Tuesday Afternoon, October 21, 2008

Biomaterial Interfaces
Room: 202 - Session BI+NC-TuA

Protein and Cells Interactions on Micro- and Nanofabricated Substrates

Moderator: H.E. Canavan, University of New Mexico

1:40pm **BI+NC-TuA1 Exploring Single Stem Cell Biology via Microarrayed Artificial Niches**, ***M.P. Lutolf***, Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland, *R. Doyonnas, H.M. Blau*, Stanford University **INVITED**

A complex mixture of extracellular cues delivered by support cells is critical for adult stem cell maintenance and regulation of self-renewal in their microenvironment, termed niche. Despite recent progress in the identification of relevant niche proteins and signaling pathways in mice, to date, hematopoietic stem cells (HSCs) cannot be efficiently cultured in vitro without rapidly differentiating. We are developing novel in vitro culture paradigms that allow fate decisions of individual stem cells to be monitored under well-controlled conditions and in real time. We have engineered microarrayed artificial niches based on a combination of biomolecular hydrogel and microfabrication technologies that allow key biochemical characteristics of adult stem cell niches to be mimicked and the physiological complexity deconstructed into a smaller, experimentally amenable number of distinct signaling interactions. Several thousand single stem cells were tracked by fluorescent time-lapse microscopy in these microarrays over a period of several days. Image analysis allowed individual cell fate changes and growth kinetics of entire populations to be statistically analyzed. Subsequent retrospective single cell RT-PCR and transplantation experiments were performed in order to correlate kinetic behavior with phenotype and function. Screening of ca. 20 putative soluble HSC regulators, including Wnt-3a and TPO, as well as surface-tethered cell-cell adhesion proteins such as N-Cadherin, allowed to identify factors that dictate distinct HSC cell cycle kinetics. Based on patterns in kinetic behavior and single cell gene expression profiles induced by stimulation with a few of these candidates, we distinguished hallmarks of self-renewal from differentiation divisions, and validated these disparate behaviors in vivo by subsequent HSC transplantation into lethally irradiated mice. Therefore, the systematic deconstruction of a stem cell niche may serve as a generalizeable paradigm for defining and reconstructing artificial niches to accelerate the transition of stem cell biology to the clinic.

2:20pm **BI+NC-TuA3 Highly Ordered Protein Patterns Generated from Self-assembly of Mixed Protein Coated Nanoparticles**, ***G. Singh***, *S. Pillai, A. Arpanaei, P. Kingshott*, University of Aarhus, Denmark

The generation of protein patterns with controllable spacing in the nanometer to micrometer range is of great interest particularly for gaining a more fundamental understanding of the molecular mechanisms associated with protein-surface interactions. Information which can provide new insights into how proteins regulate cell shape and function have great interest in many areas including medical implants, tissue engineering, sustained drug delivery devices, biofilms, and biosensors. We present here a new but simple method for generating highly ordered protein patterns that can be applied over large areas (cm^2) based on the self-assembly of mixed nanoparticles of different size, from very low volume fractions. The proof concept experiments initially involve separately coating the particles with a different type of protein. The particles are then mixed at variable concentrations at low total volume fraction. Experiments are performed with fluorescently labelled proteins. For example, FITC-labelled lysozyme is adsorbed to 2µm carboxylated polystyrene particles and rhodamine-labelled BSA is adsorbed to 0.2µm aminated polystyrene particles both in PBS at pH=7.4. In separate experiments either both types of protein-coated particles are mixed in solution, or one protein coated particle is mixed with an uncoated particle. The particle suspensions are drop-cast onto the centre of a rubber ring placed on a hydrophilic Si wafer substrate. A concave shaped layer of solvent is formed allowing for long range assembly of binary colloidal particles firstly through lateral capillary forces, and later by convective flow and entropic forces. The resultant patterns consist of highly ordered hexagonal arrays of large particles as a single layer encapsulated by crystals of the smaller particles, in which single or binary proteins patterns are generated. The coverage is simply controlled by calculating the area of particles needed to fill the rubber ring, and the spacing is controlled by the appropriate ratio of starting particles. The ordered protein patterns are shown using fluorescent microscopy combined with AFM and SEM analysis. The extent of protein adsorption to the particles is determined using XPS and zeta potential measurements. The method is very simple, fast and inexpensive and we demonstrate that the patterning of proteins covers the range for a few nanometers up to a few micrometers depending on the appropriate selection of particles.

