Biomaterial Surfaces & Interfaces
Room Naupaka Salon 4 - Session BI-MoM2

Biofunctional Surfaces and Coatings
Moderator: Buddy D. Ratner, University of Washington

10:20am **BI-MoM2-8 Structure in Lipid Films: From Biophysical Models to Drug Delivery***, **Christine DeWolf**,* Concordia University, Canada **INVITED**
Our understanding of the role of lipid membrane has evolved a long way from the Singer and Nicolson model of a fluid mosaic in which the bilayer served simply as a two-dimensional liquid providing a hydrophobic environment for membrane proteins. While inherently dynamic, membranes exhibit a wide polymorphism of co-existing structures that play important roles in cellular processes. Lipid films (monolayers and bilayers) are frequently used as models to understand and probe structural changes upon interaction with proteins, enzymes, drugs, drug delivery vehicles and foreign bodies such as pollutants and chemicals. Moreover, the understanding of such interactions and how they influence biomolecular conformation and structure, enables us to design lipid-based surface coatings that confer functionality (from simple biocompatibility to triggered drug delivery) to nanoparticles and other solid surfaces. Our work focuses on understanding the composition-structure-function relationships that govern such functionality suing a surface analysis approach that encompasses lateral and vertical structure on both the molecular and meso-/microscopic scales and employs a combination of surface x-ray scattering (GIXD, GIXOS, X-ray reflectivity), imaging (AFM, Brewster angle microscopy and imaging ellipsometry) and spectroscopic (IRRAS) techniques. The power of these methods for elucidating the inter-relationships between the structure and organization of biomembranes and their functional properties will be shown using examples of Langmuir films as model membranes. A focus of the talk will be on lipid-nanoparticle interactions. On one hand, nanoparticles can be considered pollutants or contaminants as their impacts on cellular components, including the cell membrane itself, are not well established. This will be exemplified by examining the impact of inhaled nanomaterials on the functional properties of pulmonary surfactant membranes, a lipid-protein film coating the air-alveolar surface. On the other hand, nanocarriers which are comparable in size to many cellular components and easily internalized, cause unique interactions with cells and therefore offer an intriguing route for the encapsulation and delivery of difficult to deliver drugs. In this case the nanoparticle-lipid membrane interactions have to be tuned to minimize deleterious effects and in fact using a lipid coating can not only provide the necessary biocompatibility but also to enhance the delivery of drugs using nanocarriers via a carefully selected coating composition.

11:00am **BI-MoM2-10 Ways to Synthesize Silicone Nanobodies with Complex Shape and Their Applications as Coatings***, K. Chen, **Stefan Seeger**,* University of Zurich, Switzerland
One-dimensional (1-D) silicone micro- and/or nanostructures such as filaments, wires, fibers and tubes have attracted significant attention due to their remarkable application capabilities in a large range of material and surface science /1/. The chemical synthesis is surprisingly simple and based on the Droplet-Assited-Growth and Shaping process (DAGS)/2/. Here, we demonstrate a novel, extraordinarily simple and efficient *dynamic* DAGS synthesis strategy allowing for the one-step synthesis and in situ control of the shape of nanostructures. We demonstrate bamboo-shaped silicone nanorods (SNRs) obtained by the repetitive dynamic regulation of growth conditions, concomitant with the periodic purging and injection of precursors. The new resulting nanostructures endow these newly designed SNRs with a specific number of segments and a highly regular arrangement. This approach allows the silicone micro- and/or nanorods to be customized with different heights and different segment numbers tailor-made to the requirements for various properties. With this method, various properties can be controlled, for example mechanical stiffness and water repellence. The obtained SNR coatings exhibit for example stable water-resistance under both static and dynamic wetting conditions, robust chemical and mechanical durability, and excellent performance in buoyancy promotion, self-cleaning and water harvesting. Notably, the properties are obtained by fluorine-free compounds, are very environmentally friendly, and are based on a very simple, solvent-free one-step procedure accomplishable at room temperature and normal pressure. The well-structured ultra-long rods can also be fabricated with an ultrahigh aspect ratio (~ 176), still standing straight upwards and regular even the they consist of flexible and soft silicone material /3/. Finally, the presentation will give insight in new applications which are accessible due to the precise control of the nanstructures' shape.

/1/ G. Artus, S. Jung, J. Zimmermann, H. P. Gautschi, K. Marquardt, S. Seeger, EP1644450A2 [http://www.google.ch/patents/EP1644450A2?hl=de&cl=zh]**(2003)**, *Adv. Mater. (***2006)**,18,* 2758, J. Zhang, S. Seeger, *Angew. Chem.***(2011)** 50, 6652, Stojanovic, S. Olveira, M. Fischer, S. Seeger, *Chem.Mat.*, *25*, 2787**(2013),** X. Zhang, S. Seeger, *Small*, 15(34) 1901822 (**2019**)

/2/ G Artus, S Olveira, D Patra, S Seeger, *Macromol.Rapid Comm*. **2017,** *38, 1600558*

/3/ K Chen, S Liu, Y Lau, S Seeger, *Small*, in press (**2022**)

11:20am **BI-MoM2-11 Isolation and Label-Free Detection of Circulating Tumour Cells by Fluidic Diffraction Chips with a Reflective Laser Beam System***, F. Lin,* National Taiwan University of Science and Technology, Taiwan*; H. Hsu,* National Defense Medical Center, Taiwan*; **Jem-Kun Chen**,* National Taiwan University of Science and Technology, Taiwan
A photonic crystal (PC) based line array of poly(methacrylic acid) (PMAA) brushes was grafted from a photoresist template using a trench array. The array was functionalised with anti-epithelial cell adhesion molecule antibodies (EpY). A laser beam was employed to analyse the two-dimensional (2D) and three-dimensional (3D) reflective signals of PCs at an incidence angle of 45◦. The EpY-tailed PMAA PC possessed an optical feature with a characteristic diffraction effect along two laser input configurations including the SII configuration, in which the projection of the laser beam on the plane of the SPM chip was parallel to the strips, and the ST configuration, in which they were perpendicular. A fluidic diffraction chip based on the EpY-tailed PMAA PC with 1-μm resolution was fabricated to examine the ability to detect circulating tumour cells (CTCs) along the ST configuration. The CTCs attached on the EpY-tailed PMAA PC, resulting in the change in the diffraction intensity. Dependence of change degree of the diffraction intensity exhibited a linear range of concentration of CTC from 0 to 64 cells and a limit of detection of 5 cells in 3 mL. CTC detection using both fluidic diffraction chips and a commercial IsoFlux system was carried out in clinical trials, including three healthy donors and 12 patients at various stages of colorectal cancer for comparison. Our platform provides a simple label-free method with high accuracy for rapid CTC counting, which has great potential in clinical treatment applications.

11:40am **BI-MoM2-12 Designer Silk: Plasma-Based Strategies to Customize Surface Properties of Silk Fibroin Films***, **Morgan Hawker**,* California State University, Fresno
Silk fibroin (silk) materials have promise for use in the biomedical space owing to their incredible mechanical properties. Compared to other naturally-derived polymers, silk exhibits remarkable tensile strength because of a unique antiparallel β sheet secondary structure. Additionally, silk is favorable for use in biological settings because it is non-immunogenic and degrades via enzymatic hydrolysis into non-toxic byproducts *in vivo*. Silk can be fabricated into a range of architectures to mimic biological settings, including films, porous networks, and microparticles. These aspects illustrate silk's utility in areas including biosensors, tissue engineering, and drug delivery. All biomedical applications, however, require silk to interact with biological species in specific ways. As with most polymers, silk lacks the necessary surface cues required to facilitate precise interactions with biological species. Thus, further modification is required to tailor silk materials' surface properties and enhance their efficacy as biomaterials.

