Biomaterial Interfaces
Room 210D - Session BI-MoM

In-Situ Spectroscopy of Biomolecules at Interface

Moderator: M. Grunze, University of Heidelberg, Germany

8:20am **BI-MoM1 High-Resolution Structural and Dynamic Characterization of Proteins on Biomaterial Surfaces**, ***P.S. Stayton***, *G.P. Drobny,* University of Washington **INVITED**

The development of materials with bioactive interfaces is a major focus of the biomaterials and tissue engineering communities. There is also considerable interest in the immobilization of active peptides and proteins in separations, diagnostics, proteomics, and cell culture technologies. In order to design appropriate biomaterial modification strategies where activity is retained, it is desirable to elucidate how protein structure, dynamics and orientation are related to the biomaterial surface properties and immobilization chemistries. Solid-state NMR techniques provide an opportunity to determine these molecular structure and dynamics properties of proteins and peptides on many different types of biomaterial surfaces. In particular, the high-resolution backbone conformation of proteins can be determined, the binding "footprint" - or which amino acid side-chains actually contact the surface can be determined, the role of water at the protein-material interface can be investigated, the dynamics of specific protein side-chains can be determined, and the orientation of the protein on the crystal surface can be determined. Homonuclear and heteronuclear dipolar recoupling solid-state NMR techniques, combined with dynamic studies, have been applied to determine the structure, dynamics and orientation of proteins and peptides immobilized on polymeric biomaterial surfaces, surface-modified nanoparticles, and on inorganic crystals such as hydroxyapatite.

9:00am **BI-MoM3 Protein-Surface Interactions Studied with Internal Reflection Ellipsometry**, ***H. Arwin***, *M. Poksinski,* Linköping University, Sweden

Spectroscopic ellipsometry (SE) used in internal reflection mode exhibits very large sensitivity for in situ protein adsorption on thin metal layers if used at surface plasmon (SP) resonance conditions@footnote 1@. Compared to external SE, the protein layer induced changes in the ellipsometric parameter @DELTA@ are several orders of magnitude larger. Using this high sensitivity it becomes possible to extract more details than only protein surface mass density (or film thickness) from SE data. In situ determination of the microstructure of adsorbed protein layers, e. g. in terms of mass distribution perpendicular to a surface, is within reach. Another implication is an increased sensitivity in biosensor applications. The enhanced sensitivity is here verified experimentally with adsorption studies of human serum albumin on gold and the possibility to model protein layer microstructure from SE data is demonstrated. Access to such detailed information is of relevance to understand conformation, surface interaction, dynamics and function of proteins at interfaces. The increased sensitivity is discussed in a thin film approximation of the complex reflectance ratio. It is found that the @DELTA@-sensitivity is inversely proportional to the difference between the damping @GAMMA@ of an SP if the metal is semi-infinite and the change in damping due to that the metal film is thin. The SE sensitivity is thus in principle unlimited as the metal-film induced change in damping can be selected with the film thickness and made to match @GAMMA@. However, the sensitivity becomes finite due to non-idealities of the sample, beam divergence, finite bandwidth of the light, etc. @FootnoteText@ 1. H Arwin, M Poksinski and K Johansen, Total internal reflection ellipsometry: principles and applications, Appl Opt, in press; M Poksinski and H Arwin, Protein monolayers monitored by internal reflection ellipsometry, Thin Solid Films 455-456 (2004) 716-721.

9:20am **BI-MoM4 Angle-resolved Imaging Surface Plasmon Resonance**, *D.A. Armitage,* The University of Nottingham, UK; ***P.M. Williams***, The University of Nottingham, UK, U.K.

Surface plasmon resonance (SPR) has evolved in recent years into a commercially recognized technique for analyzing surface interactions with a film thickness resolution on the sub-nanometer scale. SPR imaging can also be employed to obtain data on the spatial distribution of molecules at surfaces. However, the instrumentation currently used in SPR imaging experiments has reduced capabilities for precise SPR angle and hence thickness and refractive index measurements compared to conventional non-imaging systems. By combining a non-imaging SPR system with a micro-positioning stage we demonstrate that a 2-D image of SPR response can be produced whilst retaining high precision angle sensitivity.

9:40am **BI-MoM5 Feasibility Study of a Waveguide Excitation Fluorescence Microscope for Micro and Nanoscale Characterization of Bio-Interfaces**, ***H.M. Grandin***, *B. Städler, J. Vörös, M. Textor,* Swiss Federal Institute of Technology (ETH), Switzerland

The ability to investigate the interactions that occur between a biological system and a surface, be it a native biological surface or a synthetic surface, is of critical importance to our fundamental understanding of biomaterials and their many applications in biosensors, medical implants, and tissue engineering. Our development of a Waveguide Excitation Fluorescence Microscope (WExFM) satisfies this need uniquely by providing a means for the quantitative study of bio-interfacial interactions in-situ, e.g.: protein adsorption and cell adhesion, with both temporal and spatial resolution. Although other techniques are capable of either quantitative studies, e.g.: optical waveguide lightmode spectroscopy, or of spatially resolved imaging at the interface, e.g.: total internal reflection fluorescence microscopy, the WExFM is the only technique currently available which can provide both simultaneously. Further advantages include high target sensitivity for fluorescence detection (femtoMolar range) and high surface specificity (ca. 100 nm perpendicular to the waveguide), as well as, the capability to perform multicolour imaging, large area analysis with submicron resolution, and 'built-in' calibration of fluorescent light gain. Preliminary results from streptavidin-biotin binding studies have been obtained with sub-picoMolar sensitivity, thus, demonstrating the feasibility of this technique. In this presentation the principles and experimental set-up of the WExFM will be introduced, potential applications for in-situ, real-time quantitative monitoring of protein- and cell-surface interactions will be discussed and finally, first results demonstrating the feasibility of the WExFM will be presented.

10:00am **BI-MoM6 In situ Sum Frequency Generation Characterization of Peptide Monolayers on Hydrophobic and Charged Surfaces**, ***N.T. Samuel***, University of Washington; *K. McCrea,* The Polymer Technology Group; *L.J. Gamble,* University of Washington; *R.S. Ward,* The Polymer Technology Group; *P.S. Stayton,* University of Washington; *G.A. Somorjai,* University of California at Berkeley; *D.G. Castner,* University of Washington

Immobilization of bioactive peptides is an active research area for diagnostics, cell culture and biomedical implants. Previous studies have shown well-defined sequences of lysine (K) and leucine (L) containing peptides spontaneously adsorb onto hydrophobic substrates with either @alpha@-helix or @beta@-sheet secondary structures. In this study the adsorption of these peptides onto hydrophobic and charged surfaces has been characterized in situ with IR-Visible Sum Frequency Generation (SFG) spectroscopy. The SFG spectra in the CH, NH and OH stretch regions show the adsorption of the LK peptides onto these substrates is mediated by interactions through their leucine (hydrophobic surfaces) and lysine (charged surfaces) residues. These hydrophobic and electrostatic interactions are accompanied by ordering of the functional groups involved in the interaction. Ordering of water molecules at these interfaces is also observed. SFG spectra in the amide I region were used to examine the secondary structure of the LK peptides. For the @alpha@-helix LK peptide the @alpha@-helix secondary structure is maintained upon dehydration of the sample, even though significant changes in the side chain ordering was observed. Polarization-dependent Near-edge X-ray Absorption Fine Structure (NEXAFS) experiments were also done on the adsorbed peptides. The results demonstrate that the N1s->@pi@*@sub CONH@ feature in the Nitrogen K-edge is sensitive to the secondary structure of the adsorbed peptide. NEXAFS experiments also confirm the highly ordered nature of the adsorbed peptides.

10:20am **BI-MoM7 Measurement of Conformational Changes of Surface Bound Biomolecules: a Novel Strategy for Analytical Biosensing**, ***D.A. Russell***, *L.M. May,* University of East Anglia, UK

A large number of biomolecules change conformation upon interaction with specific substrates. Whilst spectroscopic techniques (such as CD, NMR and IR) provide sensitive measurement of secondary structure in solution, they are not amenable for the development of surface bound sensing technologies based on analyte induced conformational changes. Surface plasmon resonance (SPR) is a surface sensitive technique capable of measuring changes in refractive index (RI) that occur in proximity to the sensor interface. By depositing biomolecules onto the gold-coated sensor surface of an SPR instrument it is possible to measure changes of secondary structural conformation as a function of substrate concentration. A number of biomolecules including, polypeptides, proteins

and enzymes, have been formulated as SAMs on SPR sensor surfaces and varying concentrations of substrates or mild denaturants have been passed over the monolayer surface to elicit secondary structural changes. For example, a dramatic increase in the SPR signal (m°) was observed when polylysine was induced into the alpha-helical conformation with ethanol. Similarly, the SPR signal was related to other secondary structures (including beta-sheet and random configurations) of both polypeptides and the protein Concanavalin A. The intensity of the SPR signal being related to the RI of the secondary structural configuration of the biomolecule. Development of this sensing strategy has focused on the self-assembly of urease in order to measure the conformational change of this enzyme as a function of heavy metals. On the sensor surface, the SPR signal from the urease monolayer linearly increased in intensity as a function of cadmium concentration in the range 0 - 10 mg/L. These data show that conformational changes of surface bound biomolecules can be measured and used analytically.

10:40am **BI-MoM8 Study of Metal Film-Tethered (Bio)Molecules in Aqueous Solution by Enhanced ATR-IR Spectroscopy**, ***D.P. Land***, *J.S. Toofan, C.M. Gerth,* University of California, Davis

Data are presented illustrating the application of overlayer-enhanced attenuated transmission-Fourier transform infrared spectroscopy (E-ATR-FTIR). The presence of a thin metal film at in internal reflection interface accomplishes several important feats. It enhances and concentrates the electric field in the near-surface region. It introduces a wide range of chemical possibilities for surface modifications by which analytes can likewise be concentrated near the interface. And it minimizes interactions with the bulk solvent, facilitating the use of IR spectroscopy in aqueous (and other) solutions. The combination of effects facilitates the study of numerous solution systems, and most importantly, perhaps, aqueous solutions. The experiment has been modeled to reveal details of the dependence upon prism material (ZnSe and Ge), thin film thickness and composition (metals and oxides), as well as angle of incidence and adsorbate properties. Experimentally, several key experiments have been performed. Data will be shown which includes the study of reactions of thiols with the gold films, in aqueous and other solutions. Additionally, spectra for tethered proteins, antibodies, and protein-antibody reactions have already been acquired and will be presented. Detection limits in the tens of femtomoles for proteins and antibodies has been achieved. Experiments currently underway include reactions of enzymes with tethered inhibitors and of tethered enzymes with substrates and co-factors.

11:00am **BI-MoM9 Investigation of Fibrinogen Displacement from Oxide Surfaces**, ***P.S. Cremer,*** Texas A&M University **INVITED**

This talk will discuss the adsorption and displacement of fibrinogen at the silica/aqueous interface. It has been known since Leo Vroman's original studies in 1969 that fibrinogen is one of the first proteins to adsorb from human plasma on oxide surfaces, but it is ultimately displaced by other smaller and less abundant species in solution. We have employed a combination of vibrational sum frequency spectroscopy (VSFS), atomic force microscopy, immunoassays, and kinetic studies to unravel the molecular level details of the mechanism for this process. The results reveal that lysine and arginine residues on the protein's alpha-C domains interact with the surface via weak electrostatic binding. The rest of the protein can only make stronger hydrogen bonding and hydrophobic contacts once these domains have been displaced. In particular, the VSFS data give direct evidence for alignment of arginine and lysine residues with the surface in the protein's most displaceable configuration.

11:40am **BI-MoM11 Probing the Conformation of Hydrated Molecular Adsorbates on Solid Interfaces Using Long Period X-ray Standing Wave Fluorescence**, ***C.A. Crot***, *C. Wu, M. Schlossman,* University of Illinois at Chicago*; T.P. Trainor,* University of Alaska*; P.J. Eng,* University of Chicago*; L. Hanley,* University of Illinois at Chicago

Understanding the process of protein and biomolecular adsorption onto solid surfaces is of great importance in a wide variety of applications including biomaterials, tissue engineering, biosensors, immunoassays, and protein arrays. However, direct investigation of adsorption processes and the hydrated conformation of a molecular adsorbate is difficult since the majority of surface analysis techniques require ultra-high vacuum conditions. In this work long period x-ray standing wave fluorescence spectroscopy (XSW) is being developed as a spatial probe of molecular adsorption at the liquid-solid interface using a model surface-adsorbate system. A 25 nm thick polystyrene layer is spin coated on a thick silicon wafer, then the top of this layer is amine-functionalized via hyperthermal allyl amine ion deposition. X-ray photoelectron spectroscopy and atomic force microscopy are used to monitor the chemistry and morphology of this amine-polystyrene model surface. A thirteen residue peptide is covalently bound to a poly(ethyleneglycol) chain that is terminated with a bromine labeled amino acid and used as the model adsorbate. This Br-PEG-peptide construct is adsorbed onto the amine surface and its hydrated conformation is examined by XSW and x-ray reflectivity. Measurements of the bromine fluorescent yield as a function of incident angle provides information on the distance of the bromine layer from the silicon surface with an accuracy of several angstroms. Preliminary data analysis of the Br-PEG-peptide conformation indicates the peptide end is adsorbing directly onto the amine surface while the bromine atom on the Br-PEG end is extended ~13@+-@3 nm from the amine surface into the aqueous layer. Adsorbate configuration is probed as a function of adsorption time, amine film characteristics, and other experimental parameters. The general applicability of the XSW technique to probe the conformation of labeled adsorbates at the aqueous-solid interface is discussed.

Biomaterial Interfaces
Room 210D - Session BI-MoA

Protein-Surface Interactions
Moderator: P. Cremer, Texas A&M University

2:00pm **BI-MoA1 Thermodynamic and Kinetic Control of Protein Adsorption on Surfaces with Grafted Polymers**, ***I. Szleifer***, Purdue University **INVITED**

Grafted polymer layers modify the effective interactions between proteins and surfaces resulting in a change of the ability of the proteins to adsorb on the surface. Grafted polymers have a strong effect on both the equilibrium amount of proteins` adsorbed as well as in the kinetics of adsorption. In this talk we will review some of our understanding of the molecular parameters that determine the ability of polymer layers to reduce protein adsorption. We will present a theoretical approach that enables the study of both the kinetics and thermodynamics of adsorption. The predictions of the theory are in excellent quantitative agreement with experimental observations for the adsorption of proteins on surfaces with grafted (short and long) polyethylene oxide. We will show the role of polymer chain length and surface coverage on both the equilibrium adsorption isotherms and the kinetics of protein adsorption. For example, we find that for fixed polymer surface coverage there is a polymer molecular weight above which the equilibrium adsorption becomes independent of polymer chain length. However, the kinetics of protein adsorption depends very strongly on polymer molecular weight under all conditions. The time dependent adsorption is a very complex process due to the changes in the effective surface-protein interactions as the adsorption process progresses. Namely, the changes in the structure of the polymer layer as the proteins adsorb result in large changes in the kinetic process. We will show under what conditions the predicted equilibrium amount of protein adsorbed is finite, however, the time scale for adsorption is so slow that the layer completely prevents protein adsorption for practical purposes. Finally, we will show how chemical modifications of the polymer layer can be used to manipulate the amount, structure and time scale for adsorption and desorption of the proteins from the modified surface.

