculture & Technology, Japan

Recently, wearable health-monitoring devices based on strain sensors have been widely applied in disease diagnosis and health assessment. Various flexible materials, including polymer nanofibers [1], nanowires [2], carbon nanotubes [3], and graphene [4], have high flexibility and sensitivity, and are good potential candidates for the wearable health-monitoring devices. However, the fabrications of the wearable devices are, in many cases, complicated multistep procedures which result in the waste of materials and require expensive facilities. Therefore, we focused on a commercially available pyrolytic graphite sheet (PGS) [5] which is an inexpensive and an artificial flexible graphite sheet. Previously, we have reported that thin graphite films are simply and easily fabricated from PGSs, and are used as wearable strain sensors for monitoring human motions [6, 7]. In this study, we investigated the application of wearable devices based on the thin graphite films for wrist pulse monitoring.

First, the thin graphite films were fabricated by cutting small films from 17-μm-thick PGSs. Then, the thin graphite films were cleaved onto adhesive tapes using the mechanical exfoliation method. Finally, the thin graphite films were wired using silver conducting paste for electrical measurements. The thin graphite films used as strain sensors were attached over the radial artery to monitor the wrist pulse. The peaks of the resistance waveform were periodically observed, and therefore the wrist pulse was successfully detected using these devices. The results suggest that the thin graphite films could be applied as cost-effective health monitoring devices for human physiological signals.

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Biomaterial Surfaces & Interfaces
Room Milo - Session BI-WeM

Biomolecule/Material Interactions

Moderator: Duncan McGillivray, University of Auckland, New Zealand

9:00am **BI-WeM4 Reconstruction Process and Orientation of Membrane Proteins in Artificial Cell Membrane Systems**, ***Ryugo Tero,*** Toyohashi University of Technology, Japan **INVITED**

The lipid bilayer is the fundamental structure of cell membranes, at which the transportation of materials and signals in and out of cell membranes take place. Ion channels are one of representative membrane proteins promoting these reactions. They retain their proper structures and function only when they are incorporated in lipid bilayer membranes. It is necessary to reconstruct the membrane proteins into artificial lipid membranes to investigate the structure and function of membrane proteins out of cells. n this study we reconstructed the ion channels into solid-supported lipid bilayers (SLBs), which is an artificial lipid bilayer at solid-liquid interfaces, using proteoliposomes. We investigated the distribution and orientation of ion channels in the SLBs using fluorescence microscopy and atomic force microscopy (AFM).

9:40am **BI-WeM6 Surface Adsorbed Antibody Characterization using ToF-SIMS with Principal Component Analysis and Artificial Neural Networks**, *N.G. Welch, R.M.T. Madiona, T.B. Payten, R.T. Jones, N. Brack,* La Trobe University, Australia*; B.W. Muir,* CSIRO, Australia*;* ***Paul Pigram,*** La Trobe University, Australia

Artificial neural networks (ANNs) form a class of powerful multivariate analysis techniques, yet their routine use in the surface analysis community is limited. Principal component analysis (PCA) is more commonly employed to reduce the dimensionality of large time-of-flight secondary ion mass spectrometry (ToF-SIMS) data sets and highlight key characteristics. The strengths and weaknesses of PCA and ANNs as methods for investigation and interpretation of a complex multivariate sample set will be considered. Using ToF-SIMS, spectra were acquired from an antibody and its proteolysis fragments with three primary-ion sources to obtain a panel of 72 spectra and a characteristic peak list of 775 fragment ions. The use of ANNs as a means to interpret the ToF-SIMS spectral data is explored, highlighting the optimal neural network design and computational parameters, and considering the technique limitations. Employing Bi_3^+ as the primary-ion source, ANNs can accurately classify antibody fragments from the parent antibody based on ToF-SIMS spectra.

