Biomaterial Interfaces
Room: Milo - Session BI-MoM

Nanobio Imaging
Moderator: Sally L. McArthur, Swinburne University, Australia

8:40am **BI-MoM1 Multimodal Nano-Bio Imaging of Neuronal Cells and Tissues**, ***DaeWon Moon***, DGIST, Korea, Republic of Korea **INVITED**

Secondary ion mass spectrometry (SIMS) provides molecular specific information but for 2D imaging, SIMS needs specimens in general, to be frozen and dried for analysis in vacuum. For 3D imaging, specimens can be sputter profiled by recently developed gas cluster ions. To compensate the distortion due to cryosection and sputter profiling, we have been trying to develop a multimodal mass and non-linear optical imaging methodology of various cells and tissues for neuronal studies. In this presentation, multimodal SIMS and CARS imaging studies on neuron cells, olfactory bulb, and nematode *C. elegans* are reported.

For time-of-flight (TOF) secondary ion mass spectrometry (SIMS) imaging, 30 keV Bi_3^+ ions for 2D mass imaging and 30 keV Ar_{1000} cluster ions for depth profiling were used to analyze various tissues such as *C. elegans*, and mouse olfactory bulb. As a complementary non-linear optical imaging, coherent Anti-Stokes Raman Scattering (CARS) were used to get 3D lipid imaging down to ~50 μm with 300 nm spatial resolution in-vitro or ex-vivo.

For *C. elegans*, lipid CARS imaging was obtained for live *C. elegans* but for SIMS imaging, *C. elegans* were dried with water and sputtered with 30 keV Ar_{1000} cluster ions to get sectioned 2D SIMS images. Molecular specific SIMS imaging for lipids, neurotransmitters, and pheromones with complementary CARS lipid imaging were used to investigate the difference of molecular distributions in wild-type and various mutant *C. elegans*.

For a cryosectioned mouse olfactory bulb, SIMS imaging showed different distributions of lipid molecules and neurotransmitters which is consistent with the olfactory bulb structure of glomerulus, mitral cell layer, and granule cell layer. SIMS imaging to study the changes of neurotransmitters in an olfactory bulb upon odorant stimulus will be reported.

In conclusions, multimodal mass and non-linear optical imaging provides a practically useful platform to investigate cells and tissues for new biomedical understanding of neuronal systems. New challenges for non-cryo tissue section, plasma/fs laser based ambient mass spectrometry for live cell membrane mass imaging, and super-resolution CARS will be discussed.

9:20am **BI-MoM3 SPR Imaging Sensor for Visualization of Individual Cell Reactions and Clinical Diagnosis of Allergy**, ***Yuhki Yanase***, Hiroshima University, Japan **INVITED**

A technique to visualize individual living cell activation in a real time manner without any labeling is required in the fields of life sciences and medicine. Surface plasmon resonance (SPR) sensors detect the refractive index (RI) changes on the surface of sensor chips in label-free and on a real-time basis. We previously reported that SPR sensors could detect real-time large changes of RI in response to activation of living cells, such as mast cells, keratinocytes, basophils and B lymphocytes on a sensor chip without labeling, suggesting the potential of SPR as a new method for clinical diagnosis and drug screening. Thus, SPR sensor possesses great potential to reveal nano-scale living cell actions in evanescent field. However, conventional SPR sensors detect only an average RI changes in the presence of thousands of cells in an area of the sensor chip, and could offer only small number of sensing channels. Therefore, we developed SPR imaging (SPRI) sensor with a CMOS camera and an objective lens in order to visualize RI distribution of individual living cells and their changes upon stimuli

The sensor we developed is composed of a light source, P-polarizer, prism (RI=1.72), objective lens and CMOS camera. The SPRI sensor chips (RI=1.72, 20 mm × 20 mm × 1 mm) coated with gold thin film (1 nm Cr layer and 49 nm gold layer) by means of vapor deposition. The SPRI sensor we developed could detect reactions of individual rat basophilic leukemia (RBL-2H3) cells and mouse keratinocyte cells in response to specific or nonspecific stimuli. Moreover, the sensor could detect the reactions of individual human basophils isolated from patients in response to antigens. Furthermore, we also succeeded in distinguishing reactions of basophils activated by antigens from those of non-activated basophils spotted on an area.

The technique can visualize the effect of various stimuli, inhibitors and/or conditions on cell reactions as change of intracellular RI distribution at single cell levels. Establishment of the technique to rapidly isolate cells from patient blood should enable us to utilize SPRI system as a high throughput screening system in clinical diagnosis, such as type I allergy and drug hypersensitivity, and as a tool to reveal novel phenomena in evanescent fields around plasma membrane.

10:40am **BI-MoM7 SIMS of Cells and Tissues: Blasting Our Way to New Knowledge about Biology**, ***Lara Gamble***, *B. Bluestein, D.J. Graham*, University of Washington, USA **INVITED**

Imaging mass spectrometry can provide images of cells and tissues with chemical and molecular specificity. These chemically specific images could revolutionize our understanding of biological processes such as increasing our understanding of chemical changes in cells and tissues as a function of an applied stress or as a result of disease, and enable tracking the spatial distribution of metabolites and lipids. The mass spectral imaging capability of ToF-SIMS holds potential to achieve this goal with sub-cellular resolution. Chemistry of tumor microenvironments, lipid metabolomics relationship to cancer, delivery of nanoparticles to cells, and tissue repair could be visualized on a cellular and sub-cellular level. In this presentation, ToF-SIMS analysis of biological samples from 2D images of tissues to 3D images of nanoparticles in cells will be presented. Challenges with sample preparation for the ToF-SIMS environment and processing of the large amount of data will be discussed (including multivariate analysis of the ToF-SIMS image data). The advantage of combining ToF-SIMS images with optical images of the same samples (same slices and serial biopsy slices) will also be presented. This combination of images allows researchers to visualize a molecular map that correlates with specific biological features or functions.

11:20am **BI-MoM9 Probing the Determinants of Sphingolipid Distribution in the Plasma Membrane with SIMS**, ***Mary Kraft***, *J. Frisz*, University of Illinois at Urbana-Champaign, *P. Weber*, Lawrence Livermore National Laboratory, *R. Wilson*, University of Illinois at Urbana-Champaign, *J. Zimmerberg*, National Institutes of Health, *H. Klitzing*, University of Illinois at Urbana-Champaign

The plasma membrane is a selectively permeable lipid bilayer that separates cells from their surroundings. Numerous different lipid species, cholesterol, and a variety of different proteins form the plasma membranes of mammalian cells. One class of lipids, the sphingolipids, and their metabolites serve both as structural components in the plasma membranes of mammalian cells, and as bioactive signaling molecules that modulate fundamental cellular processes. Though segregation of the sphingolipids into distinct membrane domains is likely essential for cellular function, the sphingolipid distribution within the plasma membrane and the mechanisms that regulate it are poorly understood. To address this issue, we have combined metabolic labeling with stable isotopes and SIMS performed on a Cameca NanoSIMS 50 to image the distributions of stable isotope-labeled sphingolipids in the plasma membranes of fixed cells with ~100 nm lateral resolution. Using this approach, we previously discovered that the ^{15}N-sphingolipids were enriched within distinct domains in the plasma membranes of fibroblast cells [1]. Here we report how we have used this approach to probe the mechanisms responsible for this sphingolipid organization. To determine whether the sphingolipid domains are dependent on molecular interactions with cholesterol or protein-based barriers that are established by the cytoskeleton and its associated membrane proteins, we used SIMS to image the ^{15}N-sphingolipid distribution in the plasma membrane following cholesterol depletion and actin depolymerization. We also assessed whether these ^{15}N-sphingolipid domains were co-localized with hemagglutinin, a specific membrane protein that is thought to have an affinity for sphingolipid-enriched membrane domains. Our results indicate that the sphingolipid organizations in the plasma membrane are dependent on the cytoskeleton, but not on favorable interactions with cholesterol or hemagglutinin.

[1] J. F. Frisz, K. Lou, H. A. Klitzing, W. P. Hanafin, V. Lizunov, R. L. Wilson, K. J. Carpenter, R. Kim, I. D. Hutcheon, J. Zimmerberg, P. K. Weber, M. L. Kraft, Proc. Natl. Acad. Sci. U.S.A., 2013, 110 (8), E613-E622.

11:40am **BI-MoM10 In-Situ TOF-SIMS and SFM Measurements Providing 3D Chemical Characterization of Inorganic and Organic Nanostructures**, *R. Moellers,* ION-TOF GmbH, Germany, *E. Niehuis,* ION-TOF GmbH, *F. Kollmer,* ION-TOF GmbH, Germany, *H. Arlinghaus,* ION-TOF GmbH, *R. Dianoux,* Nanoscan AG, Switzerland, *A. Scheidemann,* Nanoscan AG, ***Nathan Havercroft***, ION-TOF USA, Inc.