Low-temperature plasma (LTP) modification represents a relatively unexplored but highly promising area for silk construct modification. LTP processing negates the use of solvents and high temperature conditions associated with wet chemical modification. Moreover, LTP treatment can be conducted with a range of precursors, translating to a myriad of attainable surface chemistries. In this talk, I will highlight three LTP approaches our group recently investigated to modulate silk film surface properties. For example, we utilized LTP strategies to enhance silk film hydrophilicity using N_2 and H_2O(g) precursors. After N_2 and H_2O(g) LTP treatments, water contact angles (WCAs) decreased by ~35° and ~50°, respectively. Notably, all LTP-modified constructs exhibited minimal hydrophobic recovery after 6 weeks of aging. In addition, we have developed plasma-enhanced chemical vapor deposition approaches to modulate silk film surface properties. We utilized LTP copolymerization, where feedgas composition was tuned using two unique precursors: acrylic acid (to produce thin films with polar functional groups on the silk surface) and pentane (to produce thin films with non-polar functional groups on the silk surface). WCA goniometry and x-ray photoelectron spectroscopy were utilized to evaluate wettability and changes in surface chemistry following

LTP treatment, respectively. We elucidated that surface properties depend on both feedgas composition and position of the film in the LTP reactor. In sum, LTP represents a promising avenue to customize silk surface properties for use in biomedical contexts.

Biomaterial Surfaces & Interfaces

Room Naupaka Salon 4 - Session BI-TuM2

Novel Biomaterials

Moderator: Michael Grunze, Max Planck Institute for Medical Research

10:20am **BI-TuM2-8 Moving Towards Intracellular and Enzyme Catalyzed Molecule Synthesis with Mesoporous Materials**, ***Brian Trewyn,*** Colorado School of Mines

As we think about advanced medicine and new biomolecule synthesis, we need to turn to cells and biological systems where drugs and precursors to new consumer goods can be synthesized. We are investigating mesoporous silica nanomaterials (MSN), functionalized with a series of catalytic active sites (inorganic and biological) that are physisorbed and covalently tethered to the pore surface and external surface of the MSN. Because catalytic active sites can be cytotoxic to viable cells, we are investigating pore properties that can be tuned and modified so single site, nanoparticle, and enzymatic catalytic sites can be active in the pores and not interfere with the health of the cell.

Our initial effort into this grand plan has been a tandem system initially using two independent metal nanoparticles to catalyze the oxidative esterification of allyl alcohol. Using separate gold nanoparticle (Au NP) and palladium nanoparticle (Pd NP) catalysts we studied the initial oxidation of the alcohol followed by ester formation to allyl acrylate. Our next generation tandem system finds us replacing the first step of the reaction (Pd NP catalyzed oxidation of allyl alcohol) with an enzyme, alcohol dehydrogenase and conducting the tandem reaction in aqueous buffer at ambient conditions. Future directions of including tethered organometallic catalysts in syntheses conducted in biological and biological simulated environments will be shared.

10:40am **BI-TuM2-9 QCM-D Characterization of Competitive Plasma Protein Adsorption on Low-Fouling Fluoropolymers for Thromboresistant Biomaterials**, ***Sherry Liu,*** *B. Ratner,* University of Washington

Blood-contacting medical devices have classically suffered from endogenous protein fouling that initiate coagulation and inflammation pathways, often leading to fatal thromboembolic complications. The temporal turnover of adsorbed plasma proteins forms the basis for materials-induced thrombosis: human serum albumin (HSA), a platelet-inert and mobile protein, forms an initial passivating layer but is rapidly displaced by fibrinogen (Fg), which is surface-activated to mediate platelet recruitment and initiate fibrin clot formation. Herein, we employ quartz crystal microbalance with dissipation (QCM-D) to monitor competitive HSA:Fg adsorption on a selected panel of superhydrophobic low-fouling fluoropolymers (FPs): poly(vinylidene difluoride) (PVDF), poly(vinylidene difluoride)-*co*-poly(hexafluoropropylene) (PVDF-HFP), and our custom plasma-polymerized C_3F_6 (ppC_3F_6). Across all FPs, ppC_3F_6 demonstrates the greatest HSA:Fg adsorption affinity ratio. Comparing Sauerbrey vs. Voigt mass models, ppC_3F_6 exhibits an uncharacteristic viscoelasticity for the HSA adlayer attributed to hydrophobic-induced reorganization and partial coupling of the hydration layer at the protein-bulk fluid interface. Additionally, binary protein exposure reduced the equilibrium areal mass (R_{max}) compared to the pure Fg condition across all FPs, indicating that HSA competes with Fg for nonspecific binding spots and is retained on the surface at different equilibrium coverages for prolonged periods. Finally, longer HSA residence time on the surface reduces total Fg adsorption upon sequential exposure, suggesting that time-dependent denaturation and packing of HSA on a surface following adsorption increases resistance to Fg displacement. Ultimately, our observations for ppC_3F_6 are attributed to its unique fluorochemistries and superhydrophobic properties created through the plasma polymerization process, while the hydration shells are subject to further study due to existing evidence that they deter cellular adhesion. With QCM-D-enabled characterization of protein adlayers, we hope to identify the optimal FP surface modifications that encourage favorable protein surface compositions and mitigate downstream thrombus formation.

11:00am **BI-TuM2-10 Hierarchical Surface Restructuring for Next Generation Implantable Neural Interfacing Applications**, ***Shahram Amini,*** University of Connecticut, Pulse Technologies Inc.*; S. Shahbazmohamadi,* University of Connecticut

Selective and targeted stimulation of neurons in close proximity to implantable electrodes is an essential prerequisite for successful application of neural interfacing devices. Additionally, the trajectory for further refinement of neural interfacing devices is in large part predicated on increased miniaturization of electrodes that enables higher spatial resolution, precision, and reliability. To achieve miniaturization, the geometric surface area of the electrodes must be reduced while the electrochemical surface area is increased. Therefore, availability of highly electroactive electrode materials or surfaces capable of improving the electrodes' electrochemical performance is paramount as it ensures delivery of enough charge across the electrode/tissue interface for stimulation as well as low impedance at the interface for sensing and recording purposes. In the past two decades, several surface treatment technologies e.g. coatings, thin films, nanomaterials, and also physical and electrochemical techniques have been vastly investigated. Despite varying degrees of improvement in electrochemical performance, most of these techniques are still facing several challenges and shortcomings, e.g. poor performance and durability, manufacturing, scalability and commercialization challenges. In this research, we introduce, for the first time, an innovative, tunable, scalable and commercially viable electrode surface treatment technology known as hierarchical surface restructuring targeted for use in next generation neural interfacing applications. In this work, we demonstrate how ultra-short pulse lasers are utilized to hierarchically restructure the surface of electrodes to create ultra-high-surface-area electrodes. In-vitro electrochemical studies such as cyclic voltammetry and electrochemical impedance spectroscopy were used to show unprecedented improvement in electrochemical performance of these electrodes compared to their untreated electrode counterparts.

11:20am **BI-TuM2-11 Blood Compatibility Assessment of Biomaterial Surface Chemistries to Reduce the Intrinsic Coagulation Pathway Activation**, ***Kyung-Hoon Kim,*** *B. Ratner,* University of Washington

Implantable biomedical devices play critical roles for patients in need of sustainable treatment. However, surface thrombus formation is still a significant complication for blood-contacting surfaces, as it can cause lethal problems. Countless attempts have been made over the past decades to overcome this challenge. However, there is still no perfect blood compatible surface yet. One efficient approach to resolve this issue is to propose different strategies depending on the hemodynamic shear rates. A single mechanism does not govern the blood coagulation of high and low shear hemodynamic flow. The low shear condition is governed by an intrinsic pathway triggered by Factor XII adsorption and activation at the surface. On the other hand, high shear is dominantly affected by an extrinsic pathway initiated by platelet aggregation. We have investigated the blood compatibility regarding an intrinsic pathway by studying diverse biomaterial groups to understand better how each molecular event of clotting varies over different surface chemistries. Two promising categories of biomaterial surface have been tested based on two different hypotheses. The zwitterionic carboxybetaine surface is one of the popular blood-compatible surfaces due to its ultralow protein fouling properties. Fluoropolymer, a popular class of biomaterial used in vascular graft, is another historical surface for improved blood compatibility. The RFGD plasma polymerized hexafluoropropylene (ppC3F6) surface has demonstrated unique properties for preferential albumin tight binding since albumin is hypothesized as a benign protein to reduce the surface blood coagulation. The tight binding of albumin can reduce the factor XII adsorption, resulting in less activation of the intrinsic coagulation pathway.

