Monday Afternoon, November 9, 2009

Biomaterial Interfaces
Room: K - Session BI-MoA

Protein and Cell Interactions at Interfaces I
Moderator: T. Boland, Clemson University, B.G. Liedberg, Linköping University, Sweden

2:00pm **BI-MoA1 Bioengineering Stem Cell Fate**, *H.M. Blau*, ***K. Havenstrite***, Stanford University **INVITED**

A major challenge facing stem cell biologists is an understanding of the mechanisms that direct stem cell fate: the delicate balance between quiescence, self-renewal, and differentiation. Adult stem cells are localized in niches, specialized microenvironments, which protect them from differentiation. Upon culture, adult stem cells lose their "stemness", or ability to self-renew. We have engineered artificial in vitro microenvironments that mimic key biochemical characteristics of adult stem cell niches in order to analyze the properties of stem cells and influence their fate. Microwell arrays are produced as topographically structured polymer hydrogel surfaces allowing exposure of single cells either to soluble or tethered niche proteins. Using this platform, phenotypic and dynamic analyses of thousands of individual cells can be monitored simultaneously by time lapse microscopy. We have found that single proteins alter proliferation kinetics and asymmetric division behavior, leading to muscle and hematopoietic stem cell self-renewal in culture. Our data demonstrate that parameters of proliferation behavior in vitro correlate with stem cell function assayed in vivo. Ultimately, the goal of these studies is to increase our understanding of stem cell biology, expand stem cells in vitro for clinical applications, and discover new drugs for stimulating a patient's own stem cells.

2:40pm **BI-MoA3 An Investigation of Human Embryonic Stem Cell Attachment on 496 Different Acrylate Polymers in a Microarray: The Importance of Surface Chemistry as Probed by ToF SIMS**, *M.R. Alexander*, ***J. Yang***, *M.C. Davies*, The University of Nottingham, UK, *Y. Mei*, *D.G. Anderson*, *R.S. Langer*, MIT, *M. Taylor*, *A.J. Urquhart*, The University of Nottingham, UK

The relationship between the surface chemistry of materials and human cellular response has great importance in existing and emerging technology areas such as tissue engineering, regenerative medicine and biosensors. Here, we investigate hESC attachment, surface chemistry (using time of flight secondary ion mass spectrometry (ToF SIMS) and XPS) and bulk properties (using confocal Raman spectroscopy) of a large set of samples with diverse chemistry. These are acrylate polymers in the form of micro-spots in an array made from 22 different acrylate monomers mixed pairwise in different proportions and UV photopolymerised to give 496 unique homo- and co-polymers.[1, 2] We do not find a correlation between the human embryonic stem (hES) cell number and wettability, or surface elemental or functional composition that holds for all the samples on the array. In contrast, surface mass spectrometric data acquired using ToF SIMS correlate strongly with cell attachment on all polymers using partial least squares (PLS) regression. The ability to predict cell attachment using the SIMS data indicated that it contains sufficient information on the surface chemistry of the polymers to describe the effect of surface chemistry on cell attachment. Some of the moieties identified using this approach are consistent with previous theories relating surface chemistry on protein adsorption and in turn to cell adhesion, whereas others are new.

We propose that in the field of cell-material interactions, this result highlights the importance of the molecular information contained in the SIMS spectra in controlling the cell attachment. Furthermore, it indicates how the PLS methodology can be used to identify the relationship between surface chemical moieties represented within the SIMS spectra to complex properties such as cellular response.

[1] D.G. Anderson, S. Levenberg, R. Langer, *Nature Biotechnology*, **2004**, 22, 863-866.

[2] M. Taylor, A.J. Urquhart, D.G. Anderson, R. Langer, M.C. Davies, M.R. Alexander, *Surface and Interface Analysis*, **2009**, 41, 127-135.

