Biomaterial Interfaces Group
Room 326 - Session BI-MoA

Cell Solid-Surface Interactions

Moderator: D.E. Leckband, University of Illinois, Urbana-Champaign

2:00pm **BI-MoA1 Cell Solid-Surface Interactions Under Flow**, ***L.V. McIntire,*** Rice University **INVITED**

Understanding the molecular mechanisms of cell-solid surface interactions is crucial for developing specific therapeutic strategies to control or modulate processes such as thrombosis, inflammation and cancer metastasis. In the vasculature or in blood contacting artificial devices these interactions occur under dynamic flow conditions. Primary adhesion or tethering of the flowing cells requires very special properties of the cell surface receptor and its ligand on the solid structure because of the very short contact times available for bond formation and the forces on those bonds due to fluid drag. Leukocytes utilize primarily members of the selectin family of receptors and their carbohydrate ligands for this first step - often resulting in a rolling interaction. For lymphocytes and monocytes, the integrin @alpha@@sub 2@ @beta@@sub 1@ is also capable of mediating primary adhesion under some flow conditions via its ligand vascular cell adhesion molecule (VCAM). Platelets utilize a receptor complex GPIb-IX-V for this tethering process. Secondary or firm adhesion is mediated by members of the integrin family on the cell surface, often after activation, in concert with their ligands on the solid. For leukocytes these are members of the @beta@@sub 2@ integrin family, while for platelets they are members of the the @beta@@sub 3@ integrin family. Methods for dissecting the specific molecular pathways involved for each step in the adhesion process for several cell types are given and the current state of our knowledge and potential applications are discussed.

2:40pm **BI-MoA3 Leukocyte Adhesion on Self-Assembled Thiol Monolayers under Flow**, ***V.A. Tegoulia,*** *S.L. Cooper,* University of Delaware

The effect of specific chemical functionalities on the attachment of neutrophils, one of the cells responsible for host response to biomaterials, was investigated using self-assembled monolayers (SAMs) containing different terminal groups. A surface modification was used to incorporate a zwitterionic phosphorylcholine group on one of the surfaces. Adhesion was studied on surfaces preadsorbed with fibrinogen or albumin, under well defined flow conditions using a radial flow chamber and automated videomicroscopy. The general formula for the thiols used was HS-(CH@sub 2@)@sub 10@-X, where X=CH@sub 3@, CH@sub 2@OH, COOH, COOCH@sub 3@ and CH@sub 2@(OCH@sub 2@CH@sub2@)@sub 3@OH (EG@sub 3@). The phosphorylcholinated monolayer (PC, X=CH@sub 2@PO@sub 4@CH@sub 2@CH@sub 2@N(CH@sub 3@)@sub 3@ was prepared by phosphorylation of the hydroxyl terminated SAM. Contact angle measurements, ellipsometry and X-ray photoelectron spectroscopy (XPS) were used to characterize the SAMs. The amount of adsorbed protein on the surfaces was quantified using radiolabelled fibrinogen and albumin. Neutrophils were isolated from fresh human whole blood. Contact angle measurements, ellipsometry and XPS confirmed the presence of the SAMs. Phosphorous and nitrogen were detected on the phosphonated SAM. Neutrophil attachment was found to be higher at the low shear rates. Cell adhesion was increased on the hydrophobic CH@sub 3@ and the anionic COOH terminated surface. Cells were more activated on the COOH surface. The presence of the EG@sub 3@ and the PC moieties led to very low cell adhesion.

3:00pm **BI-MoA4 Smart Polymers for Bacterial Release**, ***L.K. Ista,*** *V.H. Pérez-Luna, G.P. López,* University of New Mexico

Poly (N-isopropylacrylamide) (PNIPAAM) was used as a model system to demonstrate the utility of environmentally responsive, or "smart", polymers as agents for the release of bacterial biofilms. PNIPAAM was grafted onto the surface of polystyrene coupons by plasma-initiated in situ polymerization. The resultant grafted polymer exhibited the characteristic lower critical solubility temperature (LCST) of 32 @super o@C, as demonstrated by a change in water contact angle and was characterized by x-ray photoelectron spectroscopy. The surfaces were challenged with bacterial strains of marine (Halomonas marina) and medical (Staphylococcus epidermidis) importance as well as with natural sea water. Under experimental conditions, cells attached at temperatures above the LCST of PNIPAAM were released upon transfer to flow conditions below the LCST. The total release observed was greater than 90% of the initially attached cells. In addition, fouling and release could be repeated on the same sample several times, with a small loss in release efficiency upon each repetition.

3:20pm **BI-MoA5 Characterization of Biorecognition Surfaces**, ***B.D. Ratner,*** University of Washington **INVITED**

Biomaterials can now be surface-engineered to drive and control specific bioreactions in vivo and in vitro. Three examples will be presented: (1) surface-immobilized amino acids, peptides and proteins; (2) templates for protein recognition; and (3) surfaces that resist the deposition of biological materials and therefore act in a "stealth" fashion. These surfaces bring new challenges to surface analysis to deal with the molecular complexity, molecular orientation and 2D and 3D organization found on recognition surfaces. Static TOF-SIMS, XPS, IRAS, and AFM are allowing us to glean new information on such surfaces. This overview will highlight progress made in analysis of complex recognition surfaces and demonstrate relationships between surface structure and biological response.

