Biomaterial Interfaces
Room 2001 - Session BI-TuA

Cells at Surfaces
Moderator: K.E. Healy, UC Berkeley

2:00pm **BI-TuA1 Substrate Rigidity Regulates the Formation and Maintenance of Tissues**, *W. Guo,* University of Massachusetts Medical School*; M.T. Frey,* ***N.A. Burnham,*** Worcester Polytechnic Institute*; Y. Wang,* University of Massachusetts Medical School

PLEASE NOTE: YOU MUST IDENTIFY A DIFFERENT PRESENTER FOR THIS ABSTRACT. YOU MAY PRESENT ONE (1) PAPER ONLY AT THE CONFERENCEThe ability of cells to form tissues represents one of the most fundamental issues in biology. However, it is unclear what triggers cells to adhere to one another in tissues or to migrate once a piece of tissue is planted on culture surfaces. Using substrates of identical chemical composition but different flexibility, we show that this process can be controlled by substrate rigidity. The moduli of the polyacrylamide substrates were determined through analysis of atomic-force-microscopy (AFM) force curves to be a few kilopascals, the exact value depending on substrate preparation. The novel aspects of the acquisition and treatment of the force-curve data were 1) the use of log-log plots to observe where on the curve the tip was indenting the sample like a punch, sphere, or cone, and 2) the necessary metrology to bring the typical uncalibrated AFM relative uncertainty of a few hundred percent down to 13%. These methodologies can help researchers more properly interpret their results. @FootnoteText@ Biophysical Journal 90, 2213-20 (2006).

2:20pm **BI-TuA2 Compartmentalized Bioreactor: In Vitro Model for Osteogenesis and Breast Cancer Metastasis**, ***D. Ravi,*** *L.A. Shuman, A.M. Mastro, C.V. Gay, E.A. Vogler,* Pennsylvania State University

An advanced bioreactor that permits long-term study (up to 10 months) of cell/protein mediated processes such as bone accretion and cancer metastasis was developed and tested. The bioreactor based on the principle of simultaneous-cell-growth-and-dialysis, separates a cell growth chamber from a media reservoir by a dialysis membrane, compartmentalizing cell growth and cell nutrition functions. Extraordinarily stable culture conditions afforded by the reactor sustained mouse calvarial osteoblasts (MC3T3-E1, ATCC CRL-2593) for periods up to 10 months without the need for sub-culture. Months-long culture resulted in the formation of macroscopic (mm scale) sheets of bone over the inner surface of the dialysis membrane. Development of three-dimensional, tissue-like biosynthetic bone in the reactor was followed by light microscopy, scanning electron and transmission electron microscopy. Markers of osteoblast differentiation (alkaline phosphatase) and mineralization (Von Kossa Assay, SEM-EDS, XRD) were used to analyze the progression of isolated osteoblast inoculum to highly collagenous mineralizing tissue. In the second part of the study, the biosynthetic bone tissue was challenged by co-culture with GFP-expressing metastatic breast cancer cells (MDA-MB-231). Effect of the cancer cells on the morphology and organization of bone tissue was followed by confocal, scanning and transmission electron microscopy. Co-culture with breast cancer cells resulted in migration of cancer cells through the osteoblast tissue, disruption of the collagenous matrix, apoptosis and increased production of inflammatory cytokines (IL-2, IL-6). Compartmentalized bioreactor permits development of mineralizing 3-D collagenous bone tissue from isolated osteoblast inoculum over extended time periods up to 10 months and is an ideal in vitro vehicle for studying long-term cell-cell interactions involved in osteogenesis and osteopathology that are inaccessible to conventional cell culture techniques.

2:40pm **BI-TuA3 Bio-Microactuator using Cultured Cardiomyocytes**, ***Y. Tanaka,*** The University of Tokyo, Japan*; K. Morishima,* Kanagawa Academy of Science and Technology, Japan*; T. Shimizu, M. Yamato, A. Kikuchi, T. Okano,* Tokyo Women's Medical University, Japan*; T. Kitamori,* The University of Tokyo, Japan **INVITED**

