

Biomaterial Interfaces

Room 117 - Session BI-MoA

Microbes at Interfaces

Moderators: Axel Rosenhahn, Ruhr-University Bochum, Rong Yang, Cornell University

1:30pm BI-MoA-1 The Role of Surface/Interface Phenomena in The Antibacterial Action of Nano- and Microscale Gallium Oxide and Gallium Hydroxide, Yuri M. Strzhemechny, D. Johnson, J. Brannon, Texas Christian University; P. Ahluwalia, Harmony School of Innovation Fort Worth; T. McHenry, M. Smit, D. Kalluholematham, Texas Christian University; Z. Rabine, Wayne State University; P. Jadhka, Tarrant County College Northwest

Worldwide trend of increasing antibiotic resistance has spun interest in alternative antibacterial agents such as metal oxide particles. Whereas the antibacterial action of many such oxides is well established, the mechanism of this activity is largely unknown. Cytotoxicity could be mediated via such mechanisms as production of reactive oxygen species, release of toxic cations, interactions disrupting cell walls and causing osmotic stress. Targeted applications of oxide antibacterials are also hindered by a lack of understanding of the role and nature of the local bacterial environment in mediating/hindering antibacterial interactions. Surface defects in nano- and micro-crystals strongly affect performance of metal oxides in applications, necessitating elucidation and control of those defects. The beta polymorph of gallium oxide (β -Ga₂O₃) in nano- and microcrystalline form is attracting a significant research interest due to potential applications in biological therapeutics, optoelectronics, and catalysis. In our studies, we employ nano- and microparticles of β -Ga₂O₃ synthesized via a simple bottom-up hydrothermal method, which yields, as a first step, a GaOOH precursor, which then undergoes calcination to bear the final product. Such growth method, through variation of growth parameters, allows production of particles with tunable morphology and controllable relative abundances of surfaces with desired polarities. To address the nature of interactions between β -Ga₂O₃ and GaOOH crystal surfaces, cellular membranes and bacterial growth media we perform detailed systematic studies of the optoelectronic and physicochemical properties of both GaOOH and β -Ga₂O₃ samples and then evaluate their impact of on the antibacterial action of these samples. We are especially interested in the influence of surface defects and particle morphologies on the antimicrobial efficiency of the studied oxides. The biological assays with *Escherichia coli* and *Staphylococcus aureus* are used to examine the antibacterial action and also to run pre- and post-assay comparative studies of the oxide specimens themselves. For the latter we employ a variety of characterization techniques, such as electron microscopy, energy-dispersive X-ray spectroscopy, time and wavelength dependent surface photovoltage, temperature-dependent photoluminescence spectroscopy, Fourier-transform infrared spectroscopy, etc. We find in our samples a strong correlation between the growth parameters, particle morphologies, crystal surface characteristics, and antimicrobial properties.

1:45pm BI-MoA-2 Microbially-Induced Corrosion of Synthetic Granite and Dike Glass by Paenibacillus Polymyxa SCE2 Using ToF-SIMS, Gabriel Parker, University of Illinois Chicago; A. Plymale, J. Hager, J. Dhas, Z. Zhu, PNNL; L. Hanley, University of Illinois - Chicago; X. Yu, ORNL

Microbially-induced corrosion (MIC) is an important topic that focuses on material degradations over extended periods. Soil microbes are often associated with MIC of foreign objects interacting within the rhizosphere. *Paenibacillus polymyxa* SCE2 is a facultative anaerobic microbe found in soil. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a powerful mass spectrometry imaging technique that provides insights into the surface characteristics by its spectral, two-dimensional (2D) imaging, and depth profiling capabilities of biointerphases. Herein, ToF-SIMS was used to detect surface changes on synthetic granite and synthetic dike glass coupons that were treated with *Paenibacillus polymyxa* SCE2 over extensive periods of time. Confocal laser scanning microscopy (CLSM) was used to verify bacterial coverage across the granite and dike glass surfaces after three and seven months' growth in a static cell. ToF-SIMS spectral analysis shows detection of glass component related ions, such as m/z 276.84, 380.81, 418.77, 607.62, 693.48, and 721.51. Also, ions that are indicative of extracellular polymeric substance (EPS) components were observed, such as m/z 241.22, 255.23, 269.25, 297.15, 311.16, and 325.18. Clusters of unidentified peaks are detected in the biofilm treated glass coupons, which are speculated to reflect EPS components as they incorporated into the

