Wednesday Morning, November 5, 2003

Biomaterial Interfaces
Room 307 - Session BI+SS-WeM

Cell Interactions with Patterned Surfaces
Moderator: M. Textor, ETH Zurich, Switzerland

8:20am **BI+SS-WeM1 Patterned Surfaces using Masking during Plasma Deposition or Pulsed Laser Ablation**, *H. Thissen,* CSIRO Molecular Science, Australia; *J.P. Hayes,* Industrial Research Institute Swinburne, Australia; *P.G. Hartley, G. Johnson,* CSIRO Molecular Science, Australia; *E.C. Harvey,* Industrial Research Institute Swinburne, Australia; ***H.J. Griesser,*** University of South Australia, Australia

The patterning of biomaterial surfaces has attracted much recent interest for various fundamental and applied purposes, such as the control of the location and shape of attached anchorage-dependent cells. Patterned surfaces are also of interest for bio-diagnostic arrays, cell culturing and separation, some tissue engineering products, and some biomedical implants. We have used two different approaches for the fabrication of patterned surface chemistries. One approach involves the use of masks during the deposition of thin plasma polymer coatings. The other approach is based on the deposition of multilayer coating structures followed by laser ablation through a mask; the top layer is a non-adhesive coating such as PEG and the laser beam exposes adhesive regions "underneath" by ablating the PEG layer in spatially controlled areas. Cell-adhesive proteins can then adsorb only onto the exposed areas capable of adsorbing proteins. The second approach is very attractive because of its speed and ease of fabrication; ablation of the thin PEG layer using a pulsed 248 nm excimer laser is fast with nanometre thickness control by controlling the number of laser pulses. The patterned surface chemistries and their protein adsorption characteristics were analyzed by several surface analytical techniques and by antibody assay. Cell culture using bovine corneal epithelial cells confirmed that cell attachment is controlled by these surface chemistry patterns. Our work has so far focused on fluoropolymer and Si wafer substrates and the use of plasma polymer interlayers for the covalent anchoring of a cloud point grafted PEG top layer; the use of a plasma polymer interlayer has the advantage of being readily transferable to a variety of substrates both ceramic and polymeric. However, the use of laser patterning is not restricted to those coating structures and can be applied to burn adhesive "holes" into other non-adhesive coatings equally well.

8:40am **BI+SS-WeM2 Patterning Surfaces with "Nonfouling" Oligoethylene Glycol "Bottle Brushes" by Soft Lithography and Surface-Initiated Atom Transfer Radical Polymerization**, ***H. Ma,*** *A. Chilkoti,* Duke University

A "grafting from" strategy is described for creating patterned biologically-nonfouling polymer coatings. Initiators presenting a bromoisobutyrate moiety and a thiol group at two ends of the molecule were synthesized and patterned on gold by soft lithography. The patterned SAM was used as a substrate for surface-initiated atom transfer radical polymerization (SI-ATRP) of oligoethylene glycol methyl methacrylate (OEGMA). The SI-ATRP was carried out in an oxygen-free environment with CuBr/Bipy as catalysts in a water /methanol mixture. Ellipsometry showed that the thickness of the poly(OEGMA) "bottle brush" could be easily manipulated from 0 to 50 nm by control of the polymerization conditions. The patterns were characterized by imaging ToF-SIMS, imaging XPS, and AFM. This "bottle brushes" are exceptionally protein-resistant. Surface plasmon resonance (SPR) spectroscopy showed no adsorption of fibronectin (1 mg/ml), 10% or 100 % fetal bovine serum (FBS) onto those surfaces. NIH 3T3 fibroblasts were confined to regions demarcated by the patterned poly(OEGMA) brushes. The cellular patterns were maintained for over 30 days, which is significantly longer than is possible with EG-terminated alkanethiol SAMs. This "grafting from" strategy is not limited to gold-coated surfaces as demonstrated by SI-ATRP on glass and silicon, and overcomes the intrinsic limitation of low surface density of PEG chains by physisorption or the "grafting to" approach. The poly(OEGMA) grafts synthesized in situ by SI-ATRP recapitulate in a polymer brush some of the key features of oligoethylene glycol-terminated SAMs, namely the high surface density of oligoethylene glycol in a thicker and more robust coating. These patterned "nonfouling" surfaces have utility in the design of experimentally useful model system to investigate the response of cells to chemical and topographical cues, in addition to a wide range of applications in bioanalytical devices.