The zwitterionic polymer outperformed all the surface groups tested, demonstrating low thrombin generation, extended clotting time, low factor XII adsorption, and low factor XIIa activity. Besides, ppC3F6 also demonstrated second-best performance in reducing the intrinsic pathway activation compared to commercial fluoropolymers and other medical polymers. We will further investigate the various ppC3F6 surfaces with varying fluorocarbon ratios, including the CF2:CF3 portion, to discover the potential rules that can contribute to the mitigation of intrinsic pathway activation and improve the understanding of how fluorocarbon chemistry can affect the surface-blood interaction. The result of this study will offer varying strategies for reducing the factor XII-triggered surface thrombus formation, aiming to enhance the blood compatibility at the low shear hemodynamic flow condition.

11:40am **BI-TuM2-12 Innate Immune Response an Integral Part of Acorn Barnacle Surface Adhesion**, ***Kenan Fears,*** *J. Schultzhaus,* US Naval Research Laboratory

Understanding the adhesion mechanisms of marine hard foulers (e.g., barnacles and tubeworms) is of critical importance to the global maritime communitiy due to the pervasiveness of these organisms, coupled with the desire to transition away from toxic antifouling paints that have a deleterious ecological impact. Often, the biochemical processes that are crucial for the attachment and survival of hard foulers occur at buried

interfaces, which further complicates analysis. He we use confocal laser scanning microscopy protocols for imaging the adhesive interface of a model hard fouler, acorn barnacles (*Amphibalanus (=Balanus) amphitrite*), using multiple fluorescent probes to simultaneously label up to four different biochemistries. Time lapse imaging reveals cellular and extracellular processes related to cuticle development, biomineralization, and surface adhesion. More importantly, the presence of a lipidaceous secretion that phase-separates once it is exposed to seawater. This secretion oxidizes and detaches surface-adhered biofilms ahead of barnacle growth and cement deposition, and also facilitates the remodeling of the cement matrix to fill voids that may exist between the base of the barnacle and the underlying substrate. Enzymes present in this secretion generate hypochlorite ions (i.e., bleach) to oxidize organic matter at the adhesive interface and defend against microbial attack. Protein sequence analysis reveals that peroxidase enzymes found in cement collections are highly conserved across the barnacle tree of life, which includes stalked, calcareous-based, and membranous-based barnacles. However, we did not observe this surface-cleaning secretion in two membranous barnacles, *Chthamalus fragilis* and *Elminius modestus,* suggesting it is a more recent evolutionary trait.

Biomaterial Surfaces & Interfaces
Room Naupaka Salon 1-3 - Session BI-TuP
Biomaterial Interfaces Poster Session

BI-TuP-1 Cell-Mimetic Biosensors for Detecting Avian Influenza Virus Through the Viral-Fusion Mechanism, ***Jong-Woo Lim***, College of Veterinary Medicine, Research Institute for Veterinary Science, Seoul National University, Republic of Korea; *G. Park, C. Park,* Department of Chemical and Biomolecular Engineering, Yonsei University, Republic of Korea; *M. Yeom,* College of Veterinary Medicine, Research Institute for Veterinary Science, Seoul National University, Republic of Korea; *S. Lee,* Department of Chemical and Biomolecular Engineering, Yonsei University, Republic of Korea; *K. Lyoo,* College of Veterinary Medicine, Jeonbuk National University, Republic of Korea; *S. Haam,* Department of Chemical and Biomolecular Engineering, Yonsei University, Republic of Korea; *D. Song,* College of Veterinary Medicine, Research Institute for Veterinary Science, Seoul National University, Republic of Korea

Avian influenza virus (AIV) is a major respiratory disease of poultry, especially High pathogenic AIV (HPAIV) presents high morbidity and mortality. To control the spread of HPAIV, it is very important to detect HPAIV at the early stages for taking timely countermeasures such as quarantine and isolation. In this study, we developed cell mimicking nanoparticles (CMPs) for rapid detection of HPAIV as well as low pathogenic AIV (LPAIV) via a viral fusion mechanism. CMPs were polymeric nanoparticles constructed with sialic acid and FRET dye pairs, exposing FRET off signal responding to membrane fusion with AIV activated by enzymatic cleavage at endosomal condition. CMPs exhibited highly sensitive detection of various types of LPAIVs and HPAIVs in the biological environment. The developed detection system utilizing the viral infection pathway can be a potent diagnostic assay for LPAIV and HPAIV, contributing to minimizing the economic loss of the viral outbreaks.

BI-TuP-2 Using Cinnamaldehyde Plasma Treatment to Develop an Antioxidant Coating, ***Ashley N. Keobounnam***, *M. Hawker,* California State University, Fresno

When a burn injury occurs, the heat damage caused by said burn increases the permeability of the microvasculature. This increased permeability leads to the leakage of plasma into the interstitial spaces of cells, which can lead to hypovolemic shock. One approach to reduce the likelihood of hypovolemic shock is to reduce microvascular permeability. Oxidants are an agonist that increase post-burn permeability through increasing the concentration of reactive oxygen species, ultimately leading to oxidative stress. Microvascular permeability remains in an increased state so long as the rate of reactive oxygen species generation is greater than the body's rate of detoxifying. One strategy to inhibit the effects of oxidative stress is to introduce antioxidants directly to the burn site. Current studies regarding burn wounds focus on the healing process after the initial injury. However, methods to stabilize the patient before significant plasma leakage occurs remain relatively unexplored.

This work focused on developing a burn wound patch with an antioxidant coating towards the goal of reducing microvascular permeability. We opted to use a plasma enhanced chemical vapor deposition (PECVD) strategy to deposit an antioxidant coating on a model polymer substrate (chitosan). Specifically, we utilized cinnamaldehyde - an organic compound with antioxidant activity - as a plasma precursor. Chitosan substrates were modified using optimized plasma parameters, where both pulsing and continuous plasma power conditions were explored. In previous studies, continuous PECVD films exhibited limited monomer functional group retention, whereas pulsed PECVD films exhibited greater monomer functional group retention. Antioxidant activity relies heavily on chemical structure, therefore, we explored how plasma power conditions (continuous vs pulsed) impact antioxidant effectiveness. Surface wettability was analyzed using water contact angle goniometry. X-ray photoelectron spectroscopy was used to characterize surface chemistry. Additionally, radical scavenging activity of PECVD treated materials was evaluated using a 2,2-diphenyl-1-picrylhydrazyl assay. Overall, this work opens new directions for antioxidant therapy in burns.

BI-TuP-3 Modifying Commercially-Available Wound Dressing Materials with Continuous and Pulsed 1,8-Cineole Plasma, ***Mia Rose Kayaian***, *M. Hawker ,* California State University, Fresno

Hospital-acquired infections (HAIs) are one of the primary causes of death.According to Medicare data, HAIs are estimated to cost the United States anywhere from 28.1 to 96.8 billion dollars yearly. Most HAIs begin with bacterial attachment to the wound. If left untreated, the infection site can become a chronic wound. The clinical standard for existing chronic wounds are oral and topical antibiotics, but this can quickly become problematic due to the possible development of antibiotic resistance. As an alternative, designing a wound dressing to actively kill bacteria on contact would be optimal for chronic wound treatment. Plasma-enhanced chemical vapor deposition (PECVD) using 1,8-cineole as the plasma feed gas has been shown to actively kill bacteria on glass slide surfaces. Yet, 1,8-cineole-based PECVD strategies have not been extended to wound dressing materials.

