Biomaterial Interfaces Division
Room: 108 - Session BI-WeM

Cells at Interfaces

Moderator: M.R. Alexander, University of Nottingham, UK

8:00am **BI-WeM1 Real Time Analysis of Polymer Film Integrity Upon Exposure to Bacteria and Aqueous Medium**, ***D.E. Barlow***, *J.C. Biffinger*, Naval Research Laboratory, *E.R. Petersen*, Nova Research, Inc., *J.N. Russell, P.E. Pehrsson*, Naval Research Laboratory, *W.J. Goodson*, Air Force Resarch Laboratory

Polymer coatings are of great importance for protecting and imparting specific properties at the surfaces of man-made structures, but can be affected in many ways by the natural environments they must withstand. We have studied the effects of aqueous medium exposure and biofilm formation on antistatic polyurethane coatings in real time using in situ ATR-FTIR. The results show that the coatings are susceptible to water permeation and swelling, and deuterium exchange was also shown to occur within the films upon D_2O exposure. When exposed to *Pseudomonas fluorescens* in M9 minimal medium, the coating interface became compromised as the pyruvate carbon source was depleted. Reasons for these changes will be discussed, including the role of water permeation and the potential for the bacteria to use the coating as a carbon source. While ATR-FTIR has been used in the past to study biofilm growth, these results also demonstrate the effectiveness of the method for assessing substrate impact, an often overlooked factor.

8:20am **BI-WeM2 Early Stages of Bacterial Biofilm Formation – A Numerical Study of Bioadhesion on Biomaterials**, ***D. Siegismund***, *A. Schroeter, S. Schuster, M. Rettenmayr*, Friedrich Schiller University Jena, Germany

Biomaterials for implant purposes are increasingly applied in modern medicine e.g. to recover human body functions or for tissue substitution in general. Infections of these implants, called Biomaterial-centered infections (BCI), are among the fundamental challenges in biomaterials science. They are primarily initiated by adhesion of bacteria on the biomaterial's surface. The subsequent formation of a bacterial biofilm requires a total implant replacement in the majority of cases.

The adhesion of bacteria is thus the first crucial step for biofilm formation that is only incompletely understood. Interactions of bacteria with the surface are controlled by surface properties such as surface energy, surface chemistry and topography.

In the present work, a model for bacterial adhesion is introduced that describes the early stages of biofilm formation as a function of the surface properties. A two-dimensional Cellular Automaton (CA) / Finite Difference (FD) adsorption model is combined with the predictions of the extended DLVO (Derjaguin, Landau, Verwey, Overbeek) theory that accounts for the interaction energies between the bacteria and the material's surface. The model describes the mass transport of bacteria in an aqueous solution towards the material's surface and the adsorption and desorption process, depending on the surface properties.

The adhesion process of different human pathogenic bacteria (*Enterococcus faecalis, Staphylococcus aureus, Escherichia coli*) on different biomaterial surfaces (titanium, stainless steel, polyethylene, polymethylmethacrylate, polytetrafluoroethylene) has been simulated. Results are the surface coverage with bacteria and, where applicable, clustering of the bacteria due to their migration on the surface.

Excellent agreement with experimental findings from the literature and own adhesion experiments concerning the kinetics of the adsorption process is found. In addition, a realistic bond strengthening mechanism of bacteria on surfaces, as described in the literature, is reproduced by the model. By using a spatial pattern analysis of our own experimental data we show that physical processes occurring during initial stages of the adhesion process are essentially correctly incorporated in the model.