10:20am **BI-WeM8 Controlled Peptide Surfaces of Various Ratios that Guide Neural Stem Cell Differentiation.**, ***HalaShakib Dhowre,*** *C. Towlson, HS. Sahaf, N.A. Russell,* University of Nottingham, UK

Cell instructive biointerfaces represent an essential aspect for the advancement of regenerative medicine. Currently, a major issue in biointerface design is the limited ability to mimic the complex interactions of the natural processes in the extracellular matrix (ECM) with artificially designed surfaces and interfaces [1]. While biomaterial surfaces have been shown to be able to elicit specific cell responses (e.g. adhesion, proliferation, differentiation), precise control akin to that of natural cellular environments is still lacking[2].

AIM:

The present work aims to address this challenge by designing new synthetic peptide surfaces with well controlled composition and functionality able to impact control over the differentiation of neuronal stem cells with the ultimate goal to understand and control how neuronal networks function.

METHODS:

Compositionally well defined surface concentrations of two short laminin peptide sequences, Arg-Gly-Asp (RGD) and Ile-Lys-Val-Ala-Val (IKVAV) were prepared of various ratios via the "grafting from" stepwise approach and the surface modification was confirmed with surface analysis techniques to indicate successful peptide functionalisation. The neural stem and progenitor cells (NSPC) were set up from embryonic rat hippocampi (E18). Immunocytochemistry (ICC) observed cell viability and differentiation to specific NSPC lineages for Nestin, βIII-Tubulin and GFAP.

RESULTS:

Surface characterising techniques (WCA, AFM and ToF-SIMS) verified the successful amino acid build-up to peptides on the surfaces, allowing modification of the surfaces with RGD and IKVAV. Enhanced NSPC adhesion, proliferation and differentiation were observed on the peptide surfaces. ICC demonstrated Nestin expression decrease after the removal of the growth factors (EGF and FGF) and an increase in the expression of βIII-Tubulin and GFAP; thus illustrating cells differentiating from stem cells to neurons or astrocytes due to peptide surface influence.

CONCLUSION:

Well defined peptide surfaces were designed successfully, the various ratios of RGD and IKVAV surfaces demonstrated cell adhesion, proliferation and influences desirable effects in controlling different populations of stem cell fate. These surfaces may advance new insight in understanding how surface properties affect the regulation of physiological relevance in directing neural cell differentiation, which will be essential to understand how neural networks function.

References

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2. Ricoult, S.G. et al., Biomaterials., 35, 727-736 (2014)

3. Cooke, M.J. et al., J.Biomed.Mater.Res-Part A., **93**,824-832 (2010)

10:40am **BI-WeM9 Inelastic Neutron Spectroscopy Studies of Biosurfaces: the Chemistry of Hydrogen Surface Interaction**, ***Anton Stampfl,*** Australian Nuclear Science and Technology Organisation, Australia

Adsorption, surface complexation and reactivity of biological molecules on inorganic surfaces and interfaces is pervasive throughout an enormous range of fields such as chemistry (geochemistry, biochemistry), biotechnology (medical implants, biosensors, tissue engineering, bioelectronics, biomimetics and artificial photosynthesis), radiation technology (radiation damage and detection), colloid chemistry, surface chemistry and physics. Hydrogen and its interaction at surfaces clearly plays a pivotal role in the ultimate functionality of many biologically-based surfaces. Through the hydrogen's subtle interaction with the tethering surface, or interface, and the surrounding wet environment can and does lead to a multifaceted response to changes in temperature, pH, radiation etc.

Inelastic neutron spectroscopy is the domain of vibrational spectroscopic studies on bulk materials. At first sight, surface studies using such a method, with relatively low neutron flux rates and largish sample size, seems a totally hopeless task. There are, however, exceptions to this rather bullish view, where the surface dominates the scattered signal due to huge surface to volume ratios and large scattering cross-sections from adsorbate molecules, that incorporate for example, hydrogen, which neutrons are supremely sensitive to.