The goal of the present study is to use 1,8-cineole PECVD to modify commercially-available wound dressing materials: hydrofibers and hydropolymers. Our strategy was to deposit a film with antibacterial properties using plasma treatment. By adjusting plasma parameters, it is possible to control the coating chemistry. However, no attempt has been made to compare pulsing vs. continuous-wave deposition. In other PECVD systems, pulsing has enabled the plasma precursor's functional groups to be maintained within the coating. Since the functional groups give 1,8-cineole its antibacterial properties, we anticipated that pulsing would enable the functional groups to be maintained upon PECVD. This study utilized 20 W peak power and a 20 minute application time for both continuous and pulsed deposition. The pulsed deposition used 10%, 25%, and 50% duty cycles. Water contact angle goniometry was used to evaluate changes in surface wettability of the material before and after plasma treatment. Additionally, x-ray photoelectron spectroscopy was used to quantify the elemental composition of plasma-treated and control surfaces. Zone of inhibition testing was performed to evaluate antibacterial properties against both gram-positive and gram-negative bacteria. Overall, 1,8-cineole PECVD strategies were effectively applied to wound dressing materials. This work represents progress towards addressing the need to directly target chronic wound infectious sites.

BI-TuP-4 Fractional Analysis Process of Surface-Adsorbed Proteins Using Sds-Page, ***Naofumi Ohtsu***, Kitami Institute of Technology, Japan

When a biomaterial is implanted in a human body, an immediate event occurring on its surface is competitive adsorption of proteins. Such surface adsorbed proteins predominate cell attachment, proliferation, and differentiation that relate with material's biocompatibility. On the other hand, tissue fluids comprise many protein species, of which electrostatic properties are different by their structure. Compositions of the adsorbed proteins thus depend on the material's surface property. Fractional analysis of surface-adsorbed protein would thus provide significant clue for understanding the tissue-material interfacial reaction. However, separation of the surface-adsorbed proteins is difficult task; conversely, the proteins dissolved in a solution is capable to be separated by an electrophoresis, easily. Based on these, in the present study, we attempted to perform the fractional analysis of surface-adsorbed proteins through the extraction into a solution and subsequent separation by electrophoresis.

A prescribe amounts of bovine serum albumin (BSA) and lysozyme (LSZ) as model proteins were dissolved into a phosphate buffered saline (PBS) solution. Initially, a titanium (Ti) plate widely used for medical implants was immersed into the protein-contained PBS solution for 60 min at 310 K, aiming to adsorb BSA and LSZ on its surface. Next, the proteins adsorbed on the surface was extracted into a distilled water or a sodium dodecyl sulfate (SDS) solution of various concentrations. The extraction was conducted by immersing the Ti substrate into such solutions filled in a glass beaker, along with an ultrasonication for 10 min. Thereafter, the extracted proteins were separated by polyacrylamide gel electrophoresis (PAGE). Concentration of each separated protein was determined from the corresponding band intensity in PAGE gel.

When using a distilled water as the extraction solution, the proteins could not be detected in PAGE gel because almost proteins were readsorbed onto an inner-wall of glass beaker. Such readsorption was prevented when using the SDS solution exceeding 0.5 %, and actually, over 85% of adsorbed protein could be extracted. Additionally, when measuring the band intensity in the gel, we could confirm the linear correlations between the concentrations and the band intensity, in the range from 0.5 to 5 μg·mL. Based on these, we conducted fractional determination of surface-adsorbed BSA and LSZ though the analysis process above. The results demonstrated that the ratio of adsorbed BSA and LSZ was varied with the mixture ratio of these proteins, evidencing the validity of the suggested fractional analytical process.

BI-TuP-5 Advanced Surface Analysis of Porous Bioactive Polymer Coatings on a TiAl6V4 Substrate Prepared by Supercritical Foaming for Orthopedic Applications, ***Katja Andrina Kravanja**, M. Finšgar, Ž. Knez, M. Marevci Knez,* University of Maribor, Slovenia

Ti-based alloys are increasingly used as medical implants due to the combination of excellent biocompatibility, high strength, and corrosion resistance. However, their biological inertness and persistent postoperative complications such as inflammation and infection necessitate the development of bioactive coatings for orthopedic implants that enable localized, controlled release of active ingredients (AIs) at the implant site for improved osteointegration [1]. Formulation of AIs with supercritical fluids is one of the leading and well-established strategies to produce high-quality products by incorporating environmentally friendly and economically feasible properties. Supercritical (SC) foaming yields porous polymer matrices with a large specific surface area, allowing encapsulation and tailored release of AIs [2, 3].

This work aimed to develop and characterize porous bioactive coatings on TiAl6V4 surface made of biodegradable and biocompatible polymers using SC foaming technique to achieve controlled release of model synthetic and natural AIs with anti-inflammatory and antibiotic properties. The chemical structure, interactions, and morphology of the bare and coated substrates were characterized by advanced surface analysis, particularly tandem time-of-flight secondary ion mass spectrometry (ToF-SIMS), X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and 3D profilometry. Chemical information about the surface of multicomponent coatings was evaluated by determining molecular-specific signals for the coating components. The in-depth molecular distribution was determined by gas cluster ion beam (GCIB) 3D imaging by ToF-SIMS. In addition, the elemental composition, elemental environment, and depth profiling using GCIB for effective sputtering were acquired by XPS measurements. The topography of the newly prepared coatings and the agglomeration of the AIs were determined by AFM, while the large-scale study of the coatings and substrate roughness is presented by 3D profilometry. The results demonstrate that the fabricated coatings provide a good foundation for future pharmacokinetic and cellular research.

1. Kravanja, K.A. and M. Finšgar, *A review of techniques for the application of bioactive coatings on metal-based implants to achieve controlled release of active ingredients.* Materials & Design, 2022: p. 110653.
2. Knez, Ž., et al., *Industrial applications of supercritical fluids: A review.* Energy, 2014. **77**: p. 235-243.
3. Čolnik, M., et al., *Biodegradable polymers, current trends of research and their applications, a review.* Chemical Industry and Chemical Engineering Quarterly, 2020. **26**(4): p. 401-418.

BI-TuP-6 PEG-b-PLA-NHS based Self Assembled Vaccine Platform as an Adjuvant-free Influenza Virus Vaccine, ***Jaehyun Hwang,*** Chonnam National University, Republic of Korea*; G. Park,* Yonsei University, Republic of Korea*; J. Lim,* Seoul National University, Republic of Korea*; E. Ga, S. Moon,* Chonnam National University, Republic of Korea*; C. Park,* Yonsei University, Republic of Korea*; H. Kim,* Kangwon National University, Republic of Korea*; D. Song,* Seoul National University, Republic of Korea*; S. Haam,* Yonsei University, Republic of Korea*; W. Na,* Chonnam National University, Republic of Korea

Vaccines have been considered most effective tool to defend against viruses that can cause disease. However, some current vaccines are still suboptimal due to obstacles such as a risk of side effect and low immunogenicity. In the present work, we developed a self-assembled vaccine (SAV) platform based on antigen conjugated with an amphiphilic block copolymer, mPEG-b-PLA-NHS. To improve immunity of subunit vaccine, SAV was designed to display repetitive antigens in nanoscale for eliciting efficient antigen delivery and immune cell activation via multivalent recognition. SAV showed enhanced cellular uptake by dendritic cells (DCs), accelerating an efficient initiation of the adaptive immune system. SAV also showed high level of induction of IgG through in vivo analysis. These results implicate that SAV facilitate both efficient B cell activation and efficient T cell mediated immune response. In animal models, we confirmed that SAV comprising of hemagglutinin (HA) efficiently protected mice from mortality following challenge with influenza A virus. These findings suggest that the self-assembled nanosystem composed of antigen-polymer conjugate can be a potent vaccine platform with effectiveness and versatility.

BI-TuP-7 Polymersome Based Co-Delivery System of Antigen and Immunostimulant for Improvement of Humoral Immune Response, ***Eulhae Ga,*** Chonnam National University, Republic of Korea*; J. Lim,* Seoul National University, Republic of Korea*; J. Hwang, S. Moon,* Chonnam National University, Republic of Korea*; M. Yeom, D. Song,* Seoul National University, Republic of Korea*; W. Na,* Chonnam National University, Republic of Korea

Cellular uptake of antigen (Ag) by antigen-presenting cells (APCs) is vital for effective functioning of the immune system. Intramuscular or subcutaneous administration of vaccine Ag alone is not sufficient to elicit optimal immune responses. Thus, adjuvants are required to induce strong immunogenicity. Here, we developed nanoparticulate adjuvants that assemble into a bilayer spherical polymersome (PSome) to promote the cellular uptake of Ag into APCs. PSomes were synthesized by biodegradable and biocompatible block copolymer methoxy-poly(ethylene glycol)-b-poly(D,L-lactide) to encapsulate both hydrophilic and lipophilic biomacromolecules, such as ovalbumin (OVA) as a model Ag and monophosphoryl lipid A (MPLA) as an immunostimulant. After co-encapsulation of OVA and MPLA, the PSome synthetic vehicle exhibited the sustained release of OVA in cell environments and allowed efficient delivery of cargos into APCs. The administration of PSomes loaded with OVA and MPLA induced the production of interleukin-6 and tumor necrosis factor-alpha cytokines by macrophage activation *in vitro* and elicited effective Ag-specific antibody responses *in vivo*. These findings indicate that the nano-sized PSome may serve as a potent adjuvant for vaccine delivery systems to modulate enhanced immune responses.

