Tuesday Morning, November 14, 2006

Biomaterial Interfaces
Room 2001 - Session BI1-TuM

Microbe-Surface Interactions
Moderator: G.P. Lopez, University of New Mexico

8:00am **BI1-TuM1 Getting to the Root of Bacterial Hair**, ***R.J. Emerson***, *T.A. Camesano, N.A. Burnham,* Worcester Polytechnic Institute

Many bacteria use their extracellular polymers to attach to surfaces, leading to such phenomena as biofouling and biofilm-initiated infections of medical devices. One method of determining the physicochemical and physicomechanical properties of these hairlike layers is through the acquisition and interpretation of AFM force curves. Most previous researchers have defined the point of zero separation as the cell wall, and assumed the constant compliance region of the force curve to be representative of that location. With the data processed in this manner, the force at the "wall" has been used to calculate the equilibrium length of the brush, as well as the grafting density of the polymers at the point of zero separation. Here, we show: 1) that the constant compliance region frequently occurs in the middle of the layers, 2) how the layer thicknesses may be more accurately quantified, 3) a quantitative method of establishing the position of the "roots of the hairs" at the cell wall, i.e., the true zero of the separation axis, and 4) the more appropriate use of the mesh density of the polymer brush, in lieu of the polymer grafting density. For the specific case of Pseudomonas aeruginosa, a commonly found bacterium in both hospital settings and the natural environment, we have identified two discrete layers, of equilibrium thicknesses 160 ± 8 and 1700 ± 400 nm, with respective mesh densities of (1.9 ± 0.1) x 10@super 26@ m@super -3@ and (7 ± 2) x 10@super 22@ m@super -3@. AFM force curves of bacterial exopolymers can now be meaningfully interpreted.

8:20am **BI1-TuM2 Antifouling Surface Treatments for Microfluidic Applications**, ***N.E. Chang***, *M.H. Lean, S.J. Limb,* Palo Alto Research Center, Inc.

To remediate bioparticle losses from adhesion to microfluidic device surfaces, we have implemented polyethylene glycol (PEG)-type coatings by self-assembled monolayer and plasma-polymerizing deposition techniques. A variety of substrates representative of device materials in contact with bioparticle solutions were subjected to static microbial adhesion tests. We saw significant reduction in B. thuringiensis adhesion for both types of PEG coatings and reduction in B. globigii adhesion for the plasma-polymerized coating. Furthermore, we have demonstrated that both PEG-type coatings on MEMS traveling-wave arrays are effective at reducing adhesion to polystyrene beads as well as both Bacillus species. Results from both experiments have provided groundwork for in-situ experiments in flow environments for assessment of bioparticle recovery and adhesion to electrically conductive parallel flow plates. Spectrophotometry is used to gauge bioparticle concentration before and after circulation within the flow chamber. Open circuit voltage is monitored to investigate electrical behavior affected by bioparticle adhesion to the flow plates. Comparison of both measurements for parallel flow plates of different conductivities with and without our plasma-polymerized PEG coating will help corroborate and predict the degree to which bioparticle losses can be minimized.

8:40am **BI1-TuM3 Biofilm Formation on Biomaterials Implant Surfaces**, ***H. Busscher***, University Medical Center Groningen, The Netherlands **INVITED**

Biofilm formation on biomaterials implant surfaces and subsequent infectious complications are a frequent reason for failure of many biomedical devices, such as total hip arthroplasties, vascular catheters and urinary catheters. The development of a biofilm is initiated by the formation of a conditioning film of adsorbed macromolecules, such as proteins, followed by adhesion of microorganisms, where after they grow and anchor through secretion of extracellular polymeric substances. Adhesion of microorganisms is influenced by the physico-chemical properties of the biomaterial surface. Positively charge materials stimulates bacterial adhesion, but prevent growth of adhering bacteria. The use of low surface free energy materials did not always reduce in vitro adhesion of bacteria, but has been found beneficial in in vivo applications where fluctuating shear forces prevail, like on intra-oral devices and urine catheters. Polymer brushes have shown a very high reduction in in vitro adhesion of great variety of microorganisms, and AFM demonstrated weak adhesive forces. However, for clinical application, the long term stability of many types of polymer brushes is still a limiting factor.

