Biomaterial Interfaces
Room 101A - Session BI+AS-MoM

Biomolecules and Cells at Interfaces
Moderator: Joe Baio, Oregon State University

8:20am **BI+AS-MoM1 Probing the Selectivity of Antimicrobial Peptides to Cell Membranes by Sum Frequency Generation Spectroscopy**, ***Thaddeus Golbek,*** Oregon State University*; J. Franz,* Department of Molecular Spectroscopy, Max Planck Institute for Polymer Research, Mainz, Germany*; J.E. Fowler, K.F. Schilke,* Oregon State University*; T. Weidner,* Department of Molecular Spectroscopy, Max Planck Institute for Polymer Research, Mainz, Germany*; J.E. Baio,* Oregon State University

Cationic amphiphilic peptides have been engineered to target both Gram-positive and Gram-negative bacteria while avoiding lysis of other cell types. However, the exact mechanism of how these peptides target, bind, and disrupt bacterial cell membranes is not understood. One specific peptide that has been shown to selectively capture bacteria is WLBU2 (sequence RRWVRRVRRWVRRVVRVVRRWVRR). It has been suggested that WLBU2 activity stems from the fact that when interacting with bacterial cell membranes the peptide assumes an α-helical structure and inserts itself into the membrane. To test this hypothesis, we applied sum frequency generation (SFG) spectroscopy and surface tensiometry to probe the peptide-lipid-air interface and identify the structure and monitor the interaction of WLBU2 with two model lipid monolayers that mimic mammalian and bacterial cell membranes. Model mammalian cell membranes were built upon zwitterionic 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) lipids while bacterial cell membranes were constructed with negatively charged 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (DMPG) lipids. The rate at which the surface pressure reaches equilibrium is 4.3 times faster for WLBU2 interacting with the negatively charged DMPG lipid monolayer than with the zwitterionic DPPC lipid monolayer. This observed WLBU2 binding affinity preference to negatively charged membranes is likely due to electrostatic interactions between positively charged amino acids within the peptide and negatively charged lipids. SFG studies at the peptide-lipid-air interface demonstrate that binding of WLBU2 induces increased lipid monolayer order. A larger increase in acyl chain order from 2.2 to 3.4 determined by the ratio of the CD_3 symmetric (2075 cm^{-1}) and CD_2 symmetric (2100 cm^{-1}) peak amplitudes suggest that WLBU2 is found at the surface of the zwitterionic phospholipid monolayer and not inserted. The amide I region SFG spectrum of WLBU2 interacting with the zwitterionic lipid monolayer shows two peaks near 1642 cm^{-1} and 1678 cm^{-1} indicative of an inactive β-sheet structure. A peak near 1651 cm^{-1} for WLBU2 interacting with negatively charged lipids is assigned to an active α-helix structure. Altogether, we demonstrate that WLBU2 shows a higher binding affinity to bacterial cell membranes and is in an active α-helix structure, alternatively in the presence of mammalian cell membranes in an inert β-sheet structure.

8:40am **BI+AS-MoM2 Bacterial Adhesion to Immobilized Liquid Layers under Dynamic Conditions**, ***Caitlin Howell,*** University of Maine*; Y. Kovalenko, I. Sotiri,* Harvard University*; J. Overton,* University of Maine*; J. Aizenberg,* Harvard University

Immobilized liquid (IL) layers are an emerging technology shown to prevent bacterial biofouling of surfaces. In this work, we show how in one class of IL-coated materials, infused polymers, bacterial adhesion can be strongly dependent on growth conditions. Samples grown with *Escherichia coli* under more relevant dynamic conditions showed significantly increased colony-forming unit counts compared to the same system grown under static conditions. Direct visualization of the surfaces suggested that this was due to a disturbance of the IL layer when exposed to shaking conditions, which allowed more bacteria to remain on the surface after an initial rinse. However, no incorporation of the bacteria into the oil layer was detected. To further investigate the extent of this adhesion, we used sequential removal cycles to gauge the relative adhesion strength of the remaining surface-bound *E. coli*. Through this method, we found that despite no initial difference in adherent CFUs compared to control samples with no IL layers, IL samples did reduce overall adhesion of the bacteria even after incubation under dynamic conditions. Further tests on a flagella-deficient strain of *E. coli* revealed that while flagella play a significant role in adhesion to IL layers, they are not the sole adhesion mechanism for this species. Finally, tests on two other clinically-relevant species of bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, using similar methods revealed clear species-dependent differences in adhesion after growth under dynamic conditions. These results shed new light on the interaction of bacteria with IL layers, and demonstrate the importance of both relevant growth conditions and thorough analysis to obtaining clear results in these systems.