BI-TuP-8 Rapid and Effective Intradermal Application of Canine Influenza Vaccine Without Removal of Hair Using Patchless Insertion-Responsive Microneedle (Irmn) and Its *in Vivo* Efficacy Evaluation, ***Suyun Moon**, E. Ga, J. Hwang,* Chonnam National University, Republic of Korea*; A. Kang,* QuadMedicine R&D Centre, QuadMedicin, Republic of Korea*; S. Baek,* QuadMedicine R&D Centre, QuadMedicine, Inc., Republic of Korea*; H. Jun,* QuadMedicine R&D Centre, QuadMedicine, Inc., Seongnam, Republic of Korea*; S. Choi,* QuadMedicine R&D Centre, QuadMedicine, Inc., Republic of Korea*; J. Lim, M. Yeom,* Seoul National University, Republic of Korea*; J. Park,* Gachon University, Republic of Korea*; H. Kim,* Kangwon National University, Republic of Korea*; D. Song,* Seoul National University, Republic of Korea*; W. Na,* Chonnam National University, Republic of Korea

Novel tip-separable microneedle system called Insertion-responsive microneedles (IRMNs) is painless application system, compensating the existing disadvantages of conventional vaccine administration such as intramuscular vaccination. IRMNs are composed of dissolvable hyaluronic acid (HA) tips and biocompatible polycaprolactone (PCL) bases, which is immediately isolated right after needle insertion and retraction. In this study, we conducted several *in vivo* and *ex vivo* tests to prove stability, safety and efficacy of IRMNs. *Ex vivo* porcine skin injection tests confirmed IRMNs penetrates skin and successfully releases coated components with no damage on skin tissues. Immunization in Guinea pigs using IRMNs induced two times higher hemagglutination inhibition (HI) antibodies compared to intramuscular injection groups, and complete elimination of viral shedding was found at 8 days post infection challenged with influenza A/canine/Korea/01/2007 (H3N2) wild-type virus after second vaccination. Similar result has been shown in the H3N2 vaccine inoculation into dog's ears compared with intramuscular injection group. Tips of IRMN were well separated from the base, successfully delivering vaccine materials into dog's hairy skin without pain. The veterinarian assed behavior of dog during injection and compared entire response of IRMN group with intramuscular administration. Dogs treated with IRMs appeared to be more comfortable and painless compared to syringe injection group. IRMNs are potential candidate of rapid and convenient vaccination, which will be particularly useful and attractive in veterinary research fields using animal vaccination.

BI-TuP-9 Probing Bacterial Membrane Composition in the Study of Antibacterial Resistance using GCIB-SIMS, ***John Fletcher,*** University of Gothenburg, Sweden

The spread of antibiotic resistance is an increasingly difficult problem to deal with as more bacterial infections survive treatments with commercial antibiotics. One of the main routes for the spreading of resistance among bacterial population is horizontal gene transfer, mainly through conjugation where mobile genetic elements are transferred from a donor cell to a recipient cell through a conjugative pilus.

One way to deal with the increasing levels of antibiotic resistance in bacteria is to develop new antibiotics for which resistance has not yet

emerged, which can be both laborious and not always a lucrative market. An alternative is to inhibit the conjugation itself so that the rate at which new resistance genes spread between populations is reduced and the usefulness of existing and new antibiotics is extended.

A previous study, performed at the University of Gothenburg, used a high-throughput screen to identify chromosomal *Escherichia coli* genes in the donor cells that were important for conjugation of the F-plasmid and could be potential targets to reduce conjugation. Among these hits were several genes that are involved in the cell envelope through stress response pathways, biogenesis, outer membrane protein assembly and homeostasis, which formed an interest into the role and importance of the cell envelope for conjugation.

Here, recent findings on the influence of different mutations and conditions on the highlighted mutants detected by secondary ion mass spectrometry (SIMS) using a gas cluster ion beam (GCIB) are presented.

BI-TuP-10 Soft, Precision Engineered Porous, Hydrogel Scaffolds Mechanically Tailored towards Applications in the Central Nervous System, ***Ningjing Chen**, B. Ratner,* University of Washington

Largely incurable diseases and traumatic injuries to the central nervous system (CNS) demand the development of new biomaterials to improve healing and treatment options. Matching material mechanical properties to the CNS tissue and optimizing material porous structures are two central goals for improving better biomaterials for the CNS. However, biomaterials with both precision-controlled porous structure and brain-matched mechanical properties are still lacking. In this study, we developed a copolymeric hydrogel of 2-hydroxyethylmethacrylate and glycerol monomethacrylate (pHEMA-co-GMA)with mechanical properties tunable into the range of CNS tissues, and a uniform 40 µm porous structure. The two characteristics were achieved by a new fabrication process combining phase separation and sphere templating. We used scanning electron microscopy (SEM) to image their morphology and an Instron mechanical testing apparatus to examine their compressive Young's Moduli. The resulting scaffolds are non-cytotoxic and endotoxin-free according to the ISO 10993-5 standard and commercialized endotoxin testing kit. In addition, 3D culture of microglial cells within the scaffolds demonstrates cell attachment and maintenance of a rounded, quiescent morphology, potentially due to spatial confinement. These results support further *in vivo* studies and suggest broad potentials in CNS applications, such as brain-computer interfaces, neural regeneration, and basic neurobiology.

BI-TuP-11 Zwitterionic Copolymer for the Bio-Compatible Coating on Medical Devices to Prevent Protein Fouling and Complement System Activation, ***Kan Wu**, B. Ratner,* University of Washington

In the past decades, the demand for biocompatibility is increasing with the rapid development of advanced medical technologies, such as biosensors, implantable chips, and hemodialysis apparatus. Among all the biocompatible materials, zwitterionic carboxybetaine (CB) distincts itself for its superior hydration capability and stability as well as its potential for further functionalization. However, a reliable yet easy method to introduce the CB material onto the target surfaces is still challenging.

We developed a series of PCB-DOPA conjugates with different architectures of combining polycarboxybetaine (PCB) and mussel-inspired binding groups (DOPA) groups and investigated the structural effect on their coating performance. We found at a molecule with a linear PCB chain and quadruple DOPA groups at the chain end (PCB-4DOPA) can significantly increase the coating coverage and stability. Next, we applied the PCB-4DOPA conjugate on blood-contacting devices(hemodialysis membrane, catheters, respiratory devices, etc to test the performance of this method in the real application. We demonstrated that the PCB-DOPA conjugate can form a protective coating on the device surfaces and significantly reduce the fibrinogen attachment and complement system activation. The complement proteins C5b9 generated by PCB interface is only 1.96ug/ml, compared to 12.16ug/ml generated by poly(poly(ethylene glycol)methacrylate)(PEGMA) at the same incubation condition. In addition, we also propose a new standard method to detect the complement reaction activation level in the blood samples for most prevailing biomaterials. This new detection method optimized the current measuring techniques and achieved a more accurate measurement.

