Wednesday Morning, October 27, 1999

Biomaterial Interfaces Group
Room 613/614 - Session BI-WeM

Cell Solid-Surface Interactions

Moderator: W. Knoll, Max Planck Institute for Polymer Research, Germany

8:20am **BI-WeM1 Fabrication of Biologically Active Interfaces upon Self-Organization of Amphiphilic Polymers**, ***T. Nishikawa***, *J. Nishida, K. Nishikawa, R. Ookura, S.-I. Nishimura, S. Wada, T. Karino, H. Okubo, M. Matsushita, S. Todo, M. Shimomura,* Hokkaido University, Japan

Fabrication of cell culture substrates possessing micro surface morphology is one of the current topics in biomaterial research. Recently we found that two dimensional honeycomb structures can be fabricated by casting dilute solutions of amphiphilic compounds on solid supports in a humid atmosphere. The structural feature of the honeycomb films is a two dimensional single layer of hexagonally arrayed holes, whose diameter is ranging from 1 μm to 10 μm. We suggest that the honeycomb films work as artificial basal films - biologically active interfaces between cells and solid supports. The porous structure will enable the adhered cells to reach and interact with the surface of solid support as well as the exposed surface of the cast films. The pore size, porosity, and thickness of the films can be major factors which control the cell behavior on the culture substrates. In this sense cell behavior on porous surfaces can be influenced by the surface morphology as well as the chemical properties of the polymers constituting the films. In this report we describe the fabrication of the honeycomb films and the cell culturing on the films from the view point of factors affecting the cell adhesion in detail. Honeycomb films with various pore size and film thickness were fabricated by casting dilute solution of amphiphilic copolymers on water surfaces. The films were transferred onto cell adhesive supports (slide glass) or non-adhesive supports (polyhydroxyethylmethacrylate coated glass plate). Bovine aorta endothelial cells or hepatocytes were cultured on the honeycomb films. The thicknesses of the films were varied with the water temperature of the subphase (at 6°C 0.2 μm in thickness and 4 μm in hole diameter and at 20°C 1.5 μm in thickness and 4 μm of hole diameter). The cell adhesion to the honeycomb films was considerably influenced by the film thickness, which determines the distance between the adhered cells and the solid supports.

8:40am **BI-WeM2 Reactions of Biological Cells to Nanostructures**, ***A.S.G. Curtis***, *C.D.W. Wilkinson,* University of Glasgow, Scotland

The reactions of biological cells to nanostructured polymer and silica surfaces will be described. The surfaces have been prepared in a variety of materials using E-beam methods, colloidal resists and replication by embossing. The reactios include changes in adhesion and cytoskeletal organisation. The effects of topography in the ranges 20-100nm greatest plan dimension, 50-200nm repeat and 10-100nm height will be described. The degree to which the cells conform to these surfaces will be reported with details of the closeness of approach of the cells to the surfaces. The question of whether the reactions are to topography, chemistry or surface disorder at boundaries will be discussed.

9:00am **BI-WeM3 Engineering Cell Surface Chemistry**, ***C.R. Bertozzi***, University of California, Berkeley **INVITED**

Many important biological processes are initiated by cell surface molecules, such as cell-cell adhesion and communication during development, virus-host cell binding, tumor cell metastasis and immunological recognition. Consequently, the ability to chemically control the display of epitopes on cell surfaces would enable a myriad of possibilities for studying cell-cell interactions and for engineering cells with novel properties. This presentation will focus on work in my laboratory that aims to apply the principles of organic chemistry to orchestrating cell surface chemistry. We have harnessed the cell's metabolic machinery to remodel cell surfaces with reactive organic functional groups. The foundation of our approach is the unnatural substrate tolerance of several enzymes involved in oligosaccharide biosynthesis, which permits the conversion of unnatural monosaccharide precursors into cell surface-associated oligosaccharides. We have exploited these pathways as vehicles for the delivery of uniquely reactive electrophilic functional groups, such as ketones and azides, to cell surfaces. For example, we demonstrated that an unnatural analog of N-acetylmannosamine bearing a ketone group, N-levulinoylmannosamine (ManLev), is metabolized by human cells to N-levulinoyl sialosides on the cell surface, resulting in the cell surface display of ketone groups. The cell surface can then be selectively reacted with rationally-designed organic structures bearing a complementary nucleophile such as an aminooxy group which reacts to form a stable covalent adduct. The ability to engineer chemical reactivity into endogenous cell surface molecules suggests many potential applications including the engineered adhesion of cells to materials and artificial surfaces. @FootnoteText@ @footnote 1@ Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. Engineering Chemical Reactivity on Cell Surfaces Through Oligosaccharide Biosynthesis. Science 1997, 276, 1125-1128. @footnote 2@ Mahal, L. K.; Bertozzi, C. R. Engineered Cell Surfaces: Fertile Ground for Molecular Landscaping. Chemistry & Biology 1997, 4, 415-422. @footnote 3@ Lemieux, G. A. ; Bertozzi, C. R. Chemoselective Ligation Reactions with Proteins, Oligosaccharides and Cells. Trends Biotech. 1998, 16, 506-513. @footnote 4@Yarema, K. J.; Mahal, L. K.; Bruehl, R.; Rodriguez, E. C.; Bertozzi, C. R. Metabolic Delivery of Ketone Groups to Sialic Acid Residues. Application to Cell Surface Glycoform Engineering. J. Biol. Chem. 1998, 273, 31168-31179. @footnote 5@ Lemieux, G. A.; Yarema, K. J.; Jacobs, C. L.; Bertozzi, C. R. Exploiting Differences in Sialoside Expression for Selective Targeting of MRI Contrast Reagents. J. Am. Chem. Soc. 1999, 121,4278-4279.