9:20am **BI1-TuM5 Minimising of Biofilm Formation by Surfaces Coated with Fish Proteins**, ***P. Kingshott***, University of Aarhus, Denmark; *N. Bernbom,* Danish Institute for Fisheries Research; *L.M. Meyer, S. Xu, F. Besenbacher,* University of Aarhus, Denmark; *L. Gram,* Danish Institute for Fisheries Research

No surface exists that can prevent biofilm formation. Many surface treatments (e.g. use of antimicrobial agents) may help reduce biofilm formation, but the potential exists for developing bacterial resistance, and loss of function by agents getting depleted or covered up by organic matter (e.g. proteins). New surfaces that provide a barrier to bacterial attachment, but are not inhibitory and toxic, are highly desirable. We have recently discovered that attachment of bacteria found in the food industry is reduced by several orders of magnitude by coatings of an extract made from fish, compared to other organic layers (e.g. from meat, broth, milk). The coating does not per se inhibit microbial growth, the effect lasts up to 48 hours and the treatment is non-toxic. The coatings are equally effective against a range of bacteria persistent on food processing equipment including Ps. fluorescens strain AH2, E. coli strain MG1655, Vibrio anguillarum strain 90-11-287, and Aeromonas salmonicida strain Jno 3175/88. The extracts can be coated on metals (stainless steel) or polymers (polystyrene) and still be effective. Our aim is to use surface techniques (XPS, AFM, ToF-SIMS, surface-MALDI) to characterise the fish extract adlayer, and find out which component(s) reduce bacterial adhesion. Initial results demonstrate that the adlayer is protein in nature and that all surfaces adsorb high levels. Surface-MALDI shows that there are proteins of common molecular weight that adsorb to all surfaces tested, and these most likely play a role in the antifouling effect. The surface protein patterns are different to other conditioning media (e.g. TSB, chicken extract). Results will also be presented for the fish extract fractionated by chromatography and coated on surfaces, aimed at identifying more specifically the protein(s) involved. The results are discussed in terms of the potential antifouling mechanisms, such "steric-repulsive" effects or are the proteins antimicrobial.

9:40am **BI1-TuM6 Microfluidic Devices That Capture Bacteria for Growth and Kill Analysis**, ***M. Lochhead***, Accelr8 Technology Corporation

New clinical diagnostic instruments to address critical public health issues depend on a fundamental understanding of bacterial adhesion and growth at solid surfaces and the ability to control these processes. New technologies that decrease the time required for accurate identification and antibiotic susceptibility profiling of pneumonia-causing bacteria in intensive care units are one important example. Current methods require extended bacterial culture time and often force pre-mature clinical empiric antibiotic therapies, reducing positive patient outcomes and contributing to the emergence of resistant strains. Further, susceptibility testing from culture-based methods present a population-derived result and frequently can obscure the effects of minority sub-populations. We have developed a new microfluidics device that quantitatively tracks bacterial real-time growth rate and allows antibiotic susceptibility monitoring, reducing the overall time of a bacteria antibiotic challenge to less than 6 hours. Device performance with several bacterial strains and samples, and polymer coating surface characterization using protein, cell, bacterial and several surface analytical (e.g., XPS) measurements will be described. Application of this tool in the development of a fully integrated microfluidic system for automated bacterial growth and kill analysis will be demonstrated.

10:40am **BI1-TuM9 Vapor Phase Photografting of Antimicrobial Polymer Coatings**, ***T.P. Martin***, *K.K. Gleason,* Massachusetts Institute of Technology

