Biomaterial Interfaces
Room: 23 - Session BI-MoA

Cell-Surface Interactions: High Throughput Methodologies

Moderator: M.R. Alexander, University of Nottingham, UK

2:00pm **BI-MoA1 3D Niche Microarrays for Systems-Level Analyses of Stem Cell Fate**, *A. Ranga*, ***M. Lutolf***, École Polytechnique Fédérale de Lausanne, Switzerland **INVITED**

Proper tissue maintenance and regeneration relies on intricate spatial and temporal control of biochemical and biophysical microenvironmental (or 'niche') cues, instructing stem cells to acquire particular fates, for example remaining quiescent or undergoing self-renewal divisions. Despite rapid progress in the identification of relevant niche proteins and signaling pathways using powerful *in vivo* models, many stem cell types cannot be efficiently cultured *in vitro*. To address this challenge, we have been developing b iomaterial-based approaches to display stem cell regulatory signals in a precise and near-physiological fashion, serving as powerful artificial microenvironments to probe and manipulate stem cell fate. In this talk I will discuss recent efforts in my laboratory to develop three-dimensional microarrayed artificial niches based on a combination of biomolecular hydrogel engineering and liquid handling robotics. This platform allows key biochemical and biophysical characteristics of stem cell niches to be mimicked and the physiological complexity deconstructed into a smaller, experimentally amenable number of distinct signaling interactions. The systematic deconstruction of a stem cell niche may serve as a broadly applicable paradigm for defining and reconstructing artificial niches to accelerate the transition of stem cell biology to the clinic.

2:40pm **BI-MoA3 Microfluidic Gradient Systems to Generate Defined Cell Microenvironments and Study Cellular Fate Processes**, *P. Wallin, E. Bernson,* ***J. Gold***, Chalmers University of Technology, Sweden

Cell microenvironments are the main driving force in cellular fateprocesses and phenotype expression in vivo. In order to mimic specific stem cell niches, and study cellular responses under those conditions in detail, we need the ability to create and control cell micro environments in vitro. This includes the capability to modify growth substrate surface properties, liquid composition as well as cell-cell interactions in cell culture systems. Microfluidic systems offer the possibility to modify liquid mixtures on the cellular length scale in a highly defined manner. In particular, the ability to generate spatially- and temporally- controlled liquid gradients is of high relevance to study concentration dependent cell responses.

We are using a diffusion-based gradient generator that has been characterized both by computational fluid dynamic simulations, as well as experimentally. Themicro fluidic network was used to investigate HUVEC endothelial cell migration along chemotractant VEGF gradients when simultaneously grown on a continuous gradient in spacing of cell attachment peptide (cRGD) via functionalized Aunanoparticles (65-85nm spacing over 6 mm). The aim of this study is to ascertain how cell migration is affected by the spacing of attachment peptides. This has been achieved by forcing cells to migrate in a chemotrative gradient on a gradient substrate which will have portions that do not support mature focal adhesion formation or cell spreading. The same microfluidic network was also used in combination with a micro grooved growth substrate to study myoblast differentiation and alignment in response to simultaneous chemical (specifically, gradients in media composition) and topographical stimulation. This was performed in order to define the growth media and to optimize its composition.

The developed platform allows monitoring phenotype expression of cells in situ in highly controlled gradient environments of soluble factors in combination with different cell culture substrate properties. The detailed investigation of specific cellular responses to those stimuli is very difficult and timeconsuming with standard cell culture techniques.

The research leading to these results has received funding from the EU 7th Framework Programme (FP7/2007---2013) under grant agreement NMP3---SL---2009---229294 NanoCARD, and from Vinnova under contract no: 2009---00227.