Integration of chemical systems onto a microchip, sometimes referred to as micro total analysis systems (Âµ-TAS) or labs-on-a-chip, are currently a major interest due to their desirable characteristics, including reductions in reagent consumption, space requirements and analysis times. New concepts in integrated chemistry aims to create both a new academic field and a new associated (bio)chemical industry exploiting advantages of micron dimensions. As living cellular systems often exhibit complex reaction sequences and unique reagents, harnessing cell-based reactions by incorporating cells into Âµ-TAS systems is now frequently reported. However, to date, only cellular biochemical functions have been used to enhance microchip functions. Here, we have utilized cellular mechanical functions to produce more efficient biochemical processes. Our concept utilizes cardiomyocytes as microactuators, using the cellâ?Ts endogenous ability to transform chemical energy into mechanical energy. To demonstrate the concept of the cardiomyocyte bio-microactuator, we firstly actuated micropillars made of flexible polymer using cardiomyocytes.@footnote 1@ Then, we created a pump on-chip using a cardiomyocyte sheet@footnote 2@ as a prototype bio-microactuator.@footnote 3@. Our demonstrated pump could be used as a drug-delivery implant device responding body conditions. Also, that could be applied as a sophisticated in vitro micro model for research of circulatory system for revealing the mechanism of the circulatory illnesses. We anticipate our demonstrated device to be applied in various fields especially for medical usages. @FootnoteText@ @footnote 1@Y. Tanaka, K. Morishima, T. Shimizu, A. Kikuchi, M. Yamato, T. Okano, T. Kitamori, Lab Chip (2006) 6, 230 - 235. @footnote 2@T. Shimizu, M. Yamato, Y. Isoi, T. Akutsu, T. Setomaru, K. Abe, A. Kikuchi, M. Umezu, T. Okano, Circ. Res. (2002) 90, e40-e48. @footnote 3@Y. Tanaka, K. Morishima, T. Shimizu, A. Kikuchi, M. Yamato, T. Okano, T. Kitamori, Lab Chip (2006) 6, 362 - 368.

3:20pm **BI-TuA5 Integrin-Ligand Affinity Affects Neuron Outgrowth**, *Z. Zhang,* University of Delaware*; J. Zheng,* Alfred I. duPont Hospital for Children*; Y. Leng,* University of Delaware*; K.W. Dabney, J.L. Twiss,* Alfred I. duPont Hospital for Children*;* ***T.P. Beebe Jr,*** University of Delaware

The role of ligand-receptor affinity on the outgrowth of neuronal processes was studied directly on postnatal day-1 primary cultures of living neurons by atomic force microscopy (AFM). To accomplish this, micropatterned test substrates were created and analyzed using contact angle measurement, AFM, X-ray photoelectron spectroscopy (XPS), and time-of-flight secondary ion mass spectrometry (TOF-SIMS) at each step of the multistep surface functionalization process, for both substrates and AFM tips. The affinity force between an individual fibronectin molecule (Fn) and an individual growth cone integrin receptor was found to be 106 ± 8 pN, while that between the GRGDSY peptide and an individual integrin receptor was 170 ± 10 pN when measured under the same physiological conditions. In a conventional outgrowth assay on the same substrates, although both the Fn- and peptide-modified substrates supported significantly greater neurite outgrowth than controls, and outgrowth on both substrates was inhibited by the addition of soluble RGD peptide, ~30% longer neurite extension was observed on Fn-modified substrates than on GRGDSY-modified substrates. This implies that integrin-ligand affinity plays an important role in neurite outgrowth in vitro, and that adhesivity represents a balance between the formation and breakage of cell-substratum contacts. Neurite outgrowth involves the formation of new attachments at the front of the advancing growth cone, and the breakage of previous attachments now at the rear or the advancing growth cone. The higher binding affinity between a neurite's integrin receptors and the GRGDSY ligand implies a reduced rate of ligand-receptor breakage.

3:40pm **BI-TuA6 Electronically Controlled Biointerface for Neuron Growth**, ***M. Gabi,*** ETH Zurich, Switzerland*; P. Schulte, A. Offenhäusser,* Research Centre Jülich, Germany*; J. Vörös,* ETH Zurich, Switzerland

Experimental investigation of the neuronal network information processing is important for understanding how the brain performs functions such as memory and learning. The first research step is to develop novel ways for the assembly of neural networks with controlled topology. The guided growth of neurons is one basic requirement to build such defined neural networks. A variety of different surface patterning techniques have been used to achieve controlled growth, including microcontact printing, photolithograpy, ink-jet printing and topographical control, but none of these methods has been capable of controlling the "wiring" of the neurons so far. We have developed electrically responsive "smart" surfaces for controlling the growth of neurons and neurites on custom made indium-tin-oxide (ITO) microelectrodes. The substrate has a suitable microstructure to mechanically guide the out-growth of neurites from the landing spots where the soma of the neuron is located. At the same time poly(ethylene glycol) grafted polyelectrolytes are used to provide an appropriate biointerface on the connecting ITO wires between the cells. The key feature of this chemistry is that initially it inhibits the cell-attachment and neurite outgrowth but it can be switched electrically to a cell-adhesive, IKVAV peptide presenting biointerface that promotes the outgrowth of the neurites. The possibility of guiding neuron growth with the help of electronically controlled biointerfaces is an important step towards building neural networks with controlled topology. The

performance of such basic networks and network elements will be characterized using a double patch-clamp setup.

