Biofilms and Biofouling: Marine Medical Energy Focus Topic
Room: 23 - Session MB+BI-ThM

Biofilms and Biofouling in Medicine
Moderator: L. Hanley, University of Illinois at Chicago

8:20am **MB+BI-ThM2 Simple and Versatile Approaches to Design Oligoethylene Based Self-Assembled Monolayers using Thiolene Chemistry on Different Metal Oxide Surfaces: Impact on Protein Adsorption**, ***A. Galtayries***, *A. Dellinger*, Chimie ParisTech (ENSCP), France, *V. Semetey*, Institut Curie, France

The control of biomolecules adsorption (such as proteins) and other microorganisms is of high interest for various fields of biotechnology, such as bioanalytics, cell biology, tissue engineering and biomaterials. An efficient method to control adsorption includes the use of well-defined oligo(ethylene glycol)-terminated self-assembled monolayer (SAM). However, multiple processing steps are often required to prepare SAM onto substrate. To address these problems of surface modification, we have developed a simple method using the powerful thiolene reaction to prepare self-assembled monolayer on different metal oxides to control bioadhesion on surfaces starting from commercially available building blocks, and taking the advantage of the photoreaction to easily create adhesive and anti-adhesive patterns.

Such well-controlled grafting strategy has been applied to different metal oxides: from silicon substrates for the methodology set-up to model metal oxides formed on biocompatible metals and alloys. The obtained films are robust; the process is low-cost, simple, and efficient.

Surface characterization as X-ray Photoelectron Spectroscopy (XPS), Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) and Infra-Red Surface Spectroscopy (ATR-IRFT or PM-IRRAS) were used to check the surface composition at different steps of the reactions: the thiolene reaction, the PEG grafting, as well as after interaction with protein solutions: albumin for preliminary tests, and fibronectin, an adhesive protein present in the extra-cellular matrix. In addition to quantitative information, obtained by XPS, about the oxide composition and thickness at the different steps, and qualitative information, obtained by XPS and ToF-SIMS, surface patterning could be emphasized with ToF-SIMS chemical surface imaging.

8:40am **MB+BI-ThM3 Antibacterial Studies of Plasma Polymerised Cineole Thin Films**, ***A. Pegalajar-Jurado***, Swinburne University of Technology, Australia, *C.D. Easton*, CSIRO Materials Science and Engineering, Australia, *S.L. McArthur*, Swinburne University of Technology, Australia

Essential oils such as tea tree oil are known for their antibacterial properties. They have been used extensively as effective topical antimicrobial agents and are active against a wide range of micro-organisms. Traditionally, the antimicrobial properties of tea tree oil has been linked to the constituents of the oil including terpinen-4-ol and 1,8-cineole. To translate these antimicrobial properties into medical devices, methods for incorporating or coating materials are required. Recently, thin polymer films from terpinen-4-ol have been fabricated using plasma polymerisation. Initial data suggests that some antimicrobial activity was maintained. While plasma polymer films of 1,8-cineole have been fabricated previously, little focus has been placed on the antibacterial activity of this constituent.

The activity of 1,8-cineole against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) was evaluated based on the time-kill approach. Several concentrations of the oil (0.05 %, 0.2 %, 0.3 % and 0.5 % v/v) were added to broth media containing $3x10^8$ CFU/mL of each bacterium. The optical density of the resulting solutions was measured at intervals over 24-hrs to monitor bacterial growth rates. The results demonstrated that concentrations above 0.35% v/v gave a 90% kill rate in the gram-negative *E. coli* after 8-hrs. Interestingly, the oil was less effective against the gram-positive *S. aureus*, producing a lower kill rate and rapid growth recovery in a 24-hour period.

Plasma polymers thin films of 1,8-cineole and 1,7-octadiene (hydrophobic control) were prepared using a stainless steel plasma reactor as described previously, while uncoated glass slides were used as a hydrophilic, positive control. Samples were then exposed to broth media containing $3x10^8$ CFU/mL of each bacteriumto determine the effects of the coatings on bacterial attachment and growth. In this work, bacterial studies of the coating behaviour have focused on the attachment of *S.aureus* and *E. coli*, and biofilm formation of *E. coli*. Comparison of the plasma polymerised cineole (ppCineole) coating with the two controls demonstrated a reduction in bacterial attachments over 18-hrs. The reduction in bacterial attachment was more obvious in the case of *E.coli* in comparison with *S. aureus*. There were not significant differences between plasma polymerised cineole and octadiene in the inhibition of *E.coli* attachment and growth over a period of 18-hours. However, the biofilm studies indicated that only the 1,8-cineole film demonstrated antimicrobial behaviour over a period of time of 5 days.