9:40am **BI-WeM5 Biocompatibility of Cardiac Cells on Silane-Modified Surfaces**, ***J.J. Hickman***, *M.S. Ravenscroft, H. Canavan,* The George Washington University*; V. Krauthamer,* Food and Drug Administration

We are investigating the interaction of cardiac cells with select silane-modified surfaces in a defined culture system. Our goal is to examine cell reaction in vitro to the types of modified surfaces that may be useful as implant coatings with an assay that we could relate to organ function. The SAM-modified glass coverslips were analyzed using X-ray Photoelectron Spectroscopy (XPS). The morphology was determined by image analysis and the excitability of the cultured cardiac cells was examined electrophysiologically by Calcium imaging both at rest and while being stimulated. Stimulation experiments electrically pace the cells at select time intervals and monitor the resulting changes in intracellular [Ca]. We observed a significant difference in excitation-induced Ca changes on the different silanated surfaces without corresponding differences in cell morphology. This result implies a change in cardiac Ca channel function on SAMs with different functional groups that would not be detected by morphological analysis alone.

10:00am **BI-WeM6 Biomaterials That Talk**, ***P.S. Stayton***, *T.C. McDevitt, K.E. Nelson, C.M. Giachelli,* University of Washington*; R.B. Vernon, H. Sage,* Hope Heart Institute*; D.G. Castner,* University of Washington

We are working to develop a variety of biomaterials that are designed to communicate with biomolecules, cells, and tissues. This goal requires complementary surface assembly techniques, engineering of biomolecules designed for surface applications, and detailed characterization of the biomolecules at surfaces. In this talk, I will present joint protein engineering and microfabrication approaches to constructing coatings that control cell phenotype in confined dimensions on device surfaces. These coatings are designed to interact with specific receptors to control cell motility, proliferation, and protect against apoptosis.

10:20am **BI-WeM7 Biofunctionalization of Surfaces with Peptide Amphiphiles**, ***M. Tirrell***, University of Minnesota, U.S. **INVITED**

Peptides carry enormous capacity and versatility for participating in specific ligand-receptor binding interactions. As small fragments of proteins, they offer the possibility of delivering a selected activity in constructing a biofunctionalized surface or interface, absent other, undesired activities present in the full protein molecule (e.g., immunogenicity). We have been exploring the self-assembly and cell recognition properties of peptide fragments (thus far derived from extracellular matrix fragments) that we have lipidated synthetically by attaching a phospholipid-mimic, double-chain, hydrocarbon tail. Lipidation confers interesting amphiphilic and self-organization properties on the molecules and enables the stable deposition of layers of peptide amphiphiles on surfaces. Specifically, we have been using peptide amphiphiles to functionalize surfaces with peptide fragments derived from collagen and fibronectin. Deposition of these molecules by Langmuir-Blodgett methods gives a very high degree of control over the density and orientation of the surface molecules. This in turn enables us explore the effects on cell response of peptide density and molecular architecture variations with a great degree of precision. The principal results so far, which seem to have some generality for different kinds of peptides, are that there is an optimum peptide density for each kind of peptide fragment, and that the architecture of peptide presentation is a very sensitive controller of bioactivity. Examples will be given of these effects.

