Monday Morning, October 28, 2013

Biomaterial Interfaces
Room: 201 B - Session BI+AS+IS+NL-MoM

Surfaces to Control Cell Response
Moderator: H.E. Canavan, University of New Mexico

8:20am **BI+AS+IS+NL-MoM1 Modulation of Cell Behaviour using Self–assembled Binary Colloidal Crystals**, ***P.Y. Wang***, *P. Kingshott*, Swinburne University of Technology, Australia

The control of cell behaviour on surfaces is the key to a broad range of biomedical applications. Biomaterial surfaces with tuneable surface topographies and chemistries can profoundly influence the development of advanced biomaterials used in applications including tissue engineering and regenerative medicine. Recently, we developed an elaborate and feasible method to display an ordered surface topography with tuneable surface chemistry using binary colloidal crystal particles. Using this binary colloidal system, various combinations of particle size and surface chemistry can be readily employed. In this study, two combinations of binary colloidal crystals, i.e. PS-COOH (2 µm)/PMMA (0.4 µm) and SiO_2 (2 µm)/PMMA (0.4 µm) were assembled on ozone-treated silicon wafers. The preliminary results of cell attachment and morphology of L929 fibroblasts and MG63 osteoblasts were studied after 24h.

In general, cells had a small projection area rather than fully spread morphology on the crystal surfaces compared with the flat control. Fibroblasts have abundance of cell protrusions called filopodia which can be observed using scanning electron microscopy (SEM), whilst osteoblasts don't have. Fibroblasts had long and thin extended filopodia on the PS/PMMA crystal surfaces, whilst they had short and thick filopodia on the SiO_2/PMMA crystal surfaces. Regarding the surface chemistry, both SiO_2 and PMMA particles were not as favourable as the PS-COOH particles for fibroblasts attachment, and resulted in the cell projection area on the PS/PMMA being larger compared to the SiO_2/PMMA crystal surfaces. On the contrary, the cell projection area of osteoblasts didn't have significant differences between these two crystal surfaces. After fibronectin coating, cell projection area of osteoblasts on SiO_2/PMMA crystal surfaces increased significantly, whilst fibroblasts didn't, suggesting that different cell types respond to surfaces differently.

These results show for the first time that cell-substrate interactions can be easily controlled by precise positioning of different particles with various sizes and chemistries. The present results will help gain a more thorough understanding of cell-material interactions benefiting the development of advanced biomaterials and materials for tissue engineering.

8:40am **BI+AS+IS+NL-MoM2 Achieving Differential Cell Adhesion with Novel Polymer Surfaces Identified using Microarrays**, ***F.A. Simoes***, *C. Alexander, G. Mantovani, L. Buttery, M.R. Alexander*, University of Nottingham, UK

Stem cells have the ability to repair, replace or regenerate tissues. As a result their potential for regenerative medicine is vast. The processing of cells for therapeutic use and clinical diagnostics will rely on cell sorting steps to ensure a homogeneous population is obtained.[1]

Several techniques exist to achieve this, which rely on the physical properties of cells but tend to provide poor specificity.[2-4] Fluorescence Activated Cell Sorting (FACS) and Magnetic-Activated Cell Sorting (MACS) rely on specific biomarkers. However cells require labelling and label removal steps, which can affect the phenotype.[5]

There is a need for a fully synthetic, inexpensive, label-free separation system, capable of sorting cells with minimum manipulation. In order to generate robust surfaces for such a system, we have developed a method to immobilize thiol-functionalised materials to a polymer substrate using thiol-ene "click" chemistry in a high throughput format. Microarrays of these functionalised polymers comprising of 6 replicates, are fabricated using pin printing to generate a combinatorial library of materials. A mixture of differentiated cells derived from mouse embryoid bodies are then seeded onto the arrays.

Immunohistochemistry techniques are employed to track the differentiation of cells into different lineages, thus enabling the visualisation of multiple cell lines. These techniques also allow for the high throughput quantification of attachment by the means of automatic fluorescence microscopy.

Surface characterisation of the "click" immobilization procedure is performed by X-Ray Photoelectron Spectroscopy. In contrast the characterisation of microarrayed materials is performed using Time of Flight - Secondary ion Mass Spectrometry, which is followed by the ranking of materials using Partial Least Square (PLS) regression analysis. This process allows for the correlation of cell attachment with key molecular ions generated from each material by mass spectrometry.

