Tuesday Morning, November 5, 2002

Biomaterials
Room: C-201 - Session BI+SS-TuM

Platforms for Non-fouling and Patterned Surfaces
Moderator: D.G. Castner, University of Washington

8:20am **BI+SS-TuM1 Molecular Assembly and Micro-/Nanopatterning Techniques on Oxide-based Surfaces for Controlling Non-specific and Specific Interactions**, ***M. Textor***, ETH Zürich, Switzerland **INVITED**

The assembly of multifunctional molecules at surfaces has become an important technique to design interfaces for biosensor applications and model surfaces for cell-biological studies. While alkanethiol self-assembled monolayers on gold surfaces are routinely used today, corresponding systems for oxide-based surfaces had first to be developed. The objective is to produce interfaces via cost-effective, robust techniques that allow the elimination of non-specific protein adsorption and the addition of ligands in controlled density to sense the biological environment. Poly(ethylene glycol)-grafted polyionic copolymers assemble spontaneously from aqueous solutions at charged interfaces resulting in well-defined, stable monolayers. The degree of interactiveness of the resulting surface with the bioenvironment can be controlled quantitatively through the design of the polymer architecture. If the polymer is functionalized with bioligands such as biotin, biosensor interfaces with quantitative control over ligand density can be efficiently produced. Chemical patterning of surfaces into adhesive and non-adhesive areas has become an important tool to organize in a controlled manner biological entities such as cells and biomolecules at interfaces. A novel surface modification technique is presented that uses a lithographically pre-patterned, inorganic substrate, which is subsequently converted into a pattern of biological contrast via area-selective molecular assembly processes. Biologically meaningful patterns of protein-adhesive and non-adhesive areas in a size range from micrometers to as small as 50 nm could be produced. Fluorescence microscopy, XPS, ToF-SIMS and AFM were used to control ex situ each surface modification step, while the kinetics of the surface reactions including the interaction with biological media were monitored in situ with an optical sensor (OWLS) and the quartz crystal microbalance (QCM-D) technique.

9:00am **BI+SS-TuM3 Orientation in Oligo(ethylene glycol) Functionalised Self Assembled Monolayers Adsorbed on Gold Depending on the Oligomer Length**, ***M. Zwahlen***, University of St Andrews, UK, *S. Herrwerth, W. Eck, M. Grunze*, University of Heidelberg, Germany, *G. Haehner*, University of St Andrews, UK

Oligo(ethylene glycol) (OEG) functionalised self-assembled monolayers (SAMs) have attracted considerable attention due to their protein repelling properties. The underlying mechanism is of high scientific relevance for future applications but has not yet been completely resolved. 'Steric repulsion', which describes the resistance to non-specific protein adsorption in the case of the polymer PEG does not explain the mechanism in densely packed SAM structures sufficiently. It has been suggested that one cru cial parameter for the interaction of OEG-modified surfaces with their environment is the orientation in the organic adlayer. This has motivated a number of structural investigations on OEG-SAMs. As a contribution to the ongoing discussion, we present a s tudy of the orientation in OEG-functionalised SAMs adsorbed on gold. It was measured as a function of the number of EG units in the molecule using soft X-ray absorption spectroscopy (NEXAFS). The results and their implications on the vacuum structure of the OEG-films will be discussed. The data will be compared to those obtained with complementary experimental techniques under similar as well as under different environmental conditions.

9:20am **BI+SS-TuM4 DOPA: A Novel Anchor for PEGylation of Biomaterials**, ***J.L. Dalsin***, *P.B. Messersmith*, Northwestern University

