Biomaterial Interfaces
Room 307 - Session BI-MoA

Non-fouling Surfaces and Biolubrication
Moderator: D.G. Castner, University of Washington

2:00pm **BI-MoA1 Towards the Prevention of Protein Adsorption, and Bacterial and Cell Adhesion by Optimised Surface Modification**, ***P. Kingshott***, *J. Wei,* Risoe National Lab, Denmark*; H. Thissen,* CSIRO Molecular Science, Australia*; N. Gadegaard,* Univ. of Glasgow, UK*; D. Selmeczi,* Risoe National Lab, Denmark*; L. Gram, D. Bagge-Ravn,* Technical Univ. of Denmark, Denmark*; N.B. Larsen,* Risoe National Lab, Denmark*; H.J. Griesser,* University of South Australia, Australia **INVITED**

A non-fouling surface is still elusive since problems such as bacterial colonisation on medical devices and food processing equipment, and failure of implants caused by clotting and foreign body reactions are still existent. Surface modification with PEG or PEO is the most effective way of reducing protein adsorption, and bacterial and cell adhesion (also called bio-adhesion). The best reports show that protein adsorption can be reduced to a fraction of the uncoated surface or even prevented. However, so far reductions in bacterial adhesion by PEG surfaces have only been marginally successful (up to 1 or 2 orders of magnitude). Why is this the case? Can surfaces that prevent protein adsorption also prevent bacterial and cell attachment? Can theoretical predictions of a non-fouling surface ever be put into practice by design of the perfect surface? In order to answer these questions it is necessary to be able to generate stable PEG layers with sufficiently high graft density and uniformity to provide the optimal steric repulsive barrier against bio-adhesion. In addition, to make the claim that a surface is non-fouling depends on one being able to detect protein adsorption below the threshold where no subsequent events can occur (such as bacterial adhesion). In this presentation some of these issues will be discussed. Surface modification based on plasma polymerisation and wet chemical methods are used to provide reactive groups for PEG grafting, confirmed by surface derivatisation reactions and surface analysis including XPS and ToF-SIMS. PEGs are grafted at their lower critical solution temperature (LCST) for maximal surface coverage. In addition, highly sensitive and specific surface mass spectrometry analysis (i.e. ToF-SIMS and surface-MALDI) is shown to be both very useful at detecting ultra-low levels of protein on the best PEG surfaces. Furthermore, the ability of PEG surfaces to prevent protein adsorption is heavily dependent on the size and type of protein.

2:40pm **BI-MoA3 Factors that Determine the Protein Resistance of Oligoether Self-assembled Monolayers - Internal Hydrophilicity, Terminal Hydrophilicity and Lateral Packing Density**, *S. Herrwerth, W. Eck,* ***M. Grunze***, University of Heidelberg, Germany

Protein resistance of oligoether self-assembled monolayers (SAMs) on gold and silver surfaces has been investigated systematically in order to elucidate structural factors that determine whether a SAM will be able to resist protein adsorption. Oligo(ethylene glycol) (OEG), oligo(propylene glycol) and oligo(trimethylene glycol) terminated alkanethiols with different chain length and alkyl termination were synthesized as monolayer constituents. The packing density and chemical composition of the SAMs were examined by XPS spectroscopy; the terminal hydrophilicity was characterized by contact angle measurements. IRRAS spectroscopy gave information about the chain conformation of specific monolayers; the amount of adsorbed protein compared to alkanethiol monolayers was determined by ellipsometry. We found several factors that can suppress the protein resistance of oligoether monolayers. Monolayers with a hydrophobic interior such as those containing oligo(propylene glycol) show no protein resistance. The lateral compression of oligo(ethylene glycol) monolayers on silver generates more highly ordered monolayers and may cause decreased protein resistance, but does not necessarily lead to an all-trans chain conformation of the OEG moieties. Water contact angles higher than 70Â° on gold or 65Â° on silver reduce full protein resistance. We conclude that both internal and terminal hydrophilicity favor the protein resistance of an oligoether monolayer. It is suggested that the penetration of water molecules in the interior of the SAM is a necessary prerequisite for protein resistance. We discuss and summarize the various factors and the balance of forces which are critical for the functionality of "inert" organic films.

