Biomaterial Interfaces
Room 307 - Session BI-TuM

Cell/Surface Interactions
Moderator: K.E. Healy, University of California, Berkeley

8:20am **BI-TuM1 Modulation of Vascular Smooth Muscle Cell Behavior by Tuning Substrate Compliance**, *X.Q. Brown,* ***J.Y. Wong,*** Boston University

During the development of vascular occlusive disease, abnormal vascular smooth muscle cell (VSMC) proliferation and deposition of extracellular matrix leads to hardening of the vessel. Recent studies have shown that changes in substrate compliance affect cell adhesion, migration and differentiation in several different cell types. However, effects of substrate stiffness on the behavior of VSMCs have not yet been investigated. Using polydimethyl siloxane (PDMS), we are able to create substrates with Young's modulus ranging from 3x10@super 4@ Pascal (Pa) to 2x10@super 6@ Pa, which is the range of elasticity that has been reported for a human aorta. We found that changes in substrate compliance affect the behavior of VSMCs. There is a 0.7 fold increase in cell number attached to stiff substrata (2x10@super 6@ Pa) compared to soft substrata (3x10@super 4@ Pa) and a 0.8 fold increase in cell area on stiff substrata. However, the rate of VSMC proliferation on stiff substrata is only 66% that of VSMC on soft substrata. Our results show that VSMCs are capable of sensing and responding to changes in substrate compliance in a range that is physiologically relevant, and PDMS is a useful tool to study the effect of substrate compliance on cell behavior. Our results support our hypothesis that during the development of vascular occlusive disease, changes in VSMC behavior leads to changes in the biochemical and biophysical properties of the vessel, which in turn influence the behavior of VSMCs.

8:40am **BI-TuM2 Mechanical and Biochemical Analyses of Cell Adhesion Strengthening Using Micropatterned Substrates**, ***N.D. Gallant***, *A.J. García,* Georgia Institute of Technology

Cell adhesion to fibronectin (FN) involves integrin binding and subsequent adhesion strengthening, which includes integrin clustering, interactions with cytoskeletal and signaling components to form focal adhesions (FA), and cell spreading. We applied micropatterning methods to control FA size and position to analyze the contributions of FA assembly to adhesion strength. Microcontact printing was used to pattern alkanethiol self-assembled monolayers into arrays of circular adhesive islands (2, 5, 10, 20 μm dia) with a non-adhesive background.@footnote1@ NIH3T3 fibroblasts adhered to FN-coated islands and remained constrained to the patterns presenting a nearly spherical morphology. Cells assembled robust adhesive structures that localized to the micropatterned islands and contained typical components of FA. Cell adhesion strength to FN-coated micropatterned islands was quantified using a spinning disk device that applies a well-defined range of hydrodynamic forces to adherent cells.@footnote2@ Adhesion strength exhibited significant time- and adhesive area-dependent increases. Comparison of experiments for equivalent contact areas showed a 9-fold increase in adhesion strength over time, independent of cell spreading. Bound integrins were quantified using a cross-linking/extraction/reversal biochemical technique.@footnote3@ Significant area dependence was also seen in integrin binding on micropatterned substrates and a correlation between increasing integrin binding and adhesion strength was observed. These results demonstrate that FA assembly, independently of changes in cell morphology, contributes significantly to adhesion strengthening. This work provides an experimental framework for the functional analysis of FA components in adhesive interactions. @FootnoteText@ @footnote 1@N.D. Gallant et al., Langmuir 18, 5579-5584, 2002.@footnote 2@A.J. García et al., J. Biol. Chem. 273, 10988-10993, 1998.@footnote 3@A.J. García et al., Mol. Biol. Cell 10, 785-798, 1999.

