Tuesday Morning, October 21, 2008

Biomaterial Interfaces
Room: 202 - Session BI-TuM

Protein and Cell Interactions at Interfaces

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

8:00am **BI-TuM1 Multiscale Analysis of Biological Adhesion**, ***D. Leckband***, University of Illinois, *F. Li*, Pololu Corp. **INVITED**

In biological systems, the number of protein bonds mediating cell contacts varies from a few for tethering leukocytes to vessel walls to more than 105 in mature cell-matrix contacts. The characterization of the response of single bonds to a dynamic force provides insights into the physics of noncovalent bond rupture, but the more biologically relevant situation involves the rupture of multiple bonds between extended surfaces. A fundamental question concerns how adhesion between parallel surfaces bridged by multiple, parallel bonds scales with the physical chemical parameters of the protein-ligand bonds. Here I describe theoretical and experimental investigations of the forced separation of two adhesive surfaces linked via a large number of parallel noncovalent protein-ligand bonds. Specifically, we consider how the adhesive force scales with bond parameters (kinetics and affinities) as a function of dynamic loading. These results show that the separation rate relative to the intrinsic relaxation time of the bonds defines three loading regimes and the general dependence of the adhesion on kinetic or thermodynamic parameters of the bonds. In the "equilibrium regime", the rupture force asymptotically approaches the "equilibrium rupture force", which increases linearly with the equilibrium bond energy. In the near-equilibrium regime, the rupture force increases with the separation rate and increasingly correlates with the bond rupture barrier, or the logarithm of the dissociation rate. Far from equilibrium where rebinding is irrelevant, the rupture force varies linearly with the rupture barrier, and hence with the bond rupture barrier. Therefore, the adhesive strength of biological interfaces involving multiple, parallel bonds depends on the loading rate, and the loading conditions in turn determine which molecular parameters scale the strength of the junction.

8:40am **BI-TuM3 Molecular Dynamics Simulation of the Adsorption Behavior of Peptides with Secondary Structure to Functionalized Surfaces**, ***G. Collier***, *S.J. Stuart*, Clemson University, *B.R. Brooks*, National Institutes of Health, *R.A. Latour*, Clemson University

While it is well understood that protein-surface interactions are of fundamental importance for understanding cell-surface interactions, very little is understood at this time regarding the molecular level events that control protein adsorption behavior. Molecular dynamics simulations methods have enormous potential for development as a tool to help understand and predict protein adsorption behavior. We are conducting molecular dynamics simulations to simulate the adsorption behavior of peptides with secondary structure to functionalized alkanethiol self-assembled monolayer (SAM) surfaces. Two types of structured peptides are being studied: (1) an alpha-helix forming peptide with a primary sequence of Ac-L-K-K-L-L-K-L-L-K-K-L-L-K-L-NH2 (LKalpha14), where L is leucine (nonpolar amino acid), K is lysine (positively charged amino acid), and Ac represents an acetylated end-group, and (2) a beta-sheet forming peptide, Ac–L-K-L-K-L-K-L-K-L–NH2 (LKbeta9). Two types of SAM surfaces are represented, (1) a CH3-SAM (hydrophobic surface) and (2) a COOH-SAM surface (negatively charged surface). Simulations are performed with the CHARMM force field and simulation package using explicitly represented solvent (150 mM Na+/Cl- in TIP3P water) with periodic boundary conditions. An advanced sampling method, known as replica-exchange molecular dynamics (REMD), is being applied in our simulations to generate Boltzmann-weight ensembles of states for each peptide-SAM system, with the resulting ensembles providing equilibrated structures of peptide behavior, both in bulk solution, and when adsorbed to each type of SAM surface. The resulting ensembles are then analyzed to provide a theoretical understanding of how the surface influences the secondary structure of both the LKalpha14 peptide and a pair of LKbeta9 peptides. In addition, assessment is also being made to quantitatively assess how each SAM surface and the peptide-surface interactions influence the water structure at the interphase region of the system relative to bulk water conditions. Simulation results are being compared with NMR, SFG, ToF-SIMS, and SPR experimental studies that are being conducted in a collaborative effort with Profs. Castner, Gamble, Stayton, and Drobny at the University of Washington.

