Biomaterial Interfaces
Room 311a - Session BI2-WeA

Cell-Surface Interactions
Moderator: J.Y. Wong, Boston University

3:20pm **BI2-WeA5 Receptor-Ligand Interactions to Promote Endothelial Cell Adhesion and Function**, ***G.A. Truskey***, *W.M. Reichert, B. Chan,* Duke University **INVITED**

Endothelial cell adhesion and proper function are crucial for the development of tissue-engineered vessels and non-thrombogenic vascular grafts. In vivo shear stresses and mechanical forces can reduce adhesion endothelial cell function. We have systematically examined factors affecting endothelial adhesion and identified. To promote rapid and firm adhesion we developed a dual ligand system using a high affinity ligand to initiate adhesion and facilitate integrin-mediated adhesion. Avidin was used as the high affinity ligand. This ligand-receptor pair enabled endothelium to resist high shear stresses encountered in vivo. An RGD group was grafted onto avidin to permit adhesion to integrins. Interestingly, the RGD-avidin bound the endothelium in solution with high affinity and served to prime adhesion once the cells attached. Functional studies indicated that this dual ligand system promoted a nonthrombogenic phenotype. Preliminary in vivo studies of endothelium attached to ePTFE grafts indicate that the dual ligand system can promote firm adhesion but mechanical trauma to the graft is a significant limitation to complete coverage of endothelium. Current work is optimizing endothelial attachment to commercially available grafts and identifying a tissue-engineering alternative for endothelialization. (supported by NIH grants RO1 HL44972 and R21 HL72189.).

4:00pm **BI2-WeA7 Interactions Between Membrane-Bound Receptors and Soluble Ligands Measured by AFM, QCM-D and SPR**, ***Y. Lam***, *S.M. Alam, S. Zauscher,* Duke University

Many proteins in the immune system are membrane bound, presented on the cell surface. Once bound to their soluble ligands, they facilitate interactions to initiate a foreign body response. To gain a deeper understanding of these interactions we initially investigated the bonds between soluble antigen and antibodies by single molecule force spectroscopy using an atomic force microscope (AFM). We immobilized monoclonal antibodies (mAb A32) specific to HIV-1 envelope glycoprotein gp120 on a substrate, and incubated this surface with gp120. This interaction between gp120 and mAb A32 causes a conformational change in gp120, exposing an epitope for a secondary mAb (17b) to bind. We measured the strength of interaction between gp120 and 17b by single molecule force spectroscopy using a cantilever tip decorated with 17b. AFM was also used to generate an energetic landscape of the binding pocket via pulling force experiments at different pulling rates. We also report on our measurements using membrane bound receptors, providing a more native environment for the system. Quartz crystal microbalance with dissipation (QCM-D) was employed to monitor the formation of protein-lipid bilayer constructs. Finally we report on measurements using surface plasmon resonance (SPR) to elucidate changes in affinity between membrane bound and immobilized soluble receptors. This detailed knowledge of receptor ligand interactions is essential to better engineer and tailor therapeutic treatments for various diseases.

4:20pm **BI2-WeA8 A Photolithographic Method for Patterning Soft Polyacrylamide to Enhance Smooth Muscle Cell Elongation**, ***J.G. Jacot***, *J.L. Jackel, S.G. Koester, J.Y. Wong,* Boston University

Vascular smooth muscle cells (VSMCs) express a contractile phenotype in vivo that is lost as cells proliferate in vitro. The manufacture of a successful tissue engineered blood vessel requires the ability of VSMCs to proliferate and populate a scaffold, then revert to a contractile state. In vivo, VSMCs are highly elongated and previous studies from our lab have shown that cell shape influences the localization of proteins such as F-actin and calponin that have a contractile function. Further studies by others found that cell constrainment can also reduce proliferation. However, all these studies investigated cells on rigid substrates, which do not mimic the mechanical environment of the arterial wall, and cannot functionally measure contractile force generation. We have developed a soft lithography technique for patterning 10-micron lanes of collagen on soft polyacrylamide hydrogels. These patterned materials allow separate control of substrate elasticity and cell shape and also allow measurement of cell-generated forces by following the displacement of embedded fluorescent marker beads. Because these materials are fully hydrated and very compliant compared to previously patterned rigid cell culture substrates, maintaining high pattern resolution is difficult. The 10-micron patterns presented here are higher resolution than previously published protein patterns on soft polyacrylamide. Passaged bovine arterial VSMCs plated on these patterned hydrogels attach and spread only on the collagen lanes and have aspect ratios 2-fold higher than unpatterned VSMCs.