Information on the chemical composition, physical properties and the three dimensional structure of materials and devices at the nanometer scale is of major importance in nanoscience and nanotechnology. Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) is an extremely sensitive surface imaging technique which provides elemental as well as comprehensive molecular information on all types of solid surfaces. Depth profiling of multilayers with high depth resolution as well as three-dimensional analysis is performed using additional low energy sputter beams. However, the topography of the initial sample surface as well as the subsequent evolution of the topography by sputtering cannot be identified by the technique and lead to distortions of the detected depth distribution. Scanning Force Microscopy (SFM) provides the required complementary information on the surface topography with a resolution on the nanometer level.

We have combined the techniques ToF-SIMS and SFM in one UHV instrument. The TOF-SIMS analysis is performed using a new bismuth liquid metal cluster ion gun that can achieve a lateral resolution of 20 nm [1]. For the sputtering of inorganic materials the instrument is equipped with low energy oxygen and cesium beams. Sputtering of organic materials without radiation damage is performed by using large gas clusters with low energy/atom allowing molecular depth profiles of organic multilayers with a depth resolution of 5 nm as well as 3D analysis of organic nanostructures [2]. The SFM unit is mounted on a 3-axis high precision flexure stage scanner with a small out-of-plane motion for very accurate information on the surface topography. The SFM can be operated in contact mode as well as in a variety of dynamic modes to provide additional valuable information about the physical properties of the sample. In this paper we will present first results illustrating the strength of combined in-situ TOF-SIMS / SFM measurements and the potential for a wide range of applications.

References

[1] F. Kollmer, W. Paul, M. Krehl and E,. Niehuis, Surf. Interface Anal. 45, 312 (2013)

[2] E. Niehuis, R. Moellers, D. Rading, H.-G. Cramer, R. Kersting, Surf. Interface Anal. 45, (2013) 158

Biomaterial Interfaces
Room: Milo - Session BI-MoE

Biofouling
Moderator: Lara Gamble, University of Washington, USA

5:40pm **BI-MoE1 In Situ Molecular Imaging of Hydrated Biofilm Using Time-of-Flight Secondary Ion Mass Spectrometry**, ***Xiaoying Yu***, *M. Marshall, X. Hua, B. Liu, Z. Wang, Z. Zhu, A. Tucker, W. Chrisler, T. Thevuthasam*, Pacific Northwest National Laboratory

One of the most important processes in nature involves bacteria forming surface attached microbial communities or biofilms. Biofilms possess a complex structure made of a highly-hydrated milieu containing bacterial cells and self-generated extracellular polymeric substances (EPS). We report a unique approach of molecular imaging of biofilms in their native environments using time-of-flight secondary ion mass spectrometry (ToF-SIMS) to address potentially the grand challenge of complex interfacial dynamics in biogeochemistry. Biofilm is grown on a silicon nitride (SiN) membrane window in a recently developed microfluidic single channel flow reactor. Continuous imaging of complex liquid samples can be performed with high precision and sensitivity using this technique. Direct probing of the biofilm occurs *in situ* within a windowless detection area of 2 μm in diameter as soon as the hole is drilled through by the SIMS primary ion beam.

The microfluidic reactor consists of a SiN window for biofilm attachment and ToF-SIMS detection. Biofilm formation is conducted by scaling down a known protocol to the microfluidic regime. *Shewanella* with a green fluorescent protein was used so that biofilm formation can be followed in real time using confocal fluorescence microscopy. Biofilm is generally grown for 6 to 7 days before harvesting. ToF-SIMS analysis is performed immediately upon harvest. A ToF-SIMS V spectrometer (IONToF GmbH, Germany) is used.

Depth profiling is used to drill through the SiN membrane and the biofilm grown on the SiN substrate. Characteristic fatty acids fragments are clearly identified in the m/z spectra. When compared among dried biofilm sample, uninoculated medium solution, and the hydrated biofilm, principal component analysis (PCA) shows distinctions among them. 2D and 3D image reconstructions are conducted. Image PCA is done to further investigate biofilm spatial inhomogeneity. Detailed analysis of dried EPS in bound, loose, and total forms shows distinctions in their chemical makeup. PCA of hydrated biofilm, soluble total EPS, and medium solution provides new insight of the role of EPS in biofilm formation.

We show that molecular imaging of biofilm in the hydrated environment using ToF-SIMS is possible using the unique microfluidic device for the first time. Moreover, probing the natural biofilm microenvironment without drastic sample treatment such as freezing or drying makes it possible to investigate how biofilm develop metabolic and chemical heterogeneities in its hydrated state. The multimodal nature of our microfluidic reactor permits multiplexed *in situ* chemical imaging and advances mesoscale bioimaging.

6:00pm **BI-MoE2 Slime Versatility: Diverse Roles of Slimes in Bacterial Biofilms**, ***Cynthia Whitchurch***, The ithree institute, University of Technology, Sydney, Australia **INVITED**

Many species of bacteria produce extracellular "slimes" comprised of polysaccharides or DNA that provide several advantageous functions to the bacterium including protection from environmental stresses that include physical (e.g. dehydration, osmotic pressure), chemical (e.g. disinfectants, antibiotics, pH) and biological (e.g. mammalian immune system) challenges. A hall-mark feature of bacterial biofilms is the self-produced extracellular slime that provides intercellular connectivity and mediates attachment of cells and biofilms to abiotic and biotic surfaces. Slimes also participate in bacterial surface motilities that mediate the active expansion of bacterial biofilm communities.

Over the past decade, slime comprised of extracellular DNA (eDNA) has been found to be essential for biofilm formation by many species of bacteriawhere it is thought to function as an intercellular "glue" that binds cells together in mature biofilms. Interestingly, eDNA is also essential during the early stages of biofilm development by *Pseudomonas aeruginosa*, however, the precise roles of eDNA in this process have yet to be elucidated. Many species of bacteria, including *P. aeruginosa,* utilize twitching motility to actively translocate across solid and semi-solid surfaces. Twitching motility can manifest as a complex, multicellular behaviour that enables the active expansion of bacterial biofilms. We have used advanced techniques in microscopy, computer vision and image informatics to explore the roles of eDNA during early biofilm development and active biofilm expansion by *P.aeruginosa*.

6:40pm **BI-MoE4 Towards a Scalable Biomimetic Antifouling Coating**, ***MaryNora Dickson***, *E. Liang, N. Vollereaux, CA. Choe, AF. Yee*, University of California, Irvine

It has been found that the nanopillars on cicada wings are inherently antibacterial, irrespective of surface chemistry (Ivanova *et al*., Small, 2012). Thus, fabrication of devices presenting such nanostructures would obviate the requirement for any special surface chemical modification. Nano- and microstructured antibacterial surfaces have been previously proposed, including the Sharklet microstructured film (Chung *et al.*,2007), black silicon (Ivanova *et al*., 2013) and multi-scale wrinkled polymer films (Freschauf *et al*., 2012); none of these approaches can be used on ordinary polymer surfaces or easily scaled up. Thus, we endeavored to apply industrial nanostructring techniques to generate biomimetic antibacterial nanostructures at the surfaces of ordinary polymers: poly(methylmethacrylate) (PMMA) polycarbonate (PC). To begin, we replicated the nanopillars of a cicada wing utilizing a double imprinting process. First we molded the pillars in hard polydimethylsiloxane (hPDMS) and applied a backing of PDMS to produce pliable elastomeric stamps presenting a large area (diameter 15 mm) of nanoholes. Next, we utilized either dropcasting of polymer solution or thermal imprinting into a polymer thin film to generate fields of polymer pillars. Dropcasting was used for experiments that required a large area of pillars, since the natural curvature of the cicada's wing precludes large-area thermal imprinting into flat polymer thin-films. In contrast, thermal imprinting generated very flat, thin, pillared polymer films, which were more suitable for our light transmission microscopy experiments. To make the nanopatterning technique more industrially viable and generate a larger patterned area, we next employed nanoimprint lithography. A commercially available antireflective stamp (Holotools, Germany) with a nanopillared pattern very similar to that of the cicada's wing, and was used to imprint large, flat, nanostructured polymer thin films. In contaminated aqueous environments, our nanopillared surfaces 1) exhibited reduced surface adhesion of live *E. coli* determined by a standard fluorescence based viability assay, and 2) killed these bacteria, as evidenced by a decrease in colony forming units in suspension over time (up to 24 hours). Surface chemistry played a minor role. Our surfaces could be used for a wide variety of environmental and medical applications, including surgical trays / instruments and door handles (which function in air), and for implantable medical devices or catheter tubes (which function in aqueous environments).