BI-TuP-12 Enhanced Antithrombogenicity of 3D Templated Artificial Vascular Grafts Through Heparin Complex Conjugated with PEG Spacer, ***ChaeHwa Kim**,* Advanced Textile R&D Department, Korea Institute of Industrial Technology, Republic of Korea*; J. Kim,* Material & Component Convergence R&D Department, Korea Institute of Industrial Technology, Republic of Korea*; J. Lee, T. Kim,* Advanced Textile R&D Department, Korea Institute of Industrial Technology, Republic of Korea

Artificial vascular grafts to replace blood vessels are necessary in cases where irreparable damage to blood vessels occurs due to circumstances such as disasters or accidents. Furthermore, blood compatibility is also vital in the transplantation of artificial blood vessels. In this study, we designed the vascular graft with 3D printing techniques and developed heparin derivatives that have better adherence to the surface and avoid thrombosis. Heparin complex conjugated with PEG spacer was synthesized and coated on the artificial vascular graft fabricated by 3D templated printing technology. We fabricated the 3D-printed polyvinyl alcohol (PVA) templates according to the blood vessel size and shape, and these were dip-coated with salt-suspended thermoplastic polyurethane (TPU). The core template was removed to obtain a customized porous TPU graft. Next, the dopamine-PEG-heparin conjugate was prepared through NHS/EDC coupling reaction. PEG spacers were also introduced to increase the heparin surface arrangement and inhibit blood component adherence. PEGs having various molecular weights were conjugated to heparin to investigate antithrombotic properties according to the length of the PEG linker. Using an NHS/EDC coupling procedure, a heparin-PEG-dopamine complex was generated and subsequently coated onto TPU vascular grafts for robust immobilization. Then, heparin release studies, blood coagulation, and platelet adsorption tests were performed. The use of a PEG linker improved the coating stability, which lowered the release rate of heparin and reduced platelet adhesion. In addition, the longer the molecular weight of the PEG linker, the stronger the antithrombotic effect. The results demonstrated that heparin-PEG-dopamine can substantially enhance the coating strength and anticoagulant properties of porous TPU vascular grafts.

BI-TuP-13 Impact of Amino Acid Conformation on the Efficacy of Antimicrobial Cyclic Peptides Against Medically- and Industrially-Relevant Microbes, *Q. Lu, D. Regan, D. Barlow, **Kenan Fears**,* US Naval Research Laboratory

Microbial growth on surfaces, if unmitigated, poses health concerns and can accelerate the biodegradation of engineered materials and coatings. Cyclic peptides have emerged a promising class of agents to combat biofouling. Cyclic peptides are more resistant to enzymatic degradation than their linear counterparts and can be designed to interact with extracellular targets, intracellular targets, and/or self-assemble into transmembrane pores. Here, we compare the *antimicrobial efficacy of two pore-forming cyclic peptides,* α-K_3W_3 and β-K_3W_3, *against bacterial and fungal liquid cultures, and fungal biofilms. These two peptides display an identical series of side-chain chemistries, but the additional methylene group in the peptide backbone of* β-amino acids results in two structural differences: 1) β-K_3W_3 has a larger pore diameter, and 2) all backbone carbonyl (C=O) groups point in the same direction, with all amide (N-H) pointing in the opposite direction, leading to an enhancement in the dipole moment that drives self-assembly. *In liquid cultures,* β-K_3W_3 was more efficient at reducing the number *of colony forming units (CFU) when exposed to* a gram-positive bacterium, *S. aureus,* and two fungal strains, *N. albida* and *P. laurentii*. To evaluate efficacy against fungal biofilms, cyclic peptides were incorporated into surface coatings of Irogran®, a *polyester*-based thermoplastic *polyurethane. We detected no survival of N. albida* and *P. laurentii* microcolonies (10^5 per inoculation) on coatings containing either peptide a 7-day exposure. To determine if antimicrobial activity persists upon repeated exposure, we reinoculated coatings with *P. laurentii* live cells every 7 days for 4 weeks and detected no CFU after 14 and 21 days. In direct comparison, the number of CFU from Irogran® coatings without cyclic peptides was > 5 log CFU.

Biomaterial Surfaces & Interfaces
Room Naupaka Salon 4 - Session BI-WeE1

Bioimaging and Bionanotechnology

Moderator: David G. Castner, University of Washington

5:40pm **BI-WeE1-1 Machine Learning for Prediction of TOF-SIMS Spectra of Peptides**, ***Satoka Aoyagi,*** Seikei University, Japan **INVITED**

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is one of the most powerful surface analysis methods because ToF-SIMS provides molecular 3D imaging with high spatial resolution and detailed chemical structures. ToF-SIMS has extremely rich chemical information and so that it is often difficult to extract all of the important information from ToF-SIMS data by manual analysis. Multivariate analysis techniques such as principal component analysis have successfully been applied to TOF-SIMS data interpretation [1] and are generally useful for understanding TOF-SIMS results. Moreover machine learning and deep learning methods have been applied to ToF-SIMS data interpretation [2]. In order to interpret ToF-SIMS spectra, the processes of the data analysis should be opened, but most of the deep learning methods do not provide readable information on the analysis processes. Through a VAMAS (Versailles Project on Advanced Materials and Standards) interlaboratory study, the identification of peptide sample TOF-SIMS data by machine learning (Fig. 1) was investigated. In this study, unknow peptide spectra were predicted using Random Forest [3]. Moreover, this method can be applied to the prediction of other organic materials by improving the data format.

[1] M.S. Wagner and D.G. Castner, Langmuir 17 4649-4660 (2001).

[2] K. Matsuda and S. Aoyagi, Biointerphases 15 021013 (2020).

[3] S. Aoyagi, A. Takano and Y. Fujiwara, VAMAS TWA 2 A26, 2019:Identification of unknown peptide sample TOF-SIMS data by machine learning - Protocol for Analysis

6:20pm **BI-WeE1-3 Strategy for Constructing Accurate 3D NanoSIMS Depth Profiling Images of Cells Despite Lateral Variations in Surface Erosion**, *M. Brunet, B. Gorman,* ***Mary L. Kraft,*** University of Illinois Urbana-Champaign

We have developed a strategy for constructing accurate 3D NanoSIMSdepth profiling images of cells when the rate of surface erosion varies laterally. To accomplish this, we reconstruct the morphology of the cell each time a depth profiling image was acquired from the secondary electron images acquired in parallel with the negatively charged secondary ions during NanoSIMS depth profiling. Then the morphologies created for every imager plane in the depth profile are adjusted so the height of the cell at every x, y location decreases each time a new image was acquired. Finally, these reconstructions of the cell's morphology are used to shift the voxels in the 3D NanoSIMS images to the correct height. This strategy was validated by comparing morphology reconstructions for secondary electron depth profiling images acquired from a cell with focused ion beam - secondary electron microscopyand AFMdata acquired from the cell before depth profiling. The general shape and relative height of the reconstructed cell morphology was in good agreement with the AFM data. Application of this strategy to 3D NanoSIMSdepth profiling data of a metabolically labeled mammalian cell produced visually accurate 3D images of the intracellular ^{18}O-cholesterol and ^{15}N-sphingolipids distributions.Moreover, transport vesicles and organelle membranes containing ^{18}O-cholesterol and ^{15}N-sphingolipids could be more clearly visualized. Accurate 3D NanoSIMS images showing the distributions of molecules of interest within cells may now be constructed when the sputter rate varies laterally and without requiring the collection of topography data prior to depth profiling.

6:40pm **BI-WeE1-4 Multimodal Studies of Cellular Membrane Chemistry using GCIB-SIMS**, ***John Fletcher,*** University of Gothenburg, Sweden

Changes in the lipid membrane of cells has implications for cell survival, function and in the case of diseases treatment efficacy. For example lipid changes in secretory cells can alter exocytosis with implications for neuronal communication while cell membrane composition can effect permeability to pharmaceuticals, along with mechanical properties that may influence cell mobility, and in the case of cancer cells, metastatic potential.

Secondary ion mass spectrometry (SIMS) provides unique opportunities for analysing cells with both high spatial resolution, surface sensitivity and chemical specificity. The ability to characterise the cellular membrane lipid composition has been greatly enhanced by the introduction of gas cluster ion beams (e.g. $(CO_2)_{6k}^+$) that provide increased secondary ion signals for intact biomolecules. The presentation will illustrate the capabilities of GCIB-SIMS analysis of cell samples in studies of cancer and Parkinson's disease and highlight the complementarity of multimodal analysis using fluorescence microscopy and electrochemical analysis approaches.