2:40pm **BI-MoA3 Approach Towards Protein Adsorption, Desorption and Exchange**, ***M. Halter***, ETH Zurich, Switzerland; *G. Szöllösi, I. Derényi,* Eötvös University, Hungary; *J. Vörös,* ETH Zurich, Switzerland

When an artificial object is introduced into a biological environment, its surface is covered almost instantly with a protein layer. Being such a crucial issue for any biological application, the processes involved in protein adsorption, desorption and exchange are still not fully understood. Many controversial theories about the reversibility or irreversibility of protein adsorption, whether an adsorbed layer is static or forming a dynamic equilibrium and other puzzles and paradoxes exist. We present a realistic model for protein adsorption that can adequately describe the observed experimental data, such as irreversibility, history dependence, or the Vroman effect. A novel instrument, the Single Channel Grating Coupler, was used to provide new insight into protein behavior at interfaces. It is a planar waveguide technique that uses the evanescent field generated by an incoupled laser beam. Fluorescently labeled proteins within this field are excited and emit a fluorescent signal. The major advantages of this instrument are its high sensitivity (lower detection limit < 10 fmol/cm@super 2@) and the possibility to measure protein exchange by varying the labeled to unlabeled protein ratio in a solution. In situ measurements of interfacial exchange reactions provide sufficient data to develop a sophisticated protein adsorption model. It assumes that each protein molecule has several different conformations in the adsorbed state with different footprint sizes and binding energies, separated by energy barriers. Numerical simulations of large numbers of proteins - supplemented by analytical calculations - allow us to reproduce the experimental data and identify the conformations of proteins. Such a model will hopefully lead to a better understanding of protein behavior at interfaces. Beyond this, knowledge of the processes involved will help to tune the important parameters to build up and control adsorbed protein layers as desired for specific applications.

3:00pm **BI-MoA4 Mixology of Protein Solutions and the Vroman Effect**, ***A. Krishnan***, *C.A. Siedlecki, E.A. Vogler,* Pennsylvania State University

Mixing rules stipulating both concentration and distribution of proteins adsorbed to the liquid-vapor(LV) interphase from multi-component aqueous solutions are derived from a relatively straightforward protein-adsorption model. Accordingly, proteins compete for space within an interphase separating bulk-vapor and bulk-solution phases on a weight, not molar, concentration basis. This results in an equilibrium weight-fraction distribution within the interphase that is identical to bulk solution. However, the absolute interphase concentration of any particular protein adsorbing from an m-component solution is 1/mth that adsorbed from a pure, single-component solution of that protein.Applied to adsorption from complex biological fluids such as blood plasma and serum, mixing rules suggest that there is no energetic reason to expect selective adsorption of any particular protein from the mixture. Thus, dilute members of the plasma proteome are overwhelmed at the hydrophobic LV surface by the thirty classical plasma proteins occupying the first-five decades of physiological concentration. Mixing rules rationalize the experimental observations that(i)concentration-dependent liquid-vapor interfacial tension of blood plasma and serum cannot be confidently resolved,even though serum is substantially depleted of coagulable proteins(e.g. fibrinogen) and(ii) of plasma is startlingly similar to that of purified protein constituents. Adsorption-kinetics studies of human albumin (66.3 kDa) and IgM (1000 kDa) binary mixtures revealed that relatively sluggish IgM molecules displace faster-moving albumin molecules adsorbing to the LV surface. This Vroman-effect-like process leads to an equilibrium reflecting the linear combination of w/v concentrations at the surface predicted by theory. Thus, the Vroman effect is interpreted as a natural outcome of protein reorganization to achieve an equilibrium interphase composition dictated by a firm set of mixing rules.

3:20pm **BI-MoA5 Molecular Dynamics Simulation of the @gamma@ Chain Fragment of Fibrinogen on Functionalized SAM Surfaces**, *M. Agashe, S.J. Stuart,* ***R.A. Latour***, Clemson University

Protein adsorption to biomaterials surfaces is a primary governing factor of biocompatibility. While much has been learned, the molecular mechanisms involved in adsorption behavior are not understood. Empirical force field based molecular simulation methods provide an excellent approach to theoretically investigate the molecular behavior of proteins as they adsorb to surfaces. In this research, molecular dynamics simulations were conducted to investigate the adsorption behavior of a 30kDa C-terminus fragment of the @gamma@ chain of fibrinogen (Fg) as a function of surface chemistry. Simulations were conducted using the GROMACS program and force field. The surfaces were modeled to represent Au-alkanethiol self-assembled monolayers (SAMs) with 5 surface functionalities: CH3, OH, NH2, COOH, and PEG. The model system consisted of Fg in saline (explicit water with Na+ and Cl- ions) positioned over a SAM surface. Systems were contained within a 105Å x 107Å base x 80Å high simulation cell with periodic boundary conditions. 5ns simulations were performed and the effects of adsorption on Fg were analyzed. The results predict that only minor changes in Fg conformation occur during this time frame, however, Fg was observed to undergo large surface-dependent rotational and translational motions, suggesting faster kinetics for reorientation than unfolding. Although contingent on the accuracy of the GROMACS force field, which has not yet been validated for this application, these results have profound implications for surface design because they suggest that surface chemistry should be an effective means to control the orientation of adsorbed proteins.

3:40pm **BI-MoA6 The Effects of Adsorbed Proteins on the Performance of Biomedical and Biotechnological Devices**, ***T.A. Horbett***, University of Washington **INVITED**

Proteins are abundant in biological fluids, readily adsorb to most devices that contact such fluids, and often adversely affect the performance of the device. For example, adsorbed proteins are believed to lower the biocompatibility of implants in the body, non-specific adsorption of antibodies interferes in many solid phase immunoassays, and protein adsorption to the walls of microfluidic devices can cause analyte loss and/or reductions in separation efficiency. In this presentation, I will first give a series of examples illustrating the role of adsorbed proteins in device related problems. A brief review of the major mechanisms of protein adsorption affecting device performance will be given, namely variations in affinity of proteins for surfaces and differences in the ability of adsorbed cell adhesion proteins to support cell adhesion ("molecular potency"). Alterations in molecular potency have often been ascribed to denaturation of adsorbed proteins. However, studies from Norde's lab have shown that adsorbed proteins that exhibit no thermal unfolding enthalpy, and thus appear to be completely denatured, actually retain considerable structure, so these important findings will be presented. In many situations, reducing cell adhesion to a surface is desirable, but ways to accomplish this are not always clear. Towards that end, studies in my lab of platelet and monocyte

adhesion to adsorbed fibrinogen have suggested biomaterial design criteria to reduce cell adhesion that are based on the concepts of reducing molecular potency or affinity. Adsorbed proteins sometimes have low molecular potency, but the properties of the surface which cause it are unclear, so it is currently difficult to apply this criteria in designing new surfaces. In contrast, the other design criteria, a need for ultralow fibrinogen affinity surfaces, has been used to make better surfaces, as will be illustrated with glow discharge deposited tetraglyme, and with polyurethanes with added PEO.

4:20pm **BI-MoA8 The Surface Analysis and Quantification of the Electro-sprayed Fibronectin on Biocompatible Materials***, **M.J. Wang**,* Queen Mary University of London, UK, United Kingdom*; D.A. Lee, M.D. Paine, D.L. Bader, J.P.W. Stark,* Queen Mary University of London, UK

Electrospray is employed as a novel technique to incorporate biomolecules on substrates because it provides the possibility of soft landing the biomolecules. The interactions between biomolecules and substrates are, first of all, examined by surface analysis techniques such as atomic force microscopy (AFM) and Fourier Transform Infrared (FTIR) to identify the efficiency of electrospray and examine the morphology of the biomolecules. Moreover, the immunofluorescent methods provide the possibilities of gaining both quantitative and qualitative information. Fibronectin (FN) was chosen as the target molecule to be sprayed due to its functionalities such as promoting proliferation, differentiation of cells, also to promote the cell-cell and cell-substratum adhesion. Silicon wafer and medical grade stainless steel are chosen as target substrates due to their surface energy and biocompatibility. Both FTIR and AFM analysis show the effective landing of fibronectin via electrospray. The landed fibronectin shows the characteristic peaks of amide I and amide II compositions of fibronectin. Moreover, the linear relationship for the concentration of fibronectin versus the intensity of characteristic peaks of fibronectin by FTIR indicates that FTIR could serve as a semi-quantitative technique for examining the fibronectin. On the other hand, the AFM can detect the existence of Fibronectin up to the single molecule scale. The image shows the double strains of FN which is similar as the morphology of FN found in reference (Ref). Moreover, by immunofluorescence analysis, the efficiency of using electrospray to deposit FN on the substrates can be identified. And a quantitative assessment of the biomolecules on the substrates can be provided. These results provide potential possibilities of patterning array and assemblies of tissue which could be applied in the drug discovery and biosensors fields.

4:40pm **BI-MoA9 The Effect of Surface Structure and Functionality on Conformation of Surface-Adsorbed Fibrinogen***, **C.L. Berrie**, J.E. Headrick, K.L. Marchin, S. Phung,* University of Kansas

The interactions of the plasma protein fibrinogen with surfaces have been studied using atomic force microscopy (AFM). Specifically, well-characterized model substrates have been used to investigate the effect of surface chemistry and structure on the adsorption of fibrinogen. Dramatic differences in the average size and shape of fibrinogen molecules adsorbed to hydrophobic and hydrophilic substrates have been observed. These changes can be readily seen in AFM images of individual molecules with sub-molecular resolution. The differences have been quantified and correlated with the surface chemistry. In addition, new methods for patterning nanostructured substrates for use in these experiments have been investigated as well as methods for chemically functionalizing AFM probe tips in order to obtain information beyond topography. Adsorption of fibrinogen on nanostructured thin films and the effects of ionic strength and pH of the solution will also be discussed.

Biomaterial Interfaces
Room Exhibit Hall B - Session BI-MoP
Poster Session

BI-MoP1 Immobilization of Avidin on COOH-modified SiO@sub 2@/Si(100) Surface and Characterization by AFM and BML-IRRAS, ***N. Misawa***, *S. Yamamura,* The Graduate University for Advanced Studies, Japan*; T. Urisu,* Institute for Molecular Science, Japan

Nowadays bio-mimetic sensing techniques, using immobilization of intact biomolecules on solid surfaces, attract significant attentions. For solid substrates, silicon is a suitable material since precise micro-fabrication has been established. New biosensors can be combined with electronics devices on the same chip. Characterizations by IR spectroscopy and AFM observation are useful tools to investigate biomolecules immobilized on silicon surface. It is known that BML-IRRAS (Infrared Reflection Absorption Spectroscopy using Buried Metal Layer substrate [1]) is a high-resolution surface vibration spectroscopy on the semiconductor or insulator materials, which has sub-monolayer sensitivity for the wide frequency range including fingerprint regions. In this study we have immobilized avidin, which has high versatility for conjugation of biomolecules with solid surface, on the SiO@sub 2@/Si(100) surface modified with carboxyl group, and characterized the surfaces by AFM and BML-IRRAS for the first time. The -COOH modification was produced by the deposition of 2-(carbomethoxy) ethyltrichlorosilane and sequential hydrolysis by HCl. Immobilization of avidin was performed after condensation reaction by N-hydroxysucciniimide and EDC, which enhanced the reactivity of carboxyl group with amino group of avidin. AFM images showed that the roughness of the -COOH modified surface was less than 0.5 nm, and protrusions with about 15 nm diameter and 2 nm height appeared after the avidin immobilization. The BML-IRRAS measurements showed clear peaks at 1650 cm@super -1@ and 1550 cm@super -1@, which were assigned to Amide I and Amide II bands of avidin. These bands also consisted of several fine structures which might be assigned to secondary structures such as @alpha@-helix and @beta@-sheet etc. The detailed shape analysis of these bands could give the information with orientations of these immobilized proteins. [1] S.Yamamura. et al. Jpn. J. Appl. Phys. 42 3942 (2003).

BI-MoP2 An Application of Microcantilever for Biosensor using Piezoresistivity, ***K.H. Na***, *C.J. Kang, Y.S. Kim,* Myongji University, Korea

A microcantilever-based biosensor with piezoresistor has been fabricated using surface micromachining technique that allows a simple fabrication procedure and a low cost sensor. A microcantilever integrated with piezoresistive readout enables sensing even for non-transparent liquids such as blood and miniaturizing the size of biosensor compared with optical readout. Adsorptions of bio-chemical species on a functionalised surface of a microcantilever will cause surface stress. This makes microcantilever bending and results in the change of the resistance of piezoresistor in the microcantilever. The structural layer of the microcantilever is fabricated with LPCVD silicon nitride film and the sacrificial layer is LPCVD polysilicon film. The cystamine terminated with thiol was covalently immobilized on the gold-coated side of the microcantilever. The immobilization process was characterized by measurement of the microcantilever deflection in real time monitoring. We are going to present a cantilever deflection after a formation of cystamine/glutaraldehyde/protein-A/anti-protein-A composite layers and to measure a limited resolution of the cantilever-based biosensor for a concentration of biomolecule.

BI-MoP3 Architectured Surfaces for On-Probe Affinity Capture MALDI Mass Spectrometry, *G.R. Kinsel,* ***Z. Segu***, *R.B. Timmons,* University of Texas at Arlington

MALDI MS is a powerful analytical tool for the characterization of proteins and peptides and is presently used extensively for the analysis of biomolecules extracted from biological media. In these applications one or more purification / fractionation steps are typically required prior to MALDI MS analysis. The development of affinity fractionation approaches performed directly on the MALDI probe is considered attractive because of shortened analysis time, inherent simplicity and reduced sample loss. However, a significant challenge for these on-probe affinity fractionation approaches is the limited capacity of the MALDI probe surface. The significance of protein solution concentration, surface-protein binding constant, and total protein surface capacity, with respect to the MALDI limit of detection for a given protein in a mixture, is revealed in theoretical studies utilizing the systematic treatment of equilibria. Additional experimental studies confirm the predictions of the theoretical model and reveal the importance of the MALDI probe capacity on protein detection using modified probes. In addition, a novel method to increase the capacity of modified MALDI probes is presented. In this approach gold particles are attached to allyl amine RF plasma polymer modified MALDI probes and subsequently modified to incorporate affinity capture ligands through the attachment of biotinylated alkane thiols. Preliminary data demonstrates that these modified gold bead attached MALDI probes allow the selective capture of targeted biomolecules and offer significant increases in the biomolecular binding capacity of the MALDI probe surface.

BI-MoP4 Single Nanoparticle Detection of Biological Molecules via Darkfield Microscopy, ***G. Nusz***, *A. Chilkoti,* Duke University

Noble metal nanoparticles have been used as sensors for biological molecules because of their unique interactions with light due to the resonant collective oscillations of the conduction electrons known as surface plasmon resonance (SPR). The frequency at which this resonance occurs is strongly dependant upon the dielectric constant of the medium surrounding the particles. We have previously shown that gold nanoparticles chemisorbed on to glass and subsequently functionalized with a biological receptor can optically transduce analyte binding at the surface of the nanoparticles. This is because when the target analyte binds to the receptor functionalized nanoparticles, the dielectric constant of the surrounding media increases, resulting in a measurable shift of the SPR frequency that can be measured as a color change. In this study, we report the extension of this label free optical biosensor to single nanoparticles that are chemisorbed onto glass. Gold nanoparticles in the size range of 13-40 nm and gold nanorods (30 nm diameter, aspect ratio 2.7) are synthesized in solution. The nanoparticles are chemisorbed on to the surface of an amino-terminated silane monolayer under conditions that result in sparse coverage of the nanoparticles on the substrate. Darkfield microscopy is used to detect the SPR shift of a single nanoparticle by analyzing its scattering spectrum as a function of the change in the dielectric constant in the vicinity of a single nanoparticle. Applying this technique to a single nanoparticle offers the advantage of effective detection of a target analyte with detection limits on the order of a few hundred molecules.

BI-MoP5 Development of Organic Semiconductors Using Metal Doped Fish Protein, ***T. Arockiadoss***, Central Leather Research Institute, India*; F.P. Xavier,* LIFE, Loyola College, India*; B.K. Prabhu, M. Babu,* Central Leather Research Institute, India

Bioelectronics is an emerging field, which extensively uses the supra-molecular structure of proteins, DNA etc to evolve products applicable in biosensors, switchable membranes, organic thin film transistors and fuel cells. This study shows that a partial purified metal-doped muscle protein from the fish, Clarius battracus, was fabricated with poly vinyl alcohol (PVA) to form a biopolymer thin film. The initial protein characterisation was done by gel electrophoresis, followed by analysing the thin film, using circular dichorism, fourier transformed infrared spectrum, scanning electron microscopy, electrical conductivity with and without temperature dependency and finally cyclic voltameter was used to study the architectures of donor and acceptor molecules. The study concludes that the metal-doped fish muscle protein and PVA gives rise to a conducting biopolymer, leading to a versatile molecular electronic material having a unique electrical and optical property, which could be used as a semiconductor in the arena of biochip, fuel cell and nanotechnology. @FootnoteText@ KEY WORDS: FISH, CONDUCTIVITY, METAL DOPING, PROTEIN.