9:00am **BI+SS-WeM3 Molecular Assembly Patterning by Lift-off (MAPL): A Novel Approach to Produce Biologically Designed Micropatterns for Biosensor Applications and Cell-Surface Interaction Studies**, ***D. Falconnet,*** Swiss Federal Institute of Technology (ETH) Zurich; *F. Assi,* Swiss Federal Institute of Technology (ETH) Zurich, Switzerland; *A. Koenig,* Swiss Federal Institute of Technology (ETH) Zurich; *M. Textor,* Swiss Federal Institute of Technology (ETH) Zurich, Switzerland

A new chemical micropatterning technique is presented for cell-surface interaction studies. The MAPL technique allows creating patterns of bioactive molecules (such as biotin, peptides, oligonucleotides) at a controlled surface density and embedded in a background resistant to the adsorption of proteins. A simple photoresist lift-off process is exploited in conjunction with the spontaneous assembly of polycationic poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) onto negatively charged metal oxide surfaces. A positive photoresist on a metal-oxide-coated substrate (e.g. niobium oxide coated on glass) is developed resulting in a micropattern of resist and bare metal oxide areas. Bio-functionalized (e.g., biotin or cell-adhesive peptide) PLL-g-PEG is immobilized at the bare metal oxide regions by spontaneous assembly from aqueous solutions of the polymer. The photoresist is lifted off in an organic solvent without affecting the integrity of the adsorbed functionalized PLL-g-PEG monolayer. Subsequently, the background is backfilled with protein- and cell-resistant PLL-g-PEG. The resulting pattern of bio-interactive and non-adhesive areas shows an excellent contrast on the protein level, demonstrated by fluorescence microscopy using labeled streptavidin to specifically decorate the PLL-g-PEG/PEG-biotin patches. Cell attachment to such micropatterns consisting of PLL-g-PEG/PEG-RGD-peptide was tested using human foreskin fibroblasts. This lift-off-based biochemical patterning is a 'soft', robust, simple and reproducible technique that does not require specialized clean room and heavy etching facilities. It is an interesting alternative to microcontact printing because it circumvents many disadvantages of the printing technique. The high signal-to-noise ratio and the feasibility of tailoring the bioligand (capture molecule) density in the interactive patches make MAPL a promising technique also for biosensor microarray applications.

9:20am **BI+SS-WeM4 Micrometer-scale Fibronectin Patterning for Control of Focal Adhesion Dynamics in Fibroblasts**, ***D.S. Rhoads,*** *R.N. Orth, M. Wu, B.A. Baird, J.L. Guan,* Cornell University

We have developed a new method for analyzing the processes of fibroblast adhesion and spreading using micro- and nanometer-scale fibronectin patterns. Fibronectin is an extracellular matrix protein that provides mechanical stability for cells and tissues, by being a ligand for integrin cell surface receptors which anchor the actin cytoskeleton to the plasma membrane. These anchor points are referred to as focal adhesions, and are composed of numerous scaffolding and signaling proteins in addition to forming focus points of the actin cytoskeleton. Here, we patterned fibronectin using a technique previously shown to produce feature sizes as small as 700nm. The fibronectin features are used to observe small focal adhesions and the morphological effects of minimal activation by fibronectin per cell area. For fabrication of patterned surfaces, polymer-coated silicon wafers were patterned using photolithography and reactive ion etching. Fibronectin was then deposited onto the wafer samples prior to polymer removal and cell application. The resulting patterns contained features ranging from 76 µm to > 1 µm, and were used in cell adhesion and spreading experiments. Cells adhering to the pattern were fixed, permeablized and analyzed by immunofluorescence, using antibodies to fibronectin, f-actin, paxillin, and focal adhesion kinase. Fluorescence microscopy was complemented with scanning electron microscopy to image focal adhesions, stress fibers, lamellipodia and filopodia. From this analysis, we propose that this method for analyzing cellular responses to subcellular cues from their surroundings is a model system for spatially isolating and studying focal adhesions.