7:00pm **BI-MoE5 Self-Organization of Bacterial Biofilm Expansion through Surface Modification**, *E.S. Gloag*, ***Lynne Turnbull***, *CB. Whitchurch*, The ithree institute, University of Technology, Sydney, Australia

Introduction: Many bacterial pathogens have the capacity to actively expand their biofilm communities via complex multi-cellular behaviours. We have observed that when the biofilms of *Pseudomonas aeruginosa* are cultured at the interstitial surface between a coverslip and solidified nutrient media, the resulting biofilms are characterised by an extensive pattern of interconnected trails that emerges as a consequence of the active expansion of these communities.

Aim: To identify the factors governing emergent pattern formation during *P. aeruginosa* biofilm expansion.

Experimental methods: Bacterial biofilms were cultured at the interstitial space between solidified growth media and a glass coverslip. Biofilm expansion was observed using phase contrast time-lapse microscopy and the topography of the underlying media was imaged using atomic force microscopy (AFM) and 3D optical profilometry after the cells where removed by washing the samples with water.

Results: Our observations have revealed that during the migration of *P. aeruginosa* biofilms, aggregates of cells at the advancing edge forge furrows as they migrate across the semi-solid media. The formation of a series of interconnecting furrows and the re-inforcing effect of cells traversing these furrows leads to extensive remodelling of the substratum. Our analyses indicate that whilst the furrows are shallow relative to the height of the bacterial cells, this appears to be sufficient to confine cells within the furrows. We have confirmed that furrows guide the migration of biofilm bacteria using PDMS microfabricated channels. The generation and maintenance of the interconnected furrow network therefore accounts for the extensive large scale-patterning that is characteristic of these bacterial biofilms.

Conclusion: Our observations indicate that emergent pattern formation during biofilm expansion across semi-solid media occurs due to self-generated surface modification by the biofilm community.

7:40pm **BI-MoE7 Development of Micro/Nanofibrous Meshes as Smart Dressings for Chronic Wound Care**, ***Martina Abrigo***, *P. Kingshott, S.L. McArthur*, Swinburne University, Australia

Diabetic, pressure, venous and arterial ulcers are a large social, economic and healthcare burden. These chronic non-healing wounds show delayed and incomplete healing processes exposing patients to high risk of infection. The design of wound dressings that combine the necessary morphological and physical requirements for wound healing with the value-added capability to address optimal cell responses and impair bacterial proliferation represents a major challenge in chronic wound care. Polymeric nanofibrous meshes fabricated through the electrospinning process are promising candidates as wound dressings due to their high surface area, micro-porosity and non-woven structure. In this study, the parameters of the electrospinning process (such as spinning rate and electric field intensity) were optimized to fabricate nanofibrous membrane in Polystyrene (M.W. 250.000). Electrospun materials have been used as scaffolds for tissue engineering for a number of years, but there is surprisingly little literature on the interactions of fibers with bacteria. In order to understand microbial infiltration and control in wound dressings, a number of microbiological assays (MTT, MTS and live/dead) were completed using *E. Coli*, *P. Aeruginosa*, *S. Aureus* in an effort to understand how the morphological and structural properties of the electrospun meshes influence bacterial attachment, proliferation and growth. Fiber diameter was found to affect the capacity of wound bacteria to adhere onto the fibers and spread within the fibrous network. Bacterial size and shape also resulted to play a key role in regulating the interaction of bacteria with the fibers.

8:00pm **BI-MoE8 The Geno-Toxicant Reactivity of Metal-Modification on the Surface of Nanomaterials**, ***Yu-Tzu Huang***, *W.-J. Chen*, Chung Yuan Christian University, Taiwan

The metal-modification on the surface of nanomaterials are extensively used in biomedical and environmental applications recently. Numerous noval nanocomposite materials have been developed; however, reactivity of the biological effects of these nanomaterials towards living organisms is insufficient. Here, we studied the antibacterial reactivity of two kinds of metal containing nanomaterials: (1) metal organic frameworks (MOFs): iron, chromium, aluminum and (2) hydroxyapatite with metal inclusion (gold or silver). Results of the minimum inhibitory concentration (MIC), half maximal inhibitory concentration (IC_{50}), gene expression profile, quantitative gene expression levels, and scanning electron microscopy imaging were used to investigate the possible antibacterial mechanisms. The expressions of six genes (16S ribosomal RNA, DNA polymerase I, DNA polymerase II, cytochrome d complex, glucan biosynthesis protein G, and D-glyceraldehyde-3-phosphate dehydrogenase) indicated the genotoxicity is highly related to membrane or cell wall proteins. In addition, the toxic effects were dominant in iron/silver containing nanomaterials than chromium/aluminum/gold ones. Our findings have opened doors for understanding the insight reactivities of metal-modified nanomaterials, which will help their applications with controlled safety.

Biomaterial Interfaces
Room: Milo - Session BI-TuM

Biomaterial & Wet Interface Characterization
Moderator: Xiaoying Yu, Pacific Northwest National Laboratory

8:00am **BI-TuM1 Engineering of Bio-Nano Interfaces with Self-Assembled Peptides**, ***Yuhei Hayamizu***, Tokyo Institute of Technology, Japan **INVITED**

Developing elegant hybrid systems of biological molecules on two-dimensional nanomaterials is a key in creating novel bio-nanoelectronic devices. Biomolecules self-assembling into ordered structures on these nanomaterials offer a novel bottom-up approach, where organized supramolecular architectures spatially govern the electronics of nanomaterials. Despite the enormous potential in bridging nano- and bio-worlds at the molecular scale, no work has yet realized a way to control electronic properties of nanomaterials by these biomolecular structures. Our research target is the control of the interface between biotechnology and nanotechnology. In this work, we employ solid binding peptides or artificially-designed peptides which have specific binding affinities to solid surfaces and an ability to form peptide nanostructures on atomically flat surfaces [1,2]. These peptides self-assemble monolayer-thick long-range ordered nanostructures on surfaces of single-layer graphene, and on other two-dimensional materials. We observed that self-assembled peptides on a single layer graphene modify its conductivity depending on their assembled structures.

[1] C. R. So, Y. Hayamizu, H. Yazici, C. Gresswell, D. Khatayevich, C. Tamerler, and M. Sarikaya, "Controlling Self Assembly of Engineered Peptides on Graphite by Rational Mutation," ACS Nano, **6** (2) 1648-1656 (2012)

[2] T. R. Page, Y. Hayamizu, C. R. So, and M. Sarikaya, "Electrical Detection of Biomolecular Adsorption on Sprayed Graphene Sheets," Biosens. Bioelectron., **33** (1) 304-308 (2012)

8:40am **BI-TuM3 Peptide Control of Biological Membranes — A Molecular View on Lipid Structure, Peptide Folding and Hydration**, ***Johannes Franz***, *D. Schach*, Max Planck Institute for Polymer Research, *J.E. Baio*, Oregon State University, *D.J. Graham*, *D.G. Castner*, University of Washington, USA, *M. Bonn*, Max Planck Institute for Polymer Research, *T. Weidner*, Max Planck Institute for Polymer Research, Germany

The cell membrane is the most important biological surface as its interaction with peptides is an integral part of transport, communication, energy transduction and survivability. However, an intrinsic difficulty in monitoring peptide interaction with membranes is the required surface sensitivity. Sum frequency generation (SFG) vibrational spectroscopy is well suited to study protein monolayers at lipid surfaces[1] because of its inherent surface specificity and is used to investigate molecular interactions of peptides with model membranes. In this study, three different peptides are shown to interact with model membranes in very different ways.

The internalization mechanism of the negatively charged cell-penetrating peptide SAP(E) is proposed as an aggregation on the cell surface followed by an endocytic uptake. Our data suggest peptide affinity is strongly dependent on the lipid headgroup charge with phosphocholine having the strongest interaction with SAP(E). Moreover, the interaction is limited to the headgroup region with no further insertion observable proving the first step of the proposed uptake mechanism. These findings were supported with complementary surface-sensitive UHV-techniques, i.e. X-ray photoelectron spectroscopy (XPS), near edge X-ray absorption fine structure (NEXAFS) spectroscopy and time-of-flight secondary ion mass spectrometry (ToF-SIMS).

