Thursday Afternoon, November 7, 2002

Biomaterials
Room: C-201 - Session BI-ThA

Cell Patterning to Engineer Function
Moderator: G.J. Leggett, University of Sheffield

2:00pm **BI-ThA1 Analysis of Cell Adhesion Strengthening Using Micropatterned Substrates**, ***N.D. Gallant***, *A.J. García*, Georgia Institute of Technology

Cell adhesion to fibronectin (FN) involves integrin receptor binding and subsequent adhesion strengthening, which includes integrin clustering, interactions with cytoskeletal and signaling components to form focal adhesions (FAs), and cell spreading. We applied micropatterning methods to control FA size and position and decouple FA formation from gross changes in cell morphology in order to analyze the contributions of FA assembly to adhesion strength. Microcontact printing was used to pattern alkanethiol self-assembled monolayers into arrays of circular adhesive islands (2, 5, 10 μm dia.) within a non-adhesive background.[1] NIH3T3 fibroblasts adhered to FN-coated islands and remained constrained to the patterns presenting a nearly spherical morphology. Cells assembled robust adhesive structures that localized to the micropatterned islands and contained typical components of FA. Cell adhesion strength to FN-coated micropatterned islands was quantified using a spinning disk device that applies a well-defined range of hydrodynamic forces to adherent cells.[2] Adhesion strength exhibited significant time- and adhesive area-dependent increases. Comparison of experiments for equivalent contact areas showed a 9-fold increase in adhesion strength over time, independently of cell spreading. These results demonstrate that FA assembly, independently of changes in cell morphology, contributes significantly to adhesion strengthening. This work provides an experimental framework for the functional analysis of FA components in adhesive interactions.

[1]N.D. Gallant et al., "Micropatterned surfaces to engineer focal adhesions for analysis of cell adhesion strengthening," Langmuir (in press).

[2]A.J. García et al., "Force required to break $\alpha_5\beta_1$ integrin-fibronectin bonds in intact adherent cells is sensitive to integrin activation state," J. Biol. Chem. Vol. 273, pp. 1098-10993, 1998.

2:20pm **BI-ThA2 Micropatterning of Polymer Surfaces for Controlled Cell Adhesion and Spreading Processes**, ***C. Satriano***, University of Catania, Italy, *S. Carnazza, S. Guglielmino,* University of Messina, Italy, *A. Licciardello, G. Marletta*, University of Catania, Italy

The prompting of cell adhesion and spreading processes onto polymeric surfaces activated by ion beam irradiation is a phenomenon observed for several polymers. In particular, in the case of carbon-based polymers and silicon-based polymers, the enhancement of cytocompatibility of the ion-irradiated surfaces has been mainly related to the formation of amorphous phases of hydrogenated carbon or SiO_2-like clusters, respectively. In this work the physico-chemical properties of two representative polymers of the two classes above mentioned, i.e., poly(ethyleneterephtalate) (PET) and poly(hydroxymethylsiloxane) (PHMS) were modified in a graded and controlled way with a micrometric spatial resolution. Namely, irradiated patterns with stripes of width ranging between 10 and 100 microns were obtained on the two polymer surfaces by using finely focused ion beams, with a total ion dose of $1x10^{15}$ ion/cm^2. The surface chemical structure and composition of the ion-modified surfaces were characterized by TOF-SIMS and Small Spot XPS, the micro-topography and the morphology were measured by AFM, finally, the surface free energies were calculated by wettability measurements. Fibroblast cells were used to test the cell adhesion and viability on the various micropatterned surfaces. Optical Microscopy was employed to characterize the importance of the lateral resolution effect respectively in PET and PHMS. Epifluorescence Microscopy evidenced the occurrence a specific cell morphology and mitotic activity for the different patterned surfaces. Furthermore, preferential cell alignment effects were observed depending on the type of irradiated polymer.

2:40pm **BI-ThA3 Designing In Vitro Patterned Neuronal Networks**, ***B.C. Wheeler***, University of Illinois, Urbana-Champaign **INVITED**

Through the use of microstamped patterns of polylysine against covalently linked backgrounds of polyethylene glycol, we have been able to maintain patterns of neurons for up to a month in culture. We have demonstrated the ability to use patterning technology in combination with planar microelectrode arrays to confine the neurons to narrow (10 um or 40 um) tracks which intersect the electrodes and to record spontaneous electrical activity (action potentials) from them. Work is in progress to determine how sparse a network can be and still maintain functional electrical activity. This work is intended to provide a technological basis for robust, repeatable and designable neural networks from which one could study basic neuroscience or construct a neural biosensor. Supported by NIH grants R21 NS 38617-01 and R55 RR13320-01 and NSF EIA-0130828. This work is done in collaboration with Dr. Gregory J. Brewer, Dept. of Medical Microbiology, Southern Illinois University School of Medicine.

