Tuesday Evening Poster Sessions, November 4, 2003

Biomaterial Interfaces

Room Hall A-C - Session BI-TuP

Poster Session

BI-TuP2 Plasma Processing pf Polymers to Reduce Bio-Fouling for Cardiac Applications, ***M. Neumann***, *P. Fackler, D.N. Ruzic,* University of Illinois at Urbana-Champaign

Polymers are playing an increasing role in cardiac medicine as components of implants and diagnostic devices, such as tubes, diaphragms, filters, pacemaker components, blood bags, sutures, vascular grafts and shunts. Polymers exhibit high strength to weight ratio, wide range of flexibility, ease of formability, and economics of production, but processes designed to achieve desired surface properties can compromise the overall bulk material. The ability to alter the surface of the polymer while leaving the underlying bulk material unchanged has a large potential for development in the area of biomaterials. Modifying the surface of a polymeric material so as to impede water adhesion and the ability of proteins, bacteria, and cells to grow on the surface can be minimized on those surfaces that are incorporated into biological systems. This can minimize infection, thrombosis, and other undesirable interactions. Polymer surface modification was accomplished via plasma etching and deposition in a commercial size plasma-etching device, which achieves plasma densities and electron temperatures up to 10@super 11@ cm@super -3@ and 4 eV. The degree of change is controlled by macroscopic external controls, rather than invasive internal modifications. This process lends itself well for use in existing systems. Water contact measurements taken before and after treatment of HDPE that show a change from a pretreatment angle of 85° to post treatment angles of 5°, which corresponds to a dramatic change in surface energy of the polymer and biological interaction potential. This modification is both physical and chemical, but limited to a few microns of the surface of the material. Plasma analysis is done through use of Langmuir probes, microwave interferometery, and optical spectroscopy. Surface analysis is done through XPS. Blood-material interactions are studied through two-dimensional electrophoresis in order to determine the extent and nature of protein coverage.

BI-TuP3 Non-Fouling Surfaces for Biosensors and Biomaterials, ***L. Li***, *S. Chen, J. Zheng, S. Jiang,* University of Washington

Non-fouling surfaces are critical to the performance of biosensors and biomaterials. Despite of their importance and enormous effort, non-fouling mechanism is still not quite clear at present. It was shown in our previous work that the behavior of protein adsorption depended on nano-scale structures of a surface with which proteins interact. Therefore, molecular details of a surface are of great importance to protein adsorption. However, there is still a considerable lack of the fundamental understanding of how nano-scale structures affect protein adsorption at the molecular level. The objective of this work is to demonstrate that nano-scale structures of a surface are responsible for protein resistance. Polyethylene glycol (PEG) self-assembled monolayers (SAMs) are used as model systems and the nano-scale structures of the surfaces are altered by adjusting factors such as the assembly substrates, the assembly conditions, and the composition of mixed SAMs. These SAMs are characterized by atomic force microscopy (AFM)/scanning tunneling microscopy (STM) and X-ray photoelectron spectroscopy (XPS). Protein adsorption on these surfaces was investigated by surface plasmon resonance (SPR) biosensors and tapping-mode AFM. Furthermore, experimental results are directly compared with those from molecular simulations, which can also provide additional information not easily accessible to current laboratory experiments, such as adsorbed water structures at protein/SAM interfaces. Results from combined experimental and simulation studies provide insight on how nano-scale structures affect protein adsorption and shed light on non-fouling mechanisms.

BI-TuP4 Patterned Immobilization of Proteins on High-density PEG Coated Si Surfaces, ***Y. Jun***, *X.-Y. Zhu,* University of Minnesota

Spatially localized patterns of chemical and biological functions on solid surfaces are of great interest to a number of research fields, such as biochips and cell biology. We have developed a novel chemical strategy for the formation of micro-scale patterns of chemical and biological functions on silicon surfaces using soft lithography techniques. This is based on an efficient reaction between alcohol functional groups and chlorine terminated silicon surfaces. The features formed by soft lithography show exceptionally high resolution and fidelity, with edge resolution as high as 10 nm. We demonstrate the immobilization of protein molecules to spatially localized surface functional groups on high-density PEG brush coated silicon. The spatially localized activation of the PEG brush is achieved via either partial oxidation to form aldehyde groups or via attachment of efficient leaving groups. Protein molecules are covalently immobilized to these activated regions on the PEG/Si surface.