9:00am **BI-TuM4 Heat Shock Protein Expression and Cell Membrane Study of Printed Chinese Hamster Ovary Cells**, ***X. Cui***, *T. Boland*, Clemson University

Cell printing as a new cell seeding technology in tissue engineering has been attracting more and more attentions. The advantages of high throughput, automatically controlled, low cost of cell printing has a promising future for tissue engineering. Inkjet printing technology is currently widely used for cell printing. However, the heating and stress to the cells during the printing process may cause cell apoptosis or other physical or genetic changes. We present here a comprehensively study of changes in heat shock protein expression and cell membrane morphogenesis in Chinese Hamster ovary cells printed with thermal inkjet printers. We found the optimal cell concentration for cell printing using the modified HP Deskjet 500 inkjet printer using the HP 51626A ink cartridge was one million cells per ml. The heat shock protein expression of the printed cells has minor difference between the untreated cells and lower than manually heated cells. The cell membrane of printed cells developed pores which allow small molecules such as propidium iodide and dextran molecules (up to 70kD) to pass. We conclude here that cell printing technology can be used for precise cell seeding in tissue engineering fabrication with minor effect and damages to the printed mammalian cells. The printing process caused temporary pores with various sizes to appear in the membranes. This may have promising applications for small molecules transferred into cells for protein expression and drug delivery purpose.

9:20am **BI-TuM5 Bioactivity of Adsorbed Protein Layers Correlated with Adsorbed Orientation and Conformation**, ***K.P. Fears***, *R.A. Latour*, Clemson University

It has been well established that protein interactions at biomaterial surfaces are of critical importance because it is the adsorbed protein layer that dictates how the body responds to an implanted material. In general, cells do not have receptors for synthetic materials thus lack the ability to directly respond to non-biological surfaces. Therefore, the critical factors that influence the subsequent cellular responses are the protein comprised in the adsorbed protein layer, their structures, and their bioactivities. Due to the complexity of protein structure and the inherent difficulty of studying surfaces, elucidating the detailed molecular mechanisms involved in protein adsorption is a daunting challenge. We have developed experimental methods to quantitatively assess the secondary structure of adsorbed protein layers using circular dichroism along with the bioactivity of the adsorbed protein layer using spectrophotometric assays. The following model proteins were investigated on alkanethiol ($HS-(CH_2)_{11}-R$, R = OH, CH_3, NH_2, COOH) modified gold surfaces: hen egg white lysozyme (14.4 kDa, pI ≈ 11, PDB# 1LYZ), xylanase from thermomyces lanuginosus (21.3 kDa, pI ≈ 3.9, PDB# 1YNA), and glucose oxidase from apergillus niger (63.3 kDa, pI ≈ 4.2, PDB# 1CF3). Since CD only provides information about the secondary structure of proteins, amino acid specific chemical modification was used to modify solvent accessible tryptophan residues to provide information about the tertiary structure of the adsorbed proteins and their adsorbed orientations. The results from these studies provide molecular-level insights regarding how surface chemistry influences the adsorbed structure of proteins and how this influences their bioactivity.

9:40am **BI-TuM6 Development of a Novel Biodegradable and Cyto-Compatible Polyurethane for Use as a Bioink in Ink-Jet Printing**, ***C. Zhang***, *N. Brown*, *T. Boland*, Clemson University

Biodegradable polyurethanes (PUs) were synthesized from methylene di-p-phenyldiisocyanate (MDI), polycaprolactone diol (PCL-diol) and N, N-bis (2-hydorxyethyl)-2-aminoethane-sulfonic acid (BES), serving as a hard segment, soft segment and chain extender respectively. We evaluated the effects of this chain extender on the polyurethanes' degradation rate, mechanical properties, hydrophilicity, antithrombogenecity, and ability to support fibroblast cell attachment and growth. The properties were evaluated by comparing these polymers with those having a 2,2-(methylimino)diethanol (MIDE) chain extender. Mechanical testing demonstrated that the PUs containing BES have tensile strengths of about 17 MPa and elongations up to 400%, higher strength and elongation than PUs containing MIDE. In vitro degradation assays showed the presence of sulfonic acid group decrease the degradation rate of the PU containing BES than that of the PU containing MIDE. Cytocompatibility studies showed that all the PUs are nontoxic, and support cell attachment and proliferation. In vitro platelet adhesion assay showed lower platelet attachment on PU containing BES than that on either PU containing MIDE. Additionally, due to the existence of sulfonic acid groups, the BES extended PU became water-soluble in basic condition and insoluble in acidic condition, a phenomenon that is reversible at pH value of 8.7, making this a pH sensitive

polymer attractive for bioprinting applications. By adding acetic acid into an inkjet cartridge and printing it onto basic PU solution, precision fabricated scaffolds were obtained. We will show that these PU scaffolds have preprogrammed pores with fixed sizes of approximately 20 microns. After 5 days cell culture, fibroblasts are seen to attach and proliferate on the porous printed scaffolds, and a number of the cells penetrated into the pores. These results suggest that these PUs are promising candidates as synthetic inks used for customizable fabrication of tissue engineering scaffolds.

