Tuesday Morning, October 19, 2010

Biomaterial Interfaces
Room: Taos - Session BI-TuM

Cells on Surfaces
Moderator: M.R. Alexander, University of Nottingham, UK

8:00am **BI-TuM1 Co-Culturing of Osteocytes and Neurons on a Unique Micropatterned Surface: Bone Pain in Cancer**, *M.E. Boggs, W. Thompson,* University of Delaware, *M.C. Farach-Carson,* Rice University, *R.L. Duncan,* ***T.P. Beebe***, University of Delaware

Bone pain is a debilitating problem for patients with bone cancer. Bone pain is the result of communication between the neural and skeletal systems of the body, and yet the mechanisms of communication or signal transduction between cells and tissues comprising these bodily systems is poorly understood. We have developed and are now using a unique micropatterned substrate as a platform consisting of two different proteins to investigate the mechanisms of cell-cell communication between co-cultured osteocyte-like cells and neurons. We have found that an optimal matrix for neurons is laminin type-1, while an optimal matrix for osteocytes is the perlecan domain-IV peptide. The use of micropatterned lanes 40-micron-wide by 1-cm long consisting of alternating laminin type-1 and perlecan domain-IV peptide creates a unique environment in which several osteocyte-neuron meeting events can be observed and probed under controlled conditions. This presentation will build upon our prior work with neuronal cell culture substrates, starting from how they are made, characterized, and then used in experiments to study cell-cell interactions for different cell types.

8:20am **BI-TuM2 Patterned Growth of Human Neural Stem Cells on Surfaces Prepared by Microcontact Printing**, *A. Ruiz,* University Milan, Italy, *L.M. Buzanska, M. Zychowicz,* Polish Academy of Sciences, Poland, *P. Colpo,* ***F. Rossi***, Joint Research Centre, Italy

This work relates to a method of patterning human neural stem cells as a suitable platform for performing different studies of cell proliferation, migration, and differentiation. The patterning of cells has been achieved by using microcontact printing (MCP) to create micropatterns of Poly-L-Lysine (PLL) and Fibronectin (FN), on substrates coated with cell repellent poly ethylene glycol (PEG) deposited by plasma. This substrate is particularly interesting since it is anti adhesive in liquid, but protein adhesive in the dry state. More details of the preparation method are described in [1]. Briefly, microstructured polydimethilsiloxane stamps have been fabricated by casting silicon masters produced by photolithography. The PDMS stamps have been inked with a FN or PLL solution, then dried with a nitrogen stream and put in conformal contact with the PEG substrate. By this method we are able to create fouling (PLL, FN) / antifouling (PEG) contrast on the surfaces where the cells are incubated and by modification of the spot size and distance, the influence of the cell environment on stem cells maintenance and fate studied.

Human Umbilical Cord Blood - Neural Stem Cells [2] were grown on the platforms with PLL or fibronectin pattern. After incubation for 4 days on PLL patterns consisting of 105µm squares spaced 300 µm, the cells are predominantly localized within the square. Such behavior is conditioned by agents added to the incubating medium. After being exposed to dBcAMP, the cells extend neuronal projections outside the squares, but cell bodies are patterned within the active domain. Immunocytochemistry was applied to trace neuronal lineage specific markers and their redistribution on the pattern domain upon influence of differentiating agents. It is found that the maintenance and fate of stem cells can be controlled by a combination of the protein type layer deposited by MCP and culture medium composition: presence of serum, neuromorphogenes and growth factors. Proper arrangement of soluble factors and bioactive surface domains allowed to work out conditions for developmental stage-specific immobilization of neural stem cells to the surface.

1. A. Ruiz, L. Buzanska, D. Gilliland, T. Sobanski, L. Ceriotti, S. Coecke, P. Colpo, F. Rossi. *Micro-stamped surfaces for the patterned growth of neural stem cells.* Biomaterials 29 (2008) 4766-4774.

2. Buzanska, L., Jurga, M., Stachowiak, E.K., Stachowiak, M.K., Domanska-Janik, K. Stem cells Dev., 15, 391–406, 2006.

9:00am **BI-TuM4 Biomolecule Microarrays in Neural Stem Cell Assay Development**, ***D. Mehn***, *J. Nowak,* Joint Research Centre, Italy, *L.M. Buzanska,* Polish Academy of Sciences, Poland, *A. Ruiz, H. Rauscher, G.R. Marchesini, P. Colpo, F. Rossi*, Joint Research Centre, Italy

Human umbilical cord blood originated neural stem cells (HUCB-NSC) are promising candidates for developing human cell based *in vitro* neurotoxicity assays. The origin of the cells makes possible to avoid species specificity extrapolation problems and avoid ethical issues compared to the embryonic stem cell research. Besides the easy cell line like culturing procedure of these cells they were shown to be able to differentiate to neuronal, asctrocytic and oligodendroglial phenotypes. This feature makes them also excellent subjects for developmental neurotoxicity studies.

The extracellular matrix compounds (ECM) used for anchoring the cells on culture dish or device surfaces may induce various ECM-dependent stem cell developmental responses mediated by integrin receptors. Investigation of stem cell - extracellular matrix interactions on biomolecule microarrays provides fundamental knowledge on neural differentiation as well as key inputs for stem cell based assay development.