BI-TuP5 The Topography and Viscoelastic Properties of Salivary Pellicle and Their Interaction with Tannins, ***M.E. Dickinson***, *A.B. Mann,* Rutgers University

The acquired pellicle is an organic film formed by the adsorption of proteins and carbohydrates on dental enamel exposed to the oral environment. The pellicle is now understood to have several roles including lubrication and reduction of friction between teeth during mastication, as well as protection against acidic solutions. Using in vitro AFM, growth, structure and topology of pellicle grown in vivo on a clean enamel surface has been studied. Complete coverage of the enamel surface was found to occur within the first few minutes of exposure, with the layer increasing in thickness until a maximum is reached at around 2 hours. The thickness of the pellicle varies with position, but fully developed pellicle can range in thickness from 200-500nm. Tannins found in food can interact with pellicle to change its viscoelastic properties and alter the color which is a cause of extrinsic staining in teeth. Tannins are phenolic compounds capable of precipitating alkaloids, gelatin and other proteins, they are naturally found in many food stuffs especially leafy products such as tea. The topology of the pellicle surface, as studied using AFM and SEM, consists of a dense arrangement of adsorbed globular shaped proteins with no break in the structure, even when tannins are added. The viscoelastic properties of the pellicle have been studied using nanoscale dynamic mechanical analysis, this shows that substantial variations in the storage and loss modulus occur with increasing exposure to tannin containing solutions. These changes in viscoelasticity will impinge directly on the pellicle's performance as a lubricant and also its ability to act as a chemical barrier to acid attack.

BI-TuP7 Attachment of Blood Proteins to Chitosan Surfaces, ***D.W. Thompsons***, *W.H. Nosal, S. Sarkar, A. Subramanian, J.A. Woollam,* University of Nebraska, Lincoln

Chitosan is a biomaterial of interest because of the potential to modify its amine groups to control its interactions with surfaces. Though chitosan itself is thrombogenic, such modifications may lead to a surface with enhanced biocompatibility. In this work we use spectroscopic ellipsometry to monitor the attachment of three blood proteins (albumin, IgG, and fibrinogen) to a spin-cast chitosan surface in phosphate-buffered saline solution. The moisture-induced swelling of the chitosan film is characterized, and optical constants for chitosan and the proteins are determined independently for use in modelling the time-dependent data. Ex-situ infrared ellipsometry is used to characterize the chemical nature of the protein attachment and compare chitosan films with varying degrees of deacetylation. Material anisotropy due to preferred molecule orientation is apparent from ellipsometry.

BI-TuP8 Operation of the QCM-D Technique at Elevated Oscillation Amplitudes, ***M.E.M. Edvardsson***, *F. Höök,* Chalmers University of Technology, Sweden

An often raised question with respect to applications of the quartz crystal microbalance technique is whether the shear oscillation of the sensor surface influences the measured binding events. This is indeed a relevant question, especially since solid proofs for bond rupture at elevated amplitudes was recently presented.@footnote 1,2@ In part inspired by these observations, our QCM-D device has been further developed to operate at variable driving amplitudes (from 1 V to 10 V corresponding to maximum oscillation amplitude between 4nm and 40nm), while still maintaining the possibility to perform combined f and D measurements - a combination that was not implemented in previous work. In order to test the device, we choose the well established process by which intact vesicles adsorb and decompose into a planar supported lipid bilayer on SiO@sub 2@, known to be very sensitive to external perturbations. Up to driving amplitudes of 2V, the adsorption behavior is essentially unaffected, whereas it becomes significantly affected at driving amplitudes above 2V. These results thus nicely demonstrate the possibility to implement variable driving amplitudes, and proves that an entirely new dimension will be possible to explore in detail, especially when combined with f and D data at multiple harmonics and proper theoretical modeling is implemented: The amplitude variation can be used to either affect the system being studied, or to extract new information about it by going from the harmonic to the anharmonic regime, which will be discussed in this paper. @FootnoteText@ @footnote 1@Cooper MA, et al.; Direct and sensitive detection of a human virus by rupture event scanning. Nature

Biotechnology 2001, 19:833-837@footnote 2@Dultsev FN, et al.; "Hearing" bond breakage. Measurement of bond rupture forces using a quartz crystal microbalance. Langmuir 2000, 16:5036-5040.

