Biomaterial Interfaces
Room 2014 - Session BI-WeA

Bio-Interfacial Modification and Bio-Immobilization II (Honoring Marcus Textor, ETH-Zürich for Substantial Contributions to the Field)

Moderator: J. Vörös, University and ETH-Zurich, Switzerland

2:00pm **BI-WeA1 Polycationic Glycopolymers for the Molecular Assembly of Carbohydrate Functionalized Surfaces: Synthesis and Application**, ***K. Barth***, *G. Coullerez, R. Castelli, L. Nilsson, J. Moeller, M. Textor,* ETH Zurich, Switzerland

Due to their structural diversity, carbohydrates are able to mediate explicit information as markers for biomolecular interactions on mammalian cell surfaces. Usually the bindings of a monosaccharide with proteins are very weak. However, multivalent interactions between carbohydrates and target cell receptors induce strong and specific bindings mediated by clustered carbohydrates. This complexity in glycosciences complicates the study of recognition processes mediated by carbohydrates. Carbohydrate functionalized surfaces and microarrays for high throughput studies now provide versatile tools to identify and classify carbohydrate-binding proteins. A straightforward method will be presented to covalently graft mono-, di- or oligosaccharides at different densities to the polycationic brush-like copolymer poly-(L-lysine)-graft-poly(ethylene glycol) (PLL-g-PEG), which forms stable molecular assemblies on negatively charged surfaces. As the functionalized PLL-g-PEG is resistant against non-specific protein attachment, density and structure-dependent kinetic parameters for the interactions between carbohydrates and proteins can be obtained using label-free in situ bio-sensing methods. This has been proven for the protein Concanavalin A and a library of mannoside-functionalized surfaces. We were able to study the effects of the carbohydrate density, the nature of the linker and the structure of oligomannosides. In order to create structured surfaces we used a photolithography based method to form well-defined patterns with differences in size and carbohydrate density in a non-interactive background. The applicability of these substrates will be shown in studies of the adhesion behavior of type I fimbriated E. coli regarding the biological activity of the adhered bacteria. Finally, competition experiments with carbohydrates in solution will be done to remove biomolecules selectively from the functionalized surfaces in order to reuse them for several experiments.

2:20pm **BI-WeA2 Micro-Three-dimensional Structuring Platform for Cell Culturing**, ***M. Ochsner***, *M. Dusseiller, M. Grandin, M. Textor,* Federal Institute of Technology Zurich (ETHZ), Switzerland

Studies have shown differences in cell behavior when cells are cultured in a 3-dimensional matrix as compared with flat surfaces. Therefore, next to the physical and chemical microenvironment, the structural environment of a cell plays a crucial role in its shape and function. We have developed a set of tools which combines 2-dimensional chemical patterning with topographical microstructuring, thus presenting to the cells a controlled microenvironment that mimics the in vivo environment. The technique combines master fabrication in Si and replication techniques which allows us to create polydimethylsiloxane (PDMS) chips that display defined microwells of various shapes with a dimension on the order of single cells. By making use of different cross linking densities of the PDMS, tailoring of the mechanical properties of the surrounding material is possible. In addition to geometry, we are also able to control the chemistry of the microenvironments such that the surface inside the wells can present specific chemical functionality, e.g.: adhesion proteins, while the plateau surface between the wells is passivated against protein adsorption. The passivation is critical for a controlled microenvironment and we are developing a method for the wet-printing of a protein resistant graft-co-polymer, poly(L-lysine)-g-poly(ethylene glycol) using an inverted microcontact printing technique. As many proteins of interest in cell studies are membrane proteins, wherein mobility may also play an important role, we are also working toward coating the inside of the wells with protein functionalized lipid bilayers. In comparison to coating the wells with functionalized PLL-g-PEG-X,where X is some protein or peptide, we can provide an interesting platform to investigate the influence of ligand mobility. This work aims at the optimization of this technique, focusing on future applications in cell biology such as monitoring focal adhesion and actin dynamics in response to stress exposure.