3:20pm **BI-ThA5 Development of a High Throughput Cell Printing Platform**, *M.V. Deshpande, E.A. Roth,* Clemson University, *A. Gutowska,* Pacific Northwest National Laboratory, ***T. Boland***, Clemson University

High throughput cell printing has a potential to be a very valuable technique in the field of tissue engineering and genomics. A single nozzle cell pen and multi nozzle cell printer have been designed and developed to explore this area. New techniques are being developed to apply these tools for precise placement of cells with high throughput capabilities. The printer nozzles can be loaded with a known concentration of cells in solution or a pre-polymerized hydrogel solution. Pressure created by low power piezoelectric signals will push the cell solution onto a substrate in a programmed design. The cell printer was designed to print cell suspensions to media of any thickness. The body of the printer is made from PMMA with off the shelf printing components (logic board, encoder, etc.). The printer is equipped with a newly designed print head connected to sterile stainless steel hypodermic needles (gauge 30). The needles are individually addressable through piezo driven transducers. Finally, the software drivers were custom written to allow for computer-controlled delivery of single drops. In single-pass mode, the new printer is able to print 80pl drops onto substrata of varying thickness up to 1 inch. Preliminary results indicate a success in developing an array of cells. The cells are alive and healthy as determined by the green stain of the live/dead assay. This indicates the potential of printing small sheets of cells. Other techniques will be investigated to extend the use of the printer to print fluid hydrogel solutions into patterns for use as cell culture templates. Current investigation emphasizes characterizing and comparing temperature sensitive hydrogel mediums. Collagen I, a PLGA based biodegradable gel, and PIPAaM are being investigated. We will present this single cell platform technology and discuss the extension of the technology for two and three-dimensional cultivating systems of varying geometries.

3:40pm **BI-ThA6 Spatially Patterned Tissue for Retinal Cell Transplantation**, *C. Lee*, ***S.F. Bent***, *P. Huie, M.S. Blumenkranz, H.A. Fishman*, Stanford University

Patterning of tissue for selective placement of cells is currently being investigated in a novel treatment for age-related macular degeneration (AMD). The transplantation of human retinal pigment or iris pigment epithelial cells (RPE or IPE) on a carrier substrate is a proposed method for rescuing the diseased retina in AMD. We have examined the use of autologous tissue as a carrier substrate for the cells because it offers several advantages over synthetic substrates. Human lens capsule is readily available through ocular surgery and can coexist in the subretinal space without inducing immune rejection. To control the adhesion and morphology of the RPE cells, we have spatially modified the tissue surfaces using microcontact printing techniques. We have micropatterned inhibitory molecules such as poly (vinyl alcohol) (PVA) on lens capsule and have examined RPE cells subsequently cultured on the surface. We show that micropatterning these molecules via microcontact printing and related flow methodologies confines RPE cells to cuboidal structures that closely mimic the natural RPE layer. The cell inhibition by PVA was found to be stable in culture over a period of weeks. The cells have been successfully patterned on human tissue to circular patches as large as 50 microns and as small as 15 microns in diameter, separated by only a few microns. However, we find that the pattern size strongly affects the probability of cell adhesion and subsequent cell spreading. Overall, micropatterning PVA appears to be a promising and reproducible method for confining cells to high density and to a single morphology. We will discuss the potential for these methods to create a precise and organized transplanted cell layer for the treatment of patients suffering from macular disease.

4:00pm **BI-ThA7 Synaptic Connectivity in Geometrically Defined Neuronal Networks**, ***A. Vogt***, MPI for Polymer Research, Germany, *A. Offenhaeusser,* Research Center Juelich, Germany, *W. Knoll*, MPI for Polymer Research, Germany

One of the major problems in the study of neuronal network behaviour lies in the enormous complexity of the vertebrate brain. A promising approach to this problem is the creation of simplified neuronal circuits in vitro as a model system. A simplified circuit can be achieved by growing neurons on micropatterned substates which impose geometrical constraints upon the

forming network, such that the amount of possible cellular contacts is greatly reduced. Additional advantages of such a system are the clear definition of the connections formed as well as a high reproducability of the network shape. We grew rat embryonic cortical neurons on micropatterned substrates made by microcontact printing of ECM proteins onto a hydrophobic background. The pattern applied was a grid pattern with 6 µm wide lines and nodes that were 14 µm in diameter. The cells aligned with the geometry of the structure and formed simple circuits. Cell density was low enough to observe single cell contacts resulting in the formation of functional synapses along the lines of the pattern; this was shown by triple patch-clamp measurements. The synapses we found did not differ significantly from the synapses found on homogeneous control substrates in average synaptic failure and EPSP height. We therefore believe that our system is suitable as a model for neuronal networks and has multiple potential applications in basic biological research as well as in pharmaceutical testing, neurological implants, neuro-electronics and cell-based biosensors.