8:40am **BI-WeM3 A Library of Polymer Gradients for Understanding Bacteria-Material Interactions**, ***A.L. Hook***, *J. Yang, C.-Y. Chang*, University of Nottingham, UK, *D.G. Anderson, R. Langer*, 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 for biomedical devices that prevent biofilm formation would offer enormous benefits to the health industry and improve patient welfare. However, our current understanding of bacteria-material interactions limits scope for rational design of such materials. Polymer microarrays are emerging as a key enabling technology for the discovery of new biomaterials.[1] A method for forming polymer microarrays has been developed using contact printing to deposit nanolitre volumes of premixed acrylate monomer and initiator to defined locations on a poly(HEMA) coated glass slide with UV photo-initiation.[2] This platform enables a large combinatorial space to be rapidly screened by a biological assay to identify new materials that fulfil a given performance criterion.[3] A library of polymer gradients that enables the systematic investigation of biology-material interactions can be created by producing polymers from monomers mixed at hundreds of different concentrations. Utilising a high throughput surface characterisation approach the surface chemical and physical properties of each material can be characterised and related to the biological performance.[4] We have developed a high throughput bacterial attachment assay based on three pathogens (*Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli)* expressing green fluorescent protein, which is compatible with the polymer microarray format. This study provides novel insights into the bacteria-material interactions, highlighting chemical moieties that both support and resist bacterial attachment. Specifically, superior efficiacy to prevent bacterial attachment has been demonstrated for hydrophobic moieties on a polyacrylate backbone that contains weakly polar ester groups, which represent an amphiphillic chemical nature.

[1] Hook, A. L. et al., High throughput methods applied in biomaterial development and discovery. *Biomaterials* **31** (2), 187 (2010).

[2] Anderson, D. G., Levenberg, S., and Langer, R., Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells. *Nature Biotechnology* **22** (7), 863 (2004).

[3] Mei, Y. et al., Combinatorial development of biomaterials for clonal growth of human pluripotent stem cells. *Nature Materials* **9** (9), 768 (2010).

[4] Urquhart, A. J. et al., High throughput surface characterisation of a combinatorial material library. *Advanced Materials* **19** (18), 2486 (2007).

9:00am **BI-WeM4 Developing Tools to Observe Microbial Metabolic Exchange in 2D and 3D**, *J. Watrous*, University of California, San Diego, *T. Alexandrov*, University of Bremen, Germany, *W.-T. Liu, A. Lamsa, D. Gonzalez, N. Bandeira, M. Hamby, R. Kersten, K. Pogliano, B. Moore*, ***P.C. Dorrestein***, University of California, San Diego **INVITED**

From the early days of bacterial culturing over a century ago, microbiologists have known that microorganisms respond to their surroundings. Unicellular organisms rely on natural product mediated metabolic exchange to adapt to environmental stresses, sense colony density, and form biofilms. However, studies of the chemistry and phenotypes that correspond to signaling behavior have largely been disconnected and measured indirectly. To connect the chemistry and phenotypes, imaging mass spectrometry (IMS) methodologies are developed to observe metabolic exchange mediated within pair-wise interactions and microbial communities in two- and three dimensions. IMS provides the ability to correlate the presence of metabolites to phenotypic changes and to detect new biological phenotypes. Many of such phenotypes cannot be observed by the naked eye.

9:40am **BI-WeM6 Analysis of Cancer Cell Lines with ToF-SIMS and PCA**, ***M. Robinson***, University of Washington, *F. Morrish, D. Hockenberry*, Fred Hutchinson Cancer Research Center, *L.J. Gamble*, University of Washington

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) has been increasingly utilized for examining biological samples including biomaterials, cells, and tissues. The primary advantage of this MS technique is that it produces a chemical map of a sample, which includes hundreds of peaks that are detected in parallel. The advent of cluster ion sources has allowed the detection of many high mass lipid species that can be used to characterize biological surfaces [1]. In conjunction with principal component analysis (PCA), we use ToF-SIMS to determine differences in the chemical makeup of the outer membrane of two different cancer cell lines: MDA-231 and MCF-7 cells. There is similar work currently being done that uses Ultra Performance Liquid Chromatography-MS and gene sequencing to start characterizing lipid membrane metabolism in breast cancer tumor tissue [2]. The separation of the two cell lines across PC1 can be clearly seen in Figure 1. The entirety of the loads for PC1 can be seen in Figure 2, with cholesterol strongly loading towards the MDA-231 cells and many diacylglycerol (DAG) species loading towards the MCF-7 cells. Key differences were found in the levels of certain lipid constituents of the cell

membrane, which may play a role in the ability of one cell type to be more drug resistant than the other. There are a variety of lipid components that have similar trends which are not discussed in this abstract but may play an important role in understanding this system.