BI-TuP9 Protein Immobilization for Multi-Channel Sensor Detection, ***J. Ladd***, *Q. Yu, S. Chen,* University of Washington*; J. Homola,* Institute of Radio Engineering and Electronics, Czech Republic*; S. Jiang,* University of Washington

The simultaneous detection of multiple analytes is an important consideration for the advancement of current biosensor technology. Currently, few sensor systems possess the capability to accurately and precisely detect multiple antigens. The work presented demonstrates a novel approach for the functionalization of sensor surfaces for multi-channel detection. This approach combines inkjet-printing technology with self-assembled monolayer (SAM) chemistry to create a protein array. A modified commercial Epson C40UX inkjet printer is used in this work. The sensor platform is based on a layer of streptavidin immobilized on a mixed SAM of biotinylated alkanethiol (BAT) and poly(ethylene oxide) (PEO). Non-specific binding, and thus false positives, are minimized with the non-fouling background of the sensor surface. The described platform is used in a home-built surface plasmon resonance (SPR) biosensor. Results show excellent specificity in protein immobilization to the proper locations in the array, eliminating the possibility of a false detection within a channel. Analysis of multiple proteins in solution shows a similar behavior and response to pure protein solutions. The detection capabilities of a sensor using this protein array have been characterized using human choriogonadotropin (hCG).

BI-TuP10 Chemisorption of Aromatic Amino Acid Derivatives on Gold Surface, ***R.M. Petoral, Jr.***, *K. Uvdal,* Linköping University, Sweden

The interfacial property of adsorbate and thin layers of biomolecules on solid surfaces is of great significance in biomaterials and biosensor application. Understanding the binding and molecular orientation of the adsorbates is then of great importance. Amino acids with aromatic side chains such as Tyrosine and 3,4-dihydroxyphenylalanine (DOPA) is linked to a short thiol through a peptide bond and is adsorbed and self-assembled to polycrystalline gold surfaces. The molecular adsorption, chemical binding and orientation of the amino acid analogue to the surface are studied by X-ray Photoelectron Spectroscopy (XPS), Infrared Reflection-Absorption Spectroscopy (IRAS) and Near-edge X-ray Absorption Fine Structures (NEXAFS). Strong molecular binding of the amino acid derivatives on gold surface through the sulfur atom was attained. Angle dependent XPS results showed that the aromatic ring is oriented away from the gold surface. Parallel orientation of the C=O bond of the amide moiety relative to the gold surface is deduced from the IRAS and NEXAFS results. The average orientation of the aromatic ring and main molecular axis of the molecules relative to the gold surface are also determined. The aromatic amino acid derivatives are able to self-assemble and form an ordered monolayer with minimal degree of orientational disorder. Results from this experiment are valuable in our development of sensor surfaces to be used for interaction studies with other biomolecules and metal ions.

BI-TuP11 Multi-Technique Characterization of Self-Assembled Peptide Monolayers, ***N.T. Samuel***, University of Washington*; K. McCrea,* Polymer Technology Group*; L.J. Gamble,* University of Washington*; D.A. Fischer,* National Institute of Standards and Technology*; P.S. Stayton,* University of Washington*; G.A. Somorjai,* University of California, Berkeley*; D.G. Castner,* University of Washington

There is considerable interest in the immobilization of bioactive peptides for applications such as affinity separations, diagnostics, cell culture and biomedical implants. We have synthesized a series of lysine and leucine containing peptides, which are designed to attach to surfaces with different secondary structures (alpha helix, beta sheet, etc.). These peptides were attached to carboxy-terminated self-assembled monolayers and characterized with ToF-SIMS (Time-of-Flight Secondary Ion Mass Spectrometry), SFG (Sum Frequency Generation) and NEXAFS (Near Edge X-ray Absorption Fine Structure Spectroscopy). The ToF-SIMS spectra from these peptides were analyzed by principal component analysis. Principal component 1 (PC 1), which captures 89% of the variance in the spectrum, represents the variation in the bulk amino acid composition of the different monolayers. PC 2, which captures 10% of the variance, separates the peptides with different secondary structures, suggesting ToF-SIMS is sensitive to different secondary structures of the peptides. SFG spectra were acquired for the alpha helical peptide adsorbed onto both hydrophobic and negatively charged substrates. The hydrophobic surface spectrum showed strong peaks in the CH stretch regions while the negatively charged surface spectrum showed strong peaks in the NH stretch region. The SFG results indicate the alpha helical peptide binds differently to these two surfaces. These observations were also confirmed by ToF-SIMS experiments, which reveal a strong dependence of the amount of peptide adsorbed onto negatively charged substrates when the solution pH is varied. We have recently developed a simple protocol to attach a short thiol linker onto these peptides at their amine terminus. This provides an additional handle to immobilize these peptides onto surfaces and control their orientation. Polarization-dependent NEXAFS experiments on these monolayers are in progress.