Keywords: plasma polymerisation, antibacterial activity, cineole

9:00am **MB+BI-ThM4 Robustness Analysis of Biofilm Antibiotic Tolerance**, ***R.P. Carlson***, Montana State University **INVITED**

Biofilms are ubiquitous and are thought to be involved in more than half of all medical infections. Even after decades of investigation, the *in vivo* efficacy of many antimicrobial strategies is still debated suggesting a need for better understanding of biofilm antimicrobial tolerances. The robustness of biofilm antibiotic tolerance to medically and industrially relevant culturing perturbations was characterized. By definition, robust systems return similar, predictable responses when perturbed while non-robust systems return very different and potentially unpredictable responses. Biofilm antibiotic tolerance was found to vary in unpredictable manners based on modest perturbations in culturing conditions. The predictability of an antibiotic tolerance response is essential to developing, testing, and employing antimicrobial strategies. The collective data represents both challenges and opportunities for the rational design of anti-biofilm strategies. The data demonstrates that biofilms can be countered effectively with some antibiotics if the appropriate environmental conditions are applied however, if inappropriate conditions are applied, the efficacy of the treatment can be negated. The results indicate it is essential to evaluate antimicrobial strategies over a range of perturbations relevant to the targeted application so accurate predictions regarding efficacy can be made. In addition, the highly dynamic antibiotic tolerance responses observed here may explain why some current antimicrobial strategies occasionally fail.

9:40am **MB+BI-ThM6 Analysis of Force Curves of *Pseudomonas Aeruginosa* obtained by Atomic Force Microscopy**, *E.V. Anderson, R.L. Gaddis, T.A. Camesano,* ***N.A. Burnham***, Worcester Polytechnic Institute

Pseudomonas aeruginosa is extremely harmful to immune-compromised individuals. An atomic force microscope (AFM) can be used to measure the forces between the AFM tip and the bacterial exopolymers, with which the bacteria attach themselves to surfaces. These forces are characterized with a model that is a function of brush (exopolymer) layer thickness, probe radius, temperature, separation distance, and a molecular volume. Initial experiments with limited data sets are consistent with expected brush thicknesses of a few hundred nanometers. In order to progress – now with rigor – we have just developed a high throughput method for the analysis of force curves on the exopolymers of *P. aeruginosa* [1]. The above-described model is only valid for the region where the tip is in contact with the exopolymers, yet is not perturbing the bacterial membrane. MatLab code was written to determine the location of this region in each force curve, crop the curve to that region, and apply the force model in order to obtain parameters of the exopolymers. The standard deviation of the mean and Chauvenet's Criterion are then applied to the results of sets of one-hundred force curves to increase measurement precision and objectively remove outliers. This procedure removes user subjectivity in cropping, fitting, and outlier removal, decreases analysis time by two orders of magnitude, and increases the precision of fitted results by a factor of ten (for one-hundred curves), which is necessary for demonstrating the statistical significance of our data.

[1] Anderson et al., to be submitted May 2012.

10:40am **MB+BI-ThM9 Light and Dark Biocidal Activity of Conjugated Polyelectrolytes**, ***K. Schanze***, University of Florida, *D.G. Whitten, T. Corbitt, E. Ji, D. Dascier,* University of New Mexico, *A. Parthasarathy, S. Goswami*, University of Florida **INVITED**

Cationic conjugated polyelectrolytes (CPEs) are semiconducting organic polymers that contain ionic solubilizing groups. These polymers are soluble in water and they self-assemble into colloidal nanostructures in solution and layer-by-layer films at interfaces. CPEs interact strongly with bacteria in solution and on coated interfaces, and under short wavelength visible or near UV light irradition they exhibit strong biocidal activity. Mechanistic studies using photophysics and cell live/dead assays find that the CPEs efficiently sensitize the production of singlet oxygen, and this is believed to be at least partially responsible for the light-activated biocidal activity. Structure-property studies find that specific hydrophobic CPEs exhibit strong dark biocidal activity. Studies with membrane models indicate that the dark active CPEs exhibit a strong tendency to interact with and disrupt the cell membrane structure.