In contrast, viral fusion proteins can disrupt membranes and escape from endosomes when triggered at low pH. We are interested in the interaction of GALA, a peptide mimicking viral fusion proteins. While the peptide is unfolded and inactive around neutral pH, the sequence folds into its active α-helical state at lower pH and causes membrane leakage. We follow GALA activity at the molecular level and probe peptide folding as well as the disturbance and hydration of individual leaflets within model bilayers.

Besides binding to and shredding bilayers, peptides can also help stabilize lipid membranes. For example, bovine serum albumin and specific antifreeze proteins can maintain cell membrane integrity at low temperatures. We briefly discuss preliminary results about the effects of protein-lipid interactions on the temperature stability of lipid mono- and bilayers.

[1] Chen, X., Chen, Z., BBA 1758 (2006), 1257-1273.

9:20am **BI-TuM5 Development and Characterization of Tunable Porous 3D Materials for Biomedical and Environmental Applications**, *M.J. Hawker*, *A. Pegalajar-Jurado*, *M.N. Mann*, ***Ellen Fisher***, Colorado State University

Porous 3D materials are used in a range of applications from tissue engineering to water filtration to drug delivery systems. In many instances, the surface properties of these materials are not, however, ideal for the intended applications. Low temperature plasmas offer a versatile method for delivering tailored functionality to a range of materials. Despite the vast array of choices offered by plasma processing techniques, there remain a significant number of hurdles that must be overcome to allow this methodology to realize its full potential, especially with porous 3D structures such as membranes and scaffolds. Challenges include ensuring uniform composition following treatment, controlling morphology and damage, characterization of both the external and internal features as well as accurate assessment of bioactivity. Here, we present results demonstrating the relative biocompatibility of various plasma treatment strategies for polymeric membranes and scaffolds. Results from mammalian cell (human dermal fibroblasts) cytotoxicity experiments (MTS, Live/Dead, plating efficiency and morphological studies) will be demonstrated for a range of plasma treated surfaces including bio-nonreactive (e.g. fluorocarbon coated) and bio-reactive (e.g. H_2O plasma treated) 3D poly(e-caprolactone) scaffolds. All materials were characterized using X-ray photoelectron spectroscopy, scanning electron microscopy and contact angle measurements. Additional results demonstrating the efficacy of our plasma treatments in creating low fouling antimicrobial membranes and scaffolds will also be presented. Here, tunable hydrophilic surface modification strategies for different polymeric architectures are evaluated, including plasma modification of NO-releasing materials, ultrafiltration membranes, and polylactic acid constructs. Notably, many of the strategies result in 3D constructs that enhance cell growth and proliferation, retain antibacterial properties and offer promising results for applications including tissue engineering, noble water filtration systems, and advanced biomedical devices.

9:40am **BI-TuM6 The Formation of a Self-Hydrated Artificial Phospholipid Membrane on Ultra-Thin Chitosan Layer Deposited from the Gas-Phase**, *M.J. Retamal*, *M.A. Cisternas*, Pontificia Universidad Católica de Chile, Instituto de Física, Chile, *S.E. Gutierrez-Maldonado*, *T. Perez-Acle*, Fundación Ciencia & Vida, Chile, *B. Seifert*, Pontificia Universidad Católica de Chile, Instituto de Física, Chile, *M. Busch*, *P. Huber*, Hamburg University of Technology (TUHH), Germany, *U.G. Volkmann*, Pontificia Universidad Católica de Chile, Instituto de Física, Chile, ***Valeria del Campo***, Universidad Técnica Federico Santa María, Chile

The design of interfaces between solid surfaces and biological molecules such as membranes and/or proteins using $Si(100)/SiO_2$, a.k.a. *bio-silicon interfaces*, is an important and rapid developing area of both scientific and applied research. Preparation and characterization of artificial biological membranes is a necessary step for the formation of nano-devices or sensors. A soft hydrophilic polymer cushion could help to provide a "bio-mimetic" environment for the membrane and for membrane-spanning proteins. Several candidates to be used as soft-cushion polymers are currently under research, such as dextran, hyaluronic acid and other polysaccharides. Chitosan is a linear polysaccharide obtained by the deacetylation of chitin, which can be found in the shells of crustaceans, exoskeletons of insects, fungi and plants, thus being very easy to obtain from nature at low cost. In the last years, device manufacturing for medical applications adapted the so-called bottom-up approach, from nanostructures to larger devices. We describe the formation and characterization of a phospholipid bilayer (DPPC) on a mattress of a polysaccharide (Chitosan) that keeps the membrane hydrated. The deposition of Chitosan (~25Å) and DPPC (~60Å) was performed from the gas phase in high vacuum onto a substrate of Si(100) covered with its native oxide layer. The layer thickness was controlled *in situ* using Very High Resolution Ellipsometry (VHRE). Raman spectroscopy studies show that neither Chitosan nor DPPC molecules decompose during evaporation. With VHRE and Atomic Force Microscopy (AFM) we have been able to detect phase transitions in the membrane. The presence of the Chitosan interlayer as a water reservoir is essential for both DPPC bilayer formation and stability, favoring the appearance of phase transitions. Our experiments show that the proposed sample preparation from the gas phase is reproducible and provides a natural environment for the DPPC bilayer.

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M.A.C. (Master) and S.E.G.M. (Ph.D). U.G.V and T.P.A are grateful to the Anillo Científico Tecnológico ACT1107. S.E.G.M. and T.P.A. acknowledge funding from Programa Basal PFB16 (PIA CONICYT) and Centro Interdisciplinario de Neurociencias de Valparaiso (ICM-Economia P09-022-F). P.H., M.B. M.J.R., and U.G.V. were supported by a bilateral, german-chilean academic exchange project DAAD project no. 56206483 / CONICYT project no. PCCI 044.

10:20am **BI-TuM8 MP-SPR New Characterization Method for Interactions and Ultrathin Films**, ***Annika Jokinen***, *N.M. Grangvist, W.M. Albers, J.W. Sadowski*, BioNavis, Finland

INTRODUCTION

Surface Plasmon Resonance (SPR) has been used already for a few decades for label-free detection and characterization of biochemical kinetics and affinities of many different types of analytes. The physical phenomenon is not limited to biochemistry, but is applicable to other nanoscale characterization of thin films[1].

EXPERIMENTAL METHODS

Aside of the traditional interactions, Multi Parametric Surface Plasmon Resonance (MP-SPR) can be utilized to determine unique refractive index (*RI*) and thickness (*d*) of ultrathin (d 0.5-100 nm) and slightly thicker films (d 300 nm- few µm) without prior assumptions of the RI of the material. These are important properties not only for thin film coating industries and applications, but also for gaining important knowledge in biomaterials. Two methods utilizing MP-SPR to thickness and RI calculations have been introduced, either measuring in two different media (2M) with high *RI* difference, such as air and water[1-3] , or at two or more different wavelengths (2W) of light[2,3] in order to characterize properties of the thin films.

RESULTS AND DISCUSSION

MP-SPR is suitable for film deposition *in situ* or *ex situ*, which makes it compatible with several deposition methods and thereby makes it applicable to a wide range of surfaces also. Polyelectrolyte multilayer deposition *in situ* was monitored in real-time with MP-SPR. Thickness of each deposited layers was determined utilizing two wavelength method.

Similarly layer thickness and RI was determined also for *ex situ* spin coated cellulose layer. MP-SPR was used not only to determine thickness and RI of the deposited layer but also for real time monitoring of other molecules interaction to the cellulose model surface[4,5].

Recently, MP-SPR was used also to monitor polymer layer structural changes in real time, such as polymer swelling due to pH or electric potential change[6]. At pH 9 poly (acrylic acid) (PAA) brushes were extended but the brushes collapsed at acidic pH[6].

CONCLUSION

With the ability to characterize both kinetics and nanoscale layer properties, MP-SPR proves to be a versatile tool for nanomaterial, biomaterial and biochemical interactions research, which makes MP-SPR invaluable for multidisciplinary research, where both physical and interaction properties of the materials need to be characterized.