3:00pm **BI-MoA4 What Makes the Heart Grow Fonder? High Throughput Screening of Synthetic Surfaces for Cardiomyocyte Culture**, ***A.K. Patel***, *M.R. Alexander, M.C. Davies,* University of Nottingham, UK, *D.G. Anderson, R. Langer,* Massachusetts Institute of Technology, *C. Denning,* University of Nottingham, UK

Human pluripotent stem cell (hPSC) derived cardiomyocytes hold the potential to strengthen pharmaceutical toxicity testing and to provide disease models for development of treatment targets[1]. The maturation and maintenance of the cardiomyocyte phenotype may be controlled by the manipulation of the substrate supporting the cells[2]. However, the surfaces currently in use still fall short of producing cardiomyocytes of adult maturity. Standard culture-ware requires coating with biological substrates such as fibronectin which can be expensive and subject to poor reproducibility due to batch variation.

We are exploring an alternative, combinatorial materials high throughput screening approach[3] to identify novel materials that can improve cardiomyocyte culture. Polymer microarrays comprising of 6 replicates of 116 acrylates and acrylamides are fabricated using contact printing. Cardiomyocytes derived from the HUES7 human stem cell line are seeded onto the arrays. Immunostaining of nuclei (DAPI) and the cardiomyocyte specific motor protein, sarcomeric alpha actinin is performed to visually estimate cell function and maturity and enable quantification of cell attachment in a high throughput manner using automated fluorescence microscopy and image analysis software. Surface characterisation of the arrays is performed using time of flight secondary ion mass spectrometry. Partial least squares (PLS) regression analysis allows for correlation of cell attachment with key molecular ions identified from mass spectrometry[4].

Successful monomers that permit cardiomyocyte attachment, spreading and contraction are identified from the first generation homopolymer microarray and are mixed pair-wise to form second generation microarrays. This diverse library of copolymers enables unique combinations of chemical moieties to be investigated. Hit monomers and combinations identified to be synergistic can be analysed for their effect on cardiomyocyte function including electrophysiology measured by patch clamping, myofibril alignment and gene expression.

The lead materials generated by this approach are the first step in a discovery process for novel synthetic biomaterials capable of enhancing the culture of cardiomyocytes to move towards more reproducible, economical and defined conditions.

References:

1. Matsa E. *et al. European Heart Journal.* 2011;32(8):952-62

2. Engler A. *et al.The Journal of Cell Biology*. 2004;166(6):877–887

3. Hook A. *et al. Biomaterials*. 2010;31(2):187–198

4. Yang J. *et al.Biomaterials.* 2010;31(34): 8827–8838

4:20pm **BI-MoA8 High-throughput Discovery of Polymers for Stem Cell Culture**, ***A.D. Celiz***, Univeristy of Nottingham, UK, *M. Mahlstedt, A.L. Hook, D.J. Scurr,* University of Nottingham, UK, *D.G. Anderson, R. Langer,* Massachusetts Institute of Technology, *D.A. Barrett, C. Denning, L. Young, M.C. Davies, M.R. Alexander,* University of Nottingham, UK

Numerous regenerative medicine procedures are already in clinical trials or in the pipeline and if these succeed and reach the clinic, stem cell factories will be needed to meet demand for the billions of cells required per intervention. Current protocols for stem cell culture employ poorly defined biological substrates such as Matrigel™, and/or non-human feeder cell layers which exhibit batch-to-batch variability, and are a potential source of pathogens. More recently, recombinant protein surfaces have been successfully employed, but these are not always cost effective for high-throughput culture methods. Consequently, there is significant research into xeno-free culture alternatives that use fully defined culture media and synthetic substrates. To discover materials for application as substrates, we have used high-throughput polymer arrays with which to fabricate chemically defined and scalable stem cell culture systems. A combinatorial chemical polymer library can be synthesized as a microarray on-slide allowing cell response of hundreds of different materials on a single glass slide, enabling hit materials to be identified in an automated manner [1]. High-throughput surface characterization (HT-SC) can be performed on the polymer microarray to (i) rapidly evaluate the surface chemistry of each individual spot and (ii) identify surface structure-property relationships in the complex and large datasets generated. [2]