2:40pm **BI+NC-TuA4 Laminin Functionalization onto Silicon Single Crystals and Silicon Templated in Molecule Corrals**, ***S.P. Sullivan***, *X. Zhang, M.E. Boggs, H.P. Bui, A.V. Teplyakov, T.P. Beebe, Jr.*, University of Delaware

Biological and chemical surface modifications at the nanoscale have become a large area of research in response to the need for new and improved applications such as biochemical sensors and medical implants. The work describe here investigates the important extracellular matrix protein, Laminin onto Si(111) and templated silicon nanostructures. These substrates are being evaluated as biomaterial bridges for neuron outgrowth. The nanostructures are templated onto the surface of highly oriented pyrolytic graphite using "molecule corrals," which are nanometer-sized (1 – 100 nm diameter) structures etched into the basal plane of graphite. The intial defects from which molecule corrals originate are routinely produced using a low-energy cesium ion beam, followed by thermal oxidation at 650 ˚C. Using a physical vapor deposition method, silicon is then deposited onto the HOPG, leading to the formation of billions of silicon nanostructures. Previous results suggest that these structures will react similar to that of hydrogen-terminated silicon single crystal wafers. A comparison with a new protein attachment scheme, beginning with a self-assembled monolayer of 11-amino-1-undecene, was completed. XPS, TOF-SIMS, and AFM were used to characterize the substrates following each step of the reaction. To avoid deposition of physically adsorbed protein, careful rinsing and sonication procedures were optimized and used. From these results it was determined that the nanostructures react similarly to the hydrogen-terminated Si(111) surface for this covalent attachment scheme, and that protein attachment was successful on the nanostructures. To evaluate the reaction efficiency, an additional study comparing two covalent protein attachment schemes on silicon nanostructures is underway.

3:00pm **BI+NC-TuA5 Characterisation and Patterning of PEG-Supported Lipid Bilayers**, ***S. Kaufmann***, *P. Spycher, K. Kumar*, LSST, ETH Zurich, Switzerland, *G. Papastavrou*, LCSC, University of Geneva, Switzerland, *M. Textor, E. Reimhult*, LSST, ETH Zurich, Switzerland

Supported lipid bilayers (SLB) provide a basis for biotechnological applications as they constitute a simple model of cell membranes. They are of particular interest as components of future generations of biosensors based on transmembrane proteins. Two of the current limitations of supported lipid bilayers in biosensor applications are their sensitivity to air exposure and the limited aqueous space between the sensor substrate and the membrane available for large membrane proteins. Supported membranes resting on a hydrophilic polymer spacer decouple the membrane from the surface and provide increased aqueous space, but are generally more complicated to assemble than supported lipid membranes resting on an inorganic support. Recently it has been shown that poly(ethylene glycol) (PEG) can be incorporated into the membrane of liposomes through lipid molecules end-functionalized with a PEG chain and spontaneously fused to supported PEG-lipid bilayers (PEG-SLB) on glass. These membranes have been shown to possess a remarkable stability in air and would based on the length of the PEG-chains provide enough space between the SLB and the substrate to allow incorporation of functional transmembrane proteins. However, the structure of the PEG-SLB has not been characterized and important questions like whether the PEG brush is present on both sides of the membrane, its thickness, density and the kinetics of formation of PEG-bilayers have not been addressed. We present a comparison of the kinetics of PEG-SLB formation for different PEG molecular weights and densities as well as structural information. Furthermore, patterning of PEG-SLB using microspotting in glycerol-containing buffer has been done and compared to that of phosphocholine (PC) SLBs. QCM-D and FRAP measurement indicate decreased efficiency of PEG-SLB formation with increased PEG-density. This is most apparent in the initial adsorption of PEG-liposomes suggesting that POPC lipids still drive SLB formation through a mechanism similar to pure POPC SLBs and that a higher screening of the POPC lipids by PEG chains decreases the surface interaction. Force spectroscopy measurements demonstrate the presence of PEG on both sides of the SLB. SLB formation could be facilitated in glycerol-containing buffer and spotting of PC-SLBs and PEG-SLBs obtained by hydration, but with low geometrical definition. Spotting and hydration of PEG-SLBs demonstrated a weaker adhesion of PEG-SLBs than PC-SLBs.