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10:40am **BI-TuM9 *In-Situ* Analysis of Biological and Electrochemical Interfaces Using ToF-SIMS**, ***Zihua Zhu***, *X. Yu, Z. Wang, B. Liu, X. Hua, L. Yang, M. Marshall, S. Thevuthasan, J. Cowin*, Pacific Northwest National Laboratory

In-situ analysis of liquid interfaces using ToF-SIMS is challenging because ToF-SIMS is a high-vacuum technique, but liquids often generate some considerable vapor pressure. For example, the vapor pressure of water is about 20 kPa at room temperature (20 °C), thus handling samples containing water in vacuum is not easily done. We recently developed a self-contained microfluidic device for probing aqueous surfaces and demonstrated its feasibility in ToF-SIMS and SEM.[1,2] The key feature of this device is a small round aperture with a diameter of 2-3 microns, which is opened on top of a microfluidic channel. The aperture is exposed to vacuum and serves as a detection window for ToF-SIMS measurements. Our calculations and experimental data show that vacuum compatibility and possible temperature drop due to water vaporization under vacuum can be well-controlled.Performance of the microfluidic device for *in situ* ToF-SIMS analysis of selected organic molecules at aqueous surfaces has been tested.[3] This new innovation has been used in *in-situ* study of mechanism of biofilm growth[4] and electrochemical reactions[5] that occur at liquid-solid interfaces. Such in-situ chemical information at liquid-solid interfaces is very difficult to be obtained using other techniques.

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11:00am **BI-TuM10 Quantifying ToF-SIMS Depth Profiles and 3D Images for Biological and Organic Materials**, *J. Taylor, D.J. Graham,* ***David Castner***, University of Washington, USA

To process, reconstruct, and understand the 3D data from complex materials such as multi-component polymers, drug delivery scaffolds, cells and tissues, it is essential to understand the sputtering behavior of these materials. Though much is understood about sputtering characteristics of some organic materials, there is still a general lack of understanding of how organic and biological materials sputter, especially as the complexity of the materials increase. For example, in multicomponent systems each component may have a different sputter rate, resulting in differential sputtering that will distort the reconstructed depth profile. Thus, accurate reconstruction involves accounting for differential sputter rates, complex sample geometries, etc. Polystyrene and PMMA on Si were used as model systems to optimize methods for depth profile reconstruction. Depth profiling of single component and bilayer films was performed using an Ar_{1000}^+ sputter source and Bi_3^+ analysis beam on an ION-TOF V ToF-SIMS instrument. PMMA sputtered at a significantly higher rate than polystyrene, whilst sputtering of Si can be considered negligible.

Typically the z-axis of depth profiles is converted to depth using an average sputter rate based on measured film thickness and time to remove the film. However, this fails to account for sputter rate variations during the profile, leading to inaccurate film thickness, interfacial position and resolution, and the appearance of penetration into the Si substrate. Applying measured single component sputter rates to the bilayer films, and assuming a step change in sputter rate, yields more accurate film thickness and interface positions; noticeably sharpening the polymer-Si interface. The conversion from sputter time to depth can be further improved by applying a linear change in sputter rate between components across the interface. This further sharpens the interfaces, bringing overall film thickness and interface position more closely in line with expected values. We also have observed a gradual change in sputter rate with mixed polymer blends, possibly due to nanoscale interfacial mixing during sample preparation and storage or induced during the sputter process. Sensitivity analysis performed on variables in sputter rate measurements reveals further scenarios for inaccurate depth profile reconstruction.

This work with a simple laminar system highlights the need for both careful evaluation of component sputter rates and correct application of methods for conversion of sputter time to depth if accurate 3-D reconstructions of complex multi-component samples such as tissue engineering scaffolds are to be achieved.

11:20am **BI-TuM11 *In Situ* Neutron Scattering Studies of Endothelial Cells Response to Shear Stress**, ***Jaroslaw(Jarek) Majewski***, *S. Junghans*, Los Alamos National Laboratory, *L. Pocivavsek,* University of Pittsburgh, *N. Zebda, G. Birukov*, University of Chicago

Neutron reflectivity is very well established experimental tool for obtaining length-scale and density information about well-ordered, layered materials of consistent thickness and high surface occupancy, such as *model* phospholipid bi- and mono-layers, polymeric thin films, inorganic layered structures, *etc.* It is much more difficult to obtain any information about poorly stratified samples and samples that incompletely cover the surface. Measuring *living cells* adhesion and response to external stimuli like the fluid (blood) flow provided an interesting challenge because of the complexity, disordered nature, inherent inhomogeneity of the system, a difficulty in controlling and producing samples with consistent surface coverage but also *biological safety requirements*. Despite these challenges, meaningful results can be obtained. I will discuss measurements involving adhesion of human endothelial cells under fluid mechanical shear stress [1]. Understanding of the cell adhesion in dynamic conditions is connected with pathologic buildup of lipids in arterial walls: atherosclerosis . Although

atherosclerosis is responsible for hundreds of thousands of deaths each year from heart attacks and strokes its nature is not fully understood.

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11:40am **BI-TuM12 3D Collagen-Based Biomaterials Assembly: Novel Insights from Advanced Optical Characterization**, *X. Lang, M. Spousta, J. Gigante, A. Vu, Y. Hwang,* ***Julia Lyubovitsky***, UCR

Optical methods are uniquely suited for characterization of complex biological systems due to their generally non-destructive nature. The applications include characterizing biomaterials/devices employed as medical implants or tissue engineered scaffolds. We have been developing advanced optical imaging guided spectroscopy methods to study the structures of 3D collagen-based biomaterials. This talk will summarize the novel insights regarding the physicochemical controls of assembly of collagen biopolymer into the fibers within 3D hydrogels, cross-linking, digestibility and quantification of hydrogels' structural parameters. For example, our recent study indicated that ions strongly affect the aggregation of collagen into the fibers and consequently modulate the length of the fibers that can be prepared. Changing the temperature led to a multilateral response that depended on the type of ion employed. The knowledge obtained can be applied to explore the practically important and complex processes during assembly and dis-assembly of collagen in engineering of functional biomaterials.

Biomaterial Interfaces
Room: Mauka - Session BI-TuP

Biomaterial Interfaces Poster Session

BI-TuP1 Three-dimensional Conducting Polymer-based Bioelectronic Interfaces for Rare Cell Isolation and Detection, ***Yu-Sheng Hsiao***, Ming Chi University of Technology, Taiwan, Republic of China, *H.-h. Yu,* Academia Sinica, Taiwan, Republic of China, *H.-R. Tseng,* University of California, Los Angeles, *P. Chen*, Academia Sinica, Taiwan, Republic of China

Here we develop a universal solution-processing approach for producing three dimensional (3D) conducting polymer-based bioelectronic interfaces (BEIs), which can be integrated on chips for rare circulating tumor cell (CTC) isolation and detection. Based on the modified poly(dimethylsiloxane) (PDMS) transfer printing technology and bioconjugation process, the poly(3,4-ethylenedioxythiophene) (PEDOT)-based micro/nanorod array films can be fabricated with topographical and chemical control, respectively. This 3D PEDOT-based BEI film features the advantageous characteristics: (1) diverse dimensional structures (tunable from the microscale to the nanoscale), (2) varied surface chemical properties (tunable from nonspecific to specific), (3) high electrical conductivity, and (4) reversible electrochemical switching, and (5) high optical transparency. Furthermore, we integrated this 3D PEDOT-based BEI onchips, which exhibited optimal cell-capture efficiency from MCF7 cells was approximately 85%; featured highly efficient performance for the cell isolation of rare CTCs with minimal contamination from surrounding nontargeted cells (e.g., EpCAM-negative cells, white blood cells); preserved the cell viability with negligible effect on cells. According to the electric cell-substrate impedance sensing concept, the 3D BEI-based device was also demonstrated as a rapid, sensitive and specific tool for CTC detection. Therefore, it is conceivable that use of this platform will meet the requirements on developing for the next-generation bioelectronics for biomedical applications.

Keywords: Poly(3,4-ethylenedioxythiophene) (PEDOT), bioelectronic interfaces (BEIs), circulating tumor cell (CTC), epithelial cell adhesion molecule (EpCAM).

BI-TuP3 For the Development of Auto-Injection System to Cells: Coating of Inserting Pipettes, Gas-Flow Evaluation Method for Prepared Pipettes, and SPM-inspired Pipette-Top Sensing System, ***Tomohide Takami***, *J. Uewaki, H. Ochiai,* Hiroshima University, Japan, *M. Koyama, Y. Ogawa, M. Saito, H. Matsuoka,* Tokyo University of Agriculture & Technology, Japan, *Y. Ojiro, K. Nishimoto, S. Ogawa, Y. Takakuwa,* Tohoku University, Japan, *S. Tate*, Hiroshima University, Japan

Glass nanopipettes have been used as a bridge to connect macro world and micro world.[1] They can be used as an ion-selective probe,[2-6] and as an injector to deposit a small amount of materials onto a surface.[7]

Injection to cell is a hot topic for the statistical experiments on the live dynamics of injected molecules in cell as well as the application to genetic engineering. Several auto-injection systems are already commercially available. However, the fatal problem of these auto-injection systems is the viability of cells after the injection; usually less than 10%.