The deposition of amino acids and carbonyl-sulphide onto oxide surfaces is a fruitful area of discovery in the field of prebiotic formation of peptides and an example of how neutron spectroscopy makes significant contributions to the understanding of the subtle chemistry between adsorbate, substrate and surrounding environment. In this series of studies the deposition of amino-acids onto alumina from solution and in the presence of OCS is investigated by both inelastic neutron spectroscopy and high resolution photoemission which allows both the vibrational and electronic structure to be determined for these incredibly interesting systems. Studies focused on the extent of adsorption at various pH's, the character of each adsorbate (zwitterionic, basic, acidic), and the number of discrete surface sites of adsorption. Results show strong chemisorption of amino acids through an ester type bond with the alumina surface across a range of pH. Direct sorption of the amine group with alumina is observed only at pH 9. Formation of multilayers and/or peptides can also occur in conjunction with OCS absorption.

11:00am **BI-WeM10 Exploring Protein and Mesoporous Silica Nanoparticle Interactions**, ***Brian Trewyn,*** *M. Moyer,* Colorado School of Mines, USA

Tandem and cascade reactions have the potential to save time and resources, advantages not frequently observed in individual, stepwise reactions. The versatile, ordered pore structure of mesoporous silica nanoparticle (MSN) materials is an ideal support for multiple, active catalysts that potentially have orthogonal optimal conditions. Herein, we

will demonstrate that enzymes covalently tethered to MSN can be paired to inorganic species to catalyze multistep reactions. Additionally, MSN can be used to entrap large, multisubunit proteins as individual subunit monomers. Upon release, we will demonstrate that the subunits reassociate to form biochemically active proteins.

Biomaterial Surfaces & Interfaces
Room Milo - Session BI-ThM

Plasma for Biomedical Applications

Moderator: David Castner, University of Washington, USA

9:00am **BI-ThM4 Fundamentals of Plasma Interactions with Biological Systems**, ***Endre Szili,*** *R.D. Short,* University of South Australia, Australia **INVITED**

There is significant optimism that cold atmospheric plasma could play a role in the treatment of diseases and infection, particularly those that are refractory and potentially life-threatening such as non-healing chronic wounds and cancers. The medical benefits from plasma are assigned to reactive oxygen and nitrogen species (RONS) that are generated by plasma upon interaction with air and liquids. However, we still do not have a sufficient understanding of (1) what RONS are delivered by plasma, (2) the rate RONS are delivered, (3) how the RONS are perturbed by tissue and (4) how RONS interact with cellular membranes. This knowledge is essential in order to obtain a quantitative mechanistic understanding of plasma in biology and medicine. In this talk, I will discuss simple biological mimics of 3D tissue or cells membranes, which are utilized to gain new insight into the plasma generation and transport of RONS and molecular oxygen into tissue fluid, tissue and cells. Surprisingly, we discovered that plasma can directly transport RONS and molecular oxygen deep within tissue to millimeter depths and across cellular membranes without physically damaging the tissue or cell membrane. In addition, I will discuss how the combined dynamic changes in the concentrations of RONS and molecular oxygen in the biological fluid can significantly impact cell viability during and after the plasma treatment. Finally, I will discuss how the above assays can support the future development of plasma sources to deliver metered doses of RONS and molecular oxygen within tissue for treatment of diseases such as chronic wounds and cancers.

9:40am **BI-ThM6 Plasma Engineered Surface for Managing Growth Factor Release in Stem Cell Culture**, ***Jason Whittle,*** University of South Australia, Australia

The surface modification of materials used in biomedical applications is one of the earliest successful applications of gas plasma treatment. Such “Tissue Culture Plastics” are the mainstay of cell culture facilities to this day.

More recently, the ability to use plasma polymerisation to engineer novel surfaces has opened up the possibility of more advanced surfaces for cell culture. Products based around this technology are now also widely available.

In our laboratory we have developed plasma deposited surfaces that bind glycosaminoglycans from solution, and which are subsequently able to bind and release growth factors and other signalling molecules into solution. The presence of these growth factors enables the culture of primary- and stem cells without the need to add these growth factors to the media formulation. The development and application of this surface in the culture of human cells will be described, in addition to some of the challenges associated with commercialisation of plasma deposited films, where better knowledge of the physics and chemistry of depositing plasmas is needed.