2:40pm **BI-WeA3 Tunable Interfacial Hydrogels for Control of Neural Stem Cell Fate**, ***K. Saha***, *E.F. Irwin, D.V. Schaffer, K.E. Healy,* University of California, Berkeley

Highly-regulated signals in the stem cell microenvironment, such as growth factor presentation and concentration, and matrix mechanical stiffness, have been implicated in modulating stem cell proliferation and maturation. However, tight control of proliferation and lineage commitment signals is rarely achieved during growth outside the body, since the spectrum of biochemical and mechanical signals that govern stem cell self-renewal and maturation are not fully understood. Therefore, stem cell control can potentially be enhanced through the development of material platforms that more precisely orchestrate the presentation of the aforementioned signals to stem cells. Using a biomimetic interpenetrating polymer network (IPN), we define a robust synthetic and fully mechanically and chemically defined platform to regulate stem cell number and differentiation for the culture of adult neural stem cells. The IPN's properties, such as ligand type, ligand surface density, and stiffness (i.e., complex modulus 1-10 kPa), were quantitatively controlled and characterized. In this work, hydrogels modified with two cell-binding ligands, CGGNGEPRGDTYRAY from bone sialoprotein [bsp-RGD(15)] and CSRARKQAASIKVAVSADR from laminin [lam-IKVAV(19)], were assayed for their ability to regulate self-renewal and differentiation of neural stem cells in a dose-dependent manner. Media conditions, supplemented with particular soluble factors, were modulated for either stem cell self-renewal or differentiation. IPNs with bsp-RGD(15) above 5.3 pmol.cm@super-2@ supported both self-renewal and differentiation, whereas hydrogels with lam-IKVAV(19) failed to support stem cell adhesion and did not influence early differentiation. This platform is highly tunable and could potentially be used to translate in vitro control of stem cells to an implantable biomaterial that can be harnessed for tissue regeneration.

3:00pm **BI-WeA4 Immobilisation Strategies for Grafted Dendron Surfaces**, ***N.D. Pollock***, *L.J. Twyman, S.L. McArthur,* University of Sheffield, UK

Surface immobilisation of dendrimers presents an exciting opportunity for creating a wide variety of functionalised polymeric architectures suitable for the immobilisation and delivery of biomolecules. In solution, these perfectly branched monodisperse, globular macromolecules have been utilised in gene transfection, drug delivery and catalysis. In this study we investigate a range of graft-to and graft-from approaches for immobilising dendrimers for biotechnology applications. The immobilisation strategies all start with plasma polymerised acrylic acid thin films enabling the polymers to be grafted to a wide variety of substrates. The tethering of polyamidoamine (PAMAM) dendritic macromolecules to the plasma polymers was achieved via 2 routes. In the graft-to regime, water soluble carbodiimide chemistry has been used to covalently immobilise a range of PAMAM dendrons (G1-G6) possessing aniline focal points. The affect of solution pH and ionic strength on the structure of the resultant grafted layer were also investigated. In the graft-from regime plasma polymerised acrylic acid provided a platform for the physisorption of polycationic polyethyleneimine (PEI) to the surface. Michael addition was then utilised to immobilise methyl acrylate to the amine terminated surface, yielding an ester terminated surface (G0.5). Subsequent amidation with ethylenediamine generated a dendritic molecule furnished with amine groups (G1) and the process repeated to produce higher generation dendrons. Characterisation of each stage in the grafting process via XPS, ToF-SIMS and AFM illustrated the complex interactions that occur when immobilising polymers at interfaces. Successful step by step growth of immobilised dendrons with enhanced control was achieved using the graft-from regime. The results showed that the graft-to strategy gave a simple one step process for immobilisation of different generation dendrons at the interface.

3:20pm **BI-WeA5 A Poly(Vinyl Alcohol)-Based Surface Coating for Implantable Electrodes**, ***E.R. Leber***, *B.D. Ratner,* University of Washington