BI-MoP6 Biocompatibility of Microelectronic Materials, *H.D. Wanzenboeck,* ***C. Almeder***, *E. Bertagnolli,* Vienna University of Technology, Austria*; E. Bogner, M. Wirth, F. Gabor,* University Vienna, Austria

Cell-based biosensors endeavor to use microelectronic data acquisition and processing to evaluate specific signals from living cells. The potential of these bioelectronic sensors has been recognized for numerous applications in medicine, pharmaceutical research, environmental diagnostics and the food and processing industries. The interaction between living tissue and microelectronic materials is the critical issue for all those systems, as the inorganic material must neither interfer with nor harm the cells. The effects of different microelectronic materials on the growth of human colon carcinoma cells have been investigated. A systematic study of the survivability and the growth of an exemplary cell culture on various materials used in microelectronics was performed. The viability and the

adhesion of colon carcinoma cells (Caco-2) was tested on 15 different materials - metals, dielectrics and semiconductors - commonly used in microelectronics fabrication. Growth inhibiting materials such as copper and blank gallium arsenide have been identified as well as highly biocompatible materials such as silicon, silicon nitride, chromium and gold. Cells have also been cultured on a microelectrode array consisting of metal and dielectric materials on the same substrate. Neither the sub200 nm height step nor the change of the material showed to effect the cell growth. Several materials have been successfully tested to facilitate the growth of cell structures. The results allow a versatile application for microelectrode arrays and demonstrates the wide compatibility of semiconductor technology for fabrication of cell-based biosensors.

BI-MoP7 The Use of Novel Self-Assembled Monolayers for Enhancing Biosensor Performance, ***W. Laureyn***, *F. Frederix, K. Bonroy, T. Ghoos, R. De Palma, K. Jans, C. Zhou, G. Reekmans,* IMEC, Belgium*; P. Declerck, W. Dehaen, G. Maes,* KULeuven, Belgium*; C. Van Hoof,* IMEC, Belgium

The increasing miniaturisation of biochips and the demand for higher sensor detection sensitivities put severe demands on the process and methodology of coupling biomolecules to surfaces. More specifically, controlled thin film structures have to be created which allow the bio-affinity elements to be arranged and addressed in a reproducible and controlled manner. Addressing these issues, IMEC has developed promising methodologies for the construction of novel, well-defined biosensor interfaces, based on the deposition of Self-Assembled Monolayers (SAMs) of alkane thiols or disulfides on metal (e.g. gold) and alkyltrichlorosilanes on oxide (e.g. tantalum pentoxide) surfaces. In addition, polymeric biosensor interfaces have been created on gold using grafted polysiloxane-g-poly(ethylene glycol) polymers. In order to retain biological activity and to allow for the necessary accessibility, the biomolecular functional units have been immobilised onto gold and oxide surfaces derivatised with mixed SAMs. In the mixed SAM approach, the first molecule carries a functional group to firmly attach the bioreceptor molecule and the second molecule resists the non-specific adsorption of undesired biological entities. Different types of mixed SAMs have been optimised, containing e.g. molecules with a molecular backbone comprised of protein-resistant functionalities and molecules with highly reactive functional moieties. For protein detection, mixed SAMs were optimised in order to increase the amount of receptor molecules (antibodies and fragments) on the surface, while still mitigating non-specific adsorption, allowing for highly sensitive immunosensing in non-specific matrices. For the detection of small molecules, highly reproducible and tuneable immobilization protocols were developed, based on mixed SAMs. The optimisation of these (mixed) SAMs was conducted using various surface characterisation tools and using SPR and QCM-D for immunosensing experiments.

BI-MoP8 Surface Modified MALDI Probes for Affinity Fractionation of Protein Mixtures, *G.R. Kinsel,* ***M. Li***, *G. Fernando, L. van Waasbergen, R.B. Timmons,* University of Texas at Arlington

Matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) has become a powerful analytical tool for the characterization of proteins. As the effectiveness of the MALDI method has advanced, the need for high-speed separation and purification of peptides/proteins in complex mixtures (e.g. culture media, serum or urine) has increased. The approach described in this presentation focuses on the use of RF plasma polymer coated MALDI probes as platforms for peptide and protein separation based on their hydrophobicity. Pulsed RF plasma deposition of allyl alcohol directly on the MALDI probe surface is used to produce surfaces with various hydrophobicities. Control of the degree of the hydrophobicity is achieved through changes in the duty cycle of the pulsed RF plasma. Testing of the surfaces for peptide/protein separation based on their hydrophobicity is performed using various laboratory prepared control mixtures and mixtures obtained from biological sources. In all cases fractionation of the protein/peptide mixture was evaluated through the acquisition MALDI mass spectra using a Bruker AutoFLEX MALDI TOFMS or a laboratory-constructed linear MALDI TOFMS. Data has been obtained from surfaces with different hydrophobicities that demonstrate the efficacy of these modified MALDI probe surfaces for achieving on-probe fractionation of peptide/protein mixtures.

BI-MoP9 Micropatterned Substrate Screening under Shear Flow (MiSSUS): Direct Comparison of Receptor-ligand Binding, ***K.A. Burridge***, *M.A. Figa, J.Y. Wong,* Boston University

Microfluidic patterning has been combined with a parallel plate flow chamber to enable screening of combinatorial variations in targeted drug delivery carrier surface properties under tunable physiologically-relevant shear conditions. Carriers containing either drugs or imaging agents must have surface properties that promote binding to targets yet at the same time block rapid immune system clearance. In addition, ligand-receptor mediated attachment must overcome shear flow in the vasculature which decreases contact times and applies forces on bonds. Patterned bilayers which mimic the surface of liposomal delivery vehicles are created by injecting pre-mixed vesicle solutions into lanes formed by a polydimethylsiloxane stamp reversibly sealed to a glass slide. After removing the stamp in an albumin solution to form protein barriers that prevent bilayer expansion, the slide is assembled into a flow chamber for binding studies. MiSSUS provides direct quantitative comparison of the effects of variations in ligand architecture such as relative molecular weights of liganded and unliganded polyethylene glycol. Experiments using MiSSUS revealed that ligand spacer length is an important factor in maintaining adhesion under flow, i.e. that longer spacers confer higher detachment resistance.

BI-MoP10 Microfluidic Circuit Fabrication and Packaging for Surface-Controlled Bioprocesses in BioMEMS, *T.M. Valentine,* ***J.J. Park***, *G.W. Rubloff, R. Ghodssi,* University of Maryland

We have previously demonstrated that biopolymers (specifically, the polysaccharide chitosan) can be deposited in vitro at patterned, voltage-programmed electrodes, and that subsequent coupling and conjugation of biomolecules (proteins, nucleic acids) opens the door to applications such as bioassays and enzymatic catalysis. To extend these capabilities beyond simple devices to more complex bioMEMS systems, robust means for microfluidic circuit fabrication and packaging are required. We have designed and fabricated microfluidic networks to support combinatorial test site libraries for surface-controlled bioprocesses, along with packaging approaches to control fluidics and electrical inputs/outputs to the bioMEMS systems, with emphasis on robust technologies for leak-free microfluidics, use of polymer-based MEMS, incorporation of electrode structures and optical access to them, and reliable exchange of bioMEMS systems through reusable packaging which allows rapid connection of fluid and electrical inputs/outputs to external control systems. Three different approaches to microfluidic design, based on both sealing and bonding, are promising in concert with the packaging strategy. The efficacy of these fabrication and packaging approaches is demonstrated through results on fluidic sealing and on the electrically programmable deposition of NHS-fluorescein labeled chitosan at internal sites in the bioMEMS system. The impact of flow rates and residence times on biopolymer deposition and biomolecular conjugation reflect key chemical engineering questions associated with surface bioreactions in microfluidic systems, laying the groundwork for future applications in miniaturized bio-reactors and chemical and biological sensors.

BI-MoP11 Near-Field Interaction of Infrared Radiation with an Atomic Force Cantilever, ***E.S. Gillman***, NanoSpec Corporation

Infrared vibrational spectroscopy is a powerful tool for chemical identification, however most infrared spectroscopic techniques usually do not obtain spatially resolved chemical information at a nanoscopic level due to the fact that they are diffraction limited. On the other hand, a scanning near-field optical microscope (SNOM) can reveal features with spatial resolution less than the diffraction limit because it relies on near-field probing instead of optical focusing. Using an apertureless approach that is based on atomic force microscope (AFM) cantilever tip ineracting with a infrared source of sufficient intensity, spatially resolved chemical information as well as conventional topographic information can be obtained. Critical to this is the near-field interaction of the AFM cantilever tip and the surface in the presence of an electromagnetic field. Modeling of this interaction will show that this approach can result in parctical device for nanoscale chemical imaging.

BI-MoP12 Nanoscale Chemomechanical Patterning of Silicon and Germanium Surfaces Using an Atomic Force Microscope, ***R.C. Davis***, *M. Tonks, K. Barnett, M. Lee, M.R. Linford,* Brigham Young University

In nanoscale chemomechanical patterning, an atomic force microscope (AFM) probe is used to scribe a flat silicon surface under a solution of reactive molecules, attaching a layer of the molecules to the scribed line. This technique was previously used to functionalize silicon surfaces using a moderate range of scribing forces (5 μN to 80 μN). Here we will present a study focusing on the smaller forces (1μN to 5 μN) on both silicon and germanium surfaces resulting in significantly reduced tip wear and yielding lines down to 20 nm wide. We will also present the extension of chemomechanical patterning to include the attachment of proteins to the functionalized lines.

BI-MoP13 Force Spectroscopy of Mechanically Stretched Fibronectin, ***K.L. De Jong***, *P.R. Norton, N.O. Petersen,* The University of Western Ontario, Canada

Cell motility is a delicate balance between adhesion and detachment. Identifying key proteins involved (for example, fibronectin, Fn) and understanding the mechanisms employed to find this balance, will provide new insight into the means by which motility can be controlled. Mechanical forces play a key regulatory role in biological cells, and therefore to understand how cells move and adhere, ultimately relies on knowledge of how forces are generated and propagated or in essence, how the cell interacts with the surface. The stress on Fn fibres may be the deciding factor in determining the attachment of the cell to the matrix, and the adhesion of the matrix to the substrate. Studies geared toward understanding fibronectin structure, organization, and binding affinity under mechanical stretching, are providing information crucial to the understanding of the effect of mechanical forces on cell function. To test the prediction that stretching Fn reduces integrin-binding activity, an AFM compatible device is designed to apply a mechanical force to fibronectin while the change in intermolecular interactions that result is monitored. The force required to rupture the interaction between an integrin mimic and fibronectin is determined to be approximately 100 pN. After stretching fibronectin, a trend towards fewer rupture events characterized by smaller pull-off forces in each force curve is observed; this implies a decrease in potential binding sites available to the integrin mimic and concomitant weaker interactions.

BI-MoP15 Plasma Polymerization of Tetraglyme for PEO-like Surfaces and Plasma Immobilization of PEO Surfactants for Improved Blood Compatibility, ***J.L. Lauer***, *J.L. Shohet, R. Muguresan, R.M. Albrecht,* University of Wisconsin-Madison*; U.H. von Andrian,* Harvard Medical School*; S. Esnault, J.S. Malter,* University of Wisconsin-Madison*; S.B. Shohet,* University of California, San Francisco

The realization of small scale biomedical devices will be closely related to the non-fouling/biocompatibility properties of the exposed surfaces and the uniformity of the surface treatment throughout the device. PEO and PEO-like surfaces are significantly advantageous in preparing medical devices that require good blood compatibility. In this work, we explore two plasma process techniques, plasma polymerization (PP) and plasma immobilization (PI), to improve the blood compatibility of various polymer and non-polymer surfaces. Thrombus formation and embolization are significant problems for blood-contacting biomedical devices. To minimize these affects, plasma polymerized tetraethylene glycol dimethyl ether (tetraglyme) was deposited on flat Si3N4, and SiO2 samples to produce a PEO-like surface coating. In addition, a microplasma was used to immobilize a Poly(ethylene oxide) (PEO) surfactant to the lumenal surface of PE and PTFE tubing (ID 1.14mm). A microwave-cavity diagnostic was used to measure the plasma density of the microplasma inside of the polymer tube. Emitted light from the plasma during the PP and the PI processes was fed into a monochromator. Coating thickness and chemical composition of the flat surfaces was measured using ellipsometry and XPS, respectively. Contact-angle measurements were made for both the flat PP surfaces and the PI polymer tubes. To test blood compatibility, both the flat PP surfaces and the PI polymer tubes were exposed to heparinized human blood. After blood exposure, the tubes were examined with a scanning electron microscope to assess the density of adhering platelets on the flat PP surfaces and along the length of the PI polymer tubes. The plasma-treated surfaces showed fewer blood adherents than the untreated surfaces. By suitably modifying the plasma parameters, the treatment for both plasma processes can be optimized.

BI-MoP17 Nonfouling Microstructures on Hydroxylated Substrates via Chemical Vapor Deposition and Surface Initiated Atom Transfer Radical Polymerization, ***H. Ma***, *A. Chilkoti,* Duke University

The ability to covalently modify hydroxylated substrates such as glass and metal oxides with a non-fouling polymer coating is an important goal, in view of their wide application as biomaterials and in biotechnology. Most current approaches rely upon physisorption of PEG-containing polymers or grafting of the polymers from solution to the surface (â?ografting toâ? approach). We report here a â?ografting fromâ? strategy in which an oligo(ethylene glycol) functionalized monomer is polymerized in situ from the surface of glass to provide high-density polymer brushes that overcomes the intrinsic limitation of low surface density of PEG chains realized by â?ografting toâ? strategies. A silane initiator presenting a terminal bromoisobutyrate moiety was used to form a SAM on hydroxylated substrates via chemical vapor deposition (CVD). This SAM was used as substrate for surface initiated atom transfer radical polymerization (SI-ATRP) of oligo(ethylene glycol) methyl methacrylate (OEGMA). The SI-ATRP was carried out in an oxygen free environment with CuBr/bipyridine as catalysts in a water /methanol mixture. Poly(OEGMA) brushes with a tunable thickness between 2 and 10 nm can be synthesized in situ, and these brushes are exceptionally resistant to protein adsorption, even from 100% fetal bovine serum. We also report a new masking strategy to pattern the surface with the initiator silane SAM, which enabled facile patterning of the surface with the poly(OEGMA) brushes. When NIH 3T3 fibroblasts were seeded onto those surfaces, cells were confined within the regions demarcated by the polymer and were maintained within the pattern for over a week.