3:00pm **BI-MoA4 Use of QCM-D to Analyze Thin Polymer Films at Interfaces**, ***E.F. Irwin***, *J Ho, K.E. Healy,* University of California, Berkeley

An interpenetrating polymer network (IPN) of acrylamide (AAm) and poly(ethylene glycol) (pEG) was designed that can be covalently bound directly onto metal oxide and polymer surfaces via photoinitiated free radical polymerization. A p(AAm-co-EG) with acrylic acid (AA) was also designed to allow further functionalization of the IPN surface with a diamino pEG spacer arm (pEG-NH@sub 2@).@footnote 1@ In this study, we are employing a quartz crystal microbalance with dissipation (QCM-D) (qsense) to monitor the IPN swelling and protein adsorption behavior in phosphate buffered saline (PBS), pH 7.4. QCM-D crystals coated with SiO@sub 2@ and TiO@sub 2@/Ti were modified with IPNs of p(AAm-co-EG), p(AAm-co-EG/AA), and p(AAm-co-EG/AA) + pEG-NH@sub 2@. The Sauerbrey relationship was used to calculate a thickness of 48nm for a dry film of p(AAm-co-EG/AA). QCM-D thickness data can be compared to a dry IPN thickness of 17nm determined previously by spectroscopic ellipsometry.@footnote 1@ A Kelvin-Voight model of viscoelasticity was used to interpret frequency and dissipation data of the hydrated films over the swelling period. Modeling the swelling data of a p(AAm-co-EG/AAc) IPN gave an initial hydrated thickness of 101nm (after 2 minutes) and a final swollen thickness of 150nm. The shear modulus of the film ranged from 285 to 365kPa and the viscosity ranged from 6.7E-3 to 8.6E-3kg/ms according to the model. One limitation of this model is that one single density of the IPN surface is assumed (in this case a density of 1.1g/cm@super 3@) over the entire swelling period. These IPN surfaces minimize the adsorption of the protein fibrinogen that has a role in thrombosis. The QCM-D provides unique and complementary information to other surface analytical techniques (i.e. AFM, XPS) for understanding the behavior of thin polymer films at interfaces. @FootnoteText@ @footnote 1@ Bearinger, JP, et al., J. Biomat. Sci. Polym. Ed., 9 (7) 1998.

3:20pm **BI-MoA5 Non-Fouling Surfaces: Their Use and Study by Matrix-Assisted Laser Desorption / Ionization Mass Spectrometry**, *G.R. Kinsel,* ***J. Zhang***, *R.B. Timmons,* The University of Texas at Arlington

Matrix-Assisted Laser Desorption / Ionization mass spectrometry (MALDI MS) has emerged in recent years as a powerful method for the mass spectrometric analysis of a wide range of biomolecules including proteins, oligonucleotides, polysaccharides, etc. Advantages of this analytical approach include simplicity of sample preparation, high analysis speed and high sensitivity. Recently MALDI-MS has been used in the characterization of non-fouling surfaces and related mechanistic studies in our group. Specifically, non-fouling coatings are applied to MALDI sample targets using a variety of published approaches including PEO chemical modification of polyurethane and pulsed plasma deposition of tetraethylene glycol dimethylether. From a practical standpoint these surfaces are shown to significantly lower the limit of detection (to sub-femtomolar quantities) in a MALDI experiment, presumably by reducing the amount of protein lost to surface-binding interactions. This influence is revealed by the acquisition of MALDI standard curves for a variety of peptides and proteins using methods previously established in our group. Additional studies of the influence of various MALDI parameters, including matrix solvent, pH, and ionic strength and various surface properties, primarily contact angle, have been performed to reveal relationships between, for example, surface hydrophilicity and protein binding, peptide/protein size and protein binding and elution solvent properties and protein binding. These studies offer useful experimental insights into various proposed mechanisms of non-fouling behavior.

3:40pm **BI-MoA6 Comparison of Immunoassay Blocking Strategies on Metal Oxide Substrates**, ***A.N. Scribner***, *C.L. Cole, R.J. Colton, L.J. Whitman,* Naval Research Laboratory

We have developed an alumina filter-based immunosensor that is 10 times faster and ~3 orders of magnitude more sensitive than an analogous microtiter well-based format. The assay is based on a standard sandwich immunoassay but uses magnetic microbeads and magnetic forces to differentiate between specific and nonspecific interactions. The combined use of magnetic force discrimination with PEG-based surface chemistries that minimize nonspecific binding forces result in a demonstrated specificity of >98%. Additionally, a more traditional blocking agent can also be added to compensate for lot-to-lot variability in the surface chemistry of commercially available alumina membranes. However, immunoassays on metal oxide supports not based on electrochemical detection are uncommon, so comparatively little is known about the effectiveness of different blocking agents for such surfaces. We examine agents typically used to block polystyrene plates for their relative effectiveness at blocking

PEGylated and non-PEGylated alumina membranes, including detergents, proteins, hydrophobic, and hydrophilic molecules. The effectiveness of each substance as a blocker is determined quantitatively by measuring the amount of IgG-HRP remaining after incubation on a pre-blocked surface. Our results suggest that traditional reagents such as gelatin or BSA do not have the same ability to block nonspecific binding on PEGylated alumina as on polystyrene, and that casein and charged reagents such as SDS may be more appropriate choices for the blocking of modified metal oxide surfaces. Supported by ONR and the DoD JSTPCBD. ANS and CLC are employees of Nova Research, Inc., Alexandria, VA.