It is widely recognized that modification of biomaterial surfaces with biocompatible polymers is a useful strategy for controlling protein adsorption and cell interactions with materials. The physical or chemical immobilization of poly(ethylene glycol) (PEG) has routinely been used to limit biological fouling of surfaces. Many of the current PEGylation methods, however, are limited by high costs and complexity of synthesis. Most importantly, each of the present strategies vary widely depending on the characteristics of the substrate, and are typically different for metal, metal oxide, and polymer substrates. We are developing a new biomimetic strategy for anchoring PEG to biomaterial surfaces. Our approach is to utilize linear and branched PEGs end-functionalized with DOPA. DOPA is found in significant quantities in the adhesive proteins secreted by marine mussels for attachment to underwater surfaces, and recent evidence suggests that the presence of DOPA promotes strong and durable adhesion of these proteins to metal, metal oxide, and polymer surfaces. Recently, it has been shown that DOPA-containing peptides adhere strongly to gold surfaces, mediated by metal-oxygen bonds formed between the catechol group of DOPA and Au atoms at the metal surface. In this study, we report our findings on the use of DOPA as an anchor for PEGylation of biomaterial surfaces. A variety surfaces were modified by adsorption of DOPA-modified-PEGs from solution, and the presence of PEG on the surface was confirmed with a number of surface characterization techniques, including XPS and TOF-SIMS. The behavior of cells on modified and unmodified gold surfaces was evaluated in an attempt to optimize the conditions for DOPA-mediated PEGylation of metals, metal oxides, and polymers.

9:40am **BI+SS-TuM5 Characterization of Non-Fouling Surfaces by Matrix-Assisted Laser Desorption / Ionization Mass Spectrometry**, ***G.R. Kinsel***, *J. Zhang, R.B. Timmons, M. Li*, University of Texas at Arlington

Matrix-Assisted Laser Desorption / Ionization (MALDI) mass spectrometry 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. An attractive feature of this analytical approach is the relative simplicity of the sample preparation. In principle, all that is required is that the analyte of interest be mixed with an appropriate "matrix" (typically a small, functionalized aromatic compound) and the two compounds allowed to co-crystallize on some type of support. In recent work, however, we have shown that the nature of the support can have a marked effect on the magnitude of the analyte MALDI ion signal. Specifically, we have shown that as the binding affinity of the support for the analyte increases, the analyte MALDI ion signal decreases. This relationship has been used to develop a quantitative method for the determination of the protein binding affinity of various materials based on a MALDI standard additions approach. In the present studies the MALDI method has been used to quantitate the protein binding affinity of a number of "non-fouling" surfaces. These surfaces include plasma polymerized PEO, plasma polymerized CH3OH, PEO-PU block copolymers, and PEO grafted surfaces. The "non-fouling" properties of these surfaces are compared with the protein binding affinity of other conventional polymers including PTFE, LDPE, etc. In addition, the binding properties of the various surfaces are examined with relation to a variety of peptides and modest sized proteins.

10:00am **BI+SS-TuM6 Polymerized Planar Biomembrane Assemblies**, ***S. Saavedra***, University of Arizona

The utility of planar supported lipid bilayers (PSLBs) as protein-resistant coatings in molecular device technologies is hampered by the chemical and mechanical instability of these structures relative to (for example) alkylsiloxane self-assembled monolayers. We have been investigating cross-linking polymerization of diene-functionalized lipids as a strategy to enhance the inherent instability of PSLBs. The membranes are self-assembled by vesicle fusion, then polymerized in situ by a redox-initiated chemistry. In contrast to diacetylene-based materials, these new diene-based materials contain relatively few defects. They are stable to conditions that would destroy a fluid membrane (e.g. exposure to air, surfactants, solvents), yet retain the characteristic protein resistance of a fluid PSLB. Thus these structures appear to possess both the stability and inertness required for implementation of PSLBs in many technological applications. This talk will focus on preparation, characterization, and protein functionalization of diene-based PSLBs.

10:20am **BI+SS-TuM7 Protein Binding at Biomembrane Interfaces**, ***P.S. Cremer***, Texas A&M University **INVITED**

We have used a combination of lithographic patterning techniques and microfluidics to spatially address fluid phospholipid bilayers at the liquid/solid interface. These systems are capable of multivalent ligand-receptor attachment chemistry. Moreover, on-chip designs allow for high throughput temperature, concentration, pH, and ionic strength measurements in an environment which closely mimics a cell membrane interface.

