## Biomaterial Interfaces

## Room: 609 - Session BI-TuA

## Engineered Cellular Interfaces

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

1:40pm **BI-TuA1 Simultaneous Deposition of Endothelial Cells and Biomaterials for Human Microvasculature Fabrication**, *X. Cui, P.V. Kreuk,* ***T. Boland***, Clemson University

The current tissue engineering paradigm is that successfully engineered thick tissues must include vasculature. As biological approaches alone such as VGEF have fallen short of their promises, one may look for an engineering approach to build microvasculature. Layer-by-layer approach for customized fabrication of cell/scaffold constructs have shown some potential in building complex 3D structures. With the advent of cell printing, one may be able to build precise human microvasculature with suitable bioink. Human Microvascular Endothelial Cells (HMEC) and fibrin were studied as bioink for microvasculature construction. Endothelial cells are the only cells to compose the human capillaries and also the major cells of blood vessel intima layer. Fibrin has been already widely recognized as tissue engineering scaffold for vasculature and other cells, including skeleton/smooth muscle cells and chondrocytes. In our study, we precisely fabricated micron-sized fibrin channels using a drop-on-demand polymerization. This printing technique uses aqueous processes that have been shown to induce little, if any, damage to cells. When printing HMEC cells in conjunction with the fibrin, we found the cells aligned themselves inside the channels and proliferated to form confluent linings. Current studies to characterize the biology and functionality of these engineered microvascular structures will be presented. The preliminary data suggests that a combined simultaneous cell and scaffold printing can promote HMEC proliferation and microvasculature formation.

2:00pm **BI-TuA2 Cells, Surfaces, Spaces and Forces: What makes a tissue?**, ***K.D. Hauch***, *D.J. Mortisen, M.A. Laflamme, C.E. Murry, B.D. Ratner*, University of Washington **INVITED**

Tissue engineering strives to combine parenchymal and other cells with porous biomaterial scaffolds; to grow tissue like constructs that can be used to repair diseased or damaged tissues and organs. The natural course of development, as well as some (but not all) processes of wound repair and regeneration, depend upon complex parameters including: the changing composition and capabilities of the cells that populate the tissue; molecular cues from the interface between cell and its environs; the structural space wherein the cells reside; and mechanical forces. All these and more act to guide the processes that results in a hierarchical living tissue with appropriate structure and function. Here we explore these issues in the context of cardiac tissue engineering. Adult cardiomyocytes demonstrate little if any proliferative potential. However, using an appropriate schema of soluble cues, large quantities of proliferating cardiomyocytes as well endothelial cells can be derived from cultures of human embryonic stem cells, to be used for tissue engineering. Postulating the importance of scaffold geometry, novel scaffolds were constructed with appropriately sized spaces and shapes, providing an engineered support structure that mimics aspects of native muscle architecture. Molecular cues are provided by immobilizing adhesion proteins on the scaffold and delivering other soluble factors to stimulate cell survival, proliferation, and ultimately vascularization. huESC-derived cardiomyocytes populate these scaffolds and survive at high cell densities in culture. Finally, the application of cyclical mechanical stress during in vitro culture is seen to enhance cardiomyocyte size, survival and functional organization. The analysis of these engineered tissues depends on both standard immunohistochemical observations, as well as newer visualization tools, including Digital Volumetric Imaging, a microscopic 3D serial sectioning and reconstruction technique. Together, the appropriate application of proliferative cardiomyocytes to carefully engineered scaffolds featuring spaces of appropriate size and shape, in conjunction with soluble and mechanical cues can lead to the development of a robust functional unit of cardiac muscle.