The most powerful tools for microarray fabrication are microspotting and microcontact printing, offering flexibility regarding composition, concentration, spotted volumes or printed pattern geometry. Both methods are proven to be gentle enough to handle biomolecules for cell interaction studies.

In this work, the piezoelectric spotting and microcontact printing technologies were tested for rapid fabrication of biomolecule arrays on cell repellent surfaces.

Cell anchoring molecules, like extracellular matrix proteins (fibronectin and vitronectin) and poly-L-lysine (a polycationic polypeptide) were printed on anti-adhesive poly(ethylene) oxide-like films deposited by plasma-enhanced chemical vapor deposition. The microspotted and printed patterns were characterized by ellipsometry and microscopic techniques. The morphology of seeded stem cells and expression of certain differentiation marker proteins (GFAP, beta-tubulin III) were visualized by immunostaining and fluorescent microscopy, including also characterization after treatment with various concentrations of a known neurotoxic compound (MeHgCl). Toxicity results obtained on adhesive molecule spots were compared with results of a conventional neutral red uptake assay performed in coated 96 well plates.

Effect of the nature of biomolecules on cell behavior (including cell adhesion, morphology and survival) were investigated and are discussed focusing on generation long term cell-pattern stability, as well as stem cell differentiation into particular cell types.

9:20am **BI-TuM5 Imaging Conformational States of Fibronectin on Patterned Poly(dimethylsiloxane) Surfaces**, ***J. Dechene***, *A. Leclair, P.R. Norton*, University of Western Ontario, Canada

The ability to both understand and direct cellular adhesion is vital in a variety of fields including bioanalysis, medical diagnostics and implant materials design. When a cell approaches a material, its ability to adhere and proliferate will depend greatly on the surface properties of the underlying material. One such material, poly(dimethylsiloxane) (PDMS), has many ideal bulk characteristics but its surface is hydrophobic and does not inherently promote cell growth. To remedy this, many surface treatments of PDMS, particularly plasma treatments, have been used to increase the adhesion strength and bioactivity of PDMS towards cells.

We have recently reported a novel method of patterning the bioactivity of PDMS [1]. We have further exploited the patterning by spatially-selective modification with various biologically active functionalities that permit cell patterning. A different, photolithographic technique has also been developed that exploits the hydrophobic recovery of PDM S, and while we were successful at patterning hydrophobic and hydrophilic areas using this method, cell growth was unfortunately not limited to only the hydrophilic regions on the photolithographically produced patterns.

To understand why the photolithographic method was unsuccessful, we looked both at the ability of a cell to proliferate on the different surfaces, as well as the adsorption of serum proteins, the presence and conformation of which directly affect the adherence of a cell to the surface. To better understand the cell selectivity or lack thereof, fibronectin was adsorbed on the patterned surfaces. A monoclonal antibody HFN7.1 was used to identify exposed integrin binding sites. The antibody was immunofluorescently labeled using a secondary FITC conjugated antibody. The difference in relative availability of binding sites was visualized using confocal microscopy and compared to the relative adsorption of fibronectin, and cell proliferation on the patterned surfaces. The availability of binding sites was

shown to correlate with cell attachment on the stencil-masked patterned surfaces. However an increased availability of binding sites was seen on the hydrophobic recovered regions of the photolithographic patterned surfaces. We plan to directly measure the adhesion forces of chosen cells on the areas of "good" and "bad" conformation.

[1] N. Patrito, C. McCague, P. R. Norton, N. O. Petersen, *Langmuir* **2007**; 23, 2, 715 - 719

9:40am **BI-TuM6 Measuring Contractile Cell Forces on Rigid Substrates**, ***B. Müller***, University of Basel, Switzerland, *J. Köser*, University of Applied Sciences, Switzerland

Mechanical properties of substrates have been shown to be crucial factors for cell behavior, which includes the differentiation of stem cells or the malignant transformation into cancerogeneous cells (Engler et al. (2006) *Cell* **126**, 677 and Cross et al. (2007) *Nature Nanotechnol* **2**, 780). There have been published several techniques to measure contractile forces of cells, exerted onto the underlying substrate, since 1980. These approaches, however, only rely on compliant substrates and not on rigid ones as well known from load-bearing implants and culture dishes.

We present an approach to quantify the contractile forces of an ensemble of cells growing on rigid substrates based on nanomechanical cantilever sensors. In particular, we measured the relaxation of micro-cantilevers as the result of trypsin-release of about 100 fibroblasts. The optically measured change in cantilever bending, detected by means of the Cantisens Research system (Concentris GmbH, Basel), together with the number of fibroblasts counted was converted to the contractile cell force using the STONEY formula.

For the measurement of the contractile cell forces the selected cells were cultured on silicon cantilever arrays. The arrays consist of 8 micro-cantilevers each 500 µm long, 100 µm wide and 1 µm thin that enables us to detect stresses as small as 0.01 mN/m. Following adhesion and contractile force generation over night, the cantilever arrays were introduced into the Cantisens Research system to monitor the cantilever relaxations upon trypsin-mediated release of the cells from the substrate.