glass to form colonies, resulting in MIC. ToF-SIMS analysis results show that granite glass has more "corrosion related" peaks than the dike glass. The observed surface compositional and morphological differences between the two types of glass are hypothesized to be related to the glass surface hydrophobicity and ultimately its affinity to biofilms.

2:00pm BI-MoA-3 Titanium Oxynitride Thin Films Deposited in a Custom-Built ALD Reactor with Real-Time Residual Gas Probing to Enhance the Photocatalytic Activity of Polymethylmethacrylate (PMMA) and Induce Antimicrobial Activity on Its Surface, Harshdeep Bhatia, University of Illinois - Chicago; B. Nagay, V. Barão, University of Campinas (UNICAMP), Brazil; G. Jursich, C. Sukotjo, C. Takoudis, University of Illinois - Chicago

Titanium oxynitride is a novel material researched for its visible light photocatalytic activity (PCA). Polymethylmethacrylate (PMMA) is a common organic biomaterial used in the dental industry which is used in conditions where they are highly susceptible to microbial biofilm formation. In this study, Atomic Layer Deposition (ALD) was used to deposit a thin layer of titanium oxynitride on 3D printed PMMA disks. The reactor used tetrakis(dimethylamino) titanium(IV) as the titanium precursor and ammonia (NH₃) as the nitriding agent; the system was custom-designed and controlled using a Python program. A common single board computer controlled the solenoid valves connected to the pneumatic valves. The Python program could deposit a single or a bilayer of oxide and nitride and control the ALD valves manually. A Chemical Vapor Deposition (CVD) mode is also possible for faster film growth. In addition, a residual gas analyzer (RGA) was connected to the downstream to study the outgassed products from the reactor in real time. The operating pressure for the ALD reactor was 1.6 Torr while for the RGA was 1x10⁻⁶ Torr. After deposition, thickness was measured on the reference silicon sample using Spectroscopic Ellipsometry while the composition was measured using X-ray Photoelectron Spectroscopy. Post-deposition, PCA of the PMMA surfaces were also determined using the degradation of a standard methylene blue solution after irradiation by three different light sources. A similar approach was used to test the antibacterial and antifungal effect under light irradiation. Cell viability tests were also performed to test the biocompatibility of the film.

2:15pm BI-MoA-4 Isolation and Identification of Copper-Tolerant Fouling Communities, Sara Tuck, M. Kardish, US Naval Research Laboratory; B. Orihuela, Duke University; G. Vora, US Naval Research Laboratory; K. Franz, Duke University; K. Fears, US Naval Research Laboratory

Biofouling, the accumulation of unwanted organisms on submerged assets, is a fundamental problem in maritime transport and human health. Biofouling build-up increases fuel consumption, exhaust emissions, and operational costs in addition to facilitating the transfer of environmental and pathogenic bacteria from one location to another. Conventionally, biofouling is inhibited by the application of antifouling coatings, the most popular of which are copper based. In biological systems, copper is tightly regulated and, in an attempt, to exploit this, antifouling coatings contain up to 75% CuO by weight. Despite these high loadings, the efficacy of these coatings is rapidly declining with the emergence and spread of copper-tolerant species. Microbial communities resistant to copper have been found to form mature biofilms on these coatings, which could be altering the interfacial properties to create more favorable conditions for the settlement of a broader biofouling community. To gain an understanding of the mechanisms responsible for the loss of antifouling performance, coated and uncoated polyvinyl chloride panels were submerged at estuarine and marine field test sites and microbial communities were isolated. Biofouling communities were harvested from three test sites and individual species were cultured, isolated, and identified. Copper tolerance was assessed by re-exposing these cells to copper-containing coatings and traditional broth microdilutions.