4:00pm **BI-TuA7 Surface Chemical Gradients to Optimise Substrata for Self-Renewal of ES Cells**, ***R.D. Short***, University of Sheffield, UK; *P. Murray,* University of Liverpool, UK; *K. Parry,* Plasso Technology; *D. Edgar, R.S. McGreal,* University of Liverpool, UK

Various reports detail how the culture conditions for mouse ES (mES) and human (hES) may be manipulated to maintain these cells in an undifferentiated state.@footnote 1,2@ Significant differences between mES and hES cells have been commented upon, as well as common mechanisms in maintaining self-renewal. It has been recently shown@footnote 3@ that the self-renewal of mES and hES cells can be promoted by restricting the degree to which these cells spread. This result implies that the self-renewal mES and hES cells can occur when their spreading is restricted by culture on weakly adhesive substrates. Herein, we show how using surface chemical gradients, of varying carboxylic acid density,@footnote 4@ an optimal chemistry is readily identified whereby cells can be maintained in compact small colonies, retaining cell-cell contact, without loss of the key ES cell markers, alkaline phosphatase and Oct-4. Our preliminary results are strongly suggestive that the capacity of ES cells for self-renewal may be maintained by surface chemistry alone. If true, this has the important implication that geometric control (ie control over cells spreading) is an important factor in the maintenance of self-renewal. Surface chemical gradients are an ideal tool for rapid (high throughput) screening. @FootnoteText@ @footnote 1@A.G. Smith, et al, Nature (1988) 336, 688-690 @footnote 2@T. Burdon et al. Trends Cell Biol.,(2002) 12, 432-38 @footnote 3@Murray P. et al., in preparation @footnote 4@J. D. Whittle, R. D. Short et al. Chem. Comm., (2003) 14; 1766.

4:20pm **BI-TuA8 Tunable Biomimetic Artificial Extracellular Matrix Coatings**, ***E.F. Irwin***, *K. Saha, K.E. Healy,* UC Berkeley

In this work we have designed an artificial extracellular matrix that can be utilized for both in vitro cell culture and biomaterial coatings. In our system, we can modulate both ligand density and the mechanical properties of the coating. The base coating is a greater than 100 +AEA-micron+AEA- thick acrylamide (AAm) gel in which the crosslinker density is varied in order to modulate stiffness. Next, this surface is modified with a 4 nm thick poly(ethylene glycol) (pEG) based interpenetrating gel which prevents non-specific protein and cell attachment. Finally, the surface is functionalized with RGD peptides from bone sialoprotein via a 3400MW pEG spacer arm, where the surface density can be controlled by varying input peptide concentration. The mechanical properties of these coatings were measured using force-mode atomic force microscopy (AFM) and analyzed with a Hertzian mechanics model. According to the model, the Young's modulus varied linearly in the range of crosslinker used (0.3-0.03 wt percent) from 1.16 +AEAAKwBA- 0.32 kPa to 9.03 +AEAAKwBA- 1.02 kPa. The immune response to these coatings of varying ligand density and mechanical stiffness is currently being assayed by culturing THP-1 cells, a monocytic leukemia cell line, on our system. It is anticipated that softer surfaces will exhibit a reduced immune response, as macrophages will not be able to spread and activate as readily. Preliminary experiments indicate that the softer surfaces allow less THP-1 cell attachment and spreading. In summary, we have designed a tunable artificial extracellular matrix coating to modulate cell behavior.

4:40pm **BI-TuA9 Plasma Polymer Gradients and Their Use for Cellular Guidance**, *M. Zelzer, R. Majani,* University of Nottingham, UK; *J.W. Bradley,* The University of Liverpool, UK; *F.R.A.J. Rose, M.C. Davies,* ***M.R. Alexander***, University of Nottingham, UK

Plasma polymers have recently been shown to be useful in 3D tissue engineering to encourage cell ingress within porous PLA scaffolds and channels intended for applications in bioreactors.@footnote 1,2,3@ To investigate the mechanism of cell ingress we carried out experiments to form surface chemical gradients on planar substrates by diffusion through defined apertures. Consecutive depositions of plasma polymerised allyl amine and hexane on glass has been used to obtain significant variation of water contact angle from 30 to 90 deg, and corresponded to ppHex overlayer thicknesses of of 0 to 10 nm (XPS). AFM was used to identify island deposition on ppAAm at the transition zone from ppHex to ppAAm. This was supported by Tougaard background analysis of the XPS substrate signals which importantly is also applicable to non-planar substrates. It is proposed that the gradual transition may play a role in forming protein adsorption gradients to induce haptotactic cell movement. We present evidence of cellular guidance on such gradients and propose a mechanism based on model protein adsorption studies using quartz crystal microbalance invoking to Vroman effect. This mechanism relies on a ready displacement of albumin by heavier adhesive proteins (e.g. fibronectin) from the hydrophilic ppAAm surface compared with strong albumin adsorption to the hydrophobic ppHex surface. @FootnoteText@ @footnote 1@ Barry, J,et al. Using Plasma Deposits to Promote Cell Population of the Porous Interior of Three-Dimensional Poly(D,L-Lactic Acid) Tissue-Engineering Scaffolds. Advanced Functional Materials 15, 1134-1140 (2005).@footnote 2@ Barry, Jet al. Using a core-sheath distribution of surface chemistry through 3D tissue engineering scaffolds to control cell ingress. Advanced Materials (2006 in press).@footnote 3@ Dehili, C, et al. Comparison of primary rat hepatocyte attachment to collagen and plasma polymerised allylamine on glass. Plasmas Processes and Polymers (2006 in press).

Author Index

Bold page numbers indicate presenter