BI-MoP18 Plasma Grafted Anti-Fouling Films on Ethylene Oxide Base for Biosensors and Biotechnologies, ***M. Kormunda***, *G. Ceccone, A. Papadopoulou, M. Hasiwa, F. Rossi,* EU-JRC-IHCP, Italy

The generation of anti-fouling surface is a key element in the design of biosensors, medical devices and implants. Protein adsorption resistant surfaces have to avoid or reduce non-specific protein adsorption, platelet adhesion and thrombus formation, to prevent undesirable responses of the living systems to the device. The highly cross-linked anti-fouling coatings on ethylene oxide base were plasma grafted from Dimethoxydiethylene glycol vapour in mixture with reactive (N@sub 2@) or inert (Ar) gas at low-pressure 40mTorr. The plasma discharge in capacitive configuration of reactor was powered by continuous (from 10 to 100W) or pulsed RF (13.56MHz) voltage. The coatings were plasma grafted on glass, Si and PTFE substrates placed on grounded substrate holder. The film chemical composition has been analysed by XPS and FTIR as well as the surface morphology (AFM) and the protein adsorption (QCM-D). The coatings toxicity and an adhesion of Fibroblast L929 have been investigated on selected samples. The lower power plasma grafted coatings have very good antifouling properties together with low receding contact angles although the methyl function groups in the films are more populated than ethylene oxides groups. The coatings are stable with low contact angles over period about 6 months at normal conditions. No significant changes were observed on functional groups in coatings washed for 40 days in ethanol bath. The oxygen plasma etching during a nano-patterning process for biosensors fabrication has not significant influence on chemical composition, surface morphology and contact angles.}

BI-MoP19 Local Property of Linear Plasmid DNA on the Metal Wires with Different Potential, ***S.H. Jin***, *J.M. Son, N.J. Lee, C.J. Kang,* Myongji University, Korea

Linear plasmid DNA crossed on the patterned nano metal wire is characterized by scanning capacitance microscopy and electrostatic force microscopy. The metal wires processed on the silicon wafer followed by a capping thin dielectric film were used as a local potential source for the DNA molecule. By varying the voltage bias applied to the metal wire, initial stage of DNA deposition on the substrate is observed and local electrical properties, such as capacitance variation and change of surface potential are measured. With images and spectroscopy, we investigate DNA moleculeâ?"substrate interaction.

BI-MoP20 Analysis of Contaminants in Commercial Thiolated Single-stranded DNA Oligomers by XPS and ToF-SIMS, ***C.-Y. Lee***, *L.J. Gamble, D.G. Castner,* University of Washington

Commercially produced thiolated single-stranded DNA (SH-DNA) molecules are used in a variety of biotechnology applications including biosensors and DNA microarrays. The diversity of techniques used by different vendors in the synthesis and treatment lead to a significant variation in the quality of SH-DNA. In this work, we used x-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) to perform a comparative study of the relative purity of commercially-available SH-DNA from several vendors. We find that thiol-terminated 16-base pair oligomers from two of the vendors, which were self-assembled as thin layers onto gold surfaces, contain excess carbon and sulfur. By using ToF-SIMS, several contaminants including poly(dimethylsiloxane) (PDMS), lipid molecules, and sulfur-containing molecules were identified by their molecular fragments. Preliminary ToF-SIMS data indicates that the excess sulfur arises from the reductant (dithiothreitol) used to purify the SH-DNA by some of the vendors. Time dependent studies of purified versus contaminated SH-DNA were performed to determine the effect of contamination on DNA surface assembly over time. XPS results of purified SH-DNA show increased P, N, O and C atomic percentages over a 24-hour time period, confirming increasing DNA surface coverage on the gold. In contrast, XPS results of contaminated SH-DNA show that C and O atomic percentages increased over time, but no increase was observed in the P and N. This indicates that,

after the initial SH-DNA adsorption during the first five minutes, the excess material adsorbed for the rest of the 24 hours was due to contamination.

BI-MoP21 Empirical Force Field Evaluation for the Molecular Simulation of Protein Adsorption, ***V. Raut***, *S.J. Stuart, R.A. Latour,* Clemson University

Molecular simulation provides a direct method to theoretically investigate the molecular mechanisms governing the adsorption behavior of proteins to biomaterials surfaces. Because of their size, empirical force field based methods must be used for these types of simulations. Force fields (ffs), however, must be parameterized for specific molecular systems. While ffs have been designed to accurately represent the behavior of proteins in solution, none have considered peptide-surface adsorption behavior in their parameterization. Therefore, there is currently no basis to support the accuracy of simulation results. The objective of this research was to develop computational methods to complement our previous experimental studies that measured the adsorption free energy (G*) for a host-guest peptide on Au-alkanethiol self assembled monolayer surfaces (SAMs), and to use these methods for ff evaluation. Host-guest peptides were modeled in the form of SGSG-X-GSGS, where G=glycine, S=serine, and X=any selected peptide type. Molecular dynamics simulations were conducted using the GROMACS ff to calculate G* for these peptides over functionalized SAMs (CH3, OH, NH2, COOH, PEG) in a 40@Ao@ x 40@Ao@ x 60@Ao@ simulation cell with explicit solvation (water with Na+ and Cl- ions) contained within periodic boundary conditions. While simulation results for certain peptide-SAM systems are generally in agreement with experiments, others show substantial deviations from expected adsorption behavior. Parameter modifications of this ff are thus required for this application. Further work is planned for the eventual development of a validated ff for protein adsorption simulations.

BI-MoP22 Investigation of the Binding Mechanism of the Bacterial Adhesin Scp to Fibronectin, ***J.R. Hull***, The University of Washington; *D.G. Castner,* University of Washington; *G. Tamura,* Children's Hospital and Regional Medical Center

Several pathogens bind specifically to immobilized fibronectin (Fn), and not to soluble Fn, including Group B Streptococci (GBS), S. pneumoniae, S. sanguis, and p-fimbriated strains of E. coli. However, the structural basis for the specific adherence of Scp to immobilized Fn has not been resolved. There are two possible mechanisms for Scp binding to Fn. First, specific conformational determinants of Fn that allow Scp binding may be unmasked when Fn is immobilized. Second, Scp may bind to a combinatorial determinant formed by the clustering of multiple immobilized Fn molecules. The Scp-Fn binding interaction is being studied with two complementary analysis methods. Surface Plasmon Resonance (SPR) is being used to study the kinetics of the binding interactions and Atomic Force Microscopy (AFM) is being used to investigate single molecule interactions. Supporting experiments to determine surface compositions was done using X-ray Photoelectron Spectroscopy. SPR has shown that the binding affinity of soluble Scp to adsorbed Fn is approximately nano-molar and that Scp does not bind soluble Fn. A model system was set up to develop AFM methods for examining the Scp-Fn interactions. Collagen related peptides (CRPs) on the AFM tip were used to interact with adsorbed Fn. The CRPs were attached to the AFM tip via homo-functional N-hydroxysuccinimide (NHS) poly(ethylene glycol) (PEG) cross-linker. The jump heights of the force distance curves were graded based on Studen@aa t@s t-test and only events with a nominal grade were further analyzed. Analysis of unbinding events shows that there are multiple interactions centered at 75 pN and there are multiple length scales over which these interactions occur. There are at three length scales over which these interactions occur: 5 nm which is attributable to nonspecific adhesion, 15 nm which is the NHS-PEG spacer length, and 30 nm and greater which is attributable to CRP-Fn interactions.

BI-MoP23 L-Cysteine Adsorption on Cu(100) Studied by Sulfur K-edge NEXAFS and XPS, ***S. Yagi***, *Y. Matsumura, T. Nomoto,* Nagoya University, Japan; *J.A. Syed, S.A. Sardar,* Hiroshima University, Japan; *K. Soda,* Nagoya University, Japan; *E. Hashimoto, M. Taniguchi,* Hiroshima University, Japan

For the application to medical materials it is important to study the mechanism of reaction at interface between amino acid, which is the component of protein, and metal, and the influence of water on the mechanism. We have paid attention to L-Cysteine [HSCH@sub 2@CH(NH@sub 2@)COOH] and investigated the adsorption behavior of L-Cysteine/Cu(100) by Sulfur K-edge Near Edge X-ray Absorption Fine Structure (NEXAFS) and X-ray Photoelectron Spectroscopy (XPS) techniques. Sulfur K-edge NEXAFS spectrum for L-Cysteine/Cu(100) shows that the main peak of @sigma@@super *@(S-C) is decreased in comparison with the spectrum for L-Cysteine powder. This means that L-Cysteine molecule obtains some electrons from the Cu(100) surface.

BI-MoP24 Orientation of a Y-shaped Biomolecule Adsorbed on a Charged Surface, ***Y.-J. Sheng***, National Taiwan University, Taiwan, ROC; *H.-K. Tsao,* National Central University, Taiwan; *S. Jiang,* University of Washington

The adsorption and orientation properties of a Y-shaped biomolecule, which models an immunoglobulin (Ig), on a charged surface are analyzed mesoscopically by Monte Carlo simulations. The orientation is a consequence of the interplay between van der Waals interactions and electrostatic interactions. For adsorption dominated by van der Waals attraction, the molecule prefers lying flat on the surface. For weak attraction, we observe a depletion zone in the concentration profile, which can result in a negative surface excess. A secondary peak is found for strong adsorption. For electrostatically dominated adsorption, the orientation is mainly determined by electric dipole and a vertically adsorbed molecule can be attained as it possesses strong electric dipole. Our study provides an explanation for experimental observations of preferential orientation.

BI-MoP25 Interfacial Dynamics of a Gelatin Solution with Surfactant, ***H.-K. Tsao***, National Central University, Taiwan; *S.-Y. Lin,* National Taiwan University of Technology, Taiwan

In terms of dynamic surface tension, the interfacial dynamics of gelation solutions with various surfactants are investigated by pedant bubble tensiometry. On the basis of local equilibrium assumption, the thermodynamics of dynamic surface tension is analyzed. The adsorption efficiency of gelatin is low compared to that of small surfactant. However, the surface activity of gelatin may be enhanced due to intrachain and interchain rearrangement at the interface, which results in long relaxation time signature. The interplay between adsorption efficiency and surface activity categorizes our experimental results into two types of dynamic behavior. For type I dynamics, the gelatin molecule is completely displaced from the interface and the interfacial dynamics is dominated by that associated with surfactant. Nevertheless, the interaction of gelatin with surfactant in the bulk solution can alter the surfactant surface excess and hence the surface tension. For type II dynamics, the gelatin molecule is partially displaced from the surface and the dynamics displays a long relaxation characteristic. The extent of surface tension decrement due to gelatin conformational change manifests the degree of gelatin displacement from the interface. Our conclusion is able to elucidate the peculiar dynamic surface tension of a solution of gelatin and PEG.

BI-MoP26 Detergency Effectiveness with Respect to Proteins, ***M. Richard***, *T. Le Mogne,* Ecole Centrale de Lyon, France; *J. Criquelion,* Laboratoires Anios, France; *A. Perret-Liaudet,* Hopital Neurologique de Lyon, France; *J.M. Martin,* Ecole Centrale de Lyon, France

In the detergency field of re-usable medical devices, a special attention is focused on the non-conventional transmissible agent called prions which is a proteinaceous infectious agent. Few cleaning procedures are effective against prions and few techniques are available to study cleaning effectiveness with respect to proteins in general. The first part of our study shows that X-ray Photoelectron Spectroscopy (XPS) is a useful and reliable technique to evaluate detergent formulations effectiveness to remove proteins from stainless steel surface soiled with a human brain homogenate. A semi-quantitative evaluation of the detergency effectiveness could also be performed. XPS makes it possible to study chemical species remaining on surface, substrate properties after cleaning procedures and also the water quality effect on detergency effectiveness. In the second part of our study and in the light of the complexity of the previous system, a simplification of each part of the system was carried out. XPS analysis was used to study the effectiveness of some simple chemical functions to remove proteins from a native oxide layer of a pure chromium surface. The results will be presented and discussed in this paper.

BI-MoP27 Reflex-Arc on a Chip: an in Silico Cell Culture Analogue, ***K.A. Wilson***, *M. Das, L.C. Riedel, C.A. Gregory, M. Poeta, D. Damjanovich, P. Molnar, J.J. Hickman,* Clemson University

To date understanding of and development of therapies for traumatic spinal cord injury (SCI) and neurodegenerative diseases have been problematic due in part to difficulties associated with the various models used to test new drug therapies. Animal studies are expensive, time consuming, and raise ethical issues. In vitro studies are less expensive and avoid many of the ethical issues associated with animal studies, but are often poor predictors of human response. To overcome the shortcomings

of existing models we are developing a microscopic cell culture analogue (microCCA) of the spinal reflex-arc. This system should retain the cost effectiveness of in vitro systems while allowing complex tissue interactions and environmental dynamics that more realistically reflect the in vivo state. The present work draws on advances in a wide variety of technical fields including cell culture, surface chemistry, and microfabrication. These advances have allowed us to begin development of a microCCA device comprised of the basic components of the reflex-arc: a muscle fiber, a dorsal root ganglion (DRG) cell, and a motoneuron. Silicon microstructures serve as the foundation of the device. Surface modification with alkyl-silane SAMs followed by patterning with deep UV photolithography was performed to selectively control cell adhesion and growth. XPS analysis indicated monolayer formation. Furthermore, we have demonstrated the control of neuronal growth and myotube differentiation on the microstructures. Electrophysiology results have confirmed that the neurons and myotubes have physiological properties consistent with previous findings. With this system it will ultimately be possible to report on a variety of properties of the reflex-arc, thereby creating a cost-effective, predictive test bed for the development of novel drug therapies for traumatic SCI and a wide variety of neurodegenerative diseases.

BI-MoP28 Compartmentalized Bioreactor for Long-Term Culture of Bone Cells, ***R. Dhurjati,*** *E.A. Vogler, P.W. Brown, H.J. Donahue,* Pennsylvania State University

A compartmentalized bioreactor designed around the concept of continuous growth and dialysis, was used to study long-term (15-30 days) phenotypic behavior of human fetal osteoblastic cells (hFOB). This specific design separates the growth and feeding functions and permits long term culture sustaining the pericellular environment, otherwise disturbed by continuous or punctuated growth medium replacement. The attachment and growth behavior of model human osteoblasts cultured in the bioreactor was evaluated using scanning and transmission electron microscopy (SEM/TEM) and were compared to those obtained from conventional tissue culture methods. Results suggest that stable culture conditions afforded by the reactor has enhanced utility in the long-term culture of osteoblasts in terms of growth, proliferation and mineralized matrix deposition characteristics and would serve as an ideal in-vitro test system for the study of cell/protein mediated interactions with synthetic bone analog materials.

BI-MoP29 Cell-Surface Interactions between Marine Diatoms and Fouling-Release Coatings Studied with Atomic Force Microscopy, ***P.F.M. Terán Arce,*** *R. Avci,* Montana State University*; I.B. Beech,* University of Portsmouth, UK*; K.E. Cooksey, B. Wigglesworth-Cooksey,* Montana State University

Interactions between marine microorganisms, and fouling release coatings are of major significance to the Navy and maritime industry. This interaction usually occurs by means of adhesive exopolymers (EPS) secreted by the microorganisms, which allow them to attach and settle on different surfaces. In the present study, viable marine diatom cells were immobilized on tipless AFM cantilevers and used as bioprobes to investigate the adhesion forces between exopolymers, produced by the immobilized diatoms, and surfaces of Intersleek (@super TM@ International Paints) elastomers. These forces, as well as the work exerted against them, were statistically compared with the forces between the same diatoms and mica surfaces. In spite of the dissimilar character of Intersleek (hydrophobic) and mica (hydrophilic) surfaces, comparable results were obtained on both. Force vs distance curves on both surfaces presented several adhesion peaks with force magnitudes that ranged from hundreds of pNs to tens of nNs and polymer elongations up to several microns. These results demonstrate the ability of diatoms to produce hydrophobic and hydrophilic exopolymers.