10:20am **BI-ThM8 Principles for Retention of Fragile Chemical Functionality Structures in Plasma Polymer Thin Films**, ***Solmaz Saboohi,*** *B.R. Coad, A. Michelmore,* University of South Australia, Australia*; R.D. Short,* University of Lancaster, UK*; H.J. Griesser,* University of South Australia, Australia

There is a growing need for thin films which are functionalized with specific surface chemical motifs that impart new physical, chemical or biological properties. The design and fabrication of thin films with specific surface groups has the potential to provide further insights into bio-interfacial interactions as well as to yield novel coatings for products such as cell culture ware. Plasma polymerization (PP) provides a one-step, solvent-free process, irrespective of material type and format, and supports several large-scale industrial applications on the basis of advantages such as excellent uniformity and adhesion of coatings and their reproducibility. We have studied how volatile ester compounds can be plasma polymerized with retention of a high density of intact structural elements. However, in PP there usually occurs extensive fragmentation of the volatile precursor molecule ("monomer") and re-assembly of the various fragments from the plasma gas phase into a solid polymeric coating. Considerable scrambling of molecular structural elements is evident even where functional group retention has been the objective. High energy electron impact events in the plasma results in loss of specific functional groups in the plasma phase. In addition, a strong negative electric field, which develops in the vicinity of the surface, may cause positive ions to arrive at the surface with significant energy. Sputtering / reorganization at the surface due to ion impingement also results in loss of specific functional groups. Retention of chemical functional groups can be optimized by considering the pressure where the plasma transitions from the alpha to the gamma regime.[1] Operating the plasma in the collisional regime biases the deposition towards increased contributions by ions rather than neutral/radical grafting.[2] This study demonstrates that relatively complex structural motifs in precursor molecules can be retained in plasma polymerization if the chemical and physical processes occurring in the plasma phase are controlled by tuning the plasma to deliver a high flux of polyatomic ions and suitable energy of the ions to deposit films.

References

[1] Liebermann, M. A. and Lichtenberg, A. J. Principles of Plasma Discharges and Materials Processing, 2nd ed. (A John Wiley and Sons, New York), 2005.

[2] Saboohi, S.; Coad, B.R.; Michelmore, A.; Short, R.D.; Griesser, H.J. Hyperthermal Intact Molecular Ions Play Key Role in Retention of ATRP Surface Initiation Capability of Plasma Polymer Films from Ethyl α–Bromoisobutyrate, ACS Appl.Mater.Interfaces, 2016, 8, 16493−16502

10:40am **BI-ThM9 Cell-Surface Modification for Biomaterial Applications using Furfuryl Methacrylate Plasma Polymer**, ***Hanieh Safizadeh,*** *A. Michelmore, J.D. Whittle,* University of South Australia, Australia

Cell-surfaces interaction plays a significant role in biomedical applications and cell therapies. In many cases, materials which are convenient for manufacturing biomedical devices and culture ware exhibit poor cell adhesion. Therefore, it is important to modify cell-surface interactions using surface engineering. Poly(furfuryl methacrylate) (p(FMA)) is a promising polymer surface that recently has been recognized for stem cell adhesion and proliferation due to the furan ring in its structure. However controlling the thickness and topography of surface coatings of p (FMA) is difficult, which inhibits scale-up. Plasma polymerization offers a simple, solvent-free method for coating surfaces with FMA which is substrate independent, with fine control of film thickness and topography.

Herein, FMA plasma polymer coatings were prepared with different powers, deposition times and flowrates. Furan ring retention on these surfaces has been determined using chemical analysis such as XPS and ToF-SIMS. SEM demonstrated the existence of particle aggregates under certain plasma conditions. Through judicious choice of plasma polymerization parameters the formation and quantity of the particle aggregates was reduced and the fabricated plasma polymer coatings became chemically uniform and smooth and the furan ring retention was maximized. These optimised surfaces support cell proliferation, comparable to results with tissue culture plastic, while maintaining cell fate. These findings show not only the chemistry of surfaces is important but also that surface morphology plays an important role in cell adhesion and proliferation.