BI-TuP12 Molecular Simulation of Cytochrome C Adsorbed on Self-Assembled Monolayers, ***J. Zhou***, *J. Zheng, S. Jiang,* University of Washington

Cytochrome c, a membrane electron transfer protein, plays an important role not only in a wide range of basic life processes, but also in biomaterial and biosensor applications. To enable the electron transfer fast, cytochrome c should sit on the surfaces with an orientation that the heme ring close and perpendicular to surfaces. Moreover, the adsorbed cytochrome c should keep its native conformation. Self-assembled monolayers are ideal platforms for the study of protein adsorption. In this work, the orientation and conformation of cytochrome c on charged self-assembled monolayers are investigated by a combined Monte Carlo and molecular dynamics simulation approach. The effects of positively and negatively charged SAMs, degree of dissociation of COOH-terminated SAM, ionic strength are examined. CHARMM force field was used to model the protein and SAM. Both implicit and explicit solvent model were used. The root mean square deviation, gyration radius, eccentricity, Ramachandran angles, heme orientation and superimposed structures of cytochrome c were calculated during the simulation. Simulation results show that desired orientation could be obtained on a negatively charged surface. The dissociation degree of the terminal group affects the conformation of adsorbed protein. This work sheds light on the mechanism of the orientation and conformation of adsorbed proteins at the molecular level, and would be useful for the design and development of biosensors and biomaterials.

BI-TuP13 SPARC Binding with ECM Proteins and its Influence on Cell Adhesion, ***H. Wang***, *S. Chen,* University of Washington*; T. Barker, H. Sage,* Hope Heart Institute*; B.D. Ratner, S. Jiang,* University of Washington

The secreted protein acidic and rich in cystein (SPARC/osteonectin/BM-40) is associated with events characterized by changes in cell shape and mobility. Although the molecular mechanism remains unclear, it is generally believed that the counter-adhesive properties of SPARC are related to its interactions with ECM proteins. In this study, the interactions of SPARC with ECM proteins, such as collagen I and fibronectin, are characterized and quantified using atomic force microscope (AFM) and surface plasma resonance (SPR), and their influence on cell adhesion are examined. AFM can characterize the binding of SPARC with both collagen I and fibronectin at the molecular level. SPARC has been shown to interact with collagen I, but direct topographic image has not been reported. Whether SPARC interacts with fibronectin still remains inconclusive. In this work, AFM is used to determine the number and location of binding sites on individual collagen I and fibronectin. Monoclonal antibodies of SPARC are used to assist for better visualization. These studies provide direct information about how and where binding occurs. SPR is used to quantify these interactions. It was shown that these interactions have ionic nature. Furthermore, the C-terminal region of SPARC, which contains a high-affinity Ca2+-binding site, may play an important role in its binding with ECM proteins. Thus, the influence of ionic strength and concentration of Ca2+ on binding are studied in SPR experiments. Cell culture and adhesion assays are used to study SPARC as a modulator of the adhesive process of cells seeded on ECM proteins. The influence of SPARC-collagen I interaction is studied using smooth muscle cells while the influence of SPARC-fibronectin interaction is studied using endothelial cells. The number and spreading area of cells, as well as the focal adhesion plagues are obtained as a function of the relative amount of SPARC added.

BI-TuP14 Molecular Dynamics Study of Protein Adsorption on Controlled Surfaces, ***J. Zheng***, *S. Jiang,* University of Washington

Surface resistance to protein adsorption is currently a subject of great interest with potential applications in many areas, including biomaterials and biosensors. Despite its importance, there has been a lack of molecular-level understanding of protein interactions with surfaces and the mechanism of resistance to protein adsorption remains a problem to be solved. It is well known that SAMs presenting oligo (ethylene glycol) (OEG)

groups, such as S(CH2)m-(EG)nOH or -(EG)nOCH3 resist the adsorption of proteins. The molecular-level understanding of protein resistance to surfaces is needed in order to provide insights of non-fouling mechanism. In the work, we investigate interactions of protein (e.g., lysozyme) with OEG SAM (inner) surfaces using a combined Monte Carlo and molecular dynamics simulation approach in the presence of explicit water molecules. The CHARMM (version 27), an all-atom potential force field, was used to model the protein and methyl terminated SAMs. The TIP3P potential was used to describe water interactions. For the OEG terminated SAMs, we used a SJY model that reproduces very well the helix structure of OEG tails. Results from inert surfaces will be compared with those from methyl terminated SAM surfaces. The behavior of water at protein/SAM interfaces is characterized by self-diffusion coefficient, order parameter, hydrogen bonding, and radial distribution. In these simulations the effects of surface (charge, hydrophobicity, and defect), solvent, pH, and ion strength will be taken into account. Results from this work will shed a light on non-fouling mechanism at the atomic-scale level and guide the design of better biocompatible materials and biosensors.