2:40pm **BI-TuA4 Expanding Human Embryonic Stem Cells without Feeder Cells on Chitosan-Alginate 3D Porous Scaffolds**, ***M.C. Leung***, *L. Zhensheng, M. Zhang*, University of Washington

The tremendous interest in human embryonic stem (hES) cells is motivated by a wide range of potential therapeutic, diagnostic, and fundamental research applications. To preserve their undifferentiated state, two-dimensional co-culture with feeder cells is standard practice.[1,2,3] In order to develop therapeutic applications, a system for the undifferentiated expansion of hES cells in pure culture must be developed to prevent xenogenic contamination.[4,5,6] With BG01V cells as a model, porous chitosan-alginate (CA) scaffolds were studied as a three dimensional (3D) substrate for undifferentiated hES cell proliferation. It was observed that hES cells attached, proliferated, expressed relevant transcription factors, translated appropriate markers, and retained pluripotency after 21 days of cultivation. Furthermore, the 3D CA culture system replicates the structure of natural extra cellular matrix, creating additional opportunities for regenerative medicine. This method realizes the goal of expanding pure hES cell populations in vitro while preserving undifferentiated state, and represents a significant advancement in hES cultivation technique.


[1]Choo, A., Padmanabhan, J., Chin, A., Fong, W. J. & Oh, S. K. Immortalized feeders for the scale-up of human embryonic stem cells in feeder and feeder-free conditions. J Biotechnol 122, 130-41 (2006).

[2]Oh, S. K. et al. High density cultures of embryonic stem cells. Biotechnol Bioeng 91, 523-33 (2005).

[3]Richards, M., Fong, C. Y., Tan, S., Chan, W. K. & Bongso, A. An efficient and safe xeno-free cryopreservation method for the storage of human embryonic stem cells. Stem Cells 22, 779-89 (2004).

[4]Stacey, G. N. et al. The development of 'feeder' cells for the preparation of clinical grade hES cell lines: challenges and solutions. J Biotechnol 125, 583-8 (2006).

[5]Amit, M. & al., E. Feeder Layer- and Serum-Free Culture of Human Embryonic Stem Cells. Biol Reprod 70, 837-845 (2004).

[6]Hoffman, L. M. & Carpenter, M. K. Characterization and culture of human embryonic stem cells. Nat Biotechnol 23, 699-708 (2005). .


3:00pm **BI-TuA5 Stem Cell Adhesion and Proliferation Correlated with Surface Properties of Copolymer Libraries Synthesised as Micro Arrays**, *A.J. Urquhart, M. Taylor,* University of Nottingham, UK, *D.G. Anderson, R. Langer,* Massachusetts Institute of Technology, *M.R. Alexander,* ***M.C. Davies***, University of Nottingham, UK

In the field of tissue engineering, the search is on for the optimum polymer scaffold material to support the adhesion and proliferation of stem cells for organ regeneration. To accelerate this process, Anderson et al., developed a high throughput screening methodology for the assessment of stem cell interactions with a large combinatorial library of over 500 copolymers1. Initial cellular behaviour with these materials will be driven by surface-cell interactions but until very recently, there was no rapid method of measuring the surface chemistry of such spatially patterned arrays. We report on the first high-throughput screening of the surface chemistry (ToF-SIMS and XPS) and wettability (contact angle, surface energetics) of large copolymer library array spatially patterned as 300 micron islands and polymerized in-situ on a single poly(HEMA) slides. The copolymer library is designed to exhibit a range of surface phenomena and their ability to support the growth of cells (eg, endothelial stem cells, bacteria) was assessed. Statistical analysis of the large surface and biological data sets reveals important relationships linking surface properties and cell interactions that point to the key surface phenomenon that could lead to the development of optimised copolymer surfaces for the development of polymeric scaffolds.