BI-MoP30 Development and Characterization of RGD Peptide Coatings for Cell Adhesion, ***R. Canteri,*** *C. Pederzolli, L. Lunelli, P. Villani, L. Pasquardini, M. Vinante, G. Speranza, S. Forti, M. Anderle,* ITC-irst, Italy*; J.J. Park, G.W. Rubloff,* University of Maryland

The development of biomaterials able to modulate the interaction of mammalian cells with solid substrates is important for many applications, e.g., tissue replacement/regeneration and substrates for cell culture. A common mechanism that mediates cell adhesion involves the interactions between integrin receptors on the surface of mammalian cells and ligands of adhesive proteins present in extracellular matrices (ECM) and bloodstream. These proteins include fibrinogen, fibronectin, vitronectin, collagen, laminin, Von Willebrand factor. It has been demonstrated that the adhesive domains comprise a short peptide sequence, Arg-Gly-Asp (RGD), the most important recognition site for about half of all known integrins. This work describes a three-step reaction procedure for coupling a six-amino-acid (GRGDSY) fibronectin fragment synthesized with an additional cysteine (C) at the C-terminus, to solid substrates. The first step is the activation of the substrate with an amine layer, introduced by chemical modification (silanization) or by using an amino-containing biopolymer (chitosan). In a second reaction step, N-hydroxysuccinimidyl (NHS) ester polyethylene glycol (PEG) is grafted to the aminated surfaces. Fluorescence quantitative showed 1-5 x10@super 13@ PEG molecules/cm@super 2@ immobilized on the surface. The distal end of these PEG molecules carry two possible chemical groups: a maleimide or a vinylsulfone group, both selectives for reaction with sulfhydryl groups. The final step is the covalent attachment of RGD-containing peptides on the resulting terminal PEG derivatives. XPS, ToF-SIMS, AFM, SEM, fluorescence spectroscopy and microscopy were applied to characterise the surface. The RGD modified surfaces were tested using different cell lines. The results obtained on the functionalized surface showed an higher extent of cell adhesion, with mainly round-shaped cells at the initial stage of the spreading, compared to the non-modified surface.

BI-MoP31 Characterization of Nuclear Impalement by Vertically Aligned Carbon Nanofibers for Gene Delivery, *A.V. Melechko,* University of Tennessee, Knoxville*;* ***T.E. McKnight****, G.D. Griffin, D.K. Hensley, M.J. Doktycz, D.H. Lowndes,* Oak Ridge National Laboratory*; M.L. Simpson,* Oak Ridge National Laboratory, University of Tennessee

Penetration of DNA-modified vertically aligned carbon nanofibers (VACNF) into live cells provides efficient delivery and expression of exogenous genes, similar to â?~microinjectionâ?T-styled methods, but on a massively parallel basis. The efficiency of this method however depends on many factors including plasmid coverage on each nanofiber, maintaining transcriptional activity of these plasmids following immobilization, and retention or release of plasmid from the VACNF scaffold during and after insertion into cell. For DNA that remains tethered, it is believed that gene expression occurs if plasmid is delivered not only into intracellular domain but moreover into nuclear domain. In this work we report on a study of insertion and residence of VACNFs into the nuclei of mammalian cells (Chinese hamster ovary) using Laser Scanning Confocal Microscopy and Scanning Electron Microscopy.

BI-MoP32 Deposition of Lipid Bilayers on the Silicon Dioxide Surfaces Patterned by Focused Ion Beam and Synchrotron Radiation Etching, ***R. Tero,*** Institute for Molecular Science, Japan*; M. Rahman,* The Graduate University for Advanced Studies, Japan*; Z.-H. Wang,* Nagoya University, Japan*; M. Sugawara,* Nihon University, Japan*; K. Nagayama,* National Institute for Physiological Sciences, Japan*; T. Urisu,* Institute for Molecular Science, Japan

Nano-bioelectronics is one of the most attractive research fields in these days. Microfabrication and modification with biomaterials on solid surfaces have fascinated enormous attentions as important techniques for the development of new biosensors and new devices. We have fabricated fine structures on SiO@sub 2@/Si surfaces by combination of the focused ion beam (FIB) and the synchrotron radiation (SR) etching, and deposited lipid bilayers on the SiO@sub 2@ surface by the vesicle fusion method. FIB patterning was performed using JEOL-JFIB2300 with the 30 keV of Ga ion. SR etching was performed in the BL4A2 in UVSOR in IMS using Co photomask with the thickness of 400 nm under the mixture gas of SF@sub 6@ and O@sub 2@ (P@sub SF6@=5.0x10@super -2@ Torr, P@sub O2@=2.0x10@super -3@ Torr). AFM images were obtained by Picoscan (Molecular Imaging). In the deposition of lipid bilayers, the sample was incubated above the gel-liquid crystal transition temperatures in the suspension of the pure dipalmitoylphosphatidylcholine (DPPC) or the mixture of egg PC, dioleoylphosphatidylethanolamine (DOPE) and cholesterol (3:1:1), which were prepared by agitating the vacuum-dried lipid films in a buffer solution. We have patterned the Co photomask on the SiO@sub 2@/Si substrate by FIB in micrometer order. Three-dimensional micrometer-order structures were successfully obtained by one time SR irradiation (2.0x10@super 4@ mA min). After deposition of DPPC vesicles on the SiO@sub 2@ surface, flat membranes with the height of 5 nm were observed by atomic force microscopy (AFM). The thickness of the membranes well corresponded to that of the single lipid bilayer. The morphology, electronic resistance and additional effect of protein (gramicidin A) will be discussed.

BI-MoP33 Interfacing Natural and Synthetic Biomaterials: Development of a Multilayered Vascular Scaffold, *E.J. Taschner, J.B. Leach,* ***J.Y. Wong,*** Boston University

One of the greatest challenges in designing functional small diameter vascular grafts is to mimic key arterial mechanical properties (e.g., strength and compliance). Our hypothesis is that the underlying scaffold organization is a crucial factor in cellular remodeling, and ultimately, the mechanical properties of biologic vascular grafts. Thus, the overall goal of our research is to develop a multilayered or lamellar vascular scaffold biomaterial that more closely mimics the organization of native artery extracellular matrix. Our approach is to seed vascular smooth muscle cells between layers of poly(lactic-co-glycolic) (PLGA) thin films and naturally derived hydrogels (e.g., collagen, fibrin). However, the major challenge to the creation of such a multilayered scaffold is to promote stability and adhesion between the hydrogels and the relatively hydrophobic PLGA films. Therefore, to promote adhesion between the composite layers, the PLGA films were surface modified to contain specific highly reactive groups. First, the PLGA films were treated in NaOH to expose surface carboxylic acid and alcohol groups. Then, carbodiimide-mediated reactions were used to covalently bind photoreactive moieties to the PLGA films as well as the hydrogel precursor monomers. The film-cell-hydrogel composite constructs were assembled and then exposed to ultraviolet light to initiate the photopolymerization. We tested the adhesion between the layers using a modified peel/creep test that applied a constant "peel" force over time. The modified PLGA composites were associated with a significant amount of resistance to the peeling force while the unmodified PLGA controls failed instantaneously. We therefore demonstrate a promising method of creating stable, multilayered tissue scaffolds from composites of natural and synthetic biomaterials.

BI-MoP34 Immobilization of the Enamel Matrix Derivate Protein Emdogain onto Polypeptide Multilayers as studied by in situ Ellipsometry and QCM-D, ***T.J. Halthur,*** Royal Institute of Technology, Sweden*; I. Slaby, A. Lindeheim,* Biora AB, Sweden*; P. Claesson,* Royal Institute of Technology, Sweden*; U. Elofsson,* YKI AB, Institute for Surface Chemistry, Sweden

The build-up of the biodegradable poly(L-glutamic acid) (PGA) and poly(L-lysine) (PLL) multilayers on silica and titanium surfaces, with and without an initial layer of polyethyleneimine (PEI), was investigated and characterized by means of in situ ellipsometry and Quartz Crystal Microbalance with Dissipation (QCM-D). A two-regime build-up was found in all systems, where the length of the first slow growing regime is dependent on the structure of the initial layers. In the second fast growing regime, the film thickness grows linearly while the mass increases more than linearly (close to exponentially) with the number of deposited layers. The film refractive indices as well as the water contents, indicate that the film density changes as the multilayer film builds up. The change in film density was proposed to be due to polypeptides diffusing into the multilayer film as they attach. Furthermore, the use of PEI as initial layer was found to induce a difference in the thickness increments for PGA and PLL. Comparisons between ellipsometry and QCM measurements revealed that the multilayer film was highly hydrated (as much as 70-75% water) and might therefore serve as a good template for proteins and cells. The hydrophobic aggregating Enamel Matrix Derivatives (EMD) protein Emdogain was successfully immobilized both on top of as well as within the multilayer structures while measured in situ with ellipsometry and QCM and in vitro with ELISA. These polypeptidemultilayer/EMD films are thought to be able to trigger cell response and induce biomineralization and might therefore be used as bioactive and biodegradable coatings for future dental implants.

BI-MoP35 In-situ Formation of Bioactive-Titanium Coating using Reactive Plasma Spraying, ***M. Inagaki,*** *Y. Yokogawa, T. Kameyama,* National Institute of Advanced Industrial Science and Technology (AIST), Japan

A surface modification technique using reactive plasma spraying (RPS) was studied to form bioactive-titanium (Ti) coating. An in-situ surface-modification of Ti particles is conducted by making use of plasma-enhanced reactions between the Ti particles and the reactive gaseous species in the plasma flame during plasma spraying. Surface-modified Ti coatings were deposited on Ti substrates by radio-frequency (rf)-RPS using a thermal plasma of Ar gas containing 1-6% N@sub 2@ and/or 1-6% O@sub 2@ at an input power of 16 kW. As a means of surface modification, Ti powders impregnated with 0.05-0.2 mol% Ca were also sprayed. Compositional changes in the coatings' surface after soaking in simulated body fluid (SBF) were examined by Fourier transform infrared spectroscopy (FT-IR) and thin film X-ray diffraction (TF-XRD). The Ti coatings prepared with Ar-O@sub 2@ and Ar-N@sub 2@-O@sub 2@ plasma formed apatite after 3 days of soaking in SBF. This indicated that such coatings have the ability to form a biologically active bone-like apatite layer on their surface. In the TF-XRD patterns for the Ti coatings sprayed with oxygen-containing plasma, minute peaks ascribable to TiO@sub 2@ (anatase and rutile phase) were commonly observed. On the other hand, no compositional change was observed in the surface of the Ti coatings sprayed with Ar-N@sub 2@ plasma, even after 7 days of soaking in SBF. In SBF tests, we observed a retardation of apatite deposition for the Ca-added Ti coatings prepared with Ar-O@sub 2@ and Ar-N@sub 2@-O@sub 2@ plasmas. Analyses by X-ray photoelectron spectroscopy indicated that the Ca impregnated in the RPS-Ti coatings formed a Ca-O compound.

BI-MoP36 Effects of He, Ar Ion Implantation on the Surface Chemistry and Structure of Biomedical Polymers, ***M. Manso Silvan,*** Institute for Health and Consumer Protection, European Commission, Italy*; A. Valsesia, M. Lejeune, D. Gilliland, G. Ceccone, F. Rossi,* Joint Research Centre, European Commission, Italy

Ion beams have become during the last years an outstanding tool for the processing of biomedical devices due to their ability to modify the structural and chemical properties of polymers. The surface chemistry, determinant factor in the performances of biosensor and tissue engineering devices, can be tailored by exposure to ion beams in different ranges of energies and ion doses. Regarding the case of noble gas implantation (Ar, He) at energies from 25 up to 100 KeV, we have found that the surfaces of biomedical polymers such as Polymethylmethacrylate (PMMA), Polystyrene (PS), Polycaprolactone (PCL) and Polyethyleneglycol (PEG) can be notably modified by exposure to doses below 10@super 14@ cm@super -2@. These transformations were applied to adapt the polymer stability in aqueous media or the surface activity towards protein attachment. A series of physico-chemical characterization tools were used in order to follow the surface and structural changes related to the implantation conditions. Fourier transformed infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectroscopy (ToF-SIMS) were used to monitor compositional changes of the implanted samples before and after interaction in biomolecular assays at protein and peptide level. Atomic force microscopy (AFM) and Ellipsometry revealed topographic and structural changes while contact angle and Zeta potential measurements evidenced changes in the molecular interaction of the polymers surface. In particular, it is shown that relevant modifications can be observed in polymer samples exposed to identical ion doses but with beams characterized by different ion density.

BI-MoP37 Plasma Sterilisation of Thermalabile Materials, ***H. Halfmann,*** *M. Schulze, M. Czichy, P. Awakowicz,* Ruhr-Universitaet Bochum, Germany

In recent years plasma sterilisation has been developed to a certain degree that makes the corresponding results reliable. Due to the advantages of plasma sterilisation great efforts in world wide investigations are made. The dry and cold process without toxic ingredients is the large benefit of the plasma sterilisation. At the institute of Electrical Engineering and Plasma Technology (AEPT) investigations on plasma sterilisation of medical implants and PET are performed. The experiments are focused on spores and germs which are important in medical, pharmaceutical and food branches. Additionally the influence of the plasma treatment on pyrogens is examined. With the sterilisation and surface modification our attention is on medical implants made of titan, UHMWPE and degradable polylactide. Conventional procedures have several disadvantages besides the long total treatment time. The plasma process reduces germs by 6 decades in a total treatment time of less than 2 minutes. Unlike common sterilisation processes the procedure is also able to reduce pyrogens. In addition to the sterilisation the surface of UHMWPE is hardened by the plasma process. Gel content measurements indicate the improvement of abrasion resistance while the bulk material is not modified. An increasing part of non-carbonated and non-acidic beverages have been bottled in PET. For sensitive products aseptic filling must be guaranteed. A plasma process prepares bottles for aseptic filling within seconds without toxic residua. To improve the shelf-live of oxygen-sensitive soft drinks a diffusion barrier made of a SiO@sub x@ layer can be deposited on the inner side of a PET bottle in a second process step. The whole process may be performed on a plasma line microwave reactor and is done in less than 10 seconds. The future work is aimed at unterstanding the mechanisms of sterilisation in the plasma with regard to ions, neutrals and radicals to optimise the procedure.

BI-MoP38 A Scanning Small Angle X-Ray Scattering (SAXS) Study of the Nanometer Length Scale Bone Structure in Connection with Implants, ***M. Foss**, M.H. Bunger, K. Erlacher,* University of Aarhus, Denmark*; L. Haisheng, Z. Xuenong, B.L. Langdahl,* Aarhus University Hospital, Denmark*; F. Besenbacher, J.S. Pedersen,* University of Aarhus, Denmark

The understanding of the interaction between bone and orthopaedic implants is important for the development of biomaterials with improved biocompatibility. The SAXS technique has previously been applied to offer structural information on mean crystal thickness, predominant orientation and degree of orientation of mineral particles in bone. Therefore, one possible application of SAXS is to investigate the nanostructure of bone in connection with ingrowth on implants, which is not possible with conventional optical techniques. Three sections of pig vertebrae with a thickness of 190μm were examined. One sample included the neurocentral growth zone, while the other two were sections with bone and pieces of either titanium or tantalum implants. Bone provided a strong SAXS signal and relatively low transmission intensity, whereas regions within the neurocentral growth zones showed a high transmission as well as a high SAXS signal. Combining the transmission and the SAXS data, it was possible to differentiate between areas of fibrous tissue and bone. This was supported by elemental analysis performed by SEM-EDAX. The mineral particles in the cancellous bone were aligned along the trabeculae, with less orientation close to the growth zone. Also, the mineral particles tended to be aligned along the implant surfaces. Within the individual bone samples, a large variation in all SAXS parameters were observed depending on the bone position relative to the implant. Furthermore, larger particle thicknesses were found in areas of bone formation, which matches our growth zone data. The data suggests that the parameters obtained by SAXS can be used to assay the local mineral particle growth. This indicates that SAXS is a powerful tool for the characterization of the detailed mineral structure of bone in the vicinity of implants.

BI-MoP39 Photoacoustic Analysis of Bone Osteogenesis to Different Doses of Irradiation Laser, ***P. Lomelí Mejia**,* IPN SEPI-ESIME, Mexico*; J.L. Jiménez Pérez,* CICATA-IPN, Unidad Legaria, Mexico*; A. Cruz Orea,* CINVESTAV-IPN, Mexico*; G. Urriolagoitía Calderón, L.H. Hernández Gomez,* IPN SEPI-ESIME, Mexico*; H. Lecona Butron,* Centro Nacional de Rehabilitación y Ortopedia, Mexico

The photoacoustic analysis of fractured bone callus to different consolidation times in presence of the irradiation laser, was performed. In this study we take into account the fractured tibias of sacrified Wistar rats. By using photoacoustic spectroscopy (PAS) technique it was possible to determine, for different doses of laser irradiation (doses from 5 to 10 Jcm-2) the presence of characteristic absorption peaks of p-Nitrophenylphosphatase (p.Npp) in the fractured bone callus. The evolution of bone consolidation was accelerated by laser radiation when compared with non irradiated fractures bones.