11:00am **BI-WeM9 Plasma Copolymer Surfaces for Cell Culture**, ***R.D. Short***, University of Sheffield, UK

The plasma copolymerisation of a functionalised monomer with a hydrocarbon diluent comonomer can be used to fabricate surfaces of controlled chemistry. By employing low plasma power, fragmentation can be kept to a minimum and the functional group preserved from the functionalised monomer to the plasma copolymer deposit. This has been demonstrated for carboxyls, carbonyls, alcohols and amines. Changing the ratio of functionalised monomer to hydrocarbon monomer allows films of varying functional group concentration (functional groups per 100 carbons) to be prepared. Substratum surface chemistry is known to play a critical role in the attachment, spreading and proliferation and differentiation of cells in tissue culture. The culturing of different cell types - keratinocytes (primary), endothelial cells (cell line) and osteoblasts (primary and cell line) - on plasma copolymer films containing carboxyl, carbonyl and alcohol functionalities has been investigated. Cell response has been explored with functional group concentration. Optimum attachment, spreading and proliferation were obtained on surfaces containing carboxyl groups - these surfaces contained 3-5 carboxyl groups per 100 carbons. Actin-staining by direct immunofluorescence was used to visualise changes in osteoblast cytoskeleton with substratum chemistry. It was observed that as the substratum carboxyl concentration increased cell spreading was notably enhanced. As few as 5 carboxyls per 100 carbons were sufficient to support good cell attachment and a well-defined polygonal cell morphology on an essentially hydrophobic surface.

11:20am **BI-WeM10 Mechanical Properties of a Bone Marrow Cell-Knit Composite for Tissue Engineering: Evolution under Mechanical Load**, ***B. Müller***, *G. Ettel, D. Siragusano, T. Brandsberg, F. Brandsberg, M. Petitmermet, A. Bruinink, J. Mayer, E. Wintermantel,* ETH Zürich, Switzerland

Knitted textiles provide a 3D scaffold for optimal spatial and nutritional conditions in engineering biological tissue in vitro. The vital-avital composite formed by the textile fabric and the ingrown cells can be stimulated by mechanical load. Introduced by cyclic stretching it affects the proliferation and differentiation of bone cells as indicated by specific protein synthesis and cell mass increase. As an additional parameter, the evolution of the mechanical properties of the vital-avital composite is in situ measured by a piezoelectric force sensor. The system for the stimulation of in vitro cell cultures is calibrated by the use of a coil spring minimizing frictional losses. Reference measurements are performed using multifilament PET-knits as untreated ones and others saturated with serum proteins. After autoclaving and under constant load, both types of knits show an exponential run-in behavior with a time constant of about 2h. In the frequency range investigated (0.1 to 3.0Hz) the amplitude raise lies between 10 and 15%. Long-term experiments (5 days) with cyclic mobile and immobile phases of 3 and 6 hours, respectively exhibit a linear decrease of 5% in amplitude for the protein saturated knits, however. Finally, the preliminary experiments using primary adult rat bone marrow cells demonstrate that the stiffness of the vital-avital composite increases by a power law as a result of mechanical stimulation (Stretching is as low as 2%). Therefore, the successive force measurements reflect the physiological mechanical state of the cells and, consequently, enable optimizing the properties of the forming tissue. Determinants are the cell density, the stretching, the frequency of mechanical excitation, and the time span for mobile and immobile phases. A mathematical model developed on the basis of nonlinear mesoscopic elasticity theory describes the experimental observations.

11:40am **BI-WeM11 The Effect of Lipopolysaccharide Structure and Composition on Microbial Cell Adhesion**, ***S. Kim***, *J. Curry,* University of Arizona

Lipopolysaccharide (LPS), the main component of the outer membrane of Gram negative bacteria, consists of a lipid component, termed lipid A, that anchors the LPS in the outer membrane, a sugar core, and a variable O-specific polysaccharide chain. Whenever a bacterium approaches a surface, LPS predominantly mediates the interaction because of its inherent location on the cell surface. Varying size and structure of LPS molecule depending on bacterial strain appears to be an important determinant of the overall charge and hydrophobic character of the cell surface. Furthermore, some workers have shown that change in its chemical composition or pattern lead to a dramatic change in its biological activity. Those facts suggest that studying adhesion as a function of LPS structure and chemical composition may help to better understand the mechanisms of bacterial adhesion. The overall goal of our research is to understand at the molecular scale how the structure and composition of the LPS affects bacterial adhesion and biological activity by direct force measurement using the Surface Forces Apparatus (SFA). Specifically, we will measure the force of interaction and adhesion between two hydrophilic (bare mica) and hydrophobic (surfactant coated) surfaces in the presence of LPS molecules at different environmental conditions (i.e. temperature, pH, ionic strength). Samples used in this study will be several rough mutant LPS (R-form) molecules of which structures are well characterized. Along with the knowledge of their structure and chemical composition, the complete force profile will allow us to better predict adhesive properties of several different types of bacteria. This work will be very meaningful for research in many areas where microbial adhesion is important, for instance, biofilm formation and microbial transport in porous media.

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