9:00am **BI+AS-MoM3 Nitric Oxide Materials—An Approach to Creating More Hemocompatible Medical Device Coatings**, ***Hitesh Handa,*** University of Georgia **INVITED**

Blood/material interaction is critical to the success of implantable medical devices, ranging from simple catheters, stents and grafts, to complex extracorporeal artificial organs which are used in thousands of patients every day. There are two major limiting factors to clinical application of blood contacting materials: 1) platelet activation leading to thrombosis, and 2) infection. Despite a thorough understanding of the mechanisms of blood–surface interactions, and decades of bioengineering research effort, the ideal non-thrombogenic prosthetic surface remains an unsolved problem. One approach to improving the hemocompatibility of blood-contacting devices is to develop materials that release nitric oxide (NO), a known potent inhibitor of platelet adhesion/activation and also an antimicrobial agent. Healthy endothelial cells exhibit a NO flux of 0.5-4x10^{-10} mol cm^{-2} min^{-1}, and materials that mimic this NO release are expected to have similar anti-thrombotic properties. I will discuss the potential of incorporating NO donor molecules such as diazeniumdiolates or S-nitrosothiols (RSNOs) into various polymers, and their hemocompatibility and antibacterial properties in short-term (4 h) and long-term (7 d) animal models.

9:40am **BI+AS-MoM5 Why do Bacteria Stick to Some Surfaces and Not Others? Characterisation of the Behaviour of Motile Bacteria at and Above the Surface of Materials**, *A.L. Hook, A. Carabelli, N.A. Russell, P. Williams,* ***Morgan Alexander,*** The University of Nottingham, UK

High throughput screening has been used to discover a novel class of polymers with resistance to bacterial attachment and subsequent biofilm formation.[1,2] Physicochemical descriptions of the surfaces have to date been found insufficient to predict the wide range of bacterial attachment across these diverse polymer libraries, and cannot offer an explanation of the controlling phenomena. Whilst perhaps disappointing for the physical sciences, the life sciences are replete with information on how bacteria respond to their local environment, with chemotaxis being one of the most readily observed processes. Unsurprisingly, microorganisms cannot be approximated to inert spheres and rods as they possess surface responsive appendages such as flagella, which enable them to swim, pili that confer twitching motility and fimbriae that mediate surface attachment. These in turn are coupled to sophisticated signal transduction mechanisms that facilitate integration of multiple local environmental parameters at both single cell and population levels. Many of these sensory systems are postulated to contribute to surface sensing. As an example of the complexity of these processes, the opportunistic pathogen *Pseudomonasaeruginosa* has over 60 two-component sensor kinase response regulator systems involved in environmental adaptation.

We believe that bacterial decision-making is key to determining whether a surface is colonised or not. I will present the early results from our optical microscopy investigations of how individual bacterial cells respond to surfaces. We have developed a novel microscope that collects temporal 3D information on cell position using both holography and remote scanning microscopy. [3] Simultaneously surface tracking can be achieved using DIC, TIRF and TIR microscopy. This allows us to track not only the motion of single cells at the surface, but also their approach to and behaviour after contact with the surface.

We will combine these findings with our existing understanding of the surface chemistry-attachment relationships achieved for certain subsets of materials and attachment regimes,[4,5] with chemical analysis of the in situ surface to build a complete description of this complex biointerface and the response of bacteria to it. This information is crucial in determining how bacteria behave with respect to defined surfaces and has important implications for the prevention of device centred infections.

1. Hook et al. Nature Biotechnology 2012

2. Hook et al. Advanced Materials 2013

3. Botcherby et al. Circulation Research 2013

4. Epa et al. Advanced Functional Materials 2014

5. Sanni et al. Advanced Healthcare Materials 2015

10:00am **BI+AS-MoM6 Probing Adhesion of Marine Biofilm Formers by Microfluidics**, *K. Nolte,* Ruhr-University Bochum, Germany*; M. Alles, M.P. Arpa-Sancet, C. Christophis,* University of Heidelberg, Germany*;* ***Axel Rosenhahn,*** Ruhr-University Bochum, Germany

When new Materials are developed to control and influence Biofilm growth, the ability of biofilm formers to firmly adhere to the coatings is one key property. Several techniques have been developed in the past to probe attachment strength of cells [1]. Especially microfluidic test systems [2] offer several advantages, such as small sample area, small amounts of target species, and high throughput. We developed microfluidic assays that allow to test bacterial and diatom adhesion on coatings [3,4]. Cells are driven through a microchannel at a precisely controlled flow rate and at a constant concentration and both, accumulation and detachment can be monitored by video microscopy. Using self-assembled monolayers as model surfaces we were able to show that the adhesion strength correlates with the accumulation dynamics if an appropriate shear stress is applied. Based on this finding, a parallelized microfluidic system has been developed that allows simultaneous, comparative testing of materials. Due to the modular assembly of the setup, not only model surfaces and thin organic films, but also practical coatings can be analyzed.