9:00am **BI-TuM3 The Use of XPS, SIMS, and Immunostaining to Examine the Behavior of Extracellular Matrix upon Cell Detachment from a Smart Polymer**, ***H.E. Canavan***, *X. Cheng, B.D. Ratner, D.G. Castner,* University of Washington

The temperature-dependent behavior of poly(n-isopropylacrylamide) (NIPAM) is directly transmitted to cells cultured on these surfaces. At culture temperatures, cells behave similarly to those on tissue culture polystyrene (TCPS); after being cooled to room temperature, cells cultured on NIPAM spontaneously detach as contiguous sheets. In comparison, cells grown atop the TCPS surface remain attached for hours or days, requiring the use of enzymatic digestion or physical scraping to detach them. In addition, cell sheets detached from NIPAM surfaces appear to retain their function upon transfer to another growth surface, possibly due to the concurrent detachment of at least one protein of the Extracellular Matrix (ECM), fibronectin (FN). However, the extent to which ECM detaches from the NIPAM surface has remained unknown. We present a thorough examination of the cellular response to NIPAM using X-ray Photoelectron Spectroscopy (XPS), Secondary Ion Mass Spectrometry (SIMS), and immunostaining. XPS is used to make a quantitative comparison of the amount of protein atop NIPAM after cell removal. The primary proteins of the ECM (FN, laminin, and collagen) are examined using immunostaining to determine which of the ECM proteins studied lift off with the cellular layer. In addition, SIMS is used to identify the presence and identity of proteins left at the NIPAM surface after liftoff. Finally, the low-temperature liftoff technique is compared to other traditional cell removal protocols. Our SIMS, XPS, and immunoassay results suggest that low-temperature liftoff of the cell monolayer from the NIPAM surface is accompanied by the majority of the components of the ECM.

9:20am **BI-TuM4 Neurite Outgrowth on Well Characterized Surfaces: Chemically and Spatially Controlled Fibronectin and RGD Substrates**, ***Z Zhang***, *R. Yoo, M. Wells, T.P. Beebe, Jr.,* University of Delaware*; R. Biran, P. Tresco,* University of Utah*; J. Hyun, W. Jun, A. Chilkoti,* Duke University

Study of axonal growth and ligand-receptor interactions requires specificity and careful characterization of the biomaterial substrates to which the neurons bind. Without highly specific surface characterization, it would be impossible to predict the effects of ligand surface density, spatial distribution, and conformation on the outgrowth of a neuron. Here we report two different methods of surface modification (a heterobifunctional crosslinker and Pluronics @super TM@) for immobilization of fibronectin (FN) and fibronectin-derived RGD-containing peptides to the substrates. Proteins and peptides were immobilized to glass surfaces at different concentrations. Various surface analytical techniques, such as contact angle, x-ray photoelectron spectroscopy (XPS), and time-of-flight secondary ion mass spectrometry (TOF-SIMS) were used for analysis of the substrates at each step of the two different chemistries involved. After immobilization of fibronectin and RGD-containing oligopeptides, the modified surfaces were plated with dorsal root ganglia neurons of the rat. Neuron outgrowth rates on the various surfaces were measured and different bioactivity was observed on different modified surfaces. In order to spatially control neurite outgrowth on the substrates, patterned and gradiently FN-covered surfaces were synthesized and tested for bioactivity. An amphiphilic comb polymer presenting oligoethylene glycol side-chains was used to create microcontact-printed patterned surfaces because of its excellent protein repellant and cell resistance properties.

9:40am **BI-TuM5 Surfaces Engineered to Target Integrins to Direct Cell Adhesion and Function**, ***A.J. Garcia***, Georgia Institute of Technology **INVITED**

Cell adhesion to adsorbed extracellular matrix proteins and adhesive sequences engineered on synthetic surfaces plays a critical role in numerous biomaterial, tissue engineering, and biotechnological applications. Cell adhesion to adhesive motifs is primarily mediated by integrin adhesion receptors. In addition to anchoring cells, supporting cell spreading and migration, integrins provide signals that direct cell survival, proliferation, and differentiation. We have developed two biomolecular strategies for the engineering of surfaces to control integrin binding and cell adhesion in order to direct cell function. The first approach focuses on surfaces presenting well-defined chemistries that control protein adsorption to modulate integrin binding in order to potentiate cell adhesion and signaling thereby directing cell differentiation. In a second approach, we have engineered fibronectin- and collagen-mimetic surfaces presenting controlled ligand densities in a non-fouling background that promote the binding of specific integrin receptors and direct adhesive interactions. These surface engineering strategies provide a basis for the rational design of robust biospecific surfaces that tailor adhesive interactions and elicit specific cellular responses for the development of bioactive implant surfaces, 3D hybrid scaffolds for enhanced tissue reconstruction, and growth supports for enhanced cellular activities.