BI-TuP15 Creation of Surface Macromolecular Docking Sites for the Reversible Immobilization of Proteins in Active Conformation and Controlled Orientation, ***G. Zhen***, *E. Zobeley, V. Eggli, R. Glockshuber, M. Textor,* Swiss Federal Institute of Technology, Switzerland

Our contribution describes the comparative performance of two docking site concepts for the immobilization of biomolecules in active conformation and controlled immobilization. They are based on biotin-(Strep)avidin and NTA-Ni@super 2+@-histag linkage techniques, in combination with polycationic, PEG-grafted, NTA(nitrilotriacetic acid) functionalized and biotinylated copolymers. Enzyme @beta@-lactamase served as the model protein for the verification of the linkage concept and for the investigation of the activity of the surface-immobilized protein and its dependence on the molecular orientation. Five different variants of @beta@-lactamase with single cysteine site-directed mutagenesis on the surface were engineered. Two platforms were tested in order to determine how the mechanical and dynamic properties of the interface influence the enzyme-orientation-dependent catalytic activity: polymeric interface (flexible chains, soft) and functionalized alkanethiol monolayers on gold (comparatively immobile, stiff). The @beta@-lactamase variants were biotinylated at free thiol-group with a cleavable biotinylation reagent allowing for controlled release of the surface-bound enzyme. The immobilization was achieved on niobium oxide surface coated with biotinylated Poly(L-lysine)-g-poly(ethylene glycol) and on gold coated with mixture of alkanethiol-biotin and alkanethiol-OH self-assembled monolayer. The biotinylated @beta@-lactamase was subsequently bound to the surface via NeutrAvidin. The long-term stability of the immobilized proteins was evaluated. The amount of immobilized @beta@-lactamase on the chips was measured by three different techniques: OWLS, SPR and specific enzymatic activity via photospectroscopic detection of the turnover of the chromogenic substrate nitrocefin. Specific immobilization could be discriminated from non-specific adsorption. Furthermore the effect of the immobilization scheme on the biological activity was quantitatively examined.

BI-TuP16 X-ray Surface Scattering for the Structural Analysis of Adsorbed Proteins at Hydrated Interfaces, ***C.A. Pavloski***, *S.S. Lateef, M.L. Schlossman, L. Hanley,* University of Illinois at Chicago

Protein adsorption onto solid surfaces is a significant process in a wide variety of applications including biomaterials, tissue engineering, biosensors, immunoassays and protein arrays. Surface properties are altered by synthetic and naturally occurring molecular adsorbates when a biomaterial is brought into contact with a biological fluid. We are interested in determining the structural conformation of adsorbed proteins at this aqueous-solid interface. We examine the surface adsorption of bovine serum albumin (BSA), the most abundant protein in blood. We bromine label BSA to allow probes of its adsorbed conformation on an amine-functionalized monolayer on a silicon wafer. We use x-ray photoelectron spectroscopy and atomic force microscopy to study the chemistry and structure of the dry surface. We then apply x-ray reflectivity and x-ray standing wave fluorescence to probe the conformation of adsorbed BSA at the hydrated interface.

BI-TuP17 Assembly and Disassembly of Hydrogels to Entrap, Grow, and Release Cells, ***G.F. Payne***, *T. Chen, L.-Q. Wu, D.A. Small, H. Yi,* University of Maryland Biotechnology Institute; *R. Ghodssi, G.W. Rubloff,* University of Maryland; *W.E. Bentley,* University of Maryland Biotechnology Institute

Hydrogels provide a bio-friendly environment for cultivating cells. Standard methods for entrapping cells within hydrogel matrices exploit the photopolymerization of synthetic monomers (or macromonomers). The strength of photopolymerization is that standard lithographic approaches can be exploited to exert considerable spatial and temporal control of hydrogel formation. The weaknesses of photopolymerization are that these methods are not always benign to cells, and the hydrogel matrices are generally permanent. We are examining an alternative method for in situ hydrogel formation based on biopolymers and enzymes. Specifically, we mix cells with the protein gelatin, and add the protein-crosslinking enzyme transglutaminase. Gel formation occurs over the course of 1-2 hours. In situ-entrapped bacterial cells (E. coli) were observed to grow to high densities within the crosslinked gelatin matrices, and these cultured cells could sense and respond to appropriate inducers (we examined the inducible expression of green fluorescent protein). After growing the cells, they could be released from the hydrogel using the protein-degrading enzyme, proteinase K. Cells were released over the course of 1 hour and they remained viable and inducible. This study demonstrates that; one enzyme (transglutaminase) can entrap cells within a hydrogel, the cells can proliferate to high densities within the matrix, and a second enzyme (proteinase K) can "dissolve" the hydrogel to release the cells. This capability should provide unique opportunities for microfluidic biosensors.

