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.

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