4:40pm **BI2-WeA9 Identification of Residual ECM Proteins Retained at pNIPAM Surfaces using Time-of-Flight SIMS**, ***H.E. Canavan***, *M. Greenfeld, X. Cheng, D.J. Graham, B.D. Ratner, D.G. Castner,* University of Washington

Treatment of tissue culture polystyrene (TCPS) with poly(N-isopropyl acrylamide) (pNIPAM) has been developed as a technique for the harvest of intact cell layers. Recently, we demonstrated that although low-temperature liftoff removes the majority of the Extracellular Matrix (ECM) concurrently with the cells, some protein does remain at the pNIPAM surface. However, little is known about the identity of the ECM proteins retained at the pNIPAM surface after cell liftoff. In this work, we characterized the time-of-flight secondary ion mass spectrometry (ToF-SIMS) molecular fragmentation pattern of adsorbed single protein mixtures of important ECM proteins (e.g., laminin, fibronectin, and collagen). We next performed Principal Component Analysis (PCA) to distinguish between the proteins through the identification of unique amino acid fragmentation patterns in the ToF-SIMS positive ion spectra, a technique previously developed in our group. In this way, a model ToF-SIMS projection of the ECM was constructed. We subsequently compared the ToF-SIMS fragmentation pattern of the proteinaceous layer retained on the pNIPAM surface to that of the model ECM. ToF-SIMS fragmentation patterns of bovine serum albumin and serum-containing media controls were compared as positive controls as well. Using the comparison of the model ECM to that of ECM retained on pNIPAM surfaces, we discuss the identity of the proteins retained on the substrate after low-temperature liftoff from pNIPAM surfaces. We then compare our results to those obtained from analysis of the ECM using other surface analytical techniques, including immunoassay, gel electrophoresis, and matrix-assisted laser desorption ionization (MALDI).

5:00pm **BI2-WeA10 Compartmentalized Bioreactor Mitigates Culture Shock-Engenders Bone Tissue from Isolated Bone Cells**, ***D. Ravi***, *E.A. Vogler,* Penn State University

Reducing the profound gap between the physiological environment of the bone cells and in vitro cell culture models is critical for realizing the promise of tissue-engineering strategies to replace, regenerate and restore function to bone lost as a result of disease or injury. An advanced bioreactor that mitigates culture shock or the behavioral variations associated with the transition of bone cells from the in vivo to the in vitro environment was developed and tested. The bioreactor based on the principle of simultaneous-cell-growth-and-dialysis, separates a cell growth chamber from a media reservoir by a dialysis membrane, compartmentalizing cell growth and cell nutrition functions. As a consequence of compartmentalization, the pericellular environment is unperturbed by continuous perfusion or punctuated re-feeding schedules and luxury macromolecules synthesized by cells are retained in a manner that more closely simulates the in vivo condition. The stable culture conditions afforded by the bioreactor sustained model cell lines, mouse calvaria-derived MC3T3-E1 (ATCC CRL-2593) and human fetal osteoblasts (hFOB 1.19, ATCC CRL-11372) for extended time periods (30-120 days) without the need for sub-culture. The transformation of isolated osteoblast inoculum to mineralized, collagenous tissue that simulates native osteoid was followed using optical microscopy and scanning and transmission electron microscopy. Mineralization was assessed using Von Kossa assay and SEM-EDS (Energy Dispersive Spectroscopy. Development of differentiated, collagenous bone tissue (biosynthetic osteoid) from disaggregated osteogenic cells over 120 day culture was demonstrated on both 2-D polymer substrates as well as 3-D hydroxyapatite scaffolds. The compartmentalized bioreactor substantially mitigates culture shock and shows promise as an ideal in vitro tool for evaluation of orthopedic biomaterials and development of engineered bone tissue.

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