11:20am **MB+BI-ThM11 Mechanisms of Antimicrobial Activity of Quaternary Ammonium Compounds in Solution and Immobilized on a Surface**, ***H.C. Van der Mei***, University Medical Center Groningen, The Netherlands

Quaternary ammonium compounds (QAC) are potent cationic antimicrobials used in everyday consumer products like contact lens solutions and mouthrinses as well as in numerous industrial processes, like water purification. Unlike the case for many antibiotics, the development of bacterial resistance against QACs is considered unlikely, although *Pseudomonas aeruginosa* strains isolated from contact lens cases have been shown to possess resistance against QACs. The first step in the antimicrobial action of QACs is the approach of the QAC molecule towards the bacterial cell surface. This is mediated by hydrophobic and electrostatic attractions between positively charged QAC molecules and the negatively charged bacterial cell surface. Upon their subsequent adsorption, QAC molecules replace Ca^{2+} or Mg^{2+} ions from the cytoplasmic membrane in order to maintain charge neutrality in the membrane. The replacement of Ca^{2+} and Mg^{2+} ions by QACs destabilizes the intracellular matrix of a bacterium, as the hydrophobic tail interdigitates into the hydrophobic bacterial membrane causing leakage of intracellular fluid and loss of turgor pressure. Antimicrobial efficacy of QACs remains preserved when QAC molecules are immobilized on a substratum surface. It is difficult to envisage how immobilized QAC molecules can exert the same mechanism of antimicrobial activity as do QACs in solution. Immobilized QACs, especially after adsorption of a protein film as developing rapidly in the human body, are strongly hindered in their search for heterogeneously distributed negative charges on bacterial cell surfaces which is crucial for their efficacy in solution. Hence, it has been often hypothesized that QAC molecules immobilized to a substratum surface possess other generic mechanisms of action than QACs in solution, but these have never been elucidated. Immobilized QACs do not cause directly visual membrane damage. Instead, the strong adhesion forces arising from immobilized QACs enter bacterial adhesion forces into the lethal regime, i.e. where the stress exerted on the bacterial cell membrane is causing killing.

11:40am **MB+BI-ThM12 Combinatorial Discovery of Materials That Resist Bacterial Adhesion**, ***A.L. Hook***, *C. Chang, J. Yang,* University of Nottingham, UK, *R. Langer, D.G. Anderson,* Massachusetts Institute of Technology, *S. Atkinson, P. Williams, M.C. Davies, M.R. Alexander,* University of Nottingham, UK

Biofilm formation leads to a 1000 times increase in antibiotic tolerance compared with planktonic bacteria and is associated with 80% of hospital acquired infections, resulting in $3.0 billion in excess health-care costs each year in the U.S alone. Thus, new materials that prevent biofilm formation would offer enormous benefits to the health industry and improve patient welfare. However, the limited understanding of bacteria-material interactions restricts the rational design of such materials. Polymer microarrays are emerging as a key enabling technology for the discovery of new biomaterials[1] and have been utilised to identify novel polymers that resist bacterial attachment.

Polymer microarrays were formed as previously described.[2] This platform enabled a large combinatorial space to be rapidly screened by a biological assay to identify new materials that fulfil a given performance criterion.[3] In the present study a bacterial attachment assay was developed using green fluorescing protein (GFP) tagged bacterial strains (*Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli)* where the attachment of bacteria to each material was quantified by measuring the fluorescnce after incubation for 72 h.

Due to the large combinatorial chemical space available to the polymer micoarray format a strategy was devised to rapidly identify the optimal polymer composition that resists bacterial adhesion. This utilised a multi-generation microarray approach where the 'hits' from one array feed into the design of a subsequent array. Initially, an array was formed from 22 monomers with varied chemistry that were mixed to form 488 unique material compositions. Hit compositions were chosen from this array to produce a focussed second generation array containing unique materials where the hit compositions were varied incrementally. The resulting hit compositions were all amphiphilic containing both hydrophobic and hydrophilic moieties.

A methodology has been developed to screen the vast combinatorial chemical space within polymer chemistry for optimised compositions that produce novel materials with inherent resistance to bacterial adhesion. Key to this approach was the use of multi-generation microarrays.

References

[1] Hook AL, Anderson DG, Langer R, Williams P, Davies MC, Alexander MR. Biomaterials 2010;31:187-198.

[2] Anderson DG, Levenberg S, Langer R. Nature Biotechnology 2004;22:863-866.

[3] Mei Y, Saha K, Bogatyrev SR, Yang J, Hook AL, Kalcioglu ZI, Cho SW, Mitalipova M, Pyzocha N, Rojas F, Van Vliet KJ, Davies MC, Alexander MR, Langer R, Jaenisch R, Anderson DG. Nature Materials 2010;9:768-778.

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