Successful materials that selectively induce cell attachment are identified and investigated further. This is the first step in the generation of new surface-based devices that have the capacity to be fully synthetic, selective, inexpensive and disposable.[6]

1. McIntyre C. *et al*., *Bioprocess International*, 2010, 44-53.

2. Chabert M. and Viovy J., *PNAS*, 2008, **105**, 3191-3196.

3. Shim S. *et al*., *Integrative Biology*, 2011, **3**, 850-862.

4. Kose A. R. *et al*., *PNAS*, 2009, **106**, 21478-21483.

5. Bulte J. W. M. *et al*., *Blood*, 2004, **104**, 3410-3413.

6. Singh A. *et al*., *Nature Methods*, 2013, **10**, 438-444

9:00am **BI+AS+IS+NL-MoM3 Interaction of Hematopoietic and Leukemic Cells with their Microenvironment**, ***A. Rosenhahn***, Ruhr-University Bochum, Germany, *M. Hanke, C. Christophis*, Karlsruhe Institute of Technology, Germany, *I. Taubert, N. Baran, P. Wuchter, A.D. Ho*, University of Heidelberg, Germany

Especially for leukemic and haematopoietic cells, the interaction with their microenvironment is of upmost importance for extravasation and homing. One key mechanism is the interaction of the CD44 receptor with extracellular hyaluronan (HA) binding motifs. To quantitatively assess the interaction, a microfluidic experiment has been developed that allows studying the interaction of cells with interfaces under well-defined flow conditions [1]. Shear flow activated catch bond interaction is well characterized for selectin mediated extravasation of leukocytes [2]. We recently found that also the CD44 interaction with HA requires a minimum shear stress to become activated and enable cells to roll on HA surfaces [3]. Similar critical shear values were found for rolling on mesenchymal stroma cells, which are present in the bone marrow niche creating the microenvironment required for haematopoietic stem cell renewal. Interestingly not only hematopoietic stem cells but also acute leukemic blasts show a shear flow induced rolling. The proportion of rolling cells will be discussed on the basis of the pathogenesis of the disease.

[1] C. Christophis, M. Grunze, A. Rosenhahn, PCCP 2010, 12, 4498.

[2] E.B. Finger, K.D. Puri, R. Alon, M.B. Lawrence, U.H. von Andrian, T.A. Springer, Nature 1996, 379, 266

[3] C. Christophis, I. Taubert, G. Meseck, M. Schubert, M. Grunze, A. D. Ho, A. Rosenhahn, Biophys. J. 2011, 101, 585.

9:20am **BI+AS+IS+NL-MoM4 The Creation of Polymeric Biointerfaces using Non-Contact Dispensing Technology**, ***C. Dufresne***, Scienion

Polymeric surfaces of varied composition have been created in high density microarray formats. These patterned surfaces have been used to study a number of biointerface processes such as stem cell differentiation, and bacterial adhesion. Scienion offers non-contact picoliter dispensing technology that enables the creation of such surfaces. The inert glass capillaries allow for the use of a wide range of chemical reagents. Precision positioning enables drop-on-drop dispensing and mixing. Image analysis of the substrates in turns makes it possible to accurately dispense the materials onto almost any surface. This presentation will cover how Scienion technology is implemented for the production of polymeric surfaces.

9:40am **BI+AS+IS+NL-MoM5 The Role of Cell-Substrate Interactions on Cell Stiffness and Cell Volume**, ***D.A. Weitz***, Harvard University **INVITED**

Cell stiffness is often observed correlate with the stiffness of the substrate on which the cells are grown. This talk will present data which suggest that cell-substrate interactions are more diverse, and depend as well on the adhesion area. It will discuss the impact of the substrate on cell volume and the consequences of this on cell stiffness. The data presented will suggest that cell volume is a control for cell stiffness.

10:40am **BI+AS+IS+NL-MoM8 Quantitative, Predictive Models of Adhesion of Cells to Polymers**, ***V.C. Epa***, *D.A. Winkler*, CSIRO Materials Science & Engineering, Australia, *A.L. Hook, C. Chang, J. Yang*, University of Nottingham, UK, *R. Langer, D.G. Anderson*, MIT, *P. Williams, M.C. Davies, M.R. Alexander*, University of Nottingham, UK

Designing materials to control biology is an intense focus of biomaterials and regenerative medicine research. Discovering and designing materials with appropriate biological compatibility or active control of cells, tissues,

or pathogens is being increasingly undertaken using high throughput synthesis and assessment methods.