4:00pm **BI-TuA8 Hierarchical Control of Form and Function in the Heart**, ***K.K. Parker***, Harvard University **INVITED**

Expression of sarcomeric proteins is necessary, but not sufficient, for contraction of cardiac myocytes. Posttranslational processes contribute to regulation of muscle growth during cardiac development, normal function, and disease. However, little is known about the mechanisms and signals that potentiate directional muscle growth and the self-assembly and organization of sarcomeres, myofibrils, cells, and tissues. These structures appear to be optimized for their contractile function. In order to elucidate the structure-function relationships that govern contractility, we have developed computational and experimental models of self-assembly and organization in cardiac myocytes in vitro. By controlling only 2D boundary conditions imposed on the myocyte, we are able to engineer predictable myofibrillar patterns and contractility of individual myocytes. These experiments have revealed how the extracellular matrix provides an important set of instructions for self-assembly of the myocyte cytoskeleton architecture which serves as a template for myofibrillar patterning. Our results suggest the post-translational mechanisms that regulate cardiac organo- and pathogenesis.

4:40pm **BI-TuA10 BioArtificial Matrices to Control Blood Vessel Network Formation**, ***E.A. Phelps***, *A.J. García*, Georgia Institute of Technology

Vascularization of engineered regenerative constructs is a major obstacle in the development of clinically significant regenerative medicine. The ability of regenerative constructs to recapitulate normal blood vessel wiring is central to their successful integration with host tissue, proper physiological function, and long term survival. The natural formation of new blood vessel

networks is driven by spatially and temporally controlled presentation of positive and negative cues that direct cell behavior to initiate vessel sprouting, migration, and stabilization.[1] We have developed a strategy for engineering regenerative constructs with spatially patterned biomolecules to direct the formation of orderly networks of blood vessels in artificial biomaterials. Our approach employs a photopatterning technique to convalently link bioactive peptides to poly(ethylene glycol) (PEG) hydrogels to modulate and direct cell function. In this system, peptides are attached to the surface of PEG hydrogel through the use of a photoactive crosslinking agent. Peptides are patterned on the hydrodel by exposing the peptide and crosslinker solution on the surface to UV light through a Mylar photomask. We achieved sharply defined patterns of fluorescently labeled peptide with 10 µm features. We anticipate that the system can easily produce higher resolution patterns. We employed the photopatterning technique to create various patterns of the adhesive ligand RGDS on a nonadhesive PEG background. We have shown that we can constrain the adhesion and morphology of NIH fibroblast cells to the patterned RGDS with this system. We have also used RGDS functionalized PEG hydrogels to induce tubule formation of human aortic endothelial cells, and we have successfully created patterns of labeled RGDS resembling branching microvasculature. We plan to use these patterns to direct the growth of vascular sprouts from explanted sections of mouse aorta into a vascular network. Ultimately we will employ a system to pattern ligands in 3D to direct vascularization of an implanted hydrogel in vivo. The central hypothesis of this work is that spatiotemporal presentation of bioactive cues will result in directed vascularization of engineered hydrogels from the host tissue and that increased vascularization will result in improved healing, integration, and function of regenerative constructs.

[1]M. P. Lutolf and J. A. Hubbell, Nature biotechnology 23 (1), 47 (2005).

5:00pm **BI-TuA11 Cytoskeleton Structure and Focal Contact Points on a Micro 3D Patterned Film**, ***H. Sunami***, *E. Ito,* Hokkaido University, Japan, *M. Tanaka,* Tohoku University, Japan, *S. Yamamoto,* Hokkaido University, Japan, *M. Simomura*, Tohoku University, Japan

Micro fabrication of cell culture substrates is one of the most significant subjects in the field of biomaterial research. Recently we found that endothelial cells can proliferate rapidly on a micro 3D patterned film (honeycomb film). The cell shape and cytoskeleton structure on the honeycomb films were clearly different from those on a flat film. In order to elucidate the effect of honeycomb films as a 3D scaffold for cell culture, it is needed that the 3D observation of cell behaviors such as the morphological change, expression of cytoskeleton, expression of contact points on extracellular adhesion molecules, and migration on the honeycomb films during cell culture. In this research, effects of 3D honeycomb pattern on above cell behaviors were observed.

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