In this study, a polymer library substantially increased in number and diversity compared with previous studies is prepared by printing (meth)acrylate monomers in pair-wise combination on a polyHEMA coated slide. HT-SC is carried out using time-of-flight secondary-ion mass

spectrometry, X-ray photoelectron spectroscopy, atomic force microscopy and water contact angle to characterize all polymer surfaces. Human pluripotent stem cell (hPSC) attachment is probed using automated fluorescence microcopy. This suite of HT-SC techniques has allowed the identification of 'hit' materials that support the expansion and proliferation of hPSCs. Human embryonic stem cell lines (HUES7 and H9) are screened for attachment in 3 different media; mouse embryonic fibroblast-conditioned media (MEF-CM), StemPro® and mTeSR®1 without any protein preconditioning to provide an insight into the impact of material surface properties on cellular interactions. The hits identified represent novel platforms for hPSCs culture without the need for any protein preconditioning and show great potential for development into future synthetic culture systems.

[1] Anderson, D. G. *et al. Nat. Biotechnol.* **22**, 863 - 866 (2004).

[2] Mei, Y. *et al. Nat. Mater.* **9**, 768 – 778 (2010).

4:40pm **BI-MoA9 Plasma Polymer Films at the Interface: Biomaterial Applications**, ***B.R. Coad***, University of South Australia

Biomaterial research is primarily concerned with mediating the interaction of biological species at the surface of materials. Therefore, research into new ways of coating and derivatizing surfaces is advancing this field. Coating surfaces with thin plasma polymer layers is advantageous because fragmentation and recombination of organic compounds in the plasma phase allows for surface deposition leading to covalent anchoring of polymer networks that retain the functionality of the parent compounds. This is useful as a platform strategy, providing a substrate-independent path for modifying different classes of bulk materials so that they retain their bulk properties (hardness, softness, conductivity, inertness etc.).

In this presentation, we show how deposited plasma polymers form interlayers that allow further surface modification of 1) surface chemistry and 2) surface topology and relate these to their use as biomaterials.

In the first approach, we have constructed a bioconjugation platform that allows for covalent capture of proteins in a gradient fashion. Simultaneous gradient plasma polymerization of two compounds under a mask along a moving stage allows for an increasing surface density of reactive aldehyde groups to be place alongside an inert hydrophilic spacer allowing for specific, yet variable attachment to be mediated across the surface. We have used this platform to capture streptavidin on the surface to which we have bound biotinylated signaling probes to investigate T-Cell binding and specificity. This platform is shown to be useful in further immunological studies investigating T-Cell binding to gradients of assembled human major histocompatibility complex analogs.

In the second approach, the surface topology has been modified with polymer brushes. With controlled radical polymerization from a novel macroinitiator, it is possible to generate polymer brushes of variable thickness and density which in turn modify the surface interaction with proteins and cells. Polymer chains which are present in highly ordered array provide an entropic barrier to surface fouling and cell attachment. The advantage of using plasma polymer interlayers is that the substrate-independent procedure allows for coatings with the same topology to be grafted, for example, from the surface of a hard, inert metal or from a flexible, polymeric film.

We conclude by showing a few other examples of the use of plasma polymerization for biomaterials applications from our latest work.

5:00pm **BI-MoA10 A High Throughput Strategy for Studying Protein Pre-adsorption to Materials Developed for Stem Cell Culture**, ***M. Hammad***, University of Nottingham, UK, *D.G. Anderson, R. Langer*, Massachusetts Institute of Technology, *M.R. Alexander, M.C. Davies*, University of Nottingham, UK

Improved biomaterials are required for application in areas such as regenerative medicine, biosensors, and medical devices. The performance of such materials is often dependent on their surface properties which can influence factors such as cell attachment and *in-vivo* biocompatibility and assimilation. High throughput (HT) materials discovery techniques have been developed to gain a greater fundamental understanding of the nature of the cell-surface interaction[1]. We have developed polymer microarray systems using several hundred unique polymers synthesised rapidly on-slide enabling parallel assessment of cell-surface response[2]. HT materials discovery is thus possible when this platform is combined with HT surface characterisation derived structure activity relationships[3].