An all-dry vapor phase photografting process is employed to covalently attach antimicrobial polymer coatings to polymeric substrates, including both spun-cast PMMA layers and nylon fabric. Antimicrobial fabrics are of interest in military applications, such as biowarfare protection, self-decontaminating fabrics, undergarments for long term use on deployment, as well as civilian uses such as textiles in hospital environments, including bedding, draping, and scrubs. Antimicrobial coatings are also of interest for use on medical devices. Microbial colonization of medical devices is associated with significant expense and mortality. A permanent, durable non-leaching antimicrobial surface is important for both of these applications. A fabric coating must survive many wash cycles, and a medical device coating must not detach in the body. Existing strategies for imparting antimicrobial properties to surfaces commonly employ an antimicrobial agent, such as silver ions or antibiotic drugs, which leaches out from the bulk material. However, the time of effectiveness will be limited as the agent will eventually be exhausted. Additionally, the use of antibiotic drugs with medical devices has the potential to promote drug

resistant microbes, whereas the class of antimicrobial polymers under examination here has not been shown to do so. In this work, the type-II photoinitiator benzophenone is used in conjunction with methacrylate- or styrene- based monomer containing a tertiary amino group with pKa > 9. The amino group is protonated to the active cationic state at physiological conditions. The coatings were tested according to ASTM E2149-01, and demonstrated good antimicrobial activity against e. coli, with a 99.999% reduction (5 log) in viable bacteria. In addition, the coatings show no zone of inhibition against e. coli, indicating the active polymer is not leaching from the surface. Finally, the active surface is maintained through various durability testing.

11:00am **BI1-TuM10 Biocompatible Ag-doped Carbon Coatings with Biocidal Effect***, **J.L. Endrino**,* Lawrence Berkeley National Laboratory*; M. Allen,* SUNY Upstate Medical University*; R. Escobar Galindo, J.M. Albella,* Instituto de Ciencias de Materiales de Madrid, Spain*; A. Anders,* Lawrence Berkeley National Laboratory

Medical implants can be an appropriate solution to many health problems, however, any time a foreign device is implanted into one's body there is a high risk for infection. For this reason, there is an increasing interest in the development of multifunctional coatings that can provide a highly biocompatible surface while protecting from infection threats. Recent studies have shown the possibility of incorporating antibacterial elements into carbon coatings with the idea of providing medical implants with necessary infection resistance. In general, diamond-like-carbon (DLC) coatings have excellent tissue biocompatibilities and high chemical inertness; consequently they are suitable as a matrix material that can embed different drug release substances. In this interdisciplinary study, Ag/C nanocomposite coatings have been prepared using two different deposition techniques: i) dual cathode pulsed cathodic-arc (PCA) from silver and graphite cathodes and ii) Ag cathodic-arc in reactive methane (CH4) atmosphere. The silver to carbon ratio in the samples was varied from 0 to 0.1 and was controlled by adjusting the relative arc pulse frequencies of the silver and carbon pulsed sources. Chemical composition, microstructure and mechanical properties of the samples were analyzed using glow discharge optical spectroscopy (GDOES), scanning electron microscopy (SEM) and the nanoindentation technique. Morphological examinations of samples deposited on 24 well tissue culture plates confirmed that there were no adverse effects in the production of specific osteoblast proteins on low content silver-doped carbon coatings.

11:20am **BI1-TuM11 Multi-functional Ag/Plasma Polymer Coatings for Antibacterial Biomaterial Surfaces***, **D.J. Balazs**, D. Hegemann, M. Heuberger,* EMPA Materials Science and Technology, Switzerland

In the past decades much research pertaining to biomaterials surface modification was centred on modifying chemistry or wettability as a strategy to manage interactions with the surrounding environment. New demands for biomaterials surface enhancements have evolved to include multi-functionality and controlled-release. Low pressure plasma techniques represent a unique opportunity to develop tailor-made surfaces with one-step processing in the framework of an eco-friendly process. Thus, low pressure plasma processing is used to develop multi-functional coatings for smart biomaterials applications. Coatings that combine properties such as enhanced cell growth, improved wound healing, and bacterial infection prevention are the focus of the work presented. The deposition of multi-functional silver/plasma polymer (Ag/pp) nano-composites, consisting of nano-scaled Ag clusters embedded within a plasma-polymer matrix is described. Multi-functional Ag/Acrylic acid coatings are deposited to combine anti-bacterial and enhanced cell adhesion properties for wound healing applications. Likewise, Ag/amino-hydrocarbon coatings can be used to attract proteins important to cell growth and simultaneously prevent bacterial colonization. Data is reported pertaining to bacterial adhesion testing in both Gram negative and positive culture environments. A key aspect to the development of controlled-release biomaterials is the ability to characterize release or adsorption kinetics in a precise manner. In order to achieve this, we are using a novel in situ analysis technique that is equally sensitive as other commonly used in situ methods (ca 1 ng/cm@super 2@), and has the benefit that is significantly cheaper and faster. Using plasma deposition a multi-layer biosensor is built, permitting the quantification of both release and adsorption kinetics of the multi-functional coatings. The kinetics of silver release and protein adsorption to Ag/pp is also described.

