Thursday Morning, October 21, 2010

Biomaterial Interfaces
Room: Taos - Session BI1+NS-ThM

Replicating Biological Environments and Processes
Moderator: E.O. Reimhult, University of Natural Resources and Applied Life Sciences, Switzerland

8:00am **BI1+NS-ThM1 Biofunctionalized Micro- and Nano-cup Arrays by Plasma Polymer Templating**, ***R. Ogaki***, *M.A. Cole, D.S. Sutherland, P. Kingshott*, Aarhus University, Denmark

We present a novel fabrication method for creating an array of 'cups' on the micro- and nano scale by using a combination of plasma polymerization (pp), self assembled monolayers (SAMs) and colloidal lithography (CL).The method uses polystyrene (PS) particles that are first self-assembled into a hexagonal close-packed (HCP) structure onto a desired substrate over a large area via the lift-off method. The assembled particles are reduced in size by plasma etching and a plasma polymer is deposited into the interstitial spaces between the particles. The particles are subsequently removed by ultrasonication, forming an array of plasma polymerized cups with controllable sizes (through particle choice) and chemistries (through plasma monomer choice). The plasma polymer does not coat the contact region between the particles and the substrate. Thus a chemical pattern is generated, in our case, when SAMs are assembled onto the exposed substrate region. This provides a platform for site specific immobilization of biomolecules and cells with a diversity of chemistries possible. The method can be extended to other types of coatings such as those from physical vapor deposition (PVD), prior to the removal of the particles. As a result, up to three different chemistries can be presented on the array, with the first chemistry on the uppermost surface, the second chemistry on the internal wall and the final chemistry on the particle-substrate contact region inside the cup. The structural and chemical success of the cups and the patterns are determined by scanning electron microscopy (SEM), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), time of flight secondary ion mass spectrometry (ToF-SIMS). The method is adaptable to create micro- and nano-sized cups by the appropriate choice of particle sizes. The versatility of the method to tune the cup sizes and the potential to incorporate up to three chemistries is apparent, thus the presented fabrication method could be potentially utilized for immobilizing a range of multiple biological cells and molecules of different sizes inside the cups for applications such as multi-functional biosensors or for carrying out specific reactions inside the cups for biological studies.

8:20am **BI1+NS-ThM2 Fabrication of Functional Hydrogel Nano-structures for Biomolecule Immobilization**, ***R.T.S. Lam***, *J.-W. Jang, P.L. Stiles, S.R. Nettikadan*, NanoInk Inc.

Hydrogels have been used extensively for tissue engineering scaffolds and other biomedical applications because of their unique three-dimensional cross-linked polymer network that provide structural support while endowing an environment similar to natural tissue. Fabrication of hydrogels in submicron scale is greatly desirable; however structures with well-defined organization and high uniformity are not easily achievable by using traditional methods. Herein, we have demonstrated the printing of thiolated PEG hydrogels on a glass chip with precise control over the architecture and feature size using dip-pen nanolithography (DPN) techniques. This direct and reliable method for generating hydrogel patterns may serve as useful tools to explore cell-substrate interactions. In addition, conjugating different proteins through the free thiol functional groups in hydrogels is a promising approach of functionalizing the substrate with different biomolecules. This can be used as a platform for high throughput screening of protein-cell interaction studies. We have shown specific immobilization of thiol-reactive rhodamine red maleimide molecules on to the hydrogel patterns. Yellow-fluorescent was observed exclusively at the patterned area. By adjusting the ratio between the two PEG hydrogel precursors, we should be able to fine-tune the number of free thiol functional groups in the hydrogels, and hence the density of conjugated biomolecules. These hydrogels with different composition can be also printed simultaneously by DPN techniques to create gradient pattern in a single array. In a nutshell, our studies has combined the top-down approach of generating 3D nanostructures surfaces with controlled surface chemistry which creates an ideal interface for solving various fundamental questions in the field of cell biology.

8:40am **BI1+NS-ThM3 Engineering Cell Behavior in Microfabricated Substrates: Adding Dimensionality to the Sensory Toolbox**, ***M.H. Textor***, *M. Ochsner, V. Vogel*, ETH Zurich, Switzerland, *M.L. Smith*, Boston University **INVITED**

The physical properties of the local cell microenvironment regulate cell behavior in concert with soluble or matrix bound signaling molecules. *In vivo*, these properties are defined by a fibrillar ECM and adjacent cells and have implications for human health and disease. Our understanding of their role in regulating cell physiology resulted from technological advances which led to reductionist cell culture systems with tunable substrate stiffness, ligand density, or cell adhesive area and shape in two dimensions (2-D). Most of these studies were performed on flat, 2-D culture surfaces where studies have shown that these properties regulate a seemingly endless variety of observable cell responses. Regulating these processes with engineered cell culture platforms might prove useful in tissue engineering or regenerative medicine applications where a specific cell phenotype needs to be stimulated or maintained.