4:00pm **BI-MoA7 Neuronal Networks as the Basis for Computational Systems**, ***J.J. Hickman,*** *M.S. Ravenscroft,* The George Washington University

We are using patterned Self-Assembled Monolayers (SAMs) to control the intrinsic and geometric properties of cell culture growth surfaces to create in vitro circuits of mammalian neurons and their processes. The ability to control the surface composition as well as other variables, such as growth media and cell preparation, all play important roles in neuronal pattern viability and cell fate. The use of serum-free medium makes examination of the culture substratum possible by surface analysis as the serum-free medium contains very small amounts of protein, thus the protein on the surface arises primarily from the cells and can be related to their morphology. The surfaces have been characterized by X-ray Photoelectron Spectroscopy (XPS) and imaging XPS using a FISONS 220i spectrometer and we have related the intrinsic properties of the SAM surfaces and the deposited protein layer to the neuronal cellular development. The electrophysiological signals produced by the neurons in response to artificial and spontaneous electrical stimuli has been recorded by patch-clamp electrophysiology. We are using these circuits to obtain a more fundamental understanding of neuronal circuit development as well as to develop new concepts of hybrid neuroelectric devices for biological computation applications. The continuing development of this technology by our group and other groups will be discussed, as well as the application of this technology for (a) obtaining an improved understanding of neuronal synaptic development, (b) formation of neuronal circuits, and (c) biosensor fabrication. The theory behind the creation of simple hybrid devices will also be explored.

4:20pm **BI-MoA8 Growth of Central Nervous System Cells on Microfabricated Pillars**, ***A.M. Perez,*** *S.W. Turner,* Cornell University*; N. Dowell,* New York State Department of Health*; L. Kam,* Rensselaer Polytechnic Institute*; J.N. Turner, W. Shain,* New York State Department of Health*; R.C. Davis, M. Isaacson, H.G. Craighead,* Cornell University

We are investigating the influence of microfabricated micrometer-size surface features on the attachment and growth of mammalian central nervous system cells. Columnar surface structures have been fabricated using photolithography and reactive ion etching to create arrays with varying sizes and separations. Features 1 µm in height and 1.0 - 5.0 µm in diameter separated by 0.5 - 4.0 µm have been patterened on silicon wafers. The patterned wafers possess 50 µm wide regions of pillars surrounded by smooth silicon surfaces. Several pillared surfaces were also chemically modified with biological polymers including polylysine and conjugated laminin to study the behavior of cells on chemically treated topography. Cells used for these studies include LRM55 astroglial cells, cortical astrocytes prepared from primary cultures, and hippocampal neurons. Cell growth was characterized by scanning electron and fluorescence microscopy while focal contacts and cytoskeletal elements were determined using techniques of vinculin immunocytochemistry and actin cytochemistry, respectively. Astroglial cells preferentially attached to the pillars as opposed to the smooth surrounding surfaces while neurons attached randomly. Cell densities both on and off the pillars have been measured using optical microscopy. The cell densities and morphologies varied according to the geometric features of the columnar surfaces.

4:40pm **BI-MoA9 Directed Neuron Attachment and Growth by Micrometer-Scale Chemical Patterning of Glass Substrates**, ***C.D. James,*** *R.C. Davis,* Cornell University*; L. Kam,* Rensselaer Polytechnic Institute*; H.G. Craighead, M. Isaacson,* Cornell University*; J.N. Turner, W. Shain,* New York State Department of Health, University of Albany*; G. Banker, G. Withers,* Oregon Health Sciences University

Directed neuron attachment and growth is a necessary technology for long-term, in vitro studies of synaptically interactive neurons. Research has shown that chemical cues can stimulate cell attachment and neurite outgrowth in neurons when cultured on chemically-modified bioactive surfaces. Specifically, the synthetic polypeptide polylysine has been shown to induce cell attachment, and the basement membrane protein laminin has been used to initiate neuronal process outgrowth as well as cell attachment. In this paper, we attempt to produce a method for using chemical cues to control the organization of neurons into defined networks in order to facilitate long-term studies of synaptic function and inter/intra-neuronal signal processing. We demonstrate a technique for chemically patterning glass substrates with polylysine and laminin using microcontact printing, an emerging tool for micrometer-scale chemical patterning of surfaces. Further, we show that these chemically patterned surfaces are biologically active, and that cell attachment and neurite outgrowth are stimulated in culture.

5:00pm **BI-MoA10 Characterization of Cellular Interfacial Forces with AFM**, ***T.J. Boland,*** Pennsylvania State University*; Y.F. Dufrene,* Universite Catholique de Louvain, Belgium*; W.R. Barger,* Naval Research Laboratory*; D.L. Allara,* Pennsylvania State University*; G.U. Lee,* Naval Research Laboratory

Biomaterial design depends on understanding the molecular basis of material-body interactions. Much is known about the molecules and cells involved in the body's response to foreign materials but it has been difficult to characterize the physical nature of their interaction. To this end, the interfacial properties of model films have been measured at the nanometer scale with atomic force microscopy (AFM). In specific, as a model for cell surfaces, mixed, uncharged phospholipid/glycolipid monolayers have been deposited on octadecyltrichlorosilane monolayers (OTS) using Langmuir-Blodgett (LB) deposition. The lipid films phase segregate allowing us to measure the relative surface properties of the different phases. Spectroscopic ellipsometry was used to characterize optical properties and thickness of each pure layer and the mixed bilayers in air and in water. As a model for a polymeric surface, AFM probes were functionalized with SH-(CH@sub 2@)@sub 15@-R, where R=CH@sub 3@, CH@sub 2@OH, COOH groups. The height, friction, mechanical properties and surface forces of the lipid phases were measured with these probes. The force curves are purely repulsion due to a dominant short-range force indicative of steric/ hydration interaction and the range of this force is dependent on the head group of the lipid. At high loading forces the probe is observed to snap into contact with the surface which we believe is a measure of the mechanical stability of the film. These measurements demonstrate that AFM can be used to directly characterize molecular interactions between model cell surfaces and model organic surfaces.

Author Index

Bold page numbers indicate presenter