Biomaterial Interfaces
Room: 23 - Session BI-MoM

Surfaces to Control Cell Response
Moderator: A. Rosenhahn, Karlsruhe Institute of Technology, Germany

8:20am **BI-MoM1 Click Chemistry on Brominated Plasma Polymer Thin Films for Immobilizing and Patterning Biomolecules and Cells**, ***B.W. Muir***, CSIRO Materials Science and Engineering, Australia, *R. Chen*, CSIRO Materials Science and Engineering and The University of Melbourne, Australia, *G.K. Such*, The University of Melbourne, Australia, *A. Postma, R.A. Evans, K.M. McLean*, CSIRO Materials Science and Engineering, Australia, *F. Caruso*, The University of Melbourne, Australia

The development of versatile and robust strategies for the surface modification of multiple classes of materials has proven challenging, with few generalized methods. Many available methods have limitation for widespread use due to the need for specific surface chemistries and/or laborious multistep procedures[1]. A protocol to deposit brominated plasma polymer (Brpp) thin films on a variety of substrate surfaces (silicon wafers, glass, gold, Teflon) has been developed. These coatings are highly adherent and exhibit good stability in aqueous, biphasic and autoclaving conditions. The Brpp coating was found to be a useful platform for secondary reactions leading to surfaces with specific chemical properties. Following nucleophilic exchange, azide functionalized surfaces were developed and the copper catalysed azide alkyne cycloaddition (CuAAC) reaction[2]; a paradigm of click chemistry, was successful in immobilizing various acetylenes. A particular highlight is the patterning of cells via selective surface functionalisation of PEG-alkyne using a photomask.[3] This is the first known example of CuAAC reactions on pp thin films. A detailed physicochemical characterisation study of these films will also be presented.

[1] aH. Lee, S. M. Dellatore, W. M. Miller, P. B. Messersmith, *Science* 2007, 318, 426-430; bD. Y. Ryu, K. Shin, E. Drockenmuller, C. J. Hawker, T. P. Russell, Science 2005, 308, 236-239.

[2] R. A. Evans, *Australian Journal of Chemistry* 2007, 60, 384-395.

[3] Chen, R. T.; Marchesan, S.; Evans, R. A.; Styan, K. E.; Such, G. K.; Postma, A.; McLean, K. M.; Muir, B. W*.; Caruso, F., *Biomacromolecules* 2012, 13, (3), 889-895.

8:40am **BI-MoM2 Temperature-Induced Electrostatic Assembly of Poly (Ethylene Glycol) Co-Polymer for Non-Fouling Biomedical Applications: How Low Can You Go?**, ***R. Ogaki***, *O. Zoffmann Andersen, K. Kolind, D.C.E. Kraft, M. Foss*, Aarhus University, Denmark

Development of long-term stable surfaces that resist bio-adhesion continues to stimulate the field of biomedical and biological research. While numerous strategies have been developed over the last several decades, the challenge remains in the creation of surfaces that can provide long-term 'zero' bio-adhesion from a variety of biological entities that spans lengths scales from biomolecules to cells. Although the physical and chemical properties of the resisting surface itself are important in achieving this ultimate goal, assessing the extent of bio-adhesion must be accompanied by detailed surface analysis via highly sensitive analytical techniques.

We have recently discovered that increasing the temperature alone during the assembly process of poly-*l*-lysine *grafted* poly (ethylene glycol) (PLL-*g*-PEG) results in the formation of highly dense PLL-*g*-PEG brush coating. The PLL-*g*-PEG surfaces prepared at various temperatures (20 to 80 °C) have been characterized by X-ray photoelectron spectroscopy (XPS). The PLL-g-PEG surfaces prepared at the 'standard' temperature of 20 °C are found to be comparable to the previously reported literatures. Interestingly, the surfaces prepared at 80°C have shown the highest surface grafted density of PLL-*g*-PEG, with ~ 4 times denser than those prepared at 20 °C.