7:00pm **BI-WeE1-5 Self-assembling Antimicrobial Peptide Coatings for Prevention of Infections**, ***Zhou Ye,*** The University of Hong Kong

Antimicrobial peptides (AMPs) are promising candidates as antimicrobial coatings due to its broad-spectrum activity, low bacterial resistance, and good biocompatibility. Previous works have explored the coatings of AMPs by covalent immobilizations or the introduction of an intermediate layer/nanoparticles. However, the fabrication processes were costly, complicated, and non-versatile, which limited the clinical applications. We have developed new strategies to form strong physical coatings by self-assembled AMPs and determined the significant roles of self-assembly and secondary structures in forming the AMP coatings. The dominant interactions between self-assembled AMPs and the substrate surfaces were studied to be hydrogen bonding, instead of electrostatic forces. We also correlated the self-assembly dynamics of AMPs to the antimicrobial activity by comparing L and D enantiomers of one model AMP, GL13K in aqueous solutions or interacting with phospholipid double layers and other bacterial envelope components. With the understanding of the coating mechanisms, we applied the self-assembled GL13K peptide coatings on various substrates, such as etched titanium implants, dentin, enamel, or mineralized collagen/hydroxyapatite. The AMP coatings presented excellent antimicrobial activities in mouse models or on *ex vivo* tooth with potentials in clinical settings.

7:40pm **BI-WeE1-7 Development of a Process for Flame Retardant Coating of Textiles with Bio-Based Anchor Peptides**, ***Rahel Krause,*** *I. Bettermann, R. Paul, T. Gries,* Institut of Textiltechnik of RWTH Aachen University, Germany*; M. Nöth, L. Feng, U. Schwaneberg,* Institute of Biotechnology of RWTH Aachen University, Germany*; C. Hummelsheim, L. Kampas,* Klevers GmbH & Co. KG, Germany

The fire protection of materials has an important role in our everyday life and covers a highly diverse spectrum of substances, materials and fields of application. Important fields of application for fire protection, especially in public areas, are construction and transport, electronic devices, furnishings and textiles (e.g. applications for occupational safety, carpets, curtains, upholstery, insulation and technical applications in outdoor areas). The efficient and durable finishing of the materials with flame retardant additives is crucial to ensure effective fire protection. Many of the flame retardant additives currently used are based on halogens, bromides, chlorides, phosphates or antimony. However, these flame retardants are harmful to the environment and/or health. Therefore, the use of these flame retardants is already being restricted by EU directives (e.g. REACH regulation) and it is foreseeable that they will be further restricted in the future. To keep up with this development, innovative and sustainable solutions must be developed in the short term. The amount of flame retardant additives that are harmful to the environment and health must be reduced. In the medium term these harmful additives must be completely replaced by sustainable flame retardant additives that are not harmful to the environment and health. This paper describes research results to reduce the amount of additives in the short term.

In order to reduce the amount of additives used, an innovative refinement process is being developed. In a first step, the flame retardant additives are combined with bio-based adhesion promoters (anchor peptides). Anchor peptides bind with high selectivity, binding strength and occupancy density to a broad portfolio of materials (e.g. synthetic polymers, metals, ceramics, natural materials) and enable the finishing of the materials with a broad spectrum of functional units (e.g. flame retardant additives). Material functionalisation by anchor peptides is energy-efficient and resource-saving at room temperature in aqueous solution and is scalable in its production.

Based on these developments, in this paper, a finishing process is presented with which flame retardant textiles can be equipped with bio-based anchor peptides. A requirements outline for the new finishing process is described. Established processes (e.g. foulard, coating machine, roller application) are compared with each other and evaluated with regard to the requirements and their suitability. The most suitable process is then designed and a laboratory scale as well as an industry scale concept are presented.

8:00pm **BI-WeE1-8 Impact of UV-C Exposure on Single-use Mask Integrity for Reuse to Address PPE Shortages Within At-Risk Communities**, *S. Ananthakrishnan, E. Rhoades-Clark, V. Mitchell,* ***Heather Canavan****,* University of New Mexico

The COVID-19 pandemic significantly disrupted supply chains in the global economy. The American Indian populations of the Southwest United States have been particularly hard hit, due to multi-generational housing, lack of access to running water, and insufficient protective personal equipment (PPE). The limited availability and price of commercial N-95 masks led the public to reuse their masks, rather than treating them as single-use equipment. In turn, this led users to experiment on their own on how best to sterilize their masks for repeated usage, including both low-tech (UV sterilization with sunlight, washing with detergent or soap) and high-tech approaches (fogging with disinfectant, UV lamp apparatuses, etc.). Recommendations by the CDC and mask manufacturers often contradicted each other on best practices; the CDC recommended reusing single-use masks through UV sterilization, while mask manufacturers indicated otherwise due to concern of material degradation. We hypothesized that exposure to UV-C (254 nm) would damage non-woven single-use mask fibers such as those found in N-95, KN-95, and surgical masks due to spalling, which would lead to increased mask pore size, and therefore decreased filtration efficiency. However, woven fabric masks should not suffer spalling from UV exposure due to a difference in the fabrication of the fibers. To test our hypothesis, we subjected N-95, KN-95, surgical, and fabric masks to UV-C exposure for appropriate time scales (0-24 hours) to simulate repeated exposure for sterilization followed by a close inspection of layers using Scanning electron Microscopy (SEM). We examined the pore sizes and density, fiber integrity, and surface morphology of the layers that comprised each mask and compared the resulting damage. We found that limited exposure to UV-C (15 minutes/4 exposure cycles) would result in significant damage to all the masks except for fabric masks. As the acute experiences of the pandemic have receded, the cost and availability of PPE have normalized, and well-funded populations have ceased to focus on the issue of sterilization and reuse. However, members of at-risk communities are still grappling with ways to best adapt to supply chain shortages and masking needs. Therefore, these results will be of interest to those seeking to understand how UV-C affects the reusability of various mask types.

Biomaterial Surfaces & Interfaces

Room Naupaka Salon 4 - Session BI-ThM1

Bacteria Biomaterial Interactions

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

8:00am **BI-ThM1-1 Why Mechanism Matters for Antimicrobial Biomaterials**, ***Bryan Coad,*** The University of Adelaide, Australia **INVITED**

Many new antimicrobial biomaterials and coatings are being developed to address the need for implantable medical devices that prevent infections. Researchers, medical device manufacturers, and regulatory bodies are all interested in seeing "promising research results" translated to clinical use, but how are these claims evaluated? What experimental evidence is needed to improve translation of *in vitro* antimicrobial materials to the clinic?

It is illustrative to explore one of the most interesting classes of antimicrobial biomaterials: those which purport to have a contact-inhibition mechanism of action. These materials present covalently attached antimicrobial molecules from surfaces which inhibit or kill adhering microbes on contact. On the one hand, this surface design could open up new paradigms for antimicrobial therapy by virtue of making implants with potentially long-lasting antimicrobial activity. On the other hand, questions about whether these materials release antimicrobials and whether their surface activity would be nullified by protein fouling deserve serious investigation.

This presentation will delve into these issues by explaining why mechanism matters for antimicrobial biomaterials. It can also be viewed generally by biomaterials researchers from other fields who design bioactive surfaces using covalent surface immobilization and face questions about how to interpret the results of cellular assays. The presentation aims to raise awareness of the potential role of confirmation bias in antimicrobial susceptibility experiments which could lead researchers to unwittingly misinterpret antimicrobial mechanisms of action. It discusses ways to avoid this pitfall by proposing a methodological approach that emphasizes the importance of surface analysis. It is hoped that greater awareness of these issues will help "promising" *in vitro* antimicrobial surface technologies to have greater uptake in animal studies and clinical trials.