Chronically implanted neural electrodes lose the ability to record or stimulate neural activity with time,@footnote 1@ generally within a few weeks.@footnote 2@ While there are numerous potential causes of this problem, most involve an unfavorable reaction or interaction at the electrode surface.@footnote 2@ Thus, the electrode surface is of paramount importance@footnote 3@ in improving the functionality and longevity of the electrode. Our laboratory has created a platinum (Pt) electrode surface modification platform that will allow for the incorporation of a biologic at the surface, with the goal of improving electrode performance. Thus far, our data demonstrates successful use of plasma deposition of acrylic acid on Pt to generate carboxyl functional

groups. ESCA analysis of these samples consistently shows carboxyl groups to comprise around 17% of the surface carbon content. Soaking samples in H@sub 2@O at room temperature or 37° or under acidic or basic conditions does not significantly decrease carboxyl group content. EDC-activation of these carboxyl groups leads to the successful attachment of poly(vinyl alcohol) (PVA) to the Pt substrate in a concentration and temperature-dependent manner as verified by ESCA and TFAA+ESCA; this PVA layer will function as a hydratable and potentially soft intermediary layer between the Pt and the biologic. The hydroxyl groups of PVA will be activated using CDI chemistry to allow for the covalent attachment of a protein through its lysine residues; we plan to attach fibronectin. Impedance spectroscopy has been performed on bare Pt and Pt with attached PVA and preliminary results show the impedance did not increase significantly with the attachment of the PVA. @FootnoteText@ @footnote 1@ Cui X. J. Biomed. Mater. Res., 56, 261-272 (2001).@footnote 2@ Cui X. Biomat., 24, 777-787 (2003).@footnote 3@ Huber M. J. Biomed. Mater. Res., 41, 278-288 (1998).

3:40pm **BI-WeA6 Influence of pH and Ionic Strength on the Conformation of Grafted Pollallylamine**, ***L.G. Britcher,*** University of South Australia, Australia*; L. Galea, S. Griesser,* University of South Australia*; H.J. Griesser,* University of South Australia, Australia

Polyallylamine (PAA) modified surface are useful for providing a hydrophillic underlayer for subsequent covalent binding of biologically active molecules via the amino groups. However, it is necessary to control the pH and ionic strength of the polyallylamine during the grafting in order to maintain a dense coating. In this study we investigated PAA grafting onto aldehyde plasma polymer (Ald pp) treated silicon wafers under different pH and ionic strength regimes. XPS analysis showed that the nitrogen atomic % on the PAA grafted samples was greatest at pH 9.3 which, is above the pKa of the PAA (pKa 8). Less nitrogen was observed when grafting was done at pH 6 or 7. This difference in grafting density can be explained by the percentage of ionisation of the PAA chains as a function of the pH. At pH 9.3 the ionisation degree is around 30%, so there is little repulsion between the chains, allowing them to form a mushroom conformation. While at pH 6 or 7 the ionisation degree is around 70%, so the PAA forms a flat conformation on the Ald pp due to repulsion between the chains. Changes in the ionic strength did show differences in the amount of nitrogen, but pH appeared to influence the grafting density. Analysis of the high resolution N 1s XPS multiplex showed the presence of primary and protonated amines from the grafted PAA. The ratio of the primary amines to protonated amines on the dried coatings was independent of the grafting pH or ionic strength. However, after a period of time in water, the degree of protonation decreased for all samples, confirming similar results obtained with PAA layers deposited using layer-by-layer techniques.

4:00pm **BI-WeA7 Facile Synthesis of Robust Nanostructured Thin Films Containing Highly Ordered Phospholipid Bilayer Assemblies and Transmembrane Proteins**, ***G. Gupta,*** *P. Atanassov, G.P. Lopez,* University of New Mexico

We have synthesized a new generation of rugged hybrid organic/organic thin films that incorporate highly ordered supramolecular assemblies containing transporter proteins such as bacteriorhodopsin and transmembrane peptides. Such architectures have the potential to enable life-like qualities in new types of manufacturable thin film and membrane materials for a variety of technological applications. Transmission electron microscopy and X-ray diffraction were used to reveal the structure of the hybrid thin films containing 1, 2-dioleyl-sn-glycero-3-phospoethanolamine (DOPE), an unsaturated lipid, and 1, 2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), a saturated lipid in matrices of crosslinked silica obtained by sol-gel processing. While the d-spacing measured for DOPE containing films varied (from 35 Å to 48 Å) depending on the amount of DOPE added to the coating solution (10 wt% to 1 wt%), similar changes were not observed for the films containing the saturated lipid, DMPC (d-spacing ~43 Å). Incorporation of bacteriorhodopsin to the DOPE/silica coating solutions led to the formation of multi-lamellar vesicle-like structures within the thin films. Mild sonication of these solutions containing the purple membrane prior to coating led to the formation thin films with planar multi-lamellar structures that exhibit uniform d-spacing. The study further investigates the effects of incorporation of gramicidin and sonication on the structure of hybrid films and speculates on the eventual application of thin films prepared in this manner.