The degree of cell and protein adhesions on these surfaces has been stringently determined using cell culture and serum/blood adsorption assays combined with XPS and time of flight secondary ion mass spectrometry (ToF-SIMS). The temperature-induced PLL-*g*-PEG surfaces have achieved 'zero' cell adhesions from three different types of mammalian cells for at least 36 days. In addition, XPS and ToF-SIMS analysis have confirmed near-zero protein adsorptions from 10% serum/MEM (at least 36 days), whole undiluted blood (at least 24 hrs) and undiluted serum (at least 24 hrs) with the surfaces being pre-incubated in high ionic strength buffer (2.4 M NaCl for 24 hrs).

The outcome of the rigorous bio-resistance tests presented here highlights the critical importance of processing temperature on the surface graft density of electrostatically driven PLL-g-PEG. The temperature induced assembly process can be effectively and easily implemented for a range of biomedical and biotechnological applications.

9:00am **BI-MoM3 Spatially and Temporally Coordinated Processes of Cells at Molecular to Cellular Scales**, ***J.P. Spatz***, Max Planck Institute for Intelligent Systems & University of Heidelberg, Germany **INVITED**

Our approach to engineer cellular environments is based on self-organizing spatial positioning of single signaling molecules attached to synthetic extracellular matrices, which offers the highest spatial resolution with respect to the position of single signaling molecules. This approach allows tuning tissue with respect to its most relevant properties, i.e., viscoelasticity, peptide composition, nanotopography and spatial nanopatterning of signaling molecule. Such materials are defined as "nano-digital materials" since they enable the counting of individual signaling molecules, separated by a biologically inert background. Within these materials, the regulation of cellular responses is based on a biologically inert background which does not initiate any cell activation, which is then patterned with specific signaling molecules such as peptide ligands in well defined nanoscopic geometries. This approach is very powerful, since it enables the testing of cellular responses to individual, specific signaling molecules and their spatial ordering. Detailed consideration is also given to the fact that protein clusters such as those found at focal adhesion sites represent, to a large extent, hierarchically-organized cooperativity among various proteins. We found that integrin cluster have a functional packing density which is defined by an integrin-integrin spacing of approximately 68 nanometers. Such critical spacing values vary as matter of transmembrane receptor choice of interest. We have also developed methods which allows the light initiated activation of adhesion processes by switching the chemical composition of the extracellular matrix. This enabled us to identify the frequency of leader cell formation in collective cell migration as a matter of initial cell cluster pattern size and geometry. Moreover, "nano-digital supports" such as those described herein are clearly capable of involvement in such dynamic cellular processes as protein ordering at the cell's periphery which in turn leads to programming cell responses.

9:40am **BI-MoM5 Chemically Defined Synthetic Surfaces for Mesenchymal Stem Cell Expansion**, ***L. Meagher***, *H. Thissen, P. Pasic, R.A. Evans, S. Pereira, K. Tsang, V. Glattauer, K. Styan, C.L. Be, D. Haylock*, CSIRO Materials Science and Engineering, Australia

Interest in surface initiated polymerisation (SIP) for biomedical applications has increased rapidly recently, particularly the use of "living" free radical polymerisation mechanisms[1] as highly defined coating properties/architectures can be achieved. Here we demonstrate that advanced coatings can be produced using a surface immobilised macro-chain transfer agent approach[2] and that such coatings can be used for the effective control of cell-surface interactions, an essential requirement in a broad range of applications in biomaterials and regenerative medicine. In the expansion of stem cells for therapeutic applications, fully synthetic, chemically defined materials are a requirement. Polymeric coatings which contain synthetic cell signalling molecules are key to ongoing progress in the generation of cells as therapies. Coating characterization was carried out using X-ray photoelectron spectroscopy (XPS) and colloid probe atomic force microscope (AFM). Cell culture studies were carried out using bone marrow derived human mesenchymal stem cells (hMSCs) using standard techniques. Differentiation of hMSCs was carried out using standard protocols in induction medias and the presence of characteristic cell surface markers was determined using flow cytometry. Substrate materials were silicon wafers or tissue culture polystyrene (TCPS).