8:40am **BI-ThM1-3 High Throughput Screening for Antibiotics Using Droplet Microarrays**, *W. Lei, A. Popova,* Karlsruhe Institute of Technology (KIT), Germany; ***Michael Grunze,*** Max Planck Institute for Medical Research, Germany; *P. Levkin,* Karlsruhe Institute of Technology (KIT), Germany

Multidrug-resistant (MDR) bacteria are a severe threat to public health and it is urgent to identify novel antibacterial compounds or pathogen-specific mixtures of antibiotics. In a first paper we reported the application of Droplet Microarrays developed by *Aquarray* as a cost effective high-throughput screening method for the evaluation of drug resistance of *Pseudomonas aeruginosa*, an opportunistic human pathogen /1/. The DMA consists of an array of hydrophilic spots divided by superhydrophobic borders to generate arrays of hundreds of nanoliter-sized droplets containing bacteria and different drugs for screening applications. A novel simple colorimetric readout method compatible with the nanoliter size of the droplets was established. Furthermore, the drug-resistance of *Pseudomonas aeruginosa* 49, a multi-resistant strain from an environmental isolate was investigated by screening of a small library containing 18 antibiotics. We were able to show that our methology reproduces the data obtained with a 96 well microplate.

In the study reported here the search for antibiotic compounds was extended to screen over 2000 compounds for their antimicrobial properties against carbapenem-resistant *Klebsiella pneumoniae* and and methicillin resistant *Staphylococcus aureus* (MRSA). A fast single-step detection method measured the inhibitory effect of the compounds on bacterial growth on the whole array. Six hit compounds, including coumarins and structurally simplified estrogen analogs are identified in the primary screening and validated with minimum inhibition concentration assay for their antibacterial effect. This study demonstrates that the DMA-based high-throughput screening system identifies potential antibiotics from novel synthetic compound libraries, and thus offering opportunities for development of new treatments against multidrug-resistant bacteria.Due to its simplicity, the method is suitable for rapid screens in personalized medicine. If time allows, further examples of rapid screens using DMA´s which habe been designed for personalized medicine will be presented.

/1/ W. Lei, K. Demir, J. Overhage, M. Grunze, T. Schwartz, P. A. Levkin, Droplet-Microarray: Miniaturized Platform for High-Throughput Screening of Antimicrobial Compounds, Adv. Biosys., 2000073 (1-9), 2020

9:00am **BI-ThM1-4 NAP-XPS Studies of a *Pseudomonas fluorescens* Bacterial Cell-Envelope and Other Biomaterial Surfaces**, ***Paul Dietrich,*** SPECS Surface Nano Analysis GmbH, Germany; *N. Wasio, J. Hilton,* SPECS-TII, Inc.; *A. Thissen,* SPECS Surface Nano Analysis GmbH, Germany

Bacterial interactions with the environment are based on processes involving their cell-envelope. Thus, techniques that can analyze their surface chemistry are attractive tools for providing an improved understanding of bacterial interactions. One of these tools is x-ray photoelectron spectroscopy (XPS) with an estimated information depth of <10 nm for Al Kα-excitation. XPS-analyses of bacteria have been performed for several decades on freeze-dried specimens to be compatible with the classical ultra-high vacuum conditions needed. A limitation of these studies has been that the freeze-drying method may collapse the cell structure. However, recent developments in XPS enable the analysis of biological samples at near ambient pressure (NAP-XPS) or as frozen hydrated specimens (cryo-XPS) in vacuum. In this talk, we present the analysis of bacterial samples from a reference strain of the Gram-negative bacterium *Pseudomonas fluorescens* using both techniques. XPS results and reference data from the bacterial strain are provided, and we propose to use planktonic cells of this strain (DSM 50090) as a reference material for surface chemical analysis of such bacterial systems. Further selected examples of NAP-XPS on other biomaterial surfaces will be presented.

9:20am **BI-ThM1-5 Tungsten Disulfide Bio-Nanofabrication Using Dissimilatory Metal-Reducing Bacteria *Shewanella oneidensis* MR-1**, ***Lauren Brady,*** *J. Rees, S. Sawyer,* Rensselaer Polytechnic Institute

A type of bacteria known as dissimilatory metal-reducing bacteria (DMRB) can "breathe metals" to reduce heavy metal ions as part of their metabolic process. *Shewanella oneidensis* MR-1 is a type of DMRB that was first discovered in Lake Oneida, New York for its ability to reduce manganese, but has since been shown to reduce a variety of different electron acceptors including Fe(III), As(V), Cr(VI), and thiosulfate. In previous literature, *S. oneidensis* MR-1 has been grown anaerobically in media enhanced with sulfur and metal ions in order to produce several types of nanoparticles, including molybdenum disulfide, zinc sulfide, and cadmium sulfide.

This work presents the bacterial nanofabrication of tungsten disulfide particles using *S. oneidensis* MR-1. Bacterial nanofabrication synthesis, where the bacteria is the main catalyst for the production of nanomaterials, has numerous advantages compared to traditional chemical synthesis methods in that it can be conducted at room temperature and requires less chemical reagents in the reaction. One of the primary reasons to produce nanomaterials using bacteria is for a low resource input alternative to material fabrication for use in electronic devices.

Tungsten disulfide (WS_2) is a two-dimensional transition metal dichalcogenide which has a bandgap transition from an indirect bandgap in the bulk material to a direct bandgap in its monolayer form. Tungsten disulfide has a range of potential applications including photodetection, biosensing, and chemical catalysis. These applications are further enhanced when control can be exerted over crystal growth and thickness.

An anaerobic batch culture of *S. oneidensis* MR-1 was incubated at room temperature in the presence of tungsten trioxide, resulting in the production of tungsten disulfide crystalline nanostructures of varied shapes and size. Several characterization techniques were employed to identify the material, including scanning electron microscopy, transmission electron microscopy, Raman spectroscopy, absorbance spectroscopy and X-ray diffraction. In addition to confirming that tungsten disulfide can be produced by *Shewanella* bacteria, the data collected using these methods provide insight on the size, morphology, and photoresponse of nanoparticles generated this way.

9:40am **BI-ThM1-6 Nanoengineered Implant Surfaces with Enhanced Osteogenic and Antimicrobial Properties**, *R. Shahbazian ,* University of Illinois Chicago; ***Tolou Shokuhfar,*** University of Illinois at Chicago

The lack of osseointegration and implant-related infections are two major complications leading to failure of dental and orthopedic implants. Therefore, the development of effective implant surfaces able to display enhanced osteogenic activity and antimicrobial properties is required. This work aims to study the ability of bio-functionalized TiO2 Nanotube engineered surfaces to induce osseointegration, and concomitantly, to

avoid infection. TiO2 NTs were bio-functionalized with calcium (Ca), phosphorous (P) and zinc (Zn), by reversepolarization anodization. Morphological and topographical features of NTs were observed through scanning electron microscopy (SEM), while surface chemistry was investigated by X-ray photoelectron spectroscopy (XPS). Biocompatibility studies were conducted with MG-63 and human mesenchymal stem cells (hMSCs) through MTT assay. Furthermore, cell morphology and cytoskeleton organization were observed by SEM and laser scanning confocal microscopy (LSCM). The osteoblastic differentiation capacity of hMSCs was studied by real-time PCR, as well as their angiogenesis ability by measuring the total release of vascular endothelial growth factor (VEGF). Finally, viability of Staphylococcus aureus (S. aureus) was assessed by live/dead bacterial viability assay. Results show that bio-functionalized TiO2 nanotubular surfaces are biocompatible and modulated cell morphology. In particular, NTs enriched with Ca, P, and Zn, induced to significantly up-regulated levels of bone morphogenetic protein 2 (BMP-2) and osteopontin (OPN) genes of hMSCs, when compared to conventional NTs. TiO2 nanotubular surfaces induced hMSCs to release a higher amount of VEGF, and significantly reduced the bacterial viability, both when compared to adequate Ti controls. Osseointegration and antibacterial properties have been shown in vitro and in vivo to improve when implants have modified surfaces that have biomimetic nanostructures designed to mimic and interact with biological structures on the nano-scale. Pre-clinical evaluations show that TiO2 nanotubes (TNT), produced by anodization, on Ti6Al4V surfaces positively enhance the rate at which osseointegration occurs and TNT nano-texturization enhances the antibacterial properties of the implant surface. In conclusion, the superimposition of TiO2 nanotubular- textured surfaces and their enrichment with Ca, P, and Zn, is a highly promising approach for the development of novel bio-selective and multifunctional implant surfaces able to improve osseointegration and avoid infection.

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