10:20am **BI-TuM7 Atomic Force Microscopy Imaging and Force Spectroscopy of Microbial Cell Surfaces**, ***Y.F. Dufrene***, Université Catholique de Louvain, Belgium **INVITED**

The advent of atomic force microscopy (AFM) has recently opened a wide range of novel possibilities for probing microbial cell surfaces on the nanoscale.@footnote 1@ Using AFM imaging in aqueous solution, microscopists can visualize cell surface nanostructures (surface layers,

appendages), follow physiological changes (germination, growth) and monitor the effect of external agents (antibiotics, metals) in real-time.@footnote 2@ Further, using force spectroscopy, researchers can learn about local biomolecular interactions and physical properties. Spatially resolved force mapping offers a means to determine physical/chemical heterogeneities at the subcellular level, thereby providing complementary information to classical characterization methods. Force measurements allow the cell surface elasticity to be determined.@footnote 3@ Functionalizing the AFM tip with chemical groups or biomolecules enables quantitative measurements of surface charge,@footnote 4@ surface hydrophobicity@footnote 5@ and receptor-ligand interactions. Finally, single-molecule force spectroscopy can be applied to cell surface molecules to gain insight into their mechanical properties.@footnote 6@ Clearly, these new AFM-based experiments contribute to improve our understanding of the structure-function relationships of microbial cell surfaces and will have considerable impact on biotechnology and medicine. @FootnoteText@@footnote 1@Y.F. Dufrene, J. Bacteriol., 184, 2002, 5205-5213. @footnote 2@Y.F. Dufrene, C.J.P. Boonaert, P.A. Gerin, M. Asther, P.G. Rouxhet, J. Bacteriol., 181, 1999, 5350-5354. @footnote 3@H.C. van der Mei, H.J. Busscher, R. Bos, J. de Vries, C.J.P. Boonaert, Y.F. Dufrene, Biophys. J., 78, 2000, 2668-2674. @footnote 4@F. Ahimou, F.A. Denis, A. Touhami, Y.F. Dufrene, Langmuir, 18, 2002, 9937-9941. @footnote 5@Y.F. Dufrene, Biophys. J., 78, 2000, 3286-3291. @footnote 6@B.C. van der Aa, R.M. Michel, M. Asther, M.T. Zamora, P.G. Rouxhet, Y.F. Dufrene, Langmuir, 17, 2001, 3116-3119.

11:00am **BI-TuM9 Molecule Specific Imaging Analysis of Carcinogens in Breast Cancer Cells using Time-of-Flight Secondary Ion Mass Spectrometry**, ***K.J. Wu***, *J.N. Quong, M.G. Knize, K.S. Kulp,* Lawrence Livermore National Laboratory

The concentration and localization of intracellular chemical compounds such as pharmaceuticals and carcinogens are important, specifically for application in physiology and medicine. Cooked muscle meats contain small amounts of rodent carcinogens belonging to the heterocyclic amine class of compounds and are implicated in human cancers at various organ sites. 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is the most mass abundant in cooked foods. PhIP is a genotoxic carcinogen, which has been demonstrated to cause dose-dependent mammary and prostate tumor formation in rats. In this report we will present the results on time-of-flight secondary ion mass spectrometry (TOF-SIMS) studies of several MCF7 line of human breast cancer cells. Protocols for high vacuum compatible tissue and cell culture preparation have been developed. Such direct imaging approach permits an acquisition of element and molecule-specific images directly from the cell surface. The results show the intracellular concentration and distribution of low level carcinogenic compounds such as PhIP in flash-frozen MCF7 cells; the time dependent effects of heterocyclic amine carcinogens interaction with MCF7 cells. We will discuss two major efforts to further the imaging mass spectrometry applications for biological samples: ME-SIMS approach to enhance the ionization yields and multivariate analysis data reduction technique for compound distribution on cell surfaces. @FootnoteText@ This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48.