We have been developing an auto-injection system in which the distance between the injecting pipette tip top and the cell is monitored and the signal depending on the pipette-cell distance is put into the feedback system to achieve the controlled insertion/extracion motion of the pipette to the cell in order to increase the viability of cells. This system is inspired from scanning tunneling microscopy on which the tip-sample distance is well-controlled for the nanoscale observation and molecular manipulation.[8]

Also, we have developed two methods for the auto-injection. One is the coating of the pipette top with chlorobenzene-terminated polysiloxane to reduce the damage to the inserted cell. The other is the gas-flow method to evaluate the inner diameter and the shank length of the pipette before using since the pipettes after the observation with electron microscope cannot be used.

In this paper, we will show our progress to realize the auto-injection system for the use of statistic and quantitative studies. We will demonstrate the ability of manual injection system to show the limit of the manual injection study. We will also demonstrate how the surface science technologies including scanning probe microscopy (SPM), surface coating, and vacuum science can be utilized for the development of the auto-injection system.

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BI-TuP4 Correlative Imaging of Single Mammalian Cells in their Native Environments, ***Xin Hua***, *C. Szymanski, Z. Wang, B. Liu, Z. Zhu, J. Evans, G. Orr,* Pacific Northwest National Laboratory, *S. Liu,* Southeast University, China, *X. Yu*, Pacific Northwest National Laboratory

Mammalian cell analysis is of significant importance in providing detailed insights into biological system activities. Due to the complexity and heterogeneity of mammalian cell behavior and the technical challenge of spatially mapping chemical components in a hydrated environment, correlative chemical imaging from multiplexed measurement platforms is needed. Fluorescence structured illumination microscope (SIM), with super high resolution and visualization of proteins and sub-cellular structures in 3-D, provides more detailed information in cell structure and dynamics. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a unique surface-sensitive tool that provides molecular information and chemical mapping with a sub-micron lateral resolution. However, the understanding of how the spatial heterogeneity and structural difference affect the mammalian cell activities in an unperturbed, hydrated state by ToF-SIMS is severely limited due to the challenge to detect liquids with high volatility in high vacuum using surface sensitive surface techniques.

We recently developed a novel microfluidic reactor enabling correlative imaging of single mammalian cell (e.g., C10 mouse lung epithelial cell) growth by SIM and ToF-SIMS. Cells were introduced in the microchannel, incubated at 37 ^{o}C for 24 hr., fed with 5 Nm quantum dots, and then fixed with 4% paraformaldehyde before SIM imaging. In subsequent ToF-SIMS analysis, an aperture of 2 μm in diameter was drilled through the SiN membrane to form a detection window to image biological surfaces directly; and surface tension is used for holding the liquid within the aperture.

SIM images show that cells are successfully cultured on the SiN membrane, and quantum dots are uptaken by cells and dispersed in the cytoplasm. The ToF-SIMS *m/z* spectra were compared among dried cell samples, hydrated cells, and medium solution. Characteristic lipid fragments are identified. Moreover, 2D mapping of representative cell fragments were obtained. In addition, depth profiling was used to provide time- and space-resolved imaging of the single cell inside the microchannel. Furthermore, principal component analysis is conducted to evaluate the intrinsic similarities and discriminations among samples. Our results demonstrate the feasibility for *in situ* imaging of single mammalian cells in the hydrated state using ToF-SIMS for the first time. Correlative imaging using SIM and ToF-SIMS provides much sought-after information across different space scales for investigating cell dynamics. This novel approach has great potential for studying intracellular processes in the future.

BI-TuP5 Nano-Bio Interfacial Analysis using time-of-flight Medium Energy Ion Scattering, ***KwangWon Jung***, DGIST, Korea, Republic of Korea, *K.S. Park,* KMAC, Republic of Korea, *W.J. Min,* KMAC, *H.J. Lim, S.J. Moon,* DGIST, Korea, *D.W. Moon*, DGIST, Korea, Republic of Korea

We have developed a TOF-MEIS system using 70~100 keV He+. A TOF-MEIS system was designed and constructed to minimize the ion beam damage effect by utilizing a pulsed ion beam with a pulse width < 1 ns and a TOF delay-line-detector with an 120 mm diameter and a time resolution of 180 ps. The TOF-MEIS is an useful tool for interfacial analysis of the composition and structure of nano and bio systems. Our recent applications are reported.

#1) UltraShallow Junction: As doped Si ultra shallow junctions were fabricated with various annealing conditions. We measured the compositional depth profile of 2 x 10^{15} atoms/cm^2 As doped silicon (annealed/unannealed) by the random and channelling phenomenon in Si(100) lattice. The result clearly indicates that the As dopant profile depends on the annealing temperature and conditions. Monitoring of As

activated/deactivated ratio in ultra shallow junction by TOF-MEIS will be beneficial to the manufacturing processes of semiconductor industry.

#2) NanoParticles: We measured the quantitative compositional profiling with single atomic layer resolution for 0.5~3 nm CdSe/ZnS QDs with a conjugated layer. We also investigated the effect with Polyaspartic Acid (pAsp) and Osteocalcin on the initial bone growth of calcium hydroxyl appatite on a carboxyl terminated surface. When pAsp is not added to the self-assembled monolayers of Ca 2mM with Phosphate 1.2 mM, the growth procedure of calcium hydroxyl appatite cannot be monitored due to its rapid growth. When pAsp is added to the SAMs, the initial grow stage of the Ca-P can be monitored so that the chemical composition and their nucleus size can be analyzed.

#3) Liquid interface: Using a graphene as a MEIS analysis window, the electric double layer structure of liquid interface was depth profiled with atomic layer depth resolution. The electric double layer of KI solution is reported with discussions on further studies.

BI-TuP10 Enhancing Protein Adsorption Simulations by Using Accelerated Molecular Dynamics, ***Herbert Urbassek***, *X. Muecksch*, Physics Department, University of Kaiserslautern, Kaiserslautern, Germany

The atomistic modeling of protein adsorption on surfaces is hampered by the different time scales of the simulation (<< 1 ms) and experiment (up to hours), and the accordingly different 'final' adsorption conformations. We provide evidence that the method of accelerated molecular dynamics is an efficient tool to obtain equilibrated adsorption states. As a model system we study the adsorption of the protein BMP-2 on graphite in an explicit salt water environment. We demonstrate that due to the considerably improved sampling of conformational space, accelerated molecular dynamics allows to observe the complete unfolding and spreading of the protein on the hydrophobic graphite surface. This result is in agreement with the general finding of protein denaturation upon contact with hydrophobic surfaces.

Biomaterial Interfaces
Room: Milo - Session BI-WeM

Biomaterials, Interfaces, and Cells
Moderator: Keith McLean, CSIRO, Australia

8:20am **BI-WeM2 Self-Assembly of Macroscopic RNA Membrane**, *Y. Park, H. Kim,* ***Jong Bum Lee***, University of Seoul, South Korea

DNA and RNA have gained attention as powerful materials for bio-nanotechnology. Although, a variety of DNA structures have been developed, structures based on RNA are extremely rare. Here, we developed the robust and free-standing RNA membrane using an enzymatic synthetic method. This macroscopic RNA structure was fabricated by following two steps, complementary rolling circle transcription (cRCT) and evaporation-induced self-assembly (EISA). In addition, properties of the membrane can be controlled by adjusting base-pairing of RNA strands and the concentration of template circular DNA. In this research, we fabricated three types of RNA membranes and used these membranes for controlled drug release systems.

8:40am **BI-WeM3 Cytocompatible Mineralization on Jurkat T Cell Surfaces with Titania Composites**, ***EunHyea Ko***, KAIST, Republic of Korea, *W.G. Youn,* KAIST*, I.S. Choi*, KAIST, Republic of Korea

The artificial shells of organic/inorganic materials on living cells would give new properties to the encapsulated cells. For example, the encapsulate cell could live against physical deformation and chemical hazards, and control the cell division. Also, functionalization would be easier than native cell surface. Recently, our paper said that the $(RKK)_4D_8$ peptide had both the TiO_2-inducing and cytocompatible units for *Chlorella* cell. However, due to the fragile property of mammalian cells, there are few studies on the mammalian cell encapsulation.

In this work, individual Jurkat T cells were encapsulated within peptide/TiO_2 composite shells by layer-by-layer assembly and bioinspired mineralization. The cell viability and shape were maintained during the encapsulation processes, and the division of the encapsulated cells was changed by the artificial TiO_2 shell. There are cluster of differentiation 3 (CD3) antigens on the Jurkat T cell surface. After encapsulation, anti-CD3 antibody was hindered to bind CD3 antigens on the cell. For the functionalization of cell surface, TiO_2 composites made it possible to anchor the ligands of interest to the shell. After formation of the TiO_2 shells on cell surfaces, the shells were functionalized via catechol chemistry in a cytocompatible fashion. We believe that these new properties on the Jurkat T cell surface could be apply to cell-based sensors and assays as well as for fundamental studies such as immunology.