[1] L. Marcotte, M. Tabrizian, ITBM-RBM 2008, 29, 77

[2] D.P. Bakker, A. van der Plaats, G.J. Verkerke, H.J. Busscher, H.C. van der Mei, Appl. Envir. Microbiol. 2003, 69(10), 6280

[3] M. Arpa-Sancet, C. Christophis, A. Rosenhahn, Biointerphases 2012, 7, 2

[4] M. Alles, A. Rosenhahn, Biofouling. 2015, 31, 469–480.

10:40am **BI+AS-MoM8 Protein Control of Materials Nucleation Probed by Sum Frequency Generation***,* ***Tobias Weidner,*** Max Planck Institute for Polymer Research, Mainz, Germany

Proteins can act as Nature's engineers at interfaces and manipulate both hard and soft tissue – they shape biominerals, manipulate cell membranes and nucleate materials. Despite the apparent importance for engineers working in the fields of surface engineering, drug delivery, or diagnostics, the molecular mechanisms dictating interfacial protein action have remained largely elusive. Our goal is to probe the structure and structural dynamics of such active proteins – in action at the surface.

Mineral proteins have the ability to control and steer the growth of hard tissue by binding specific mineral facets and precipitating silica and phosphates. They control the intricate mineral morphologies found in diatom cell walls, mollusk nacre, but also human teeth and bone. Inspired by diatom silification we used amphiphilic peptides consisting of leucine and lysine (LK peptides) to investigate biomineralization at surfaces. These peptides can adopt helical or beta sheet structures at the air-water interface. Upon addition of a silica precursor we obtained freestanding peptide-silica hybrid sheets with thicknesses of ~4 nm. We have followed the biomineral composition and interactions between peptides and silica at different early stages of biomineralization using a combination of surface spectroscopies and microscopies. Our experimental findings were complemented with molecular dynamics simulations. Our data shows that the peptide surface folding dictates the nanometer scale morphology of the prepared silica film.[1]

A particularly fascinating example of protein driven nucleation and phase transitions are ice-nucleating proteins. These proteins are used by specific bacteria to attack plants and cause frost damage by growing ice crystals at temperatures that would otherwise not allow ice formation. A recent survey by the NASA found large amounts of biological ice nucleators in the troposphere where they may affect global precipitation patterns. We have followed the interaction of freeze proteins with surrounding water molecules – how specialized protein sites lock water molecules in place and manipulate the flow of energy within the surrounding layers of water.[2]

1 H. Lutz, V. Jaeger, R. Berger, M. Bonn, J. Pfaendtner, T. Weidner

Biomimetic growth of ultrathin silica sheets using artificial amphiphilic peptides

Advanced Materials Interfaces, 1500282 (2015).

2 R. Pandey, K. Usui, R. A. Livingstone, S. A. Fischer, J. Pfaendtner, E. H. G. Backus, Y. Nagata, J. Fröhlich-Nowoisky, L. Schmüser, S. Mauri, J. F. Scheel, D. A. Knopf, U. Pöschl, M. Bonn, T. Weidner

Ice-nucleating bacteria control the order and dynamics of interfacial water

Science Advances, 2 (2016).

11:20am **BI+AS-MoM10 Regulation of Cell Surface Access and Mechanics at the Interface***,* ***Jennifer Curtis,*** *P. Chang, W. Wei, L.T. McLane,* Georgia Institute of Technology*; J. Scrimgeour,* Clarkson University **INVITED**

A polymer brush-like structure decorates the cell surface of many cell types ranging from fibroblasts to mesenchymal stem cells to cancer cells. This sugar-rich pericellular matrix (PCM) plays physical and chemical roles in biological processes ranging from brain plasticity, to adhesion dependent processes like cell migration, to the onset of cancer. Here I will report on biophysical and mechanical assays that characterize the structure of the pericellular matrix and its impact on the transport of nanoparticles and molecules to the cell surface. Further, I will present compelling quantitative evidence that hyaluronan polymer expression at the cell-substrate interface tunes cell adhesion strength, working in concert with focal adhesions.

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