2:30pm BI-MoA-5 Surface-Cleaning Mechanisms Used by Acorn Barnacles (Amphibalanus Amphitrite) to Prevent Microbial Colonization at Their Adhesive Interface, Q. Lu, E. McGhee, W. Hervey, D. Leary, C. Spillmann, Kenan Fears, US Naval Research Laboratory

Barnacles have long been admired, or hated, for their robust underwater adhesives that allow them to tenaciously adhere to surfaces and endure harsh marine environments. Previously, we revealed that acorn barnacles evolved a remarkable surface cleaning fluid that removes microorganisms ahead of expansion of their base and the deposition of a new ring of cement. This process involves the secretion of a lipidaceous material that phase separates in seawater, into a phenolic laden gelatinous phase that presents a phase rich in lipids and reactive oxygen species to the seawater interface. Biofilms in close proximity to this material rapidly oxidize and lift

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off the surface as the secretion advances. Proteomics analysis of the adhesive interface reveals the presence of a haloperoxidase, a class of enzyme known to participate in the innate immune response of a wide variety of organisms, which converts chlorine ions to hypochlorite ions (bleach) in the presence of hydrogen peroxide. We performed agar well diffusion assays to assess the susceptibility of marine and terrestrial micro-organisms to hydrogen peroxide with and without the presence of a haloperoxidase. While yeast cells (*P. larentii*) were shown to be quite susceptible to hypochlorite ions at low doses, the oxidation of biofilms of marine bacterium (*V. natrigens* and *M. atlanticus*) by hypochlorite ions did not result in significant cell death. Confocal microscopy of different barnacle species revealed that the surface cleaning mechanisms employed by acorn barnacles is not ubiquitous to all barnacle species. Microbial colonies were present in the basal region of barnacle species in which the secretion of this surface cleaning fluid was not observed, in stark contrast to barnacle with this surface cleaning fluid. Knowledge of these processes could enhance the efficiency of synthetic underwater adhesives and lead to novel environmentally benign antifouling technologies.

2:45pm **BI-MoA-6 BioSAXS - a Tool to Enrich and De-Risk Antimicrobial Drug Development**, *Axel Rosenhahn*, *C. Rumancev*, *A. Gräfenstein*, Ruhr University Bochum, Germany; *K. Hilpert*, University of London, UK **INVITED**

Antimicrobial resistance is a worldwide threat to modern health care. Low-profit margin and high risk of cross-resistance resulted in a loss of interest in big pharma, contributing to the increasing threat. Strategies to address the problem are starting to emerge. Novel antimicrobial compounds with novel modes of action are especially valued because they have a lower risk of cross-resistance. Up to now determining the mode of action has been very time and resource consuming and will be performed once drug candidates were already progressed in preclinical development. BioSAXS is emerging as a new method to test up to thousands of compounds to classify them into groups based on ultra-structural changes that correlate to their modes of action. BioSAXS experiments in gram-negative *E. coli* and a correlation with transmission electron microscopy have demonstrated that using conventional and experimental antimicrobials a classification of compounds according to their mode of action was possible. Further work showed that the approach can also be used for gram-positive bacteria (*S. aureus*) and the effects of novel antimicrobial peptides on both types of bacteria were studied. Preliminary experiments will be presented that show that BioSAXS can also be used to classify antifungal drugs, demonstrated on *Candida albicans*. The data will be discussed in view of the perspective of how BioSAXS can accelerate and enrich the discovery of antimicrobial compounds from screening projects with a novel mode of action and hence de-risk the development of urgently needed antimicrobial drugs.

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