4:00pm **BI+NC-TuA8 Nano-Rough Surfaces Produced by Glancing Angle Deposition (GLAD) for Protein Adsorption Measurements and Cellular Assays**, *A. Dolatshahi-Pirouz,* Univ. of Aarhus, Denmark, *C.P. Pennisi,* Aalborg University, Denmark, *S. Skeldal,* ***M. Foss****, J. Chevallier, P. Kingshott,* Univ. of Aarhus, Denmark, *V. Zachar,* Aalborg University, Denmark, *K. Yoshida,* Indiana University and Purdue University, *F. Besenbacher*, Univ. of Aarhus, Denmark

Currently, there is a strong focus on the fabrication of nanostructured artificial surfaces in order to tailor the biological response of artificial materials. The nanostructures are mainly used for more fundamental protein and cell studies, but in some cases also for applications like implants and cell/tissue engineering. Here a simple method to generate nano-rough platinum surfaces with varying morphological characteristics and a well-controlled surface roughness has been employed. The surfaces were fabricated by glancing angle deposition (GLAD) with varying angles and deposition times. Afterwards the biological response of the characterized nanorough samples were examined by protein adsorption and cell adhesion/proliferation assays in order to evaluate their potential as biomaterials surfaces. The effect of the deposition angle, θ, and deposition time, t, on the morphological characteristics of the thin films was investigated by utilizing Atomic force microscopy (AFM) and analyzing the images in order to determine the surface roughness and the size of the nano-rough surface features. The chemical composition of the platinum coatings were examined by X-ray Photoelectron Spectroscopy (XPS). From the AFM images it is observed, that the surface nano-features residing on the substrates can be changed by varying the deposition angle: as the deposition angle approaches grazing incidence sharp columnar protrusions are grown, while more smoothly shaped surface features appear for the thin films fabricated at higher deposition angles. The surface root-mean-square roughness, w_{rms}, increased from 1.49 nm to 15.2 nm as grazing incidence was approached. The surface roughness was additionally enhanced from w_{rms} = 6.6 nm to 26.3 nm for films grown at θ = 5° by increasing the deposition time. It is found that the blood fluid protein, fibrinogen, is influenced by the nano-rough substrates as compared to a flat control surface. Furthermore, the proliferation of primary human fibroblasts is almost completely inhibited on the nano-rough substrates. A maximum difference of almost 200% is observed between the tallest columnar surface features (44 ± 5 cells/mm2) and the flat platinum reference (125 ± 6 cells/mm2). These results show that GLAD is a versatile technique for fabrication of varying nano-rough model surface morphologies capable of influencing both the protein and cell behavior on the surface.

4:20pm **BI+NC-TuA9 HaloTag™ Protein Arrays: An Integrated Biomolecular Interaction Analysis Platform**, ***N. Nath****, R. Hurst, B. Hook, K. Zhao, D. Storts, B. Bulleit*, Promega Corporation

Protein arrays are emerging tools geared toward proteome wide detection of protein-protein, protein-drug, protein-DNA or protein-antibodies interactions. Wide application of protein array technology however faces significant challenge due to lack of high-throughput method for protein expression and purification. Here we present a new integrated approach for creating protein arrays that combines in-vitro protein expression system with HaloTag™ capture technology. The method allows for rapid and covalent capture of HaloTag™ fusion proteins in an oriented fashion directly from complex protein matrices without any prior purification. Multiple fusion proteins can be rapidly synthesized (90min) and immobilized in parallel for high throughput studies. We also demonstrate that arrayed fusion proteins are functionally active and can be used for protein-protein and protein-nucleic acid interaction studies. Furthermore, we show that by using a HaloTag-Protein G fusion we can fabricate antibody arrays directly from ascites fluid without any prior purification of antibodies. Unlike current antibody array platforms, antibodies on our platform are oriented on the surface for maximum biological activity. HaloTag™ protein arrays thus provide a single platform for multiple-biomolecular interaction studies.