11:20am **BI-TuM10 Preparation and Characterization of Chemically Patterned Surfaces for Cell-Surface Interaction Studies**, ***D. Marton***, *E.A. Sprague,* The University of Texas Health Science Center at San Antonio*; K. Cho,* University of Michigan Medical Center*; J.C. Palmaz,* The University of Texas Health Science Center at San Antonio

There is significant evidence that surface chemistry plays a major role in the way proteins and living cells interact with biomaterial surfaces. These phenomena could be best studied on surfaces with designed and identifiable chemically different areas. For this purpose we developed surface patterning techniques that combine vacuum deposition (dc sputtering or evaporation) and sputter removal of material. Four types of patterned surfaces were developed. Substrates were made of either pre-treated hydrophilic silicon or hydrophobic Teflon. Two types of specimens were created using each substrate type. The first set of patterned surfaces, called "dot pattern" comprised of carbon, stainless steel and gold dots of nominal 25 micron diameter directly deposited on the clean substrates through a stainless steel mask with a polka-dot type hole arrangement. The second set of patterned surfaces, called "hole pattern" were produced by first depositing a continuous layer of carbon, stainless steel or gold. The specimens were then sputtered through the holes of the mask using 1-2 keV Ar ions until the underlying Teflon or silicon became exposed. Typical film thicknesses were 5-10 nm. All specimens were analyzed to verify the patterning on five areas using ToF-SIMS imaging and some using XPS imaging. Pattern definition depends on mask apposition, and, in the case of insulating specimens, on charging effects. In general, the hole patterns have sharper boundaries than the dots. Using an in vitro cell migration model, human aortic endothelial cells were observed to respond to the different patterns with respect to cell shape and cell migration rate. Pattern dependent protein adherence was also observed.

11:40am **BI-TuM11 Drug Testing and Environmental Toxin Detection using Cell-based Biosensors**, ***A. Natarajan***, *P. Molnar, K. Sieverdes, A. Jamshidi, J.J. Hickman,* Clemson University

In the last decade the threat of environmental pollution, biological warfare and new diseases has increased research into cell-based biosensors. The need for more advanced methods to evaluate candidates has pushed this area of research even further. Cells are natural sensors in the body and react to different bioactive compounds in specific ways. Our research exploits this feature, using the ion channels of electrically active cells like cardiac myocytes, to create a database of specific responses. These responses can then be used to detect the acute presence of a substance. A traditional method of studying drug and toxin effects has been low throughput patchclamp electrophysiology. Our cell-based biosensor consists of a uniform monolayer of cardiac myocytes on a microelectrode array (MEA) with 60 substrate-integrated electrodes. The microelectrode arrays are surface modified and the media used for the cells is defined. Surface analysis was used to verify surface modifications. Long-term recordings of beating cells produced extracellular field potentials in the range 100 µV to 1200 µV, with a beating frequency of 0.5 to 4 Hz. The toxins tested were classified as drugs (epinephrine), heavy metals (Cadmium) and pyrethroids, a group of synthetic pesticides. Pyrethroids modify sodium channels thus disrupting nerve cells in insects. Epinephrine is a well-known stimulant for the heart. Cadmium chloride causes serious illnesses with the same symptoms as lead poisoning. Concentrations used were mainly 1, 10 and 100 µM. Each of the above substances produced specific responses in the action potential signals, changing amplitude, frequency and shape. 10 µM Epinephrine increased spike frequency from 4 Hz to 6 Hz. Our goal is to replace patchclamp electrophysiology with microelectrode arrays as a means to testing many drugs and toxins. Future work involves making this system more stable, creating a larger database and extending the use for chronic detection of compounds.

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