11:00am **BI+SS-TuM9 Surface Characterisation of Supported Lipid Layers**, ***S.L. McArthur***, *M.W. Halter, V. Vogel, D.G. Castner*, University of Washington

The boundaries of biological cells and organelles are defined by complex and dynamic membranes constructed from an array of lipids, proteins and carbohydrates. These interfaces have a range of specific functions and properties, one of which is their ability to prevent non-specific protein adsorption, making membrane mimics an attractive option for a variety of in vivo and in vitro biomedical implant and diagnostic applications. The

development and characterization of complex biomimetic surfaces presents a challenge in terms of their initial formation, long-term stability and integrity in a variety of environments and the maintenance of bilayer fluidity. In this study we detail the development and chemical characterization of supported lipid monolayers. The structure was formed by coupling HEMA to a glass support and subsequently activating it with CDI to couple the headgroups of the lipid, dimyristoyl ethanolamine (DMPE). The success of the immobilization procedure was investigated by XPS and ToF-SIMS. A number of different lipid transfer regimes were explored. Results illustrated that the samples produced using Langmuir-Blodgett transfer at high pressure (20 mN/m) had the largest fraction of the transferred lipids remaining at the surface after 5 minutes sonication in ethanol. Fluorescence microscopy of the lipid layers showed that the presence of this limited number of anchored lipids acted to stabilize the monolayer and maintain its integrity without having a detrimental effect on layer fluidity.

11:20am **BI+SS-TuM10 Purification of Mobile, Membrane-tethered Proteins in Micropatterned Supported Lipid Bilayers**, ***L. Kam***, *T.D. Perez*, *W.J. Nelson*, *S.G. Boxer*, Stanford University

Supported lipid bilayers are a unique system for studying fluidic membranes in a controllable in vitro format. A variety of methods for tethering proteins to supported bilayers provide a powerful reductionist model of cell-cell recognition and activation. However, contemporary methods for preparing membrane-tethered protein systems typically incur an immobile fraction; this population complicates and, at worst, subverts interpretation of experimental results. Here, we present a method for separating a population of mobile, GPI-tethered protein from an immobile fraction in a supported lipid bilayer. A GPI-modified protein based on the cell-cell adhesion protein E-cadherin was introduced into Egg PC vesicles by detergent dialysis. On a glass substrate, two adjacent and connected regions of supported lipid bilayer were created using a converging flow configuration. One region contained both mobile and immobile populations of GPI/cadherin while the other contained Egg PC alone. An electric field applied tangentially to the surface induced migration of the mobile, but not immobile, protein into the region of Egg PC, generating a purified population of these proteins which may then be isolated for analysis or further experimentation free from the immobile fraction. Importantly, this method is independent of the specific factors influencing protein mobility and thus generally applicable.

11:40am **BI+SS-TuM11 Spatial Control of Cell Attachment Using Micropatterned Plasma Polymers**, ***S.A. Mitchell***, *N. Emmison*, The Robert Gordon University, Scotland, UK, *A.G. Shard*, Sheffield University, England, UK

In recent years, there has been increased interest in the spatial control and regulation of cellular attachment and growth. Several techniques have been developed to produce surfaces with a well-defined chemical heterogeneity that are suitable for the rapid adhesion, spreading and proliferation of cells. Spatial control and sub-cellular pattern resolution has been successfully demonstrated by techniques such as micro-contact printing of self-assembled monolayers.[1] However, the labour intensive, time consuming preparation and ready oxidation of these surfaces limit the utility of these devices. Additionally, they are only applicable to substrates that are rarely used in biomedical devices. We have employed plasma polymerisation as an alternative method for the chemical patterning of surfaces, although the chemical composition of these surfaces is more difficult to control, this one-step procedure is rapid and cost effective.[2] The resulting surfaces have both a chemical functionality and a pattern resolution comparable to alternative techniques.[3] They may be applied to virtually any substrate, including relatively rough surfaces such as tissue culture polystyrene, greatly increasing their applicability. We describe the patterned deposition of plasma polymers onto a variety of substrates and outline some of the advantages and limitations of the technique. Physicochemical characterisation of the plasma polymers is performed with XPS, AFM and contact angle analysis. The culture of mammalian cells on patterned substrates demonstrates their ability to spatially regulate cell attachment and spreading.

[1]M Mrksich and G M Whitesides, Tibtech, 13, 228-235(1995)

[2]N A Bullett, R D Short, T OLeary, A J Beck, C W I Douglas, M Cambray-Deakin, I W Fletcher, A Roberts, C Blomfield, Surf. Interface Anal., 31, 1074-1076(2001)

[3]L Dai, H J Griesser, A W H Mau, J. Phys. Chem. B, 101, 9548-9554(1997).

Authors Index

Bold page numbers indicate the presenter