In this study, we focus on a surface initiated Radical Addition-Fragmentation chain Transfer (RAFT) approach and present data demonstrating that dense polymer brushes can be prepared via surface immobilized macro-RAFT agents. The brush nature of the coatings was confirmed using a combination of XPS analysis and direct interaction force measurements with the AFM colloid probe technique. The properties of the coatings could be fine tuned using a variety of parameters such as the RAFT agent surface density, the polymerisation conditions, the monomer feed composition and the conjugation of cell attachment motifs such as cyclic peptides which interact with cell surface integrins. For example, the combination of a low cell adherent, low protein adsorbing polymer brush coating containing a conjugated peptide which interacted with $alpha_v beta_3$ integrins resulted in a surface which supported the expansion of hMSCs in a xeno-free, chemically defined, serum replacement media. In addition the expanded cells expressed cell surface markers typical of undifferentiated

hMSCs and the expanded cells were able to differentiate along adipogenic, osteogenic and chondrogenic pathways.

[1] Edmond, S., Osborne, V.L. and Huck, W.T.S., Chem. Soc. Rev. 2004, 33, 14.[2] Meagher, L., Thissen, H., Pasic, P., Evans, R.A. and Johnson, G., WO2008/019450.

10:00am **BI-MoM6 Binary Colloidal Crystal Structures Combined with Chemical Surface Modification to Achieve Superior Control Over Biointerfacial Interactions**, ***P. Koegler***, Swinburne Univ. of Tech., Australia, *P. Pasic, J. Gardiner, V. Glattauer,* CSIRO Materials Science and Eng., Australia, *A. Clayton,* Swinburne Univ. of Tech., Australia, *H. Thissen,* CSIRO Materials Science and Eng., Australia, *P. Kingshott*, Swinburne Univ. of Tech., Australia

Biointerfacial interactions play a major role in the field of biomedical materials and regenerative medicine and are of tremendous importance to *in vivo* and *in vitro* applications. Cell-material interactions are mediated by surface parameters including the materials surface chemistry and topography. Colloidal lithography represents a promising tool to modify surface topographies at the nanoscale with precision and over large areas while at the same time not requiring complex instrumental set-ups or rigorous experimental conditions. The creation of nanostructured surfaces in this way can also be combined with sophisticated surface modification techniques such as polymer grafting techniques via functional groups (grafting-to) or initiating groups (grafting-from) on the particle surface. This platform, which provides control over surface chemistry and topography, offers great flexibility in regard to the design of advanced surface coatings.In the current study we have generated highly ordered binary colloidal crystal structures using surface functionalized particles. This approach allows precise control over particle size, spacing, and thus pattern morphology. In order to minimize undesired non-specific protein adsorption which can mediate cell attachment, graft polymer coatings were applied to particles using heterobifunctional poly(ethylene glycol) (PEG) to render the surfaces non-fouling. In addition, colloid crystal modified surfaces were modified with specific bioactive signals, such as the cyclic RGD peptide (cRGDfK) to promote cell attachment. Surface characterization was carried using scanning electron microscopy (SEM), atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS). Cell culture experiments were carried out using L929 mouse fibroblasts up to 24 hours.The unprecedented control over the surface chemistry and topography provided by this simple coating platform is of significant interest for the study of biointerfacial interactions and the development of new and improved biomedical devices.

11:20am **BI-MoM10 Influence of Ca^{2+} Binding to Titania on Platelet Activation Profiles**, *S. Gupta,* ***I. Reviakine***, CIC biomaGUNE, Spain

Surface properties of implant materials are known to influence biological responses they elicit. However, complex processes operating at the interface remain poorly understood. To get an insight into these processes, we investigated the role played by surface ion equilibrium in defining interactions between an implant material (TiO_2) and components of blood (in this case, platelets), because blood is the first tissue that foreign materials come into contact with when inserted into the body and because platelet response is crucial in defining the implant's fate.

Titanium is a widely used biomaterial. Its success is in part due the favorable biocompatibility properties conferred by its oxide, TiO_2. We have previously shown that Ca^{2+}-TiO_2 interactions affect the distribution of phospholipid phosphatidyl serine (PS) in model lipid membranes prepared on TiO_2. This allowed us to hypothesize that platelet activation will be affected by these interactions as well.