BI-TuP18 Customized Tissue Engineering Using Photopolymerizable Hydrogels and Stereolithography Techniques, ***B. Dhariwala***, *E. Hunt, T. Boland,* Clemson University

The power of tissue engineering can be enhanced using customizable scaffolds to repair defects caused by birth or accidents. For customized tissue engineering, one of the variables accessible and tunable by the engineer is the form and aspects of the scaffold onto which cells are seeded. Here we are studying hydrogels as materials that can be used for custom designed scaffolds. For this study, we employed photopolymerizable hydrogels fabricated by crosslinking polyethylene oxide (PEO) with polyethylene dimethacrylate (PEGDM) monomer using 2-methyl-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (IRGACURE) as the photoinitiator. Several hydrogels have been prepared including custom shaped hydrogels made using polypropylene molds. Initial characterization of these gels will be presented. Use of stereolithography technique has been carried out to make customized scaffolds. This is a computerized technique where a high intensity UV laser beam is used to form the hydrogel according to a 3D image of the defect. The laser scans in the X-Y plane and there is a movable platform which serves as the Z plane. Different scaffold shapes were made using this technique. Some mechanical tests were carried out on the polymer will also be overviewed and their results will be shown. Toxicity studies of the photoinitiator were carried out to determine adequate amount of initiator to be used. Initial cell studies were carried out to ensure cell viability in the polymer. Cells were mixed with the polymer, which was then photopolymerized, and their viability was studied and results will be discussed. We would further investigate cell viability & cell growth over extended periods of time. Stereolithography is a very efficient method of preparing 3D scaffolds and holds a promising future for tissue engineering.

BI-TuP19 Cell-Based Biosensors - A Tool for High Throughput Toxin Detection, ***K. Varghese***, *A. Jamshidi, K. Sieverdes, P. Molnar, M. Das, C.A. Gregory, J.J. Hickman,* Clemson University

Cell-based biosensors incorporate a cellular sensing element that detects a change in the cells immediate environment and converts the cellular signal with an electrical impulse that is conducive for integration to a silicon environment. Cell-based electrophysiology can be broadly divided into two categories - those based on intracellular potentials (eg. using glass microelectrodes as in patch clamping) and those based on extracellular potentials. Our research focuses on the latter, wherein extracellular microelectrode arrays are used as a noninvasive and long-term approach for the measurement of biopotentials. The objective of this study is to culture cardiac myocytes on Metal Microelectrode Arrays (MEAs) and Field Effect Transistors (FETs) and test them upon exposure to various toxins. The culture conditions (serum vs serum free), were also investigated to compare and contrast the results obtained therein. In the first and present part of this study, cardiac myocytes from Day 6 chicken embryos were cultured on MEAs and tested for their response to different concentrations of Cadmium Chloride and Cesium Chloride, which are known

environmental toxins. Studies also were carried out to study the effect of various adhesive surfaces on the health and response of these cardiac myocytes. DETA, fibronectin and Entactin-Collagen-Laminin (ECL) matrix were the surfaces studied. Preliminary results have shown a healthier monolayer and higher beat frequency with serum free conditions. In the comparative study between different surfaces, best results have been obtained on DETA for beat frequency.

BI-TuP20 In-vitro Electrophysiological Comparison of Embryonic Hippocampal Neurons Grown in 2D and 3D Environments, ***T. Xu***, *P. Molnar, C.A. Gregory, M. Das, J.J. Hickman, T. Boland,* Clemson University