9:40am **BI+SS-WeM5 Microengineering Surfaces to Interface with Mammalian Cells**, ***C.S. Chen,*** Johns Hopkins University **INVITED**

The interactions between cells and their surroundings provide the basis for the coordinated functions of tissues. To understand and control these interactions, we have developed several microfabrication-based approaches to provide model environments for cells. We will describe these approaches, and how they are beginning to elucidate how cells probe and make sense of their environment through biochemical and mechanical means. Integrating microfabricated devices and cells will pave the way for a new era in biomedical research and medicine.

10:20am **BI+SS-WeM7 Analyzing Lymphocyte Adhesion, Membrane Receptors and Cytoskeletal Rearrangement on Micron Scale Mitogen Patterns**, ***R.N. Orth***, *M.J.B. Flaminio, J. Kameoka, T.G. Clark, H.G. Craighead,* Cornell University

In this study, we investigated an in vitro immune reaction on a planar surface between T cells, B cells, and micron scale patterned mitogens as a model system for analyzing cell surface ligand responses. To form functionalized biomaterial microdomains, a polymer-coated substrate was patterned using photolithography and reactive ion etching. The samples were incubated in antibody and mitogen solutions prior to polymer removal and cell application. Uniform mitogen patterns ranging from 76 µm to <1 µm were created to target cell surface receptors, upregulate intracellular signaling cascades and cell activity, and stimulate proliferation. Several methods were used to analyze the patterned mitogens' effects on the lymphocytes. Carboxy-fluorescein diacetate, succinimidyl ester (CFSE)-stained lymphocytes harvested from the substrate demonstrated a proliferative response when assayed by flow cytometry. Mouse monoclonal antibodies against equine membrane cell receptors (anti-major histocompatibility (MHC) class II, anti-CD4, anti-CD3, and anti-leukocyte function associated-antigen (LFA)-1) provided a view of stimulated cells' surface receptor distribution. Secondary anti-mouse antibodies with a conjugated 1.5 nm gold sphere were bound to the primary antibodies. The samples were incubated in a silver solution to form 10-100 nm spheres as the silver nucleated off the gold particles. Scanning electron microscopy (SEM) imaging provided high resolution images of the cell surface ligands' spatial distribution as marked by the silver spheres. This patterning technique provided a precise and reproducible means to structure biomaterial surfaces at subcellular resolutions.

10:40am **BI+SS-WeM8 Directed Motoneuron Growth on Self-Assembled Monolayer (SAM) Patterned Surfaces**, ***M.G. Poeta***, *M. Das, C.A. Gregory, P. Molnar, D.C. Henry, L.M. Riedel, J.J. Hickman,* Clemson University

We are investigating the directed growth of embryonic rat motoneurons on glass substrates and are determining if they exhibit proper morphological and electrophysiological characteristics in this defined environment. This is the first step in recreating the reflex arc, one of the fundamental controls circuits in the body, with biological components on a MEMS chip. Recreating this system in vitro could have significant implications for improving treatment for people with spinal cord injuries, which affect 10,000 people every year in the United States. Throughout the last century, many methods have been developed to direct the growth of different cell types. These include fibroblasts, glial cells and hippocampal neurons grown on spider webs adhered to coverslips, grooves scratched in polystyrene and palladium deposited on petri dishes. In order to direct the growth of the motoneurons, we are using two recently developed patterning techniques, microcontact printing and laser ablation. Microcontact printing is a patterning method where a polydimethyl siloxane (PDMS) stamp is cast from a mold. It is inked in our case with a hydrophilic silane, diethyltriamine trimethoxysilane (DETA), and brought into contact with a substrate. The substrate is then backfilled with a hydrophobic silane, tridecafluoro-1,1,2,2-tetrahydroctyl-1-trichlorosilane (13F). Laser ablation is a patterning method where, again in our system, a substrate is coated with DETA and exposed with an excimer laser fitted with a beam homogenizer through a photomask. The exposed regions are then backfilled with 13F. Embryonic rat motoneurons are plated on these patterned substrates. XPS and contact angle are used to verify the surface modification procedures. We have found that the motoneurons orient themselves along the hydrophilic patterns. We will report on the characterization of these patterns using patch-clamp electrophysiology to measure the electrophysiological characteristics of the cells.

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