9:20am **BI-WeM5 Selective Cell Adhesion to Surface Nanotopography**, ***Elena Liang***, *MN. Dickson, N. Vollereaux, AF. Yee*, University of California, Irvine

Understanding cell interactions with material surfaces is critical to the performance of medical devices. Of particular interest to our research, such understanding could lead to simple and durable ways to control cell adhesion without chemically modifying the surface of biomaterials used in implantable devices. Recently, it was found that the nanopillar structures on cicada wings are inherently antibacterial irrespective of surface chemistry (Ivanova *et al*. Small. 2012). Such nanostructures can eventually be incorporated on surfaces of medical devices, but first, we need to ensure that patient's own cells would not be adversely affected by these structures. Hu *et al*. showed that nanopillars of widely varying aspect ratios and surface energies had strong effects on cell morphology, discouraging cell spreading (Hu *et al.* 2010). Kong *et al*. discovered that human embryonic stem cells grown on nanopillar structures have a significantly reduced number of focal adhesions per cell and concordantly exhibit increased cell motility on the nanopillars (Kong *et al*. 2013). Based on these findings, we hypothesized that the pillar nanostructures on the cicada wing would prevent cells from adhering. To show this, we first created a library of nanostructures, beginning with a biomimetic cicada wing replicate. We molded a negative hPDMS stamp of the cicada wing and pressed the stamp into polymethylmethacrylate and polystyrene films to create the polymer replicates. We also fabricated pillar arrays of different spacings from commercially available silicon molds using nanoimprint lithography. To evaluate cell adhesion, we counted the number of fibroblasts adhering to flat polymer and the nanopillars, and we determined the number of focal adhesion sites from immunostaining for vinculin, a major protein in the focal adhesion complex. In addition, we examined cell morphology on the various surfaces. After 24 hours, we observed that the cells adopted different cell morphologies, possibly indicating changes in adhesion dynamics. Fibroblasts showed a spread-like morphology on the flat film while the cells on pillars were more equiaxed. Our study has shown that nanostructures in the 100-500 nm-size range do affect cell adhesion dynamics. We found that structure dimensions modulate the adhesion of cells, which may provide researchers a useful means of controlling cell adhesion on material surfaces.

9:40am **BI-WeM6 Polysaccharide Films at an Air/Liquid and a Liquid/Silicon Interface: Effect of the Polysaccharide and Liquid Type on their Physical Properties**, ***Cathy McNamee***, Shinshu University, Japan, *Y. Taira*, Tohoku University, Japan

Chitin and chitosan show biocompatibility, biodegradability, and non-toxity, and are therefore used in pharmaceutical and biomedical applications. The successful applications of chitin and chitosan require the ability to create well-defined films that display the required properties in the working environment. This ability requires an in-depth understanding on the physical properties of the films created using chitin or chitosan and the way to control these properties in different environments. The polysaccharide type, its conformation and packing in the film, and the surrounding liquids in the working environment contribute to the forces and friction of the system, which affect the properties of the polysaccharide films. We investigated the effect of the polysaccharide type, the subphase on which the chitin or chitosan Langmuir monolayers were prepared, and the liquid in which the properties of the transferred monolayers were measured on the physical properties of the polysaccharide films at an air/aqueous interface and at a liquid/silicon substrate interface, and the forces and friction of the polysaccharide transferred films when measured in solution against a silica probe.

Chitosan was modified with a silane coupling agent to make chitosan derived compounds with a low and a medium molecular weight. Chitin and the chitosan-derived compounds were used to make Langmuir monolayers at air/water and air/pH 9 buffer interfaces. The monolayers were transferred to silicon substrates via a Langmuir-Blodgett deposition, and the chitosan-derived compounds subsequently chemically reacted to the silicon substrates. Atomic Force Microscope force and friction measurements were made in water and in the pH 9 buffer, where the water and the pH 9 buffer acted as a good and a bad solvent to the polysaccharides, respectively.

The polysaccharide type affected the friction of the polysaccharide film, where the physically adsorbed chitin gave the lowest friction. The forces and friction of the polysaccharide films changed when the subphase on which the Langmuir monolayers were formed was changed or when the liquid in which the properties of the films adsorbed at the silicon substrate were being measured was changed. The friction increased significantly when the liquid was changed from water to the pH 9 buffer.

10:20am **BI-WeM8 Engineered Surfaces for Stem Cell Expansion**, ***Laurence Meagher***, CSIRO, Australia **INVITED**

Control over biomolecule-material and cell-material interactions is critical to the performance of designed surface coatings in a broad range of applications including cell culture materials, implantable biomedical devices and biosensors. Three key design features for materials used in the expansion of cells is that the materials should have very low non-specific protein adsorption, the coatings should be covalently attached to the substrate and should contain covalently attached, highly specific ligands to mediate cell attachment. For cell therapy applications, these materials should be able to function effectively in cell culture media which is chemically defined and animal product free (i.e. serum-free). We have developed a platform coating approach[1], which in one step, results in coatings with very low non-specific protein adsorption, i.e. no initial chemical functionalisation or priming steps are required. In addition, the coatings also contain functional groups onto which cell attachment ligands such as peptides can be chemically attached. The approach can be used to produce coatings on many different formats of interest, such as multiwall plates, tissue culture flasks and microcarrier particles. Microcarrier particles are particularly attractive for application in stirred tank and wavebag-type bioreactors

In this study we have prepared a number of synthetic polymer coatings using a platform grafting from approach to produce materials for the culture of cells. Coatings were formed using a grafting from approach from a monomer feed comprising 10 mole percent acrylic acid and 90 mole percent acrylamide. Coatings were found to be similar in composition to the monomer feed ratio, highly swelling. Characterisation was carried out using X-ray photoelectron spectroscopy and atomic force microscopy. Coupled to these coatings was a cyclic peptide (cRGDfK) which interacts in a highly specific manner with αvβ3 integrins only. These surfaces were found to be

highly suitable for the attachment and growth of murine L929 fibroblasts, bone marrow derived human mesenchymal stem cells (hMSCs) and human embryonic stem cells (hESCs). Furthermore, in the case of hMSCs the surfaces were used to expand the cells over three passages in three different media (two were serum free). The hMSCs were characterised by their ability to differentiate into adipocytes, osteocytes and chondrocytes as well as maintenance of cell surface markers typically used to define hMSCs.

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11:00am **BI-WeM10 Why Biointerface Science is Important for Stem Cell Research**, ***Kevin Healy***, University of California, Berkeley, USA **INVITED**

Highly regulated signals in the stem cell microenvironment such as ligand adhesion density, matrix stiffness and architecture, growth factor presentation and concentration have been implicated in modulating stem cell differentiation, maturation, and ultimately function. Therefore, it is desirable to have independent control over both the biochemical and mechanical cues presented to stem cells to analyze their relative and combined effects on stem cell function. Accordingly, we have developed a range of materials systems to study stem cell function. This presentation will discuss our progress in developing: 1) self-organizing human cardiac microchambers mediated by geometric confinement; and, 2) *in vitro* disease specific tissue models (e.g., 'organs on a chip') to be used for high content drug screening and patient specific medicine. Examples of how biointerface science is important in these applications will be highlighted. For example, in the former we used PEG-patterned polystyrene substrates to geometrically confine human pluripotent stem cell colonies and spatially present mechanical stress. Upon chemical modulation of the Wnt/b-catenin pathway, biochemical and biophysical cues synergistically induced self-organizing lineage specification and creation of a beating human cardiac micro-chamber confined by the pattern geometry. In the second theme, we employed microfabrication technologies to form cardiac and liver micro-tissues from patient-specific human induced pluripotent stem cells (hiPSC), to be used for high content drug screening and patient specific medicine. Ideally, the use of human disease specific tissues organized into a single integrated physiological system could have an enormous impact on the early screening of candidate drugs.