This work is the foundation for future studies using human tumor biopsy samples that will help elucidate the link between fatty acid composition within a tumor and the potential drug resistance of that tumor.

10:40am **BI-WeM9 Engineering Stem Cell Differentiation via Material Properties**, ***T. McDevitt***, Georgia Institute of Technology **INVITED**

Stem cell differentiation is sensitive to a variety of global and local environmental cues that impact cell fate decisions. Pluripotent stem cells (i.e. ESCs & iPSCs) are capable of recapitulating many aspects of early development and can serve as a robust cell source for the development of cell-based diagnostics and regenerative medicine therapies. ESCs are commonly differentiated as three-dimensional multi-cellular aggregates referred to as "embryoid bodies" (EBs), because of their ability to mimic the early morphogenic transformation of pluripotent cells into derivatives of the three germ lineages (ecto-, endo-, and mesoderm). In order to better understand and ultimately control ESC morphogenesis, w e have focused on systematically engineering biochemical and biophysical parameters of the 3D EB microenvironment via the integration of different biomaterials and examining the emergent results on stem cell differentiation. Microparticles (MPs) of varying size (1-20 µm) and chemistries (i.e. PLGA, agarose, gelatin) were incorporated within 3D stem cell aggregates in a dose-dependent manner (~1 particle / 10 cells) without adversely affecting cell viability. Interestingly, the mere presence of relatively small numbers of different types of materials alone could modulate stem cell phenotype as evidenced by gene expression profiling and immunophenotype analyses. D elivery of morphogenic factors, such as retinoic acid (RA), bone morphogenic protein 4 (BMP4) or vascular endothelial growth factor (VEGF), to ESCs from incorporated MPs significantly impacted the differentiation of the cells to different lineages and was more efficient than comparable soluble treatment methods. Altogether these results suggest that engineered biomaterials can direct the differentiation of stem cells through modulation of biochemical and/or biophysical properties of the 3D microenvironment. It is expected that the development of inherently scalable techniques to direct pluripotent stem cell differentiation will benefit the biomanufacturing of stem cell derivatives for regenerative cellular therapies and *in vitro* cell based diagnostic technologies, as well as enable engineering of tissues directly from stem cells.

11:20am **BI-WeM11 Adhesion and Rolling of Leukemic Cells on Immobilized Hyalurons**, ***A. Rosenhahn***, Karlsruhe Institute of Technology, Germany, *C. Christophis, I. Taubert, G.R. Meseck, A.D. Ho, M. Grunze*, University of Heidelberg, Germany

Adhesion and rolling on vessel walls are two processes which are relevant for the homing of hematopoietic cells. Especially in the case of acute leukemia, one key in successful therapy is the homing of the hematopoietic stem cells (HSC) to the bone marrow after transplantation. We investigated the interaction of HSC with the hyaluron binding motive and quantitatively studied the interaction of different leukemic cells with synthetic polysaccharide surfaces. For the experiments we applied a microfluidic shear force assay recently developed in our group [1]. Leukemic Jurkat and Kasumi-1 cells lacking CD44-expression showed no adhesion or rolling on the polysaccharides whereas CD44 expressing leukemic cells KG-1a, HL-60, and K-562 attached and rolled on hyaluronan. We find that at weak flow cells have a poor tendency to adhere and only if shear forces above a treshold are present, adhesion is mediated. While this effect is well known for leukocytes on hyaluronan expressing feeder layers, it is the first demonstration that the mechanism also occurs in leukemic progenitor cells towards synthetic hyaluronan coated surfaces. We also extended the study to hematopoietic progenitor cells and saw for the first time that also HPCs with high degree of stemness show a flow induced interaction with hyalurons.

[1] C. Christophis, M. Grunze, A. Rosenhahn, Phys. Chem. Chem. Phys. (2010) 12, 4498

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