11:00am **BI-ThM10 Quasi-zwitterionic Glow-Discharge Radio Frequency Plasma Coatings Reduce IgG Protein Adsorption**, ***Marvin Mecwan,*** *B.D. Ratner,* University of Washington, USA

Introduction: Glow discharge plasma-treated surfaces have been used to create non-fouling surfaces, and can be readily applied to implants. For successful plasma polymerization it is important that the monomer of interest be easily volatilized. Zwitterionic polymer hydrogels in mice have shown to resist foreign-body reaction. However, zwitterionic polymer precursors, such as carboxybetaine methacrylate (CBMA) and sulfobetaine methacrylate (SBMA) are solids with high boiling points which would not make them ideal candidates for glow-discharge plasma treatment to coat surfaces. This study investigates the preparation of quasi-zwitterionic surfaces via glow-discharge plasma treatment prepared by the simultaneous deposition of a positively charged (allylamine or AAm), and negatively charged (acrylic acid or AAc) monomer, and its ability to act as a non-fouling surface.

Methods: Glass substrates were cleaned with MeOH in a sonication bath for 10 mins x 2. Substrates were loaded into the reactor, Ar etched (40W for 10 min), followed by a CH4 layer (80W for 5 min). The monomer of choice—AAc and AAm—was introduced into the chamber either by itself or simultaneously and plasma deposition was carried out at 150mT pressure; 80W for 1 min (adhesion) followed by 10W for 10 mins (deposition). Samples were quenched for 5 mins before venting the chamber and retrieving coated samples resulting in 3 treatment groups: AAc, AAm and

AAc-AAm. Plasma-treated samples were washed using DI water x 3 and ESCA was used to assess coating composition before and after washing. ESCA analyses were done using an S-Probe ESCA (with monochromatic Al K-alpha X-rays focused to 800µm spot size) using survey and detailed C1s scans. Protein adsorption studies were performed using bovine IgG, and the amount of IgG adsorbed was determined by substrate based ELISA. Cytotoxicity studies were performed using NIH3T3 mouse fibroblast cells.

Results: ESCA scans of plasma-treated substrates showed absence of substrate associated peaks implying that plasma coatings on substrates are at least 10 nm thick (coating thickness will be measured using AFM). Furthermore, experimental and theoretical elemental compositions of the surfaces align well. Moreover, AAc-AAm coatings were able to reduce protein adsorption by 50% compared to untreated controls, and were non-cytotoxic.

Conclusions: The preliminary data demonstrates that quasi-zwitterionic surfaces can be successfully created, reduce IgG protein adsorption and are non-cytotoxic. Further optimization is required to reduce protein adsorption further in order to create a new generation of materials that perform efficaciously as non-fouling surfaces.

11:20am **BI-ThM11 Titanium Films Deposited by HiPIMS for Medical Applications**, *K. Thorwarth,* Empa, Switzerland*; S. Jin,* Sungkyunkwan University, Korea, Republic of Korea*; S. Gauter,* Christian-Albrechts-University Kiel, Germany*;* ***Joerg Patscheider,*** Empa, Switzerland

The metallization of polymer substrates by metallic titanium films provides many attractive applications, e.g. in biomedical applications. In contrast to conventional techniques in orthopaedic implants such as plasma spraying, which lead to a high thermal load of the substrates' surface and thereby to the undesired loss of surface structural feature, magnetron sputter deposited films maintain the surface topography of PEEK substrates

In this work Ti coatings were deposited on PEEK (polyether ether ketone) by chopped HiPIMS, a technique where HiPIMS pulses are decomposed into a sequence of short micropulses. The combination of these pulse trains distinctly influence the properties of titanium coatings prepared by this technique. The plasma was characterized using voltage/current measurements, optical emission spectroscopy and Langmuir probing, along with caloric measurements during deposition. The prepared coatings were examined using X-ray diffraction, scanning electron microscopy and X-ray photoelectron spectroscopy. It is shown that the pulse sequence is decisive for the applicability of Ti coatings on polymeric substrates, as it strongly influences properties such as process stability, deposition rate, morphology and thermal load during deposition, which can be improved with respect to standard HiPIMS and DC sputter deposition. The coatings' microstructure shows increased smoothening of the coating surface and shallower surface oxidation for samples deposited using chopped HiPIMS

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