To compare electrophysiological differences between neurons cultured in 2D and 3D environments, neurons dissociated form embryonic rat brain were seeded onto type-I collage thin coating, collagen gel surface, and were entrapped randomly into collagen gel, which were set up for imitating a 2D culture environment, a defined 3D environment and a random 3D environment for neuron culture respectively. Double-immunostaining for MAP-2, a neuronal cell body and dendritic marker, and anti-neurofilament antibody, an axonal marker, was used to identify neuron morphology. Hippocampal neuron polarities and outgrowth of neurites were evaluated by confocal microscope images. The gels were optimized for neuronal 3D cultures by varying concentrations, porosities, and glial cell densities. Surface properties of the gels will be characterized by AFM. Whole-cell patch clamp experiments were carried out to investigate electrophysiology of hippocampal neurons cultured in different conditions. Improvements of patch clamp technique for neuronal 3D culture over traditional 2D culture were applied. Membrane and synaptic properties of neurons in response to their different culture conditions were recorded and compared. Our preliminary results show that hippocampal neurons cultured in 2D and 3D environments exhibited similar passive membrane properties and sodium and potassium currents. Repetitive firings of action potential were found in neurons cultured in 3D environment over 14 days. Results will be presented on functional synapse formation for neurons cultured in 3D environment as measured by the combination of the patch clamp technique and the sharp microelectrode technique. The results of this study indicate that embryonic hippocampal neurons retain a clearly neuronal electrophysiological phenotype in a engineered in-vitro 3D culture condition, which is holding potential in applications ranging from neural tissue engineering to providing active neuronal networks for neuro-computing.

BI-TuP21 Detection of E. Coli O157:H7 with Surface Plasmon Resonance Biosensor in Complex Matrices, ***A. Taylor***, *Q. Yu, S. Jiang,* University of Washington

There is an urgent need for fast, sensitive, and reliable methods for detecting biological warfare agents and food contaminants. Large analytes (i.e. Escherichia coli, Salmonella enteritidis, or Listeria monocytogenes) are difficult to detect and quantify at low concentrations without time-consuming amplifications (i.e. culturing and PCR). E. coli O157:H7, an important food contaminant, was detected with a surface plasmon resonance (SPR) biosensor. However, with amplification, the detection limit was 5x10@super 7@ cfu/ml. In this work, we detect E. coli in both buffer and complex matrices using a home-built SPR. Antibody specific to the O antigen protein expressed on the membrane of the E. coli cell was immobilized on sensing surface via self-assembled monolayers. Atomic force microscopy (AFM) is combined with SPR to optimize surface chemistries and antibody immobilization at the molecular level. Transport of large charged bacteria to the antibody functionalized surface is one important factor limiting the ability to detect low concentrations. Thus, we study the effects of flow rate and pattern on detection. The biosensor was proven to differentiate between E. coli strains O157:H7 and K12 based on the antibody chosen. Furthermore, immunomagnetic separation methods using antibody functionalized magnetic particles were used to separate analytes from complex matrices (i.e. ground beef). The objectives of this work are to improve low detection limit and to minimize non-specific binding for SPR detection of larger-sized analytes in complex matrices.

BI-TuP22 A Comparative Study of Bone Cell Attachment and Spreading on Various Metal Surfaces by Cryo-SEM and QCM-D, ***M. Foss***, *J. Justesen, M. Duch, A.-L. Stranne, J. Chevallier, C. Modin, F.S. Pedersen, F. Besenbacher,* University of Aarhus, Denmark

The detailed understanding of the attachment of bone-forming cells on surfaces is crucial for the development of new generations of orthopaedic implant materials. The goal of these studies is to establish methods for a more quantitative measurement of biocompatibility. Here, traditional methods of cell counting and cell area measurements are correlated with data obtained by the Quartz Crystal Microbalance technique (QCM-D, Q-Sense AB) of cell attachment and spreading. The spreading and attachment of the murine preosteoblastic MC3T3-E1 cells were quantified by cryo-SEM. Cells were seeded and allowed to attach for various periods of time, fixed and snap-frozen in liquid nitrogen. The number of attached cells and the mean area were determined using a standard image analysis program. After attachment the shape of the cells changes from round to an initial maximum spread at the surface. The time-scale for maximum spread at serum-free conditions has been determined to 40 - 50 min, which is in good agreement with QCM-D data where the maximum shifts in both frequency and dissipation are reached at similar time points. The examinations have been applied to several relevant metal surfaces including tantalum and chromium. At cell concentrations ranging from 50,000 cells/ml to 300,000 cells/ml, a variation in the degree of cell spreading is observed between these two metals implying differences in the cell attachment at the chemically different surfaces. However, the viscoelastic properties of the cells are independent of the substrate material. The results point to the establishment of a fast and accurate general method for screening biomaterials with QCM-D. The methods will furthermore be applied to surfaces functionalized by prototype proteins including BSA and fibronectin.