Biomaterial Interfaces
Room 210D - Session BI+NS-TuM

The Nano-Bio Interface

Moderators: G.J. Leggett, University of Sheffield, UK, M. Textor, ETH Zurich, Switzerland

8:20am **BI+NS-TuM1 Programmed Macromolecular Synthesis*, D.A. Tirrell,*** Caltech **INVITED**

We have developed three approaches to the synthesis of proteins and protein-like macromolecules containing novel amino acids. In the first approach, we replace every copy of one of the natural amino acids by an analogue, in effect building proteins from an altered set of twenty starting materials. This approach is most useful when one is interested in changing the overall physical properties of the protein, or in de novo design of protein-based biomaterials. A second method, which has also been implemented successfully by Schultz and coworkers, allows site-specific incorporation of a single copy of an amino acid analogue in response to a stop codon. Such methods are useful in probing protein structure and function. The third approach, developed most recently, uses mutant transfer RNAs to break the degeneracy of the genetic code, and offers the prospect of a protein chemistry based on a substantially expanded set of amino acid building blocks. This lecture will describe the most important elements of each of these strategies as well as some thoughts on the design of wholly artificial proteins with potential application in biotechnology and materials science.

9:00am **BI+NS-TuM3 The Art of Mechano-Transduction within the Extracellular Matrix*, V. Vogel,*** Swiss Federal Institute of Technology (ETH), Switzerland **INVITED**

While engineered matrices allow asking well defined questions of how cells interact and respond to their environment, it remains unclear whether a minimal set of cues exists by which synthetic matrices can be engineered that mimics biological matrices in their essential functions. Here we address how mechanical force can alter the conformation of extracellular matrix proteins and consequently regulate the display of the protein's functional states. The function of cells is tightly controlled by their interaction with the surrounding extracellular matrix to which they are coupled via the transmembrane integrins. Using intramolecular fluorescence resonance energy transfer (FRET), we studied the extent to which fibronectin is stretched and partially unfolded by the traction forces generated by fibroblasts in 2d and 3d matrices. We then derive structural models of the unfolding pathways of ECM proteins by computational techniques (steered molecular dynamics simulations), and gain insight how tension applied to extracellular matrix proteins affects the exposure of their molecular recognition sites. The consequences of our findings to the field of biomaterials and tissue engineering will be discussed. @FootnoteText@ V. Vogel, G. Baneyx, The tissue engineering puzzle: a molecular perspective, Annual Review Biomed. Eng., 5 (2003) 441-463. G. Baneyx, L. Baugh, V. Vogel, Co-existing conformations of fibronectin imaged in cell culture by fluorescence resonance energy transfer, Proc. Natl. Acad. Sci. USA, 98 (2001) 14464-14468. G. Baneyx, L. Baugh, V. Vogel, Fibronectin extension and unfolding within cell matrix fibrils controlled by cytoskeletal tension, Proc. Natl. Acad. Sci. USA, 99 (2002) 5139-5143. D. Craig, M. Gao, K. Schulten, V. Vogel, Structural insights how sequence variations tune the mechanical stability of fibronectin type III modules, Structure, 12 (2004) 21-30.

9:40am **BI+NS-TuM5 Capture and Release of Proteins on the Nanoscale by Surface-Confined Biomolecular Switches*, J. Hyun,*** Seoul National University, Korea*; W.K. Lee, N. Nath, A. Chilkoti, **S. Zauscher**,* Duke University

In this paper we describe the fabrication and characterization of stimulus-responsive elastin-like polypeptide (ELP) nanostructures grafted onto ï?·-substituted thiolates that were patterned onto gold surfaces by dip-pen nanolithography (DPN). We exploited the hydrophilic-hydrophobic phase transition of ELP in response to a change in ionic strength as a switch in order to reversibly immobilize a thioredoxin-ELP fusion protein onto the ELP nanopattern above the lower critical solution temperature. We demonstrated the biological activity of the Trx-ELP nanoarray by binding an anti-thioredoxin monoclonal antibody. Furthermore, we showed that the resulting Trx-ELP/anti Trx-mAb complex could be released below the LCST. Our research demonstrates proof-of-principle that "smart," surface-confined biomolecular switches can be built at the nanoscale. Our method of fabricating switchable surfaces is attractive because it is entirely modular and generic, in that it only requires an ELP-modified or patterned surface and a protein that can be appended with an ELP tag. ELP synthesis is easily achieved through genetic engineering techniques. The nanoscale miniaturization of on-chip separation and the presentation and triggered release of the captured proteins made possible by this methodology should be integrable into nanoscale bioanalytical devices that are currently under development.

10:00am **BI+NS-TuM6 Micro- and Nanopatterns of DNA-Tagged Vesicles*, B. Städler,*** *D. Falconnet,* Laboratory for Surface Science and Technology, Switzerland*; F Höök, I Pfeiffer,* Chalmers University of Technology, Sweden*; H Solak,* Paul Scherrer Institute, Switzerland*; J. Vörös,* Laboratory for Surface Science and Technology, Switzerland

A new approach for the creation of vesicular micro-and nanoarrays is presented based on a novel patterning approach termed Molecular Assembly Patterning by Lift-off (MAPL) in combination with the immobilization of DNA-tagged intact vesicles. This technique is shown to be a promising platform for future studies of enzyme and membrane protein activity in a controlled, native nanoenvironment. Fabrication of DNA microarrays by spotting is state-of-the-art today. This arraying technology, however, cannot be directly applied to membrane-based microarrays because the contact with the ambient environment damages the membranes. Our approach starts with conventional single stranded DNA arrays, which are subsequently converted into a membrane protein array by using phospholipidic vesicles tagged with the complementary DNAs. These functionalized vesicles specifically couple to the surface through hybridization of the DNA strands. The MAPL process was used to provide a surface with a background resistant to the nonspecific adsorption of vesicles and active spots (diameter between 1 and 200 µm) for the immobilization of the single stranded DNAs. The surface chemistry of the active spots and background consisted of biotinylated PEG and non-functionalized PEG, respectively. Complexes of biotin-terminated DNA and neutrAvidin, preformed in solution, were immobilized to the biotinylated, active spots. POPC vesicles tagged with complementary cholesterol-terminated DNA could then be specifically coupled to the surface through the hybridization of the DNA strands. Quartz crystal microbalance and optical waveguide technique were used to monitor in situ and optimize the multistep surface modification process. The micropatterns of DNA-tagged, fluorescently labeled vesicles were investigated by fluorescence microscopy. X-ray Interference Lithography was successfully used to downscale the patterning process to the nanometer scale in order to produce single vesicle arrays.

10:20am **BI+NS-TuM7 Label-Free Biosensor Based on the Surface Plasmon Resonance of Gold Nanoparticles*, S.M. Marinakos,*** *N. Nath, A. Chilkoti,* Duke University

The optical properties of gold nanoparticles immobilized on a surface were used in a label-free biosensing scheme. The sensing modality is based on the change in the local refractive index associated with receptor-ligand binding at the particle surface which shifts the surface plasmon resonance (SPR) peak in the absorbance spectra of the nanoparticles. In previous work, we have shown that solid, spherical gold nanoparticles with a size in the range of 13-50 nm could be self-assembled on amine-functionalized glass. These chemisorbed nanoparticles were then functionalized with a biotin derivative. We showed that this scheme enabled single wavelength monitoring of streptavidin binding at the surface by single wavelength measurements of the change in intensity that was caused by binding of streptavidin at the nanoparticle-solution interface. In this study, we extend these measurements to anisotropic gold nanorods, in an effort to further improve the analytical sensitivity and detection limits of this label-free transmission optical sensor. Results will be presented that compare streptavidin-biotin binding with sensors fabricated from gold nanorods with previous results on spherical gold nanoparticles.

10:40am **BI+NS-TuM8 Activation of Integrin Function by Nanopatterned Adhesive Interfaces*, J.P. Spatz, M. Arnold,*** University of Heidelberg, Germany **INVITED**

To study the function behind molecular arrangement of single integrins in cell adhesion, we designed a hexagonally close-packed rigid template of cell adhesive gold nano-dots coated with cyclic RGDfK peptide by lithographic means of diblock copolymer self-assembly. The diameter of the adhesive dots is = 73nm between the adhesive dots results in limited cell attachment and spreading and dramatically reduces the formation of focal adhesion and actin stress fibers. We attribute these cellular responses to restricted integrin clustering rather than insufficient number of ligand

molecules in cell-matrix interface since "omicro-nanopatterned" substrates consisting of alternating fields with dense and no nano-dots support cell adhesion. We propose that the range between 58-73 nm is a universal length scale for integrin clustering and activation, since these properties are shared by a variety of cultured cells.

11:20am **BI+NS-TuM10 Lifetime of Biomolecules in Hybrid Nanodevices: The Aging Process of Motor Protein-based Molecular Shuttles**, *H. Hess,* University of Washington*; C. Brunner,* ETH Zurich, Switzerland*; K.-H. Ernst,* EMPA Duebendorf, Switzerland*;* ***V. Vogel,*** University of Washington and ETH Zurich, Switzerland

Prolonging the lifetime of biomolecules in their functional states is critical for applications where biomolecules are integrated into synthetic materials or nanodevices. A simplified molecular shuttle system, which consists of fluorescently labeled microtubules propelled by kinesin motor proteins bound to the surface of a flow cell, served here as a model system for such a hybrid device. In this system, the functional decay can easily be assayed by utilizing optical microscopy to detect motility and disintegration of microtubules (MTs). We found that the lifetimes of these hybrid systems were mainly limited by the stability of MTs, rather than of kinesin. To determine the biocompatibility of polymers widely used in microfabrication, we assembled flow cells with glass bottom surfaces and covers fabricated from glass, poly(urethane) (PU), poly(methyl-metacrylate) (PMMA), poly(dimethylsiloxane) (PDMS), and ethylene-vinyl alcohol copolymer (EVOH). Without illumination, only PU had a substantial negative impact on MT stability, while PMMA, PDMS and EVOH showed stabilities comparable to glass. Under the influence of light, however, the MTs degraded rapidly on PDMS or PMMA. A similar effect was observed on glass if oxygen scavengers were not added to the medium. Strong bleaching of the fluorophores was again only found on the polymer substrates and photobleaching coincided with an accelerated depolymerization of the MTs. The presented data provide a benchmark for the lifetime of motor protein-based bionanodevices which utilize glass as the primary synthetic material, and test the impact of a variety of polymer materials on the longevity of microtubules, the most fragile biological structure in the device. This study demonstrates that our definition of biocompatibility evolves, as we progress towards architectures engineered on a molecular level, which integrate multimeric proteins and protein assemblies.

11:40am **BI+NS-TuM11 Analysis of Collision Events of Self-Propelled Biomolecular Shuttles Carrying Cargo**, ***B.C. Bunker,*** *A.K. Boal, S.B. Rivera, G.D. Bachand,* Sandia National Laboratories

Collision events between cargo carrying biotinylated microtubules (MTs) laden with 0.56 mm diameter streptavidin coated polystyrene beads (SBs) while being transported across kinesin coated surfaces were observed. Six distinct actions resulted from such collisions: no interaction, SB transfer between MTs, one MT deforming as a result of the collision, co-joining of the two MTs through mutual attachment to the SB, the SB being dislodged from the MT, or one of the MTs being severed. Interactions were studied both as a function of percent biotin-tubulin used to prepare the MTs and temperature. While biotin percent was observed to have a negligible effect of the percent chance of the various outcomes, heating the system from 24ËsC to 30ËsC decreased the likelihood of a SB transfer event while increasing the rate of MT bending and dragging events. Two important factors are proposed to determine the outcome of these collisions: the geometry of the collision event and the nature of the binding site that the SB is originally attached to.

Biomaterial Interfaces
Room 210D - Session BI-TuA

Biomembranes on a Chip
Moderator: J.Y. Wong, Boston University

1:40pm **BI-TuA2 Binding and Aggregation of @alpha@-Synuclein on Supported Lipid Bilayers**, ***J.S. Hovis***, *E.A. Gamble, M.C. Hull, J.-C. Rochet,* Purdue University

Interest in @alpha@-synuclein was initiated with the observation that two mutations in the @alpha@-synuclein gene are linked to an early onset form of Parkinson's disease. @alpha@-Synuclein was further implicated in Parkinson's disease when the protein was found to be the main component of Lewy body inclusions in the brains of Parkinson's disease patients. In solution @alpha@-synuclein is natively unstructured while in the Lewy bodies it is primarily @beta@-sheet in character. It has been shown that @alpha@-synuclein readily binds lipid vesicles containing negatively charged lipids and that upon binding the protein adopts an @alpha@-helical conformation. Interestingly, the aggregation of @alpha@-synuclein into @beta@-sheet fibrils appears to be enhanced in the presence of negatively charged lipids. Due to the small size of the vesicles used in the previous studies the growth of the aggregates could not be observed directly. To provide more insight into the necessary conditions for the aggregation of @alpha@-synuclein we have observed the binding of @alpha@-synuclein to supported lipid bilayers using epi-fluorescence microscopy and infrared spectroscopy. The extent of aggregation was observed to depend on time, salt concentration, protein concentration and lipid composition. Results will be presented highlighting the necessary conditions for aggregation and comparing the conditions needed for wild-type aggregation with those of two mutant proteins (A30P and A53T) which have been linked with early onset Parkinson's.

2:00pm **BI-TuA3 Bioanalytics in the Nanometer and Attoliter Range**, ***H. Vogel***, Swiss Federal Institute of Technology Lausanne, Switzerland **INVITED**

Spatial compartmentalisation is a prerequisite for the creation of living matter. Without the existence of clearly defined borders, differentiation and diversity at the cellular level would not be possible. Most scientific disciplines that deal with dissolved molecules are concerned with the same problem of subdividing solutions in miniaturised autonomous units, either to increase the functional complexity of a system, reduce reagent consumption, monitor fast chemical kinetics or even to study single-molecules. I will report on our recent progress that allows the massively parallel isolation of attoliter- sized artificial and native, cell-derived vesicles and their self-assembled positioning with 100-nm precision in ordered arrays on surfaces. The broad application for investigating (bio)chemical reactions and cellular signaling processes in individual containers by electrical and optical techniques will be discussed. The biological processes which will be presented are mediated on and across cellular membranes via transmembrane receptors such as transport-, channel-proteins or G protein-coupled receptors to mention some important examples. Our novel approaches are important for the elucidation of the molecular basis of receptor function and signal transduction processes as well as for applications in the field of screening for novel therapeutic compounds.

2:40pm **BI-TuA5 Construction and Characterization of Planar Lipid Bilayers Supported on Conductive Thin Polymer Films: Toward Artificial Photosynthetic Supramolecular Devices**, ***L. Wang***, *T. McBee, S. Marikkar, C. Ge, N. Armstrong, S. Saavedra,* The University of Arizona

We are developing a new type of biomimetic photosynthetic device based on a photoactive lipid bilayer supported on a planar optical waveguide electrode overcoated with indium-tin oxide (ITO). The bilayer serves as a host for incorporation of artificial photosynthetic centers, which are prepared by Moore, Gust, and Moore (Arizona State University). A water-swollen, conductive polymer cushion is used to couple the bilayer to the ITO surface. The polymer cushion is required to planarize the ITO support and render it compatible with vesicle fusion, as well as act as a transducer of light-generated proton flux across the lipid layer, so that changes in flux can be detected both electrochemically and spectroscopically. In this presentation, we will summarize recent progress in preparation and characterization of lipid bilayers deposited on conductive polymer films composed of poly(aniline) (PANI) and poly(acrylic acid) (PAA), which are deposited by layer-by-layer self-assembly on ITO. A variety of lipid compositions, polymer compositions, and assembly conditions have been compared. An array of methods has been used to characterize these assemblies, emphasizing the diffusive properties of the lipid components, the spectroscopic and electrochemical responses of the PANI/PAA film, and the barrier properties of the lipid layer. Different lipid systems exhibit different diffusive properties; these appear to be correlated with the degree to which the potentiometric response of the PANI/PAA is blocked by lipid bilayer deposition. Egg phosphatidylcholine/cholesterol appears to form a continuous, nearly pinhole free bilayer on 2(PAA/PANI)/ITO, which is attributed to the role of cholesterol as a stabilizer in supported lipid films.