4:00pm **BI-MoA7 Lubricating with Water: Biomimetic Additives**, *M. Müller, S. Lee,* ETH-Zürich, Switzerland*; X. Yan, S.S. Perry,* University of Houston*; **N.D. Spencer,*** ETH-Zürich, Switzerland **INVITED**

Nature often relies on surface-bound, brush-like structures to impart lubricity to natural surfaces (joints, G.I. tract, lungs) in an aqueous environment. These generally consist of polysaccharides, which are frequently charged and coordinate a large amount of water. We have found that another heavily hydrated brush-forming system: poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG), can impart lubricity to inorganic surfaces, such as silicon, glass and steel, in an aqueous environment. A clear dependence on polymer architecture can be observed, which is manifested on both nano- and macro-scales, as determined by AFM and tribometer measurements, respectively.

4:40pm **BI-MoA9 Boundary Lubrication Properties of Bio- and Synthetic Polymers Containing Poly- and Oligosaccharides**, ***S. Lee**, G. Kilcher, N.D. Spencer,* ETH-Zürich, Switzerland

In this study, we have investigated the boundary-lubrication properties of aqueous solutions of natural and synthetic polymers possessing poly- and/or oligosaccarides as an additive to reduce interfacial friction forces. As natural polymers containing poly- and oligosaccarides, we have chosen porcine gastric mucin (PGM) as a standard material responsible for bio-lubrication. Mucins are large and complex glycoproteins composed of a linear polypeptide and polysaccarides side-chains. Due to their aggregation or polymerization, often involving gel formation, mucins are known to form a protective layer between the lumen and the cell surface. Mucins possess a structure involving a combination of hydrophilic and hydrophobic domains with high molecular weight. The boundary lubrication properties of PGM-containing aqueous solutions have been investigated on hydrophobic tribo-pairs, such as self-mated poly(dimethylsiloxane) (PDMS). To more systematically investigate the role of poly- and/or oligosaccarides for water-based lubrication, we have synthesized block copolymers consisting of a polypeptide backbone, e.g. poly(L-lysine), and oligosaccarides with well-defined structure and chemistry. For this model system, we have selected various oxide surfaces as a tribo-pair. Both macroscopic- and molecular-scale sliding contact have been investigated employing pin-on-disk tribometry and atomic force microscopy respectively. The frictional properties of the selected tribosystem have been measured as a function of pH and ionic strength/type of the aqueous lubricant solution. The observed changes of the lubrication properties of both bio- and synthetic polymers as a function of pH and ionic strength are discussed in terms of the corresponding changes of conformation and adsorption behavior.

5:00pm **BI-MoA10 The Role of Polysaccharides in Bacterial Adsorption: A Chemical Perspective**, ***K.T. Queeney**, J.W. Clemens, C. Royce,* Smith College

While it is well known that extracellular polysaccharides influence the adhesion properties of a range of encapsulated bacteria, studies of the adsorption properties of these polysaccharides have been largely limited to investigations of their conformational and/or mechanical properties.@footnote 1@ Xanthan, a model bacterial polysaccharide, has been well studied in the solution phase and therefore provides a useful starting point for understanding, at a molecular level, what influences the adsorption properties of these large and complex molecules. We have used surface infrared spectroscopy to investigate the adsorption of xanthan on a variety of surfaces that exhibit both varying hydrophobicity and a range of chemical terminations. While a previous study of polysaccharide adsorption on oxide surfaces focused only on hydrogen-bonding behavior as evidenced by the OH-stretching region,@footnote 2@ we find that the carbonyl stretching region shows marked changes in the local chemical environment of these moieties, suggesting that they interact strongly with the surface. Furthermore, xanthan's similar affinity for hydrophobic and hydrophilic surfaces provides evidence that polysaccharide/surface interactions must include non-hydrogen bonding effects. @FootnoteText@ @footnote 1@See for example T. A. Camesano and K. J. Wilkinson, Biomacromolecules 2, 2001, 1184-1191.@footnote 2@B. A. Jucker, H. Harms, S. J. Hug and A. J. B. Zehnder, Coll. Surf. B 9, 1997, 331-343.

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