BI-TuP23 Control of Osteopontin Behavior on Surfaces for Cell Adhesion, ***L. Liu***, *S. Chen, B.D. Ratner, S. Jiang,* University of Washington

Osteopontin (OPN) is an important extracellular matrix protein shown to function in wound healing, inflammation and foreign body reaction and has been identified as a potential target for engineered biomaterials. It contains RGD moiety that has been shown to mediate cell adhesion through interaction with integrins. In preferred orientation and conformation, the RGD tripeptide of OPN will be presented to the cells to the greatest extent. However, control of OPN orientation/conformation is seldom investigated so far. In this work, we investigate OPN adsorption and cell adhesion on self-assembled monolayers (SAMs) of alkanethiols terminated with different functional groups to tailor surface properties. Four different alkanethiols terminated with -CH3, -OH, -NH2 and -COOH were used to form surfaces representing hydrophobic, hydrophilic, positive and negative surfaces. Atomic force microscopy (AFM) is used to characterize the adsorption of OPN on various SAM surfaces. Our AFM results show that the amount of adsorbed OPN on -COOH surface is slightly less than that on -NH2 surface. In vitro cell adhesion assay of bovine aortic endothelial cells (BAEC) was performed to test OPN function on various SAMs. Our results show that on -NH2 surface BAEC adhesion is the most and cell appears most spread. Both cell counts and average cell spreading area on -COOH and -CH3 surfaces are much less than those on -NH2 surface. The -OH surface is resistant to both OPN adsorption and cell adhesion. By comparing results from AFM and cell adhesion experiments, it is suggested that the orientation/conformation of OPN on -NH2 positively charged surface is more favorable for cell interaction than on -COOH negatively charged surface. This is consistent with our molecular simulation prediction. Our studies clearly show that surface properties will alter OPN behavior on surfaces, thus cell interactions with OPN. In addition, we use atomic force microscopy (AFM) to image the binding of OPN onto type I collagen monomer. Interactions among extracellular matrix (ECM) proteins are important in many aspects such as orientating ligand proteins and correctly delivering signals into a cell. Recent experiments show that osteopontin (OPN)-incubated collagen I chemically immobilized on poly(HEMA) promotes cell adhesion in vitro. It is speculated that bound OPN on a collagen matrix may have better orientation/ conformation and thus influence its cell-binding ability. Although it has been reported that OPN is able to bind with type I collagen, direct visualization of OPN-collagen binding complexes has not been reported and OPN binding sites on collagen I are still unknown. In our work, AFM is used to image the binding of OPN onto individual triple-helical collagen I monomer on freshly cleaved mica for the first time. Analysis of AFM results clearly shows binding patterns of OPN to collagen I. This work provides a direct means to identify binding among ECM proteins and a better understanding of ECM proteins on cell adhesion.

BI-TuP24 Tissue Formation of Endothelial Cells on a Microporous Film of Biodegradable Polymer, ***T. Nishikawa***, *T.A. Ohzono, J. Hayashi, M. Hara, M.A. Shimomura,* The Institute of Physical and Chemical Research, Japan

Micropatterned surface is considered to be a promising biointerface that can control both surface chemistry and surface morphology of cell culture substrates. The biointerface to be issued in this report is a microporous film of biodegradable polymers. Honeycomb films are microporous films of polymers which are formed by applying moist air to a spread polymer

solution. We report the tissue formation of endothelial cells (ECs) on self-supporting honeycomb films. The tissue formation was studied in regard to cell-matrix adhesion, proliferation, and movement. Honeycomb films were prepared from mixtures of biodegradable polymers (poly(L-lactic acid) (PLLA) and poly(@epsilon@-caprolactone) (PCL)) and amphiphilic polymers). Adhesion behavior of ECs was characterized by formation of stress fiber of actin and localization of focal adhesion proteins at the interface between cells and culture substrate. ECs did not form focal adhesions on self-supporting microporous films. The modulated cell adhesion on the microporous films influenced cell-division cycle of ECs. Doubling time represents an average period of cell-division cycle. The doubling time of ECs estimated from the proliferation curves was 20 hrs on flat cast film of PCL and 27 hrs on microporous films of PCL. Micropores can be considered to be pathways connecting two sides of a self-supporting honeycomb film of PLLA. ECs were seeded onto a top side of a honeycomb film having an average poresize of 5 μm and an average thickness of 3 μm. At the day 11 of culturing, the cell culture was observed by confocal microscopy after staining filamentous actin of ECs and a honeycomb film with fluorescent probes. Monolayer of ECs was confirmed at each side of the honeycomb film. This suggests that ECs attached onto the top side pass through the micropores, appear on the bottom side of a honeycomb film, grow, proliferate, and finally cover the both sides of the honeycomb film.

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