4:40pm **BI+NC-TuA10 Use of Aligned Polymer Microfibres for Peripheral Nerve Repair**, *C. Murray-Dunning, R. McKean, A.J. Ryan, S.L. McArthur,* ***J.W. Haycock***, Sheffield University, UK

Nerve guidance conduits (NGC) have considerable potential for repairing peripheral nerve gap injuries caused by trauma, with basic entubulation designs encouraging limited reinnervation of nerve fibres. Following transection injury, Schwann cells are essential for repair as they proliferate rapidly, clear debris and secrete growth factors. We have designed a closed loop bioreactor enabling us to seed Schwann cells into experimental NGCs comprised of uniaxially aligned poly-L-lactide microfibres. Cells were introduced in fibres (5-10µm diameter) varying in length from 10-80mm, contained within 1.2mm diameter silicone tubes and grown under static and flow conditions (0-5.0ml/min) for 24 - 96 hours. MTT and confocal live/dead analysis data showed that cell viability was considerably improved when given an initial 4 hour adhesion time followed by a 0.5ml/min flow rate. To optimise Schwann growth within aligned fibre scaffolds, we then investigated seeding cells onto aligned fibres which were surface modified by acrylic acid plasma deposition. Schwann cells were stained with live/dead and phalloidin-FITC fluorophores and analysed by confocal microscopy in 3D. Microfibre scaffolds revealed a high degree of uniaxial cell alignment and a 50% increase in cellular viability on acid surface treated fibres, verse uncoated PLLA fibres. In conclusion, the following NGC approach is readily adaptable for autologous and stem cell delivery methods for the pre-clinical investigation of 3D tissue models for peripheral nerve repair.

5:00pm **BI+NC-TuA11 The Synthesis of Smooth PZT Thin Films and the Effects of Self-Assembled Monolayers and Ferroelectric Polarization on Surface Properties**, ***R.E. Ducker****, A. Garcia, B.B. Yellen, S. Zauscher*, Duke University

Lead zirconium titanate (PZT) thin films have attracted a great deal of interest in recent years due to their piezoelectric and ferroelectric properties. Recent applications for these films are in microelectromechanical systems (MEMS) and ferroelectric non-volatile random access memories. Here we present the formation of self-assembled monolayers (SAMs) on thin polarizable PZT films for potential biological applications such as biosensors. PZT Pb(Zr0.5Ti0.5)O3 thin films were prepared by a sol-gel deposition on platinum coated silicon substrates. The surface properties of the platinum substrate were modified using SAMs and the effects on the final crystal structure was investigated. These films were characterized by X-ray diffraction, scanning electron microscopy, X-ray photoelectron spectroscopy (XPS). We also present the formation of SAMs of alkylphosphonic acids and alklysilanes on the surface of these PZT films. Monolayers on metal oxide surfaces are an important way of changing the surface chemistry of functional materials. The formation of SAMs on PZT is not well understood compared to other metal oxide systems. These monolayers were formed with varying surface chemistries. These were characterized by contact angle goniometry, XPS and atomic force microscopy (AFM). Thirdly we present the ferroelectric polarization of the thin PZT films. The ferroelectric domains of PZT can be polarized using a conductive AFM tip. A DC bias voltage was applied between the tip and the bottom Pt electrode on the substrate to achieve features ranging from 400nm to several microns. The characterization of the polarization was performed using scanning Kelvin probe microscopy, electric force microscopy and scanning polarization force microscopy. These techniques confirmed the presence of an out-of-plane polarization component due to the reorientation of the ferroelectric domains in the PZT. The ability to change the polarization of PZT back and forth by applying opposite polarities to the AFM tip is also demonstrated. The effect of the polarization on the monolayer is also discussed.

5:20pm **BI+NC-TuA12 Patterned Protein Gradients of Extracellular Matrix Protein Affect Cell Attachment and Axonal Outgrowth**, *W.M. Theilacker, A.L. Styer,* University of Delaware, *D.E. Willis, J.L. Twiss,* Alfred I. DuPont Hospital for Children, *M.E. Boggs, S.P. Sullivan,* University of Delaware, *Z. Zhang,* Spansion, Inc., ***T.P. Beebe, Jr.***, University of Delaware

We have developed a method to control the local surface density of peptides and proteins that are covalently attached to various test substrates, for cell culture assays ranging from cell attachment propensity, to cell attachment density, to cellular behavior and signaling, to cell-cell interactions, for a variety of cell types and for a variety of proteins and peptides. This degree of control has recently been extended to step and continuous gradients in local protein and peptide concentrations from the micron to the centimeter length scale. Our use of these test substrates has focused mainly on neuronal cell types, for the development of new biomaterial bridging applications in brain and spinal cord injury patients, and on cell-cell interactions between osteocytes and neurons, for an understanding of "bone pain" in cancer patients. This presentation will focus on the production of these test substrates, their characterization by a variety of surface analytical and optical microscopy techniques, including XPS, TOF-SIMS, AFM, and epi-fluorescence microscopy with immunostaining, and the results of cell culture studies using these test substrates.

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