3:20pm **BI-TuA7 Nanoscale Dissection of a T Cell Immunological Synapse**, ***J.T. Groves***, University of California, Berkeley **INVITED**

Coordinated rearrangement of cell membrane receptors into large-scale patterns is emerging as a broadly significant theme of intercellular signal transduction. In an effort to help unravel the mechanisms governing protein organization at intercellular synapses and the role of this organization in signal transduction, we have dissected living T cell immunolgical synapses in a hybrid live cell - supported membrane configuration. Nanometer-scale patterns of fluid lipid membranes, displaying cell recognition and signaling molecules, have been constructed on solid substrates by a combination electron-beam and scanning-probe lithographic techniques, along with membrane self assembly. When doped with appropriate proteins, supported membranes mimic and antigen presenting cell and can form synapses with living T cells. The substrate nanostructures guide the mobility of membrane-linked proteins and, correspondingly, the motion of their cognate partner proteins within living cells. A critical feature of this strategy is that proteins displayed in the supported membrane exhibit diffusive mobility. This enables formation of functional synaptic structures with living cells by permitting the necessary protein rearrangements. The manner in which precisely defined geometrical restrictions frustrate or facilitate synapse formation and signaling in living cells can be used to elucidate the mechanisms and functional consequences of molecular patterns at intercellular synapses.

Biomaterial Interfaces
Room 210D - Session BI1-WeM

Cell-Surface Interactions
Moderator: A. Chilkoti, Duke University

8:20am **BI1-WeM1 Study of Confluent Cell Culture Monolayers by XPS and SIMS**, *M. Greenfeld, H.E. Canavan, X. Cheng, B.D. Ratner,* ***D.G. Castner,*** University of Washington

Adhered cells transform the surfaces on which they are cultured by excreting and remodeling the underlying extracellular matrix (ECM) proteins. As the ECM is known to play a vital role in the processes of differentiation, motility, and proliferation, the characteristics and identity of the ECM proteins excreted by different cells, or by the same cells throughout its lifetime, are of a great deal of interest in biology and surface science alike. Until now, traditional high vacuum techniques such as X-ray Photoelectron Spectroscopy (XPS) and Secondary Ion Mass Spectrometry (SIMS) have played only a minor role in the analysis of the ECM, as traditional cell removal techniques are destructive to both the cells and the underlying ECMâ?"in effect damaging the structure of analytical interest. Recently, poly(n-isopropylacrylamide) (pNIPAM) treated tissue culture polystyrene (TCPS) has been developed as a method to non-destructively harvest intact cell monolayers. Using the low-temperature liftoff technique, cell sheets may be non-destructively removed from surfaces and re-deposited atop new surfaces, achieving multilayer structures of different cell types, such are used for tissue engineering. To date, studies of cells harvested via this method have primarily utilized traditional biological techniques to track the morphology of the cells and the location of their ECM proteins. Previously, we have used XPS and SIMS to examine culture surfaces after cell liftoff and identify the ECM proteins retained. In this work, we present the first application of high vacuum techniques to an examination of cell monolayers harvested by low-temperature liftoff. We find that the presence of proteins in the basal surface of the ECM is easily detected via XPS and SIMS. We then compare the identities and relative amounts of ECM proteins at the apical and basal surfaces of the cell sheet to those retained by the underlying surface.

8:40am **BI1-WeM2 Modeling of Bacterial Attachment Using Lewis Acid-Base Models of Colloidal Adhesion**, ***L.K. Ista***, *K. Artyushkova, T.M. Madrid, J.E. Fulghum, G.P. Lopez,* The University of New Mexico

Understanding the processes involved in primary bacterial adhesion to solid surfaces is an important step in development of surfaces on which biofilm formation can be controlled. The relationship of the interfacial tensions between the attaching organism, the liquid medium and the solid substratum determines whether or not attachment can proceed. Control of bacterial attachment is most easily addressed, therefore, by control of substratum surface energy. The relationship between surface energy and attachment can be described qualitatively using colloidal models of adhesion, although a definitive quantitative model is still elusive. Most attempts at modeling bacterial attachment have been made using data generated from attachment to commercially available substrata or their derivatives, many of which are chemically ill-defined. Investigation of substratum physicochemistry on the attachment of a marine bacterium is described. Model solid substrata were generated using mixed self-assembled monolayers of @omega@-terminated alkanethiolates on gold. These substrata varied systematically in Lewis acid-base, dispersive and polar characteristics, while controlling for other surface factors that may affect bacterial adhesion. The test bacterial strain was the gram negative, marine bacterium Cobetia marina. The surface energetics of this organism were determined by partition into organic solvents that differed in their surface energy . Attachment of this organism to SAM surfaces was then quantified and modeled using standard Lewis acid base models of colloidal attachment and multivariate analysis.

9:00am **BI1-WeM3 Deconstruction the Cell-Biomaterial Interface**, ***J.Y. Wong,*** Boston University **INVITED**

Cells respond to three main categories of physicochemical cues: chemical, topographical, and mechanical. While surface chemistry and topography have been studied extensively, substrate mechanics has only recently been appreciated. Recent technologies of creating surfaces with well-defined chemistry and topography combined with sensitive surface characterization techniques have unquestionably deepened our understanding of surface chemical and topographical effects on cell behavior. In contrast, much less is known about substrate mechanics effects on cell behavior. This talk discusses the types of substrata and characterization methods that have been used to investigate substrate mechanics effects on cell behavior. We also speculate on the relationships between changes in substrate elasticity that occur naturally in vivo (e.g. wound healing) and cellular response. We present recent developments in creating substrata with well-defined mechanical properties in our own laboratory and the major challenges and issues of determining whether substrate mechanics effects are a material-independent phenomenon. We also discuss the effects of combining multiple physicochemical cues on cell behavior. The use of model systems in which chemistry, topography, and mechanics can be independently controlled will facilitate the quest for design principles and material selection rules to control cell response.

9:40am **BI1-WeM5 Supramolecular Structure of Adsorbed Collagen Layers and Influence on Endothelial Cells Behavior**, ***C.C. Dupont-Gillain***, *E. Gurdak, Z. Keresztes, P.G. Rouxhet,* Universitat Catholique de Louvain, Belgium

The aim of this study is to examine the supramolecular organization of adsorbed collagen and to evaluate its influence on endothelial cells, thereby increasing our understanding of cell-material interactions. Collagen was adsorbed on polystyrene (PS) and plasma-oxidized PS (PSox) in different conditions, likely to affect the supramolecular structure of the adsorbed layers. The collagen layers and their mechanism of formation were examined using atomic force microscopy, quartz crystal microbalance, X-ray photoelectron spectroscopy and radiolabeling. On PS, the adsorbed collagen molecules leave protruding segments in solution, allowing fibril formation at the interface; this increases with concentration and with time. Dewetting of the collagen layer leads to the formation of discontinuous layers with a net-like nanopattern. On PSox, collagen mainly forms a felt of lying molecules. The adhesion of human umbilical vein endothelial cells (HUVEC) was studied on collagen layers adsorbed on PS or PSox and presenting a diversity of supramolecular structures. In presence of serum, HUVEC cells could not adhere to PS. After adsorption of a smooth collagen layer, cell adhesion became high, and increased with the adsorbed amount. However, the formation of fibrils at the interface provoked a decrease of cell spreading. The last trend was also observed on PSox. This may be related to the accessibility of recognition sites, which could be hidden once collagen forms fibrils. In contrast, the spreading of HUVEC cells was enhanced on discontinuous collagen layers compared to smooth, continuous ones. In this case, collagen association was triggered by dewetting, which could change the availability of recognition sites. Moreover, the discontinuous pattern could stimulate the organization of cell surface receptors, or allow coadsorption of proteins secreted by the cells. Further work includes antibody assays to assess the availability of recognition sites on adsorbed collagen.

10:00am **BI1-WeM6 Using Thin Films of Fibrillar Type I Collagen to Investigate a Signaling Mechanism that Mediates Growth Arrest in Smooth Muscle Cells**, ***J.T. Elliott,*** National Institute of Standards and Technology **INVITED**

Smooth muscle cells (SMC) on fibrillar collagen activate different signaling pathways, have a minimally spread morphology and appear growth arrested compared to SMC cultured on non-fibrillar native collagen. Because studies suggest that SMC interact with both matrices through the same integrin receptors, it appears that it is the supramolecular fibrils that are responsible for the phenotypic response. We used thin films of fibrillar collagen assembled on hexadecanethiol monolayers to investigate which properties of the collagen fibrils control the proliferation signaling. The films are on average 30 nm thick and composed of collagen fibrils that are microns long and as large as several hundred nanometers in diameter. They also have optical properties that are ideal for both phase and fluorescence microscopy. When the fibrillar films are kept hydrated, they induce a growth arrest response in SMC that is similar to the response that is observed on fibrillar collagen gels. If the thin films are dried for several hours before rehydration, the SMC exhibit a well-spread proliferative phenotype and begin to proliferate. Atomic force microscopy (AFM) analysis of these fibrillar films indicates that they are nearly identical in topography, density of fibrils and size of fibrillar structures. These data suggest that the presence of collagen fibrils alone is not sufficient to induce the growth-arrested phenotype. AFM imaging of the fibrillar films under aqueous conditions suggest that the flexibility of the collagen fibrils is reduced during the drying process. We hypothesize that the mechanical properties of the fibrils are an essential determinant of the SMC growth-arrest response. We are currently using live-cell microscopy to understand how these cells interrogate the mechanical properties of collagen fibrils when deciding their phenotypic state.

10:40am **BI1-WeM8 Engineering of Functional Three Dimensional Cell Structures by Inkjet Printing**, ***T. Boland***, *P. Kesari, T. Xu,* Clemson University*; D. Varghese,* Southampton General Hospital

Tissues and organs exhibit distinct shapes and functions nurtured by vascular connectivity. In order to mimic and examine these intricate structure-function relationships, it is necessary to develop efficient strategies for assembling tissue-like constructs. Many of the top-down fabrication techniques used to build microelectromechanical systems including photolithography are attractive due to the similar feature sizes, but are not suitable for delicate biological systems or aqueous environments. A bottom-up approach using inkjet printers has been proposed to pattern functional cell structures in three dimensions. The freeform cell structures created by the inkjet method are viable and show mature character as exemplified by the contractile responses of smooth muscle cell tubes. These results show promise of the inkjet method for vascular tissue engineering and other applications.

11:00am **BI1-WeM9 Nanofabrication of a Novel Cell Array on Ultrathin Hydrophilic Polymer Gels Utilizing Electron Beam Irradiation and UV Excimer Laser Ablation**, ***M. Yamato***, Tokyo Women's Medical University, Japan **INVITED**

Many methods for surface patterning presented to date are based on lithography techniques and microfabrication onto silicon or glass substrates. Here, we show a novel method to prepare patterned surfaces on polystyrene substrates by grafting ultrathin cell-repellent polymer layers utilizing both electron beam (EB) polymerization and local laser ablation techniques for microfabrication. Polyacrylamide(PAAm) was grafted onto tissue culture polystyrene (TCPS) dishes using electron beam irradiation. Water contact angles for these PAAm-grafted TCPS (PAAm-TCPS) surfaces were less than 10° with grafted amounts of PAAm of 1.6 mg/cm@super 2@ as determined by FT-IR/ATR method. UV excimer laser (ArF: 193 nm) ablation resulted in the successful fabrication of micropatterned surfaces by exposure of the basal polystyrene layers. Many cell types adhered only to the ablated domains after pretreatment of the patterned surfaces with optimized concentration of fibronectin solution. The ablated domain sizes have significant influence on the number of cells occupying each domain. Cell patterning functionality of the patterned surfaces was maintained for more than 2 months without losing pattern fidelity. Utilization of these surface fabrication techniques are also presented for basic cell biology as well as preparation of cell-based biosensors.

11:40am **BI1-WeM11 Evaluation of PDMS as a Model Substrate to Investigate Effects of Substrate Compliance on Cell Behavior: Interplay of Surface Chemistry and Substrate Mechanics**, ***X.Q. Brown***, *J.Y. Wong,* Boston University

Polydimethylsiloxane (PDMS) is an attractive model system for studying the effects of tissue mechanical properties on cell behavior, because the elastic modulus of PDMS can be tuned to achieve a physiologically-relevant range. However, it has been suggested that altering crosslink density can also modulate surface properties. Both the chemical and mechanical properties of a substrate can affect cell behavior: while the importance of surface chemistry and substrate mechanics have been studied independent of each other, few studies have considered their integrated effects. In this study, we characterized the mechanical and surface properties of PDMS substrata with different crosslink density and systematically investigated the effect of PDMS crosslink density on vascular smooth muscle cell (VSMC) attachment, spreading and proliferation. We find that after the same surface treatment, the water contact angle of PDMS decreases with decreased crosslink density, whereas the amount of protein adsorbed onto the material surface remains the same. We also find that in the absence of serum, there is a 39% decrease in cell attachment and a 42% decrease in projected cell area as the Youngâ?Ts modulus decreases from 1.79 to 0.05 MPa. Although these differences in VSMC adhesion are diminished in the presence of serum or adsorbed fibronectin, the rate of serum-stimulated cell proliferation is significantly lower on PDMS with higher crosslink density. We conclude that for the range of crosslink density we investigated, the surface properties of PDMS play a major role in controlling the initial attachment and spreading of VSMC, whereas the mechanical properties of PDMS influence the long term growth of VSMC.

Biomaterial Interfaces
Room 213C - Session BI2-WeM
Oligo Nucleotide - Surface Interactions

Moderator: M.J. Tarlov, National Institute of Standards and Technology

9:00am **BI2-WeM3 Characterization of Surface Order and Structure of Thiolated Single-stranded DNA Oligomers on Gold by XPS and NEXAFS**, ***L.J. Gamble***, *C.-Y. Lee, N.T. Samuel, H.E. Canavan, D.G. Castner,* University of Washington

The hybridization efficiency of DNA microarrays and biosensors is determined in part by variables such as the density and orientation of the single stranded DNA oligomers used to build the devices. In this study, we have used x-ray photoelectron spectroscopy (XPS) and near-edge x-ray absorption fine structure spectroscopy (NEXAFS) to characterize the surface order and structure of thiol-terminated ssDNA on gold. While NEXAFS provides an investigation of the order and orientation of the DNA oligomers at a surface, XPS provides a quantitative measure of the amount of DNA at a surface. We find that when thiolated DNA oligonucleotides (T)@sub 16@, (A)@sub 16@, and (A)@sub 8@(C)@sub 8@ are adsorbed to gold surfaces, their surface orientations vary differently with increased adsorption time. Time dependent studies of thiolated (T)@sub 16@ and (A)@sub 16@ oligomers by NEXAFS showed opposite polarization dependence between the two sequences possibly due to differences in their interactions with the gold surface. XPS results also indicate variations in the phosphorus to gold ratios among the three thiolated DNA oligomers over time. Furthermore, we explain these contrasting observations by examining the interaction of unmodified DNA strands that are non-specifically adsorbed to gold. Although polyT does not interact with the gold surface, polyA binds to gold possibly through the amine groups of the bases.