11:40am **BI1-TuM12 Detection and Mapping of Individual Adhesins on Living Bacteria using Atomic Force Microscopy***, **V. Dupres**,* Université Catholique de Louvain, Belgium*; F.D. Menozzi,* Institut Pasteur de Lille, France*; Y.F. Dufrene,* Université Catholique de Louvain, Belgium

Bacterial pathogens adhere to host cells via the specific interaction between surface proteins, referred to as adhesins, and host surface receptors. Although much progress has been made in the identification and characterization of adhesins borne by pathogenic bacteria, the molecular details underlying such interactions remain largely unknown owing to the lack of appropriate probing techniques. In this work, we used atomic force microscopy (AFM) with tips bearing biologically active molecules to measure the specific binding forces of individual adhesins and to map their distribution on the surface of living bacteria.@footnote 1@ First, we determined the adhesion forces between the heparin-binding haemagglutinin adhesin (HBHA) produced by Mycobacterium tuberculosis, the etiologic agent of tuberculosis, and heparin, used as a model receptor. We obtained a bimodal distribution of the adhesion forces with average forces of 50 pN and 117 pN, which could be attributed to one and two binding events between HBHA and heparin. Both the adhesion frequency and adhesion force increased with contact time, indicating that the HBHA-heparin complex is formed via multiple intermolecular bridges. We then mapped the spatial distribution of single HBHA molecules on the surface of living mycobacteria using heparin-modified tips. Strikingly, adhesion events were observed in about half of the locations and were concentrated into nanodomains over the mycobacterial surface. @FootnoteText@ @footnote 1@ Dupres V., Menozzi F.D., Locht C., Clare B.H., Abbott N.L., Cuenot S., Bompard C., Raze D. and Dufrene Y.F., Nat. Methods, 2, 515-520 (2005).

12:00pm **BI1-TuM13 Dynamic Interactions of the Streptococcal C5a Peptidase with Fibronectin***, **J.R. Hull**, G. Tamura, D.G. Castner,* University of Washington

Group B Streptococci (GBS) are a leading cause of sepsis and meningitis in newborns, and an emerging cause of serious bacterial infections in immunocompromised adults and the elderly. The streptococcal C5a peptidase (ScpB) of GBS is found in virtually all clinical isolates of GBS. ScpB inhibits neutrophil chemotaxis by enzymatically cleaving the complement component C5a. ScpB is a known Fibronectin (Fn) adhesin; however, it only binds to immobilized Fn and not soluble Fn. Therefore, it is unknown whether or not ScpB binds to a conformational determinate of Fn or multiple adjacent Fn molecules. For this study, Surface Plasmon Resonance (SPR) was used to investigate the interactions of soluble Fn with Scp bound the sensor surface. Scp was made as a GST fusion and bound to the sensor surface through a self-assembled monolayer of glutathione. It was found that binding of soluble Fn with Scp is significantly lower than the binding of Scp to immobilized Fn (KD ~4.0 nM). Next, immobilized Fn was probed with Scp attached to an AFM tip via the bifunctional crosslinker pyridyldithio poly(ethylene glycol) succinimidylpropionate. Each step of the tip functionalization was verified by X-ray photoelectron spectroscopy, and static secondary ion mass spectrometry. It was found that the interaction force between immobilized Fn and Scp is roughly 100 pN. With this force value, a force map was made showing where the Fn/ScpB interactions occurred.

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