Platelets are anuclear cell fragments circulating in blood. Activated at wound sites, they aggregate and provide a catalytic surface for the formation of a fibrin-based clot that stops the bleeding. Recently, platelets have been recognized to participate in inflammation, wound healing, tissue regeneration, and immune responses. Activation of platelets by foreign surfaces is detrimental to blood-contacting implants but beneficial for osteoimplants. Upon activation, platelets expose on their surface and secrete a number of markers. These include PS, activated form of GPIIb-IIIa, and proteins CD62P and CD63 that are found in the membranes of the intracellular α- and dense granules of quiescent platelets. To assess the state of platelet activation on TiO_2, we assayed for the expression of these markers. In order to isolate a clear cause-and-effect relationship between Ca^{2+}-TiO_2 interactions and platelet activation, we focused on purified platelets.

Our main finding is that the platelet activation profile on TiO_2 depends on the presence of Ca^{2+}. Furthermore, in the absence of Ca^{2+}, the α- and dense granule secretion is differentially regulated on titania. The differential granule secretion by platelets, as regulated by the surface properties, can be applied towards controlled release of molecules from platelets by nanoparticles or implants in drug delivery applications.

11:40am **BI-MoM11 Enhancing the Osseointegration of Titanium Dental Implants by Magnetron-Sputtered Strontium Containing Coatings**, ***O.Z. Andersen***, Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Denmark, *V. Offermanns*, Medizinische Universität Innsbruck, Universitätsklinik für Mund-, Kiefer- und Gesichtschirurgie, Austria, *M. Sillassen,* Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Denmark, *D.C.E. Kraft,* Aarhus School of Dentistry, Denmark, *J. Bøttiger, F. Besenbacher,* Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Denmark, *F. Kloss,* Medizinische Universität Innsbruck, Universitätsklinik für Mund-, Kiefer- und Gesichtschirurgie, Austria, *M. Foss*, Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Denmark

Introduction: Strontium (Sr) has been shown to have a beneficial influence on the subsequent remodelling of the bone structure in relation with implant osseointegration. Both decrease of the osteoclast driven bone resorption and enhancement of the osteoblast driven process of bone formation has been shown. Furthermore, Sr has proven to have an anti-inflammatory effect.

Methods: The coatings used in this study were either prepared on Ti implants (rods with diameter = 1.1 mm and length = 6 mm) or on silicon wafers. The Sr containing surface modifications were prepared by co-sputtering in a setup with a pure Ti and a sintered composite target. The samples were characterized using SEM, AFM, XPS and RBS. ICP-AES was used to investigate the amount of Sr released from the samples as a function of time. Human dental pulp stem cell (hDPSC) cultures were used to assess the *in vitro* cellular response: Cell attachment and proliferation was studied along with the cells ability to mineralize. Quantification of osteogenic expression markers and specific cytokines was performed via RT-PCR. Human blood derived monocyte cultures were carried out to investigate the *in vitro* differentiation of these into osteoclast-like cells in response to Sr. *In vivo* experiments were carried by inserting implants into the femur of Wistar rats and evaluation was done by assessing bone-to-implant contact and new bone volume.

Results: The amount of Sr incorporated in the surfaces was found to be between 0 and 8.7 at. %. The Sr release profile showed that the most Sr was released from samples incorporating 5.5 at. % Sr. In relation with the *in vitro*experiments, the hDPSC proliferation and mineralization was found to correlate with the surface Sr concentrations. Moreover, the Sr concentration also affected the differentiation of monocytes into osteoclast-like cells. In relation with the *in vivo* experiment it was found that the incorporation of Sr had a beneficial effect on implant osseointegration, where an increase in direct bone contact and in new bone volume was observed with an increasing Sr release.

Discussion: From the *in vitro* and *in vivo* Sr release experiments it was found that a more dense surface structure developed as the Sr concentration were increased. We therefore speculate that the peak in the Sr release around 5.5 at.% can be ascribed to an optimal correlation between the morphology and the amount of incorporated Sr. The results from the *in vitro* and *in vivo* models shows that the coating process we have developed for modifying implants is an interesting candidate in relation with shortening the healing period when inserting osseointegrating implants.

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