When rat2 fibroblasts are seeded on the silicon cantilevers they adhere and develop the morphology indistinguishable from that on standard culture dishes. Upon trypsin-induced release of the cells from the silicon substrate bending, the free end of the cantilever changes its position according to the following function $D = 0.5D_0 (1-\tanh(t/\tau))$, where D_0 corresponds to the deflection amplitude and τ the time constant of the cell release. While τ is apparatus and process specific, D_0 directly relates to the contractile forces of an ensemble of cells. The contractile force of an individual rat2 fibroblast on silicon corresponds to (17±7) µN. This value is reasonable, but high compared to the contractile forces of fibroblasts exerted on compliant substrates, a behavior expected from the studies on differently stiff compliant substrates. The contractile cell forces are strongly dependent on the state of the cell that explains the rather large error bar.

The method will support the fundamental understanding of cell-materials interactions with implications for cell-based biosensing and implant design.

10:40am **BI-TuM9 Biological Cell Detachment from Poly(*N*-Isopropyl Acrylamide) and its Applications**, ***H.E. Canavan***, University of New Mexico **INVITED**

Over the past two decades, poly(*N*-isopropyl acrylamide) (pNIPAM) has become widely used for bioengineering applications. In particular, pNIPAM substrates have been used for the non-destructive release of biological cells and proteins. In this work, we review the applications for which pNIPAM substrates have been used to release biological cells, including for the study of the extracellular matrix (ECM), for cell sheet engineering and tissue transplantation, the formation of tumor-like spheroids, the study of bioadhesion and bioadsorption, and the manipulation or deformation of individual cells. The work reviewed includes that of our own group, as well as from those performing research in the field world-wide.

11:20am **BI-TuM11 Rare Earth Nanoparticles - Biocompatibility Studies: Interaction with Human Neutrophil Granulocytes**, *N. Abrikossova,* ***C. Skoglund***, *M. Ahren, L. Selegård,* Linköping University, Sweden, *T. Bengtsson,* Örebro University, Sweden, *K. Uvdal*, Linköping University, Sweden

Rare-earth metal nanoparticles are among the most promising candidates to be used as probes for visualization and targeted drug delivery. Compared to the ion-based gadolinium containing complexes used clinically as contrast agents today, gadolinium oxide (Gd_2O_3) nanoparticles show a considerably improved relaxivity and thus enable an increased resolution and an increased contrast enhancement. However, surface modification of these nanoparticles is essential in order to improve the biocompatibility and diminish any potential toxic effects. In the present study we have evaluated the impact of Gd_2O_3 nanoparticles (as synthesized, dialyzed, and functionalized with polyethyleneglycol, PEG) on the production of reactive oxygen species (ROS) from human neutrophils.

Gd_2O_3 nanoparticles were synthesized (via the polyol route), functionalized with PEG and characterized as previously described in Ahren *et al.*[1]. Neutrophil granulocytes were isolated from heparinized whole blood using density gradient centrifugation. Generation of ROS by neutrophils upon addition of IgG-opsonized yeast, in presence and/or absence of as synthesized, dialyzed and functionalized Gd_2O_3 nanoparticles was studied with luminol-dependent chemiluminescence. In addition, the morphology of neutrophils after interaction with Gd_2O_3 nanoparticles was evaluated by fluorescence microscopy.

The ROS production from neutrophils challenged with IgG-opsonized yeast after exposure to as synthesized Gd_2O_3 nanoparticles was significantly decreased compared to control without nanoparticles. This indicates that the as synthesized nanoparticles are not well suited to be directly used in a living system without further modification. However, after dialysis and functionalization with PEG, no inhibitory effects were observed, possibly indicating that the high concentration of diethylene glycol (DEG) present in the as synthesized nanoparticle preparation is responsible for the inhibitory effects. Indeed, we in the present study also show that even a low concentration of DEG (0.3%) inhibits neutrophil ROS production. Our results indicate that dialyzed and PEG-functionalized Gd_2O_3 particles may be suitable in an in vivo situation as they do not impair the neutrophils capacity to produce ROS in response to a pray.

[1] Ahren M, Selegård L, Klasson A, Söderlund F, Abrikossova N, Skoglund C, Bengtsson T, Engström M, Käll P.O, and Uvdal K, *Synthesis and characterization of PEGylated Gd2O3 nanoparticles for MRI contrast enhancement*. Langmuir 2010, 26 (8) 5753-5762

11:40am **BI-TuM12 Synchrotron Radiation X-Ray Fluorescence Mapping of Cobalt Ferrite Nanoparticles in BALB 3T3 Fibroblast Cells**, ***G. Ceccone***, *P. Marmorato, J. Ponti, F. Rossi,* EC-JRC-IHCP, Italy, *B. Kaulich, A. Gianoncelli, M. Kiskinova,* Elettra Sincrotrone Trieste, Italy, *M. Salomé,* ESRF Grenoble France, *R. Ortega, G. Deves, A. Carmona,* University of Bordeaux, France, *L. Pascolo*, Elettra Sincrotrone Trieste, Italy

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