10:40am **BI-TuM9 Surface Immobilization and Characterization of Proteins**, *F. Cheng, P.-C. Nguyen, L. Baugh, P.S. Stayton, L.J. Gamble,* ***D.G. Castner***, University of Washington

Immobilized proteins mediate the interactions between a material and its biological environment. We have used XPS, ToF-SIMS, NEXAFS and SPR to investigate protein immobilization onto surfaces containing nitrilotriacetic acid (NTA), N-hydroxysuccinimide (NHS) and maleimide headgroups. NHS surfaces were prepared by self-assembly of NHS ester oligo(ethylene glycol) thiols (NHS-OEG) onto gold. Protein immobilization onto NHS surfaces occurs primarily through the amine groups on the side chains of lysine residues present on the protein surface, resulting in the proteins being immobilized in a random orientation. Mixed monolayers containing NTA headgroups and OEG chains were self-assembled onto a gold surface. The surface concentration of NTA headgroups was 0.9-1.3 molecule/nm^2 in the mixed NTA/OEG monolayers, compared to 1.9 molecule/nm^2 in pure NTA monolayers. The NTA headgroups were slightly reoriented toward an upright position after OEG incorporation. Histagged, proteins were specifically and reversibly immobilized onto Ni(II)-treated mixed NTA monolayers in well-defined orientations. For a humanized anti-lysozyme Fv fragment the amount of reversible, site-specific adsorption varied from 108 - 205 ng/cm^2 with dissociation rates (k_{off}) between $1x10^{-4}$ and $2x10^{-5}$ s^{-1}, both depending on the NTA surface concentration and orientation. The monolayers without Ni(II) treatment exhibited low nonspecific adsorption. ToF-SIMS was used to compare the controlled orientation of histagged proteins on NTA surfaces with the random orientation of proteins on NHS surfaces. Previously studies have characterized the composition and structure of maleimide-ethylene glycol disulfide (MEG) monolayers on gold for the immobilization of single-stranded DNA oligomers (Lee, et al., Analytical Chemistry 79 (2007) 4390.). These same MEG surfaces were used to covalently immobilize cysteine mutants of the Protein G B1 domain. Two mutants were prepared with cysteines located at opposite ends of the Protein G B1 domain. XPS and SPR were used to quantify the amount of each cysteine mutant onto both bare gold and MEG covered gold surfaces. The ToF-SIMS intensity ratios of amino acid fragments with asymmetric distributions in the Protein G B1 domain (ala, asn, gly, leu, met and tyr) were used to show the two immobilized cysteine mutants had opposite orientations. This difference in orientation was observed on both the gold and MEG surfaces.

11:00am **BI-TuM10 Block-oligonucleotide Brushes: Controlled Structure and Recognition Properties**, *A. Opdahl*, University of Wisconsin, *L.J. Whitman,* Naval Research Laboratory, ***D.Y. Petrovykh***, Naval Research Laboratory and University of Maryland, College Park

DNA brushes with unique properties can be prepared using a new immobilization method that is based on the intrinsic affinity of adenine nucleotides for gold (Opdahl et al., PNAS, 104, 9-14, 2007). The general method uses block-oligonucleotides with sequences that follow a d(A_k-T_m-N_n) pattern: a block of k adenine nucleotides [d(A_k)], followed by a block of m thymine nucleotides [d(T_m)], and a short sequence of n (arbitrary) nucleotides [d(N_n)]. These block-oligonucleotides attach to gold via the d(A) blocks and present the rest of the strand for hybridization or for attachment of other molecular recognition ligands. The range of grafting densities produced by this immobilization method can be further extended by co-immobilizing the d(A_k-T_m-N_n) probe DNA with short d(A_l) DNA that act as lateral spacers. We present two applications of this immobilization strategy. In the first, we use d(A) as a means to immobilize d(N_n) probes for hybridization. We find that the strategy results in reproducible and reversible hybridization behavior, offering practical advantages including low cost and resistance to nonspecific adsorption. Moreover, the high degree of control of probe spacing inherent to the method allows us to observe and quantify by both x-ray photoelecton spectroscopy (XPS) and surface plasmon resonance (SPR) the effects that surface density and conformation of DNA probes have on hybridization efficiencies. In the second application, the d(N_n) portion of the strand is replaced with a biotin functionality [d(A_k-T_m-biotin)]. Altering the length or mole fraction of the d(A_l) lateral spacer systematically changes the surface coverage of biotin, allowing control over the amount of streptavidin (SA) that can be linked to the surface. The SA captured by the d(A_k-T_m-biotin) layer is stable and maintains activity towards addition of a subsequent layer of biotin-functionalized molecules. Control experiments also indicate that gold surfaces covered by d(A) oligos exhibit resistance to nonspecific adsorption of SA, both as a molecule and as SA-functionalized microbeads. Since many types of molecules can be functionalized with biotin, compatibility with biotin-SA opens the door to a broad range of applications based on DNA immobilization via d(A) blocks.