3:40pm **BI-MoA6 Design of Protein Polymers as Novel Tissue Engineering Scaffolds**, ***D. Sengupta***, *S.C. Heilshorn*, Stanford University

The ability to tailor specific cell-matrix interactions in biomaterials is now recognized as an important method to control cell behaviour. Biomaterial adhesivity and elasticity are important determinants of cell adhesion, proliferation, and differentiation; and a coordinated cell response to these different material inputs results in complex signaling crosstalk. Independent modification of these biomaterial properties is thus extremely important, but difficult to achieve with current synthetic as well as natural biomaterials. While natural biomaterials such as collagen and Matrigel do not allow for the independent tuning of multiple biomaterial properties, synthetic biomaterials such as PEG and acrylates can be toxic and immunogenic. An alternative approach to natural as well as synthetic materials is the use of protein polymers made with recombinant protein engineering technology. By templating protein synthesis using the genetic code, we have exact molecular-level control over our material. Using this strategy, we have engineered a family of tunable and biodegradable protein-engineered biomimetic materials that incorporate critical elements of the natural extracellular matrix. The materials are manufactured using a modular design strategy, resulting in a fusion protein comprised of multiple peptide domains that provide cell adhesion and matrix elasticity. Specifically, the elastic modulus of the material can be tuned (from ~43-1200 kPa) independently of RGD ligand density (from 0-9300 cell adhesion sites/μm2), enabling optimization of the biomaterial interface for specific tissue engineering applications. Additionally, these interfaces can be easily micro-molded to incorporate micro- or nanoscale topographical features that induce cell alignment. Human embryonic stem cell-derived cardiomyocytes as well as mouse embryonic stem cells cultured on our protein-engineered biomaterials demonstrate viability, proliferation, differentiation, and morphology comparable to positive gelatin controls, providing a viable alternative to commonly used materials. The molecular-level design strategy of these protein polymers allows for unprecedented control over the biomaterial-cell interface for regenerative medicine applications.

4:00pm **BI-MoA7 Axon Guidance on Patterned Gradients of Extracellular Matrix Proteins**, *W. Theilacker*, *H. Bui*, University of Delaware, *S. Sullivan*, Alcoa Technical Center, *L. Capriotti*, University of Delaware, *D. Willis*, *J. Twiss*, Alfred I. duPont Hospital for Children, *N. Zander*, Army Aberdeen Research Laboratory, *Z. Zhang*, Excellatron, ***T. Beebe Jr***, University of Delaware

This presentation will focus on axonal extension experiments made possible by recent developments in a general platform for substrate patterning of protein and peptide gradients using covalent attachment schemes, and employing cell- and protein-resistant lanes of PEO-like comb polymer. The platform uses step gradients and continuous gradients in local protein and peptide concentrations from micron to centimeter length scales. Control of the local surface density of proteins and peptides allows cell culture assays involving competition of cells for different extracellular matrix (ECM) proteins, propensity of axons to cross from one ECM protein lane into a different ECM protein lane, neuron attachment propensity, axon extension direction and rate, and controlled studies of cell-cell interactions between different cell types. This presentation will address the relationship between the local protein coverage and the "bioactivity" or "bioavailability," using a variety of surface analytical techniques including XPS, TOF-SIMS and AFM, and optical microscopy techniques including epi- and confocal fluorescence microscopy.

4:20pm **BI-MoA8 Aligned Highly Porous Electrospun Scaffolds for Nerve Tissue Engineering**, ***N. Zander***, Army Research Lab/University of Delaware, *J. Orlicki*, *A. Rawlett*, Army Research Lab, *T. Beebe*, University of Delaware

Spinal cord injuries are one of the most catastrophic and costly types of injuries since damaged axons in the central nervous system are unable to spontaneously regenerate. Although reconstruction of damaged and diseased neural pathways remains a major hurdle, recent research has shown that aligned electrospun fiber mats can provide contact guidance cues to direct axon growth by acting as a bridging device. However, due to the nanometer sized fiber diameter and highly aligned nature of the scaffolds, the low interfiber distance limits penetration of the cells into the scaffold.

To study the effect of fiber mat porosity on cellular infiltration, aligned fiber mats were fabricated via co-electrospinning polycaprolactone with polyethylene oxide (a water soluble polymer). Variation of the fabrication parameters allowed for control of the porosity of the scaffold with a full range of sacrificial (PEO) fiber composition. As the surface composition is also critical in providing biochemical signals to direct neurite growth, the surfaces of the fibers were functionalized via air plasma treatment followed by attachment of several extracellular matrix proteins. The surface chemistry was characterized by X-ray Photoelectron Spectroscopy, Time of Flight Secondary Ion Mass Spectrometry, and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy. Cellular infiltration, proliferation and neurite outgrowth of PC12 cells were evaluated for fiber mats of varying porosity and surface composition.