The extent to which observations made in 2-D Petri dishes can be transferred to predict cell behavior in a 3-D environment is a focus of current research. However, only a limited number of studies investigated the different microenvironmental parameters as a function of dimensionality, often with no or limited control of cell shape and substrate stiffness, and thus cannot be directly compared to observations made on (patterned) 2-D culture systems.

The focus of this talk is to demonstrate how the surface area of adhesive contact and substrate rigidity differentially regulate actin cytoskeleton assembly in 2-D versus 3-D environments, and how this impacts cell phenotype and function. PDMS polymeric substrates (compatible with inverted stage microscopy) for the organization of single cells in engineered quasi-3-D microenvironments were fabricated presenting arrays of microwells of different shape/aspect ratios, and stiffness (typically 1 MPa to 10 kPa Young's Modulus). The walls and bottom of wells were backfilled with extracellular matrix proteins such as fibronectin or mobile lipid bilayers.

On rigid substrates cytoskeleton assembly within single fibroblast cells was found to occur in 3-D microwells at shapes that inhibited stress fiber assembly in 2-D. In contrast, cells did not assemble a detectable actin cytoskeleton in soft 3-D microwells (20 kPa), but did so on flat, 2-D substrates that were otherwise equivalent. These data indicate that neither cell shape nor rigidity are orthogonal parameters directing cell fate. The sensory toolbox of cells seems to integrate mechanical (rigidity) and topographical (shape and dimensionality) information differently when cell adhesions are confined to 2-D or occur in a 3-D space.

9:20am **BI1+NS-ThM5 Uniform Spheroid Formation Using a "Smart" Polymer**, ***J.A. Reed***, *J.P. Freyer, H.E. Canavan*, University of New Mexico

Spheroids are small (~50-1000 µm diameter) sphere-shaped aggregates of cells that have been developed as 3D models for tumors. In addition to providing a model that more closely approximates the microenvironments of tissues and tumors than 2D cultures, spheroids can be more easily controlled than tests preformed on animal models. Current approaches for spheroid formation result in spheroids with a wide size distribution (>25% standard deviation), requiring the use of secondary sorting to obtain a uniformly-sized population. To increase the efficacy of these models for drug discovery in cancer therapeutics, it is necessary to develop an efficient way to fabricate a large number of uniform spheroids. Using a thermoresponsive polymer, poly(*N*-isopropyl acrylamide) (pNIPAM), cell aggregates of reproducible size and cell density can also be obtained. In this work, we pattern pNIPAM on an anti-fouling substrate to direct cell attachment, and ultimately cell sheet detachment for uniform spheroid formation. Using plasma polymerization, pNIPAM is patterned on Pluronic® F-127, which is nonfouling, to form 300µm diameter reversibly cell adhesive "islands" in non-fouling Pluronic® F-127 "seas." EMT6 cells are grown to confluence on the islands in 2 days, at which time their growth media is exchanged to stimulate cell detachment to form 70 spheroids of ~100µm from each 35mm diameter substrate. To verify pattern fidelity, X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), atomic force microscopy (AFM), and contact angle goniometry are used. In addition to developing a novel technique for the formation of tumor analogs, we also find that use of the larger surface area: volume ratio accelerates the speed of cell release.

9:40am **BI1+NS-ThM6 Parylene Peel-Off Technology: A Tool for Nano- and Microengineering Biological Environments**, ***C.P. Tan***, *B.R. Cipriany, B.R. Seo, D.J. Brooks, E.M. Chandler, C. Fischbach, D.M. Lin, H.G. Craighead*, Cornell University

Spatial manipulation of biomolecules and cells on a surface with nano- and micrometer scale precision is important in engineering biological microenvironments for tissue engineering, micro total analysis systems (biosensors, microfluidics and microarrays), and fundamental biophysical studies. We present Parylene Peel-Off, a simple and adaptable tool that can be used to improve current patterning/engineering of biological environments. In this work, we describe the fabrication process for creating a polymer (parylene-C) template to serve as a stencil for printing nano- and microscale regions of nucleic acids, proteins, lipids and cells. Afterwards, the parylene template can be easily peeled away to yield arrays of highly uniform biomolecular features in a large area format. We demonstrate the use of our Parylene Peel-Off technology to micropattern tumor cell arrays, for investigations into the role of cell-cell interactions in angiogenesis and cancer progession. By combining Parylene Peel-Off with current inkjet printing technologies, we have also generated multi-component, combinatorial protein arrays with array feature sizes down to 90nm. We anticipate that Parylene Peel-Off will be useful for enabling high-resolution studies of subcellular biological processes, integrating biochemical functionalities with miniaturized sensors, and engineering cellular and tissue microenvironments. Beyond basic science, our Parylene Peel-Off technology can be a useful tool to pattern chemically sensitive materials that are difficult to manipulate on the nano-scale, improve drug screening, and enable current inkjet printing technologies to extend their resolution to the sub-micrometer scale.

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