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11:20am **BI-TuM11 Surface Initiated Polymer Coatings for the Control of Cell-Surface Interactions**, ***L. Meagher***, *H. Thissen, P. Pasic, R.A. Evans, S. Pereira, G. Johnson, G. McFarland, K. Tsang, T. Gengenbach, K. McLean*, CSIRO Molecular and Health Technologies, Australia

Interest in surface initiated polymerization (SIP) to generate surface coatings for application in biomaterials has increased rapidly in the last few years, particularly those coatings generated using "living" free radical polymerization since they result in coatings with very well defined properties and architectures.[1] Biomaterials applications have largely been in the control of cell attachment, achieved by varying the type of monomer used (e.g. neutral, hydrophilic coatings can be used to reduce protein adsorption and cell attachment). In this study, we present an approach to preparing such coatings which relies on the covalent attachment of a polymer containing controlled free radical initiators onto amine surfaces.[2] This approach allows for maximum flexibility with respect to the substrate (unlike those using self assembled monolayers). Whilst earlier studies have relied on passive coatings, we have included biological signals by building activated functional groups into the coatings as well as drugs and molecules for the specific binding of proteins. These coatings therefore have the potential to interact with biological systems in a much more dynamic manner. XPS and colloid probe AFM were used for coating characterisation. Cell culture studies (24 hours) were carried out with either HeLa, bovine corneal epithelial or L929 fibroblast cells using standard techniques. Protein adsorption was quantified using time resolved fluorescence and proteins labeled with an Eu chelate. Examples presented in this study are the use of iniferters, ATRP initiators and RAFT agents to form SIP coatings with well defined properties. For example, dense polymer brushes which have low or high cell attachment depending on the type and amount of protein adsorption. In addition, the incorporation of cell attachment peptides into coatings via reaction with activated NHS esters will be discussed. Furthermore, the use of click chemistry to conjugate biological signals into coatings formed using our approach will be presented as well as the incorporation of molecules which allow for specific protein binding (e.g. polymerisable biotin) will be discussed in the form of application to ELISAs.

[1] Edmond, S., Osborne, V.L. and Huck, W.T.S. Chemical Society Reviews, 33, 14, 2004.

[2] Meagher, L., Thissen, H., Pasic, P., Evans, R.A., Johnson, G., Polymeric coatings and methods for forming them, WO2008019450-A1, 21 Feb 2008.

11:40am **BI-TuM12 The Effects of Plasma-Polymerized Surface Interactions on R1 Mouse Embryonic Stem Cell Differentiation**, ***E. Hanley***, *J.L. Lauer, G.E. Lyons, J.L. Shohet*, University of Wisconsin-Madison

Plasma polymerized tetraglyme coatings have been studied for their non-fouling and biocompatibility properties due to their inhibition of non-specific protein binding. In this work, we explore how embryonic stem (ES) cell differentiation patterns are affected by surface interactions with various plasma-processed materials. In order to identify a surface as a potential scaffolding material for ES cells in the development of an artificial blood vessel, at least two design criteria must be met. First, the ES cells must adhere to the surface. Second, the surface must facilitate, rather than inhibit, the process of vasculogenesis. ES cells were plated on the following surfaces to compare their effectiveness: 1.) glass cover slips onto which a coating of tetraglyme was deposited by plasma polymerization, 2.) vacuum gas plasma treated polystyrene (Falcon), and 3.) unprocessed control glass coverslips (Corning). In order to characterize the progression of differentiation of the ES cells, each sample was fixed three and seven days after cells had been plated on each surface, and then stained for immunofluorescence analysis. Two genetic markers were used for the antibody staining procedure. PECAM (platelet endothelial cell adhesion molecule: CD31) is an early marker for endothelial cell differentiation. vWF (von Willebrand Factor) is a cytoplasmic protein only expressed in mature endothelial cells. It has been previously reported by using an RT-PCR/southern hybridization blot analysis that ES cells in embryoid bodies first express PECAM near day 5 of differentiation and will first express vWF near day 11 of differentiation. ES cells that were plated on tetraglyme surfaces showed expression of PECAM after 3 days and vWF after 7 days of being plated on the surface. The positive results seen by ES cell derivatives precociously expressing the vWF and PECAM genetic markers on the surfaces suggest a directed differentiation of ES cells into endothelial cells. Furthermore, by using confocal microscopy, it was shown that the endothelial cells that express the vWF genetic marker are those cells closest

to the tetraglyme coating. We conclude that the nature of the surface does influence R1 stem cell differentiation. It has also been shown that higher mole fractions of tetraglyme in the plasma during processing increases the percentage of endothelial cell expression.

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