11:40am **BI-WeM12 Characterization of Tethered Phospholipid Bilayers by the Electrochemical Impedance Spectroscopy**, ***Gintaras Valincius***, *M. Mickevicius, T. Penkauskas*, Vilnius University, Lithuania

We discuss the characterization of tethered phospholipid bilayer membranes (tBLMs) utilizing the electrochemical impedance spectroscopy (EIS). An emphasis is put on applications of tBLMs in protein (peptide)/phospholipid membrane interaction studies. Such interactions modulate the dielectric properties and affect the integrity of phospholipid bilayer. Because of highly asymmetric structure tBLMs exhibit a unique EI response, which cannot be modeled by simple equivalent circuits consisting of capacitors and resistors. We discuss the special functions that describe the characteristic features of the EI spectra. Those analytical functions obtained by solving problem of voltage-current distribution at the interface take into account the structural and dielectric properties of tBLMs. Also, our analysis of the EIS provides the theoretical background for the utility of tBLMs as bioanalytical sensors for the membrane damaging agents, such as pore-forming toxins. We demonstrate that the magnitude and frequency of the negative of the impedance phase minimum, as well as the magnitude of impedance are the parameters indicative of the extent of the membrane damage, and may be used to estimate the defect density in bilayers, as well as the activity of the membrane damaging proteins and peptides. The precision of such estimates is highly dependent on the knowledge of the electric properties of the submembrane electrolyte reservoir separating phospholipid bilayer and solid surface. We present an algorithm, which allows to make estimates of the submembrane specific resistance. Clustering of the defects affects EI response in a unique way, which may be used for the qualitative analysis of the protein membrane interactions.

Biomaterial Interfaces
Room: Milo - Session BI-ThM

Plasma Bio, Medicine & Agriculture
Moderator: Ellen Fisher, Colorado State University

8:40am **BI-ThM3 Diagnostics of Nonthermal Atmospheric Pressure Plasma Jet and Dielectric Barrier Discharge Sources for Plasma Bioscience and Medicine by Collisional Radiative Model and Stark Broadening Method**, ***Eun Ha Choi***, *Y.J. Hong, G. Cho, H. Uhm*, KwangWoon University, Korea **INVITED**

Nonthermal biocompatible plasma (bioplasma) sources and their characteristics operating at atmospheric pressure have been introduced and overviewed for plasma bioscience and medicines, especially used in Plasma Bioscience Research Center (PBRC), Korea. The electron temperature and density have been measured, respectively, by the atmospheric collisional radiative model and Stark broadening method in this experiment. The electron temperatures and plasma densities are measured to be 0.2 ~ 0.7 eV [1] and 1×1014 ~ 2×1015 cm-3, respectively, for the nonthermal bioplasma jet and DBD plasma sources in PBRC. Herein, we have also introduced the basic generation mechanism of reactive hydroxyl radical OH species and hydrogen peroxide H2O2 by plasma-initiated ultraviolet photolysis of water[1] inside the biological solutions, which are main species of interactions with microbial[2] and mammalian cells resulting in apoptotic cell death [3].

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9:20am **BI-ThM5 Surface Modification of Drug-Delivery Systems to Optimize Device Performance**, ***Adoracion Pegalajar-Jurado***, *M.J. Hawker, B.S. Neufeld, M.M. Reynolds, E.R. Fisher*, Colorado State University

Current medical device are often affected by biofouling issues, including blood clot formation and bacterial infection. To combat undesirable side effects and severe medical complications related to the fouling of the devices, a dual approach is required where tuned surface properties and localized delivery of specific biomolecules are combined to enhance device performance. To accomplish this, advanced material platforms are needed to achieve localized therapeutic action and customizable surface properties. Although several wet chemical methods have been explored for surface modification, these methods can produce irreproducible surface modifications resulting from non-uniform coatings and/or can interfere with drug delivery mechanisms. An alternative technique that alleviates many of these issues is plasma surface modification, which offers a tunable and versatile parameter space for tailored and reproducible surface modifications for specific applications while retaining the bulk properties of the material. Herein, we describe the surface modification of a variety of drug delivery systems (including S-nitrosated polymer derivatives) via plasma treatment, resulting in a device that maintained their releasing capabilities (i.e. release of nitric oxide (NO)), but exhibited tailored surface properties for specific applications. As a prototype drug delivery system, we have used *S*-nitrosated poly(lactic-*co*-glycolic acid)-based hydrophobic polymer to achieve a material capable of releasing the therapeutic agent NO. The *S*-nitrosated polymer derivative was modified via H_2O plasma treatment, resulting in a superhydrophilic material (water droplet spread completely in <100 ms) that retained 90% of its initial *S*-nitrosothiol content. Under thermal conditions, NO release profiles were identical to controls. Under buffer soak conditions, the NO release profile was slightly lowered for the plasma-treated materials; however, they still result in physiologically relevant NO fluxes. Correlations between this data and those recorded from other plasma treated drug delivery systems will also be discussed.

9:40am **BI-ThM6 Generation and Transport of Reactive Oxygen Species in Plasma Irradiated Liquid**, *I. Ikuse,* ***Satoshi Hamaguchi***, Osaka University

Numerical simulations of chemical reactions and diffusion of reactive species in water exposed to an atmospheric-pressure plasma (APP) have been performed based on one-dimensional reaction-diffusion equations. When a living tissue is exposed to a low-temperature APP, there is almost always a liquid layer, such as blood, lymph, or other body fluid, that separates the gas phase and the tissue. Therefore charged and chemically reactive species generated by the plasma are transported through the liquid before reacting with the tissue surfaces. The aim of this research is to understand how and where such chemically reactive species that affect biological matters are generated and transported through a liquid. While a variety of ions, excited atoms and molecules as well as chemically reactive charge-neutral species (including free radicals) are generated in the gas phase, the majority of highly reactive species may decay or be converted to more stable species before reaching the liquid surface. On the other hand, charged species and highly reactive charge-neutral species generated in the gas phase near the plasma-liquid interface are likely to be adsorbed by the liquid surface and to generate highly reactive species in a very thin layer (with a thickness 10 ~ 100 nm) of liquid just below the liquid surface. In the simulation, gas phase species generated by APP are assumed to enter pure water at their thermal velocities and dissolved without any barrier. The model incorporates 37 species and 111 chemical reactions in water at room temperature. The simulation has indicated the presence of such a thin liquid layer (which we call a "reaction boundary layer") at the plasma-liquid interface, only in which highly reactive species such as OH radicals and solvated electrons exist and rapidly generate less reactive species such as H2O2, which are then transported to the bulk liquid by diffusion.

10:20am **BI-ThM8 Short-Pulsed Uniform Atmospheric Pressure Dielectric Barrier Discharges in Medical and Biological Surface Treatment**, ***Gregory Fridman***, Drexel University, USA **INVITED**

Engineering innovation has produced startling advances in healthcare. Lasers, ultrasound, ionizing and electromagnetic radiation are examples of life saving diagnostics and treatments that originated in engineering disciplines outside of medicine. In this vein, it was demonstrated that specific types of strongly non-equilibrium nanosecond pulsed atmospheric air plasmas have unique therapeutic effects and hold the promise for new medical diagnostic tools. For example, this presentation will be focused on therapeutic effect of plasmas based on their ability to deactivate pathogens directly in the wound bed, stop bleeding without damage to healthy tissue, promote cell migration and proliferation into the wound bed, angiogenesis, growth factor release, stem cell differentiation and specialization, and other effects leading to the improved healing of wounds and diseases. Mechanisms of plasma-tissue interaction through liquid medium, nanosecond plasma uniformity, and generation and control of reactive oxygen and reactive nitrogen species in plasma will be discussed in this presentation, based on results of the current DARPA and NIH-funded projects.

11:00am **BI-ThM10 Plasma Applications to Agriculture: Plasma Farming**, ***Sukjae Yoo***, National Fusion Research Institute, Korea, Republic of Korea **INVITED**

Plasmas have been applied to various fields: The surface modification and thin film deposition, semiconductor and display fabrication, development of new energy sources, and environmental improvements, plasma medical treatments, etc. In addition to the above mentioned fields, the plasma can be well applied to the agriculture and food.

In case of the semiconductor industry, the first technical innovation was caused by invention of the transistor and integrated circuit (IC) based on chemical wet processes, and the second technical innovation with the very large scale integrated circuit (VLSI) has been realized by adopting plasma processes.

We can draw an analogy between the semiconductor industry and agriculture: The traditional agriculture was innovatively replaced by the chemical agriculture with much higher productivity owing to the invention of agricultural pesticides and chemical fertilizers. Due to the pesticide residue, however, the chemical agriculture has been increasingly replaced by the inorganic agriculture which has even the disadvantage of lower productivity. The problems of both the pesticide residue of chemical agriculture and the low productivity of the inorganic agriculture can be innovatively overcome by adopting the plasma technology.

In this paper, a new concept of the plasma application to the agricultural phases, 'Plasma Farming', will be introduced and some case studies of how to apply the plasma technology to the agriculture will also be given

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