4:20pm **BI-WeA8 Bridging Interactions beween Silica and Grafted PEO Surfaces**, ***L. Meagher,*** *P. Hamilton-Brown, A. Tarasova,* CSIRO Molecular and Health Technologies, Australia*; H.J. Griesser,* University of South Australia, Australia

Covalently grafted, functionalized polymer layers are of increasing technological interest, e.g. attaching molecules or proteins to the terminal functional group. One of the most widely studied is biotin terminated poly(ethylene oxide), which allows for specific attachment of biotin binding proteins. If densely grafted, these layers have the additional benefit of low non-specific interactions with proteins. One way of characterizing these layers is by direct interaction force measurements. Generally, it is assumed that the interactions between solid surfaces and such polymer layers are repulsive due to confinement of the polymer layer by the probe surface. In this study, we have used a combination of XPS and AFM interaction force measurements to characterize surfaces with covalently grafted PEO layers of different grafting density and functional end group. The surfaces were prepared using cloud point grafting of functionalised PEO molecules onto amine plasma polymers. Where the PEO coupling density was low, long ranged attractive polymer bridging forces were obtained between the PEO coated surfaces and AFM tips modified with silica particles in 0.15 M NaCl solutions. The range and magnitude of the forces were correlated with the grafting density of the PEO molecules, lower densities giving longer ranged, more attractive forces, and the molecular weight of the covalently attached PEO molecules. Long ranged attractive forces were also observed between silica and densely grafted layers with a small proportion of incorporated larger molecular weight chains, in an analogous fashion that those obtained for low grafting density, mono-dispersed layers. The origin of the attractive forces was related to the adsorption of PEO molecules onto the silica surface on approach, with more molecules adsorbing at smaller separation distances. This mechanism was verified by modification of the opposing surface and by specific coupling of NeutrAvidin to the grafted layer.

4:40pm **BI-WeA9 Investigating the Protein Repellent Properties of Highly Crosslinked Oligo Ethylene Glycol-Like Plasma Polymer Films through Surface Force Measurements with Colloidal Probe Atomic Force Microscopy.**, ***B.W. Muir,*** *A. Tarasova, T.R. Gengenbach, L. Meagher, F. Rovere, K. McLean, A. Fairbrother, P.G. Hartley,* CSIRO, Australia

Interaction forces of highly protein resistant polyethylene glycol (PEG)-like plasma polymer films were investigated with colloid probe atomic force microscopy (AFM). The technique of radio frequency glow discharge plasma polymerisation was used to deposit protein resistant films from diethyleneglycol dimethylether (DG) on a heptylamine (HA) plasma polymer (pp) layer. Films were characterised using a combination of techniques including X-ray Photoelectron Spectroscopy, Electrokinetic Streaming Potential Measurements, Secondary Ion Mass Spectroscopy and AFM. The interaction force measurements of a bare silica probe with the plasma polymer films are discussed in relation to observed bovine serum albumin fouling in phosphate buffered saline. A DGpp film was produced with a high ether content which resulted in sub 10 ng/cm@super 2@ levels of protein on these films. We have found that a compressible DGpp with a steric repulsive interaction on the order of only 3 nm provides a non-fouling PEG-like surface. The steric behaviour of the protein resistant and fouling DGpp films was equivalent in high ionic strength solution with differences due to van der Waals and electrostatic attractions detected in low ionic strength solution. We have shown that thin DG films retain their protein repellent properties regardless of the net surface charge and potential. We therefore deduce that surface free energy and potential is not a key determinant in the protein resistance of inert DGpp surfaces nor is the hydrophilicity and roughness of the films. We believe that it is the density of the residual ether functionality and a short range steric repulsive interaction in these films which is the key factor determining their protein resistance and not long range electrostatic or steric interactions. These experiments further highlight the utility of aqueous surface force measurements toward understanding the protein repellent properties of highly crosslinked PEG-like pp films.

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