4:20pm **BI-ThA8 Oral Keratinocyte Attachment to Chemical Surfaces**, ***R.E. Rawsterne***, UMIST, UK, *G.J. Leggett*, University of Sheffield, UK, *S. Kothari*, UMIST, UK

The control of surface chemistry and topography are key factors in the design and development of next generation biomaterials and prostheses. The importance of surface chemistry has been well established for a variety of cell types, and the importance of surface topography is also gaining momentum. Whilst these are now recognised as being influential in initial cell attachment and growth, and both have been studied independently, there has been little work on examining their combined effects. Furthermore, the effect of these parameters on the behaviour of oral keratinocytes has not been studied. In order to ascertain which chemical functionality would best promote oral keratinocyte attachment, self-assembled monolayers (SAMs) of alkanethiolates on gold with varying chain lengths and acid (COOH), alcohol (OH) or methyl (CH_3) terminal groups were used. To introduce chemical cues to these surfaces SAMs were exposed to UV light through a mask resulting in selective oxidation of specific regions. Following photooxidation, samples were placed in a solution of a contrasting thiol, resulting in the displacement of the oxidised SAM in the exposed region with fresh thiols from solution. Samples exhibiting both single functionality and patterned chemistry were incubated with an oral keratinocyte cell line. For samples with a single functional group, the numbers of attached cells were counted at various time points up to 24h. Attachment to all surfaces was also observed using an inverted microscope and images recorded using a digital camera. It was found that hydroxyl terminated SAMs were the preferred surface for attachment of oral keratinocytes, in contrast to results for the attachment of fibroblasts. This was further investigated by observing the attachment of keratinocytes to patterns comprising of OH/CH_3 and OH/COOH terminated SAMs.

4:40pm **BI-ThA9 The Adaptation of Hydrogel Scaffolds to Three Dimensional Tissue Construction of Cylindrical Vessels**, ***E.A. Roth***, Clemson University, *A. Gutowska*, Pacific Northwest National Laboratory, *T. Boland*, Clemson University

This study investigates the ability of hydrogels to establish patterns for cell growth and their application to the construction of three-dimensional tissues. A variety of hydrogels are being investigated for this application including a collagen based hydrogel and Poly-N-Isopropyl Acrylamide (polyNIPAAm) based copolymers, which undergo liquid-gel transformations in response to temperature changes. The end goal is to construct viable cylindrical vessels that maintain stability, after hydrogel absorption or removal has occurred. A high-throughput cell printing system is under development that allows for accurate cell placement in pre-designed patterns. In this system, bioabsorbable hydrogels and cellular solutions are precisely deposited by needles connected to piezo electric pumps programmed through a software interface. A second method employs a mold consisting of two concentric cylinders, which has been designed to create vessels consisting of smooth muscle cells propagated in a hydrogel matrix. The outer surface of the annulus acts as a structural component during hydrogel stabilization and cellular proliferation. To allow for mold removal, this surface is grafted with polyNIPAAm so that upon slight cooling, the tissue can detach with all cellular junctions intact due to a decrease in hydrophobicity of the polymer. The inner surface of the annulus, composed of an inert nanoporous material, allows for nutrient diffusion from a media reservoir contained in the center of the mold. After sufficient culture time the mold is removed leaving a freestanding cylindrical vessel. Results from both of these methods will be discussed.

5:00pm **BI-ThA10 Integration of Cells and Silicon Devices via Surface Microengineering**, ***J. Hickman***, *M. Das*, *P. Molnar*, Clemson University

The long-term research goal of our group is to learn how to handle and prepare biological cells as components for microdevices and engineered tissues, and then to demonstrate the practicality of this approach by manipulating them to build hybrid systems and engineer functional tissues. The idea is to integrate microsystems fabrication technology and surface modifications with cellular components, with the aim of initiating and maintaining self-assembly and growth into biologically, mechanically and electronically interactive functional multi-component systems. The ability to control the surface composition of an in vitro system, as well as controlling other variables, such as growth media and cell preparation, all play important roles in creating a defined system for hybrid device fabrication. We are using self-assembled monolayers (SAMs) to control the intrinsic and geometric properties of surfaces in contact with these cellular systems. We have used the geometric control of the surface composition afforded us by SAMs to create in vitro circuits of rat hippocampal neurons. We have also demonstrated functional control of these systems by recording the electrophysiological signals on the patterned SAMs in response to stimuli and demonstrated geometric control of synaptic development. We have used geometric only cues to define axonal/dendrite polarity in developing hippocampal neurons which is a key step in creating engineered neuronal networks. Summed together these all represent a growing set of tools for building hybrid cellular systems. We are using this ability to integrate biological systems with silicon-based systems to create cell-based sensors for high throughput drug discovery and functional genomic assays as well as for hybrid neuronal/silicon systems to study biological computation. We are also using what we learn for a more fundamental understanding of cellular development and neuronal regeneration.

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