In particular, culture of multipotent cells such as stem cells is a major research focus in regenerative medicine. Much research effort is focused on designing chemically defined, serum-free, feeder-free synthetic substrates and media to support robust self-renewal of pluripotent cells. Changes in cellular properties such as adhesion, morphology, motility, gene expression and differentiation are influenced by surface properties of the materials on which cells have been cultured. Similarly, designing new materials to control the growth of pathogens on implantable and indwelling devices such as pacemakers, and catheters, is critical given the high level of device-centred infections.

We report a relatively simple but powerful machine-learning method of generating models that link microscopic or molecular properties of polymers or other materials to their biological effects. We illustrate the potential of these platform modelling methods by developing the first robust, predictive, quantitative, and purely computational models of adhesion of human embryonic stem cell embryoid bodies, and three clinically important pathogens, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and uropathogenic *Escherichia coli*, to the surfaces of 496 polymers.

11:00am **BI+AS+IS+NL-MoM9 Smart Surfaces for Studies of Real-Time Dynamic Cell Behavior**, ***M.N. Yousaf***, York University, Canada

Active migration, local tissue invasion and seeding of distant metastases are all characteristics of malignant cells. These complex cellular events require the integration of information derived from soluble growth factors with positional information gained from interactions with the extracellular matrix and with other cells. The biochemical events of the signaling cascades occur in a spatially and temporally coordinated manner that then dynamically shape the cytoskeleton in specific sub-cellular regions. Therefore cell migration and invasion involve a precise but constantly changing subcellular nano-architecture. To fully understand the complex signaling and cytoskeletal aspects of the cellular nano-architecture during migration requires a multidisciplinary coordinated effort. The long-term goal of this research program is to develop new surface chemistry and cell biological tools to generate a class of tailored dynamic nanopatterned substrates for a variety of cell adhesion and migration experiments. The combined application of dynamic smart substrates, molecular surface gradients and in vivo biosensors will potentially allow for the analysis and quantitation of the events of cell migration at each step from initial engagement with extracellular matrix ligands, to localized activation of signaling proteins, to organization and activation of the cytoskeleton, to overall movement of the cell.

11:20am **BI+AS+IS+NL-MoM10 What Makes the Heart Grow Fonder? Chemically Diverse Polyacrylate and Polyacrylamide Surfaces for Human Cardiomyocyte Culture and Their Effect on Phenotype**, ***A.K. Patel***, University of Nottingham, UK, *D.G. Anderson*, *R. Langer*, Massachusetts Institute of Technology, *M.C. Davies*, *M.R. Alexander*, *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 targets1. The maturation and maintenance of the cardiomyocyte phenotype may be controlled by the manipulation of the substrate supporting the cells2. 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 approach3 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 spectrometry4.

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

11:40am **BI+AS+IS+NL-MoM11 Selectivity in Platelet Activation by the Titania Surface: A Model System for In Vitro Modulation of Platelet Activity**, *S. Gupta*, CIC biomaGUNE, Spain, ***I. Reviakine***, Karlsruhe Institute of Technology, Germany

Platelet are anuclear cell fragments circulating in blood. Their major function is haemostasis: they catalyze the formation of the fibrin clot that stops the bleeding. Recently it was shown that they have a multitude of other functions in processes such as the immune response, inflammation, angiogenesis, implant rejection or integration.

Platelets circulate in the blood in a quiescent form. They become activated at wound sites, implant surfaces, or through the action of soluble agonists secreted by activated platelets or produced in the blood as a result of the clotting process. Activated platelets express on their surface a variety of protein and lipid receptors that catalyze the clotting process, interact with other platelets, leukocytes, and endothelial cells, and adhere to the extracellular matrix exposed at the wound sites. The also secrete a variety of active substances, including growth factors, that are stored inside special granules within the platelets.

Recently discovered diversity of platelet functions implies a tight regulation of the activation processes. Indeed, there is evidence to suggest that platelet activation is a selective process with a spectrum of activated states, rather than a two-state process involving quiescent vs. pro-coagulant platelets. In this context, we have previously shown that platelet activation profile on TiO_2 depends on the surface-bound Ca. Here, we measure intracellular calcium currents in surface-adsorbed platelets in order to understand how this manifestation of platelet activation selectivity is related to the internal signaling pathways. Such an understanding is a prerequisite for designing new, platelet-based approaches to the treatment of haemostatic and inflammation-based disorders, to enhancing implant integration and wound repair, and to tissue engineering applications.

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