The response of cells to these materials is controlled by the identity and conformation of the proteins adsorbed to the surface, which is in turn controlled by the chemistry of the underlying substrate. The complex nature of protein adsorption and their diversity in typical culture conditions makes this a difficult process to follow in-situ. Flaim et al. illustrated how printing of extracellular matrix (ECM) proteins can be used to investigate their role in stem cell differentiation and adhesion on a hydrogel surface[4]. We use an adaption of this methodology to analyse proteins adsorbed to a range of polymer surfaces in the form of spots on the array using piezo dosed solutions of ECM proteins. We have achieved this on polymer microarray systems, illustrating the ability to control both the pre-adsorption and also surface protein composition. Surface chemical analysis techniques including X-ray photoelectron spectroscopy and secondary ion mass spectrometry are used to characterise the protein identity and distribution at the surface. Polymerisation was achieved using the deposition of monomer solutions by piezo dispensing nozzles in an array format onto pHEMA coated substrates before the slides were irradiated with a long wave UV source. ECM protein solutions were then printed on the polymer spots allowing cell response to be correlated with protein surface composition using the same apparatus.

1. Hook, A.L., et al. Biomaterials, 2010. (2): p. 187-198.

2. Anderson, D.G., S. Levenberg, and R. Langer Nature Biotechnology, 2004. (7): p. 863-866.

3. Mei, Y., et al Nature Materials, 2010. (9): p. 768-778.

4. Flaim, C.J., S. Chien, and S.N. Bhatia Nature Methods, 2005. (2): p. 119-125.

5:20pm **BI-MoA11 Combinatorial Development of Biomaterials for Pluripotent Human Stem Cell Culture**, ***Y. Mei***, Clemson University

Pluripotent human stem cells include human embryonic stem cells (hESCs) and more recently developed human induced pluripotent stem cells (hIPSCs). These cells can replicate indefinitely in culture and can differentiate into all types of cells in human body. Thus, they hold remarkable promise as cell source for regenerative medicine and tissue engineering applications. However, hESCs and hIPSCs are currently cultured on a feeder cell layer of mitotically inactivated mouse embryonic fibroblasts (mEFs) or Matrigel^{TM},extracellular matrix (ECM) protein mixtures secreted by mouse carcinoma cells. The utilization of mouse feeder cells and Matrigels as substrates leads to cell populations unsuitable for replacement therapy.

To address the challenge, we recently developed a high throughput polymer microarray technology to rapidly synthesize and test thousands of microscale substrates for hESC and hIPSC culture. In this study, 22 acrylate monomers were used to construct polymer arrays containing 496 different materials with diversified properties. Material properties including surface wettability, indentation elastic modulus, surface roughness and surface chemistry of each polymeric substrate in the array were quantified using high throughput methods. The results were then used to establish structure-function relationships between material properties and biological performance. Surface chemistry was shown to have controlling effects on hES cell undifferentiated growth while indentation elastic modulus or roughness had less pronounced effects on growth. The optimal ("hit") surface was defined as certain oxygen containing ions and hydrocarbon ions in time of flight secondary ion mass spectrometry [ToF-SIMS] analysis. The "hit" surfaces can effectively enhance adsorption of vitronectin and engagement with integrin a_vb_3 and a_vb_5 to promote self-renewal of hESCs and hIPSCs.

Based on the structure-function relationship, favorable substrates for hESC and hIPSC culture was developed by exposing polystyrene (PS), a typical cell culture plastic, to an optimized dose of short wavelength ultraviolet (UV) light. In this way, key chemical moieties supporting self-renewal of hESCs and hIPSCs (e.g. hydrocarbons and ester/carboxylic acid) can be introduced onto the surface of PS. PS surfaces treated with the optimal dose of UV (i.e. UVPS) can support more than three times more cells per area than traditional mouse embryonic fibroblast (mEF) feeder cells, the current gold standard. As "hit" polymers, UVPS can promote adsorption of vitronectin to support self-renewal of hESCs and hIPSCs.

Authors Index

Bold page numbers indicate the presenter