4:40pm **BI-MoA9 Study of GPIbα and vWF Interations under Blood Flow**, ***X. Cui***, *HB. de Laat, J. Orje, Z.M. Ruggeri*, The Scripps Research Institute

The mechanism of platelet-adhesion plays an important role in hemostasis and thrombosis. When blood vessels are injured or disrupted, the platelets membrane presented glycoprotein Ibα (GPIbα) will bind to von Willebrand Factor (vWF), which is a large multimeric plasma protein immobilized on collagen fibers. The binding interactions are regulated by the applied fluid shear rates. In this paper, we conducted a study of blood platelets adhesion on vWF A1 domain coated glass slides at different shear rates. The platelets movements are recorded by a high speed camera at 30fps. The recorded videos are analyzed using video/image processing software in order to calculate the platelet velocities at different shear rates as well as different protein coating concentrations. We found the average velocity decreased when the shear rate increased. This showed the binding requires high fluid shear rate in the flow. PLGA beads with the shapes of ellipsoid and sphere of about 1μm diameter were also studied using this method. However, the beads were coated with vWF A1 domain and the flow chambers were coated with GPIbα. We observed the decrease of the velocity with the increased shear rates, which showed increased binding strength between vWF A1 domain and GPIbα protein at higher shear rates. The ellipsoid beads had lower velocity comparing with the sphere beads at the same shear rate due to the larger contact area to the coating surface.

5:00pm **BI-MoA10 Platelets Adhere to Adsorbed Albumin through a Receptor-Mediated Process**, ***B. Sivaraman***, *R.A. Latour*, Clemson University

Since albumin (Alb) lacks known sequences recognized by platelet receptors, it is not supposed to support platelet adhesion. However, studies have suggested that platelets may be able to adhere to adsorbed Alb (*1, 2*) with adhesion related to adsorption-induced Alb unfolding (*1*), although the mechanisms of this remain unclear. To address this issue, we conducted studies to definitively determine if platelets adhere to adsorbed Alb, whether adhesion is related to adsorbed Alb conformation,and if it occurs by a receptor-mediated process. Alb was adsorbed at 0.1, 1.0, and 10 mg/mL on various alkanethiol SAM surfaces to vary the degree of unfolding in the adsorbed Alb. The adsorption-induced conformational changes in Alb was quantified by CD spectropolarimetry (*3*). Platelet adhesion studies were carried out and the platelet response determined by LDH assay and SEM. A series of platelet adhesion inhibitors and protein modification agents were used to probe the mechanisms of platelet adhesion. Platelet adhesion to adsorbed Alb was negligible when adsorbed Alb retained most of its native structure (< 34% loss in alpha-helix), but began to linearly increase with the degree of adsorption-induced unfolding thereafter (r^2=0.92). SDS-PAGE results showed that the platelet suspension was free of residual proteins and anti-Alb polyclonal antibodies completely inhibited platelet adhesion to adsorbed Alb, but had negligible effect on adsorbed Fg (used as a control); thus confirming that the platelets were adhering to adsorbed Alb and not some other residual protein in the system. Addition of an RGDS peptide to the platelet suspension strongly inhibited platelet adhesion to adsorbed Alb (~60% reduction on CH_3 SAM; $p < 0.01$), while the addition of RGES peptide had no inhibitory effect. Neutralization of arginine residues in the adsorbed Alb layer using 2,3-butanedione reduced platelet adhesion to a similar degree as exposing the platelets to the RGDS peptide in solution. These results indicate that the adhesion of nonactivated platelets to adsorbed Alb is primarily mediated by RGD-specific receptors and the degree to which the binding domains in adsorbed Alb are exposed and/or formed is directly proportional to the degree of adsorption-induced unfolding of the protein. Further studies will be carried out to identify the specific platelet receptors and Alb domains that mediate adhesion. These results indicate that we have much yet to learn about the mechanisms that influence platelet adhesion to adsorbed proteins.

1. Hylton et al, *J. Biomed. Mater. Res.* 73A, 349 (2005).

2. Rodrigues et al, *Biomaterials* 27, 5357 (2006).

3. Sivaraman et al, *Langmuir* 25, 3050 (2009).

Authors Index

Bold page numbers indicate the presenter