9:20am **BI2-WeM4 Detection of DNA Hybridization by Infrared Absorption Spectroscopy**, ***K. Miyamoto***, *Y. Kimura, H. Ishii, M. Niwano,* Tohoku University, Japan

We have previously proposed a new, label-free method of in-situ (in-vitro) determining the chemical bonding conformation of DNA in aqueous solution, by infrared absorption spectroscopy in the multiple internal reflection geometry (MIR-IRAS). In our method, a Si prism, through which infrared lights penetrate, internally reflecting a number of times, serves as an electrochemical electrode. By applying a positive or negative potential to the electrode (prism), we can manipulate negatively-charged DNA molecules in aqueous solutions. In this study, we have selected the chemical system of complementary DNA as an appropriate template for testing the possible application of our method to biosensors for detecting DNA hybridization. We first collect IRAS spectra for complementary single-stranded (ss) DNAs that are comprised of 30 bases, and then analyze the hybridization by observing the spectral changes in the IRAS spectra caused by mixture of the two complementary DNAs in D@sub 2@O solution. We observed significant spectral changes in the frequency region of 1600-1750 cm@super -1@, where the bases of DNA have specific vibration modes (C=O stretching and -NH@sub 2@ scissoring modes) that are quite sensitive to base-paring. On the other hand, no significant spectral changes were observed for mixture of non-complementary DNAs. This confirms that the observed spectral changes were specifically induced by DNA hybridization. Our method would be capable of detecting and classifying other biomolecules such as proteins and peptides.

9:40am **BI2-WeM5 XPS and SIMS Characterization of Oligonucleotide Immobilisation via Patterned Plasma Polymerized Interlayers**, ***P-C. T. Nguyen***, University of South Australia, Australia*; R. Metz, S. Kumar,* University of South Australia*; M. DeNichilo,* TGR BioSciences, Australia*; N. Voelcker,* Flinders University of South Australia*; M. Jasieniak,* University of South Australia*; S. Coultas, S. Hutton,* Kratos Analytical Ltd, UK*; H.J. Griesser,* University of South Australia, Australia

Surface immobilised oligonucleotides are an attractive choice as recognition elements in microarrays for parallel, multidimensional, high throughput analysis in many biosensing applications. We investigate the physico-chemical factors affecting efficient oligonucleotide immobilisation and patterning. Furthermore, by using patterned plasma polymer coatings, we implemented a direct, one-step method of fabricating microdot arrays with specific surface chemistries onto which oligonucleotides can be covalently immobilised. Amino terminated oligonucleotides such as 15-T are immobilised via surface aldehyde groups of plasma polymerised polymers. Propanal plasma polymer coatings have been applied to a wide variety of substrates including silicon and Teflon. A mask containing 100

µm diameter holes was used during plasma polymerisation to produce an array of distinct aldehyde surface chemical regions for oligonucleotide immobilisation. XPS analysis of the plasma polymer and the immobilised oligonucleotides gave rise to the expected C, O, N and P peaks. Using atomic concentration, surface coverage is calculated as a measure of immobilisation efficiency. SIMS spectra revealed characteristic thymine containing fragments from the immobilised oligonucleotides. MALDI-MS verified the covalent attachment of the oligonucleotides and their ability to hybridise a complementary strand of 15-A oligonucleotide. Hybridisation kinetics studies are underway. SIMS imaging is used to document the spatial patterns formed by masked plasma deposition and to assess the spatial selectivity of oligonucleotide immobilisation. Small spot XPS will be used to quantify the composition of the microarray dots. Results to date demonstrate that the plasma deposition step, both patterned and unpatterned, is readily transferable to various substrates including Si wafer, Teflon PTFE and Teflon PFA.

10:00am **BI2-WeM6 Molecular Recognition in 2D Binary Mixtures of DNA-Base Molecules Studied by STM***, **M. Schöck**, R. Otero, L.M. Molina, E. Laegsgaard, I. Stensgaard, B. Hammer, F. Besenbacher,* University of Aarhus, Denmark

Molecular recognition events between complementary nucleic acid bases are fundamental for many biological processes, like DNA replication, and is currently being exploited for self-assembling DNA-based nanostructures. The DNA replication fidelity in living organisms is maintained by a complex molecular machinery of polymerases, exonucleases, etc. On the other hand, in the case of replicating NA molecules in the prebiotic soup, the basic physico-chemical mechanism to steer the replication process is the hydrogen-bonding between DNA bases. The fidelity of this replication process implies that Watson-Crick pairing must be favored over others, like "wobble" or "deviant" pairing. By means of a combination of STM experiments and DFT calculations, in this contribution we compare the 2D molecular networks formed on Au(111) upon deposition of the binary mixtures G-C (purine-pyrimidine pair of complementary bases) and A-C (purine-pyrimidine pair of non-complementary bases). We show that, after a gentle annealing to 80°C the non-complementary bases segregate into islands of pure A and a network of pure C, whereas the complementary bases G and C form a network that cannot be separated by annealing up to the desorption temperature for C. High-resolution STM images allow us to identify the structures for these enhanced thermal stability as structures that contain G-C bonds possibly with the same structure as the Watson-Crick pairs in DNA molecules. This result shows that the hydrogen-bonding interaction alone can steer the molecular recognition process necessary for high-fidelity DNA replication even in the absence of polymerases, exonucleases, etc.,a result that could be relevant to understand the origin and nature of the first self-replicating molecules in the prebiotic soup.

10:20am **BI2-WeM7 Formation of ssDNA Brushes with Controlled Length and Spacing on Gold***, **A. Opdahl**,* National Institute of Standards and Technology*; D.Y. Petrovykh,* University of Maryland*; H. Kimura-Suda,* National Institute of Standards and Technology*; L.J. Whitman,* Naval Research Laboratory*; M.J. Tarlov,* National Institute of Standards and Technology

A method is presented that uses block-oligonucleotides to generate single stranded (ss)DNA brushes with controlled length and density on gold surfaces. The method is based on previously reported observations that adenine oligonucleotides (dA) have a higher affinity than thymine oligonucleotides (dT) for adsorbing on gold substrates (Kimura-Suda, H.; Petrovykh, D. Y.; Tarlov, M. J.; Whitman, L. J.; J. Am. Chem. Soc.; 2003; 125(30); 9014-9015). In this study, adenine/thymine block-oligonucleotides, d(T@sub m@-A@sub n@), with specific (dT) and (dA) sequence lengths, m and n, were adsorbed on gold substrates from aqueous solution and were characterized by FTIR and by XPS. The FTIR and XPS experimental results support a model where the (dA) nucleotides preferentially adsorb on the gold substrate and the (dT) sequences extend away from the substrate. The surface density of the (dA@sub n@) blocks decreases with their length n, such that the overall surface density of (dA) nucleotides adsorbed on the gold is approximately independent of the (dA@sub n@) block length. Therefore d(T@sub m@-A@sub n@) oligonucleotides with long (dA) sequences (larger n) have lower densities of (dT) brush strands in the adsorbed layer. Oligonucleotides with long (dT) sequences, m, are observed to have longer brush strands extending away from the substrate. The n and m dependent adsorption behaviors and the stabilities of the brush layers will be compared to the behavior of alkanethiol derivatized ssDNA monolayers on gold.

10:40am **BI2-WeM8 Coverage and Stability of ssDNA on Gold: Effects of Temperature and Displacement by Alkanethiols***, D.Y. Petrovykh,* University of Maryland and Naval Research Laboratory*; A. Opdahl, H. Kimura-Suda, M.J. Tarlov,* National Institute of Standards and Technology*; **L.J. Whitman**,* Naval Research Laboratory

We characterize self-assembled films of thiolated and unmodified single-stranded DNA (ssDNA) on polycrystalline Au films using Fourier transform infrared (FTIR) and X-ray photoelectron (XPS) spectroscopy. We use homo-oligonucleotides to study the film stability as a function of the DNA-base under conditions used in hybridization experiments. One common method of controlling the ssDNA probe spacing and availability is post-deposition exposure to alkanethiols. Another common post-deposition treatment is exposure to buffer solution at elevated temperature during the hybridization step. In both cases, we find strong base-dependence in agreement with the previous results for film structure and relative adsorption affinities of thiol-modified and unmodified ssDNA [JACS 125, 5219 (2003); 125, 9014 (2003)]. The use of these post-deposition treatments also allows us to compare the relative effects of DNA-DNA vs. DNA-Au interactions for each of the bases. For the three bases that we examined, a wide range of DNA-DNA and DNA-Au interactions is observed: both interactions are weak for oligo(dT); oligo(dA) exhibits a strong affinity for Au surfaces but weak DNA-DNA interactions; and oligo(dC) represents the opposite case, with strong DNA-DNA but weak DNA-surface interactions.

11:00am **BI2-WeM9 Biomolecular Immobilization in a Sugar Polyacrylate Hydrogel***, **M.S. Spector**, P.T. Charles, B.D. Martin, C.M. Soto, C.H. Patterson,* Naval Research Laboratory

A novel sugar polyacrylate hydrogel has been developed as a substrate for high density microarrays. Copolymerization of chemo-enzymatically synthesized 6-acryloyl-@beta@-O-methyl-galactopyranoside with acrylate monomers containing terminal amine or carboxyl functionalities allows for covalent attachment of the oligonucleotides or proteins. The hydrogels show extremely low non-specific adsorption of biomolecules leading to increased signal-to-noise ratio and enhanced immunoassay sensitivity over two-dimensional surfaces. High density microarrays containing oligonucleotides and protein toxins have been obtained using a non-contact microdispensing system onto thin hydrogel films. The large pore size and solution-like environment of these hydrogels allow for easy penetration of large biomolecules and detection reagents. Confocal microscopy was used for three-dimensional visualization of the gel, immobilized biomolecules, and hybridized DNA. Results indicate that DNA diffuses into the hydrogel as discrete spots with higher concentration near the middle of the gel. Hybridization of 70-mer nucleotides was readily observed in these gels.

Applied Surface Science
Room 210A - Session AS+BI-WeA

Biological Applications of Surface Analysis
Moderator: A. Belu, Medtronic

2:00pm **AS+BI-WeA1 G-SIMS-MS: Towards Molecular Structure at Surfaces**, ***I. Gilmore***, *F. Green, M. Seah,* National Physical Laboratory, UK

SSIMS is a powerful technique for the analysis of complex surfaces. However, many view SSIMS as an excellent research tool but unreliable as an analytical method. This is changing. Modern instruments have superb repeatability and reliability. In the VAMAS 2002 SSIMS inter-laboratory study, the average repeatability of 27 instruments was already 2%. Accessibility to SSIMS measurements is increasing rapidly. However, the complexity of mass spectra makes identification and quantification far from straightforward, even for the experts! This is a major barrier to the wider take-up of SSIMS, especially in new fields. One way around this problem is G-SIMS. G-SIMS or gentle SIMS is a library independent method providing a straightforward way to simplify SSIMS spectra@footnote 1 @@footnote 2 @@footnote 3 @. SSIMS spectra are composed of parent fragment ions amongst a large number of high intensity degradation products. In G-SIMS, this fragmentation may be quantified in terms of the partition functions of the fragments emitted from a surface plasma with effective temperature, T@sub p@. By extrapolation of the data to low T@sub p@, the intensity of the degradation products rapidly reduces, revealing the parent fragments. The latter peaks are directly characteristic of the material without rearrangement and can enable direct interpretation and identification. This is fine for smaller molecules but, within the plethora of possible larger molecules for which a total mass is insufficient to provide adequate characterisation, an extension of G-SIMS has exciting prospects to elucidate the required structure. Here we use, G-SIMS-MS, to explore the re-building of parent molecules using the fragmentation pathways that are mapped out as T@sub p@ is varied. @FootnoteText@ @footnote 1@ I S Gilmore and M P Seah, Appl. Surf. Sci., 161 (2000) 465. @footnote 2@ I S Gilmore and M P Seah, Appl. Surf. Sci., 187 (2002) 89. @footnote 3@ I S Gilmore and M P Seah, Appl. Surf. Sci., 203-204 (2003) 551.

2:20pm **AS+BI-WeA2 Functional Molecular Surfaces for Healthcare - Characterisation, Analysis and Understanding**, ***M.C. Davies***, *S.J.B. Tender,* University of Nottingham, UK*; P.M. Williams,* University of Nottingham, UK, U.K.*; C.J. Roberts, S. Allen,* University of Nottingham, UK **INVITED**

The characterisation of the surface structure of both conventional and advanced biomedical systems can be an important step in understanding the performance and optimising the function of such healthcare devices. A number of advanced biophysical analytical techniques have emerged for the study of pharmaceutical and biomedical systems. In this talk, we shall explore the role of these analytical tools as complimentary techniques, in the study of surface structure and function of advanced polymeric materials. The value and limitations of visualisation of surface topography and morphology of polymeric devices will be discussed and will include the condensation of polymeric constructs for gene therapy to the single molecule imaging of micro-patterned proteins on nanoengineered tissue-engineering substracts. The role of the force microscope in determining interparticulate and inter-molecular forces in order to explore its potential for the study of biomolecular interactions at polymer interfaces through to the macromolecular stimuli response hydrogels will be reviewed. The potential of the biophysical methodology of high-resolution imaging and force spectroscopy to aid research in biorecognition, development of gene delivery systems and understanding interparticulate and molecular forces, will be highlighted. The ability to identify the chemical structure of the molecules laterally across of a surface for both model and complex multiplayer patterned macromolecular assemblies shows promise but faces major challenges to low surface density ligands and high throughput array systems. The need for new approaches for handling large data sets of surface information and their relationship to surface functionality remains a significant challenge. The talk will aim to provide an objective assessment of current status, future challenges and opportunities.

3:00pm **AS+BI-WeA4 Model Calculations for the Quantification of XPS-Results; Application to Self Assembled Monolayers on Gold**, ***C. Van der Marel***, *J.H.M. Snijders, H.R. Stapert,* Philips Research, The Netherlands

XPS-analysis is widely applied for the characterization of surfaces and multilayers of thin films. In order to obtain quantitative results, the XPS peak areas generally are divided by appropriate sensitivity factors and normalized to 100 at% to obtain the apparent concentrations. Within the model developed by us, the sample is assumed to consist of a substrate on top of which a number of homogeneous layers are present. Starting from the apparent concentrations, the model calculation provides the thickness, the number of sulphur atoms per surface area and the composition of all layers. The proposed method requires only one measurement at one measuring angle to obtain these results. The method has been verified for a large series of self assembled monolayers made of mercaptoalkyl-polyethylene glycol compounds with various chain lengths on gold-plated Si. The influence of deposition time upon the obtained SAM-layers was examined; also SAM-layers deposited from mixtures of molecules with different chain length were investigated. RBS analysis was applied to determine in an independent way the amount of sulphur at the gold surface (expressed in number of Sulphur atoms per unit area); the RBS results correlated well with the XPS data. Also XPS-results obtained from mixtures of mercaptodeca-triethylene glycol and biotinylated thiols were analyzed in this way. The method resembles the calculation method proposed recently by Petrovykh et al@footnote 1@. Yet, in the latter method the analysis is restricted to only one layer on top of a substrate. Moreover, ion-etching is required to obtain a reference signal of the gold substrate; the consequence is, that elastic scattering of Au4f-electrons in the top layer is partly neglected in Ref.@footnote 1@. @FootnoteText@ @footnote 1@ D. Y. Petrovykh, H. Kimura-Suda, M.J. Tarlov and L.J. Whitman, Langmuir 20 (2004) 429.

3:20pm **AS+BI-WeA5 Surface Analysis by Friction Force Microscopy**, ***G.J. Leggett***, University of Sheffield, UK*; N.J. Brewer,* Dundee University, UK*; K.S.L. Chong,* University of Sheffield, UK

The characterisation of surface chemical structure on the nanometre scale still presewnts significant challenges. Friction force microscopy (FFM) is a widely accessible technique typically provided as standard on commercial atomic force microscoopes. It is capable of providing significant insights into variations in surface chemical composition and molecular organisation. The sensitivity of FFM to changes in molecular organisation will be illustrated with data from studies of self-assembled monolayers (SAMs) on Au and Ag. It will be shown that unexpected packing density differences, revealed by FFM, correctly predict the variation in the photo-oxidation kinetics of these materials. FFM suggests that while SAMs of methyl temrinated adsorbates on Ag are more closely packed on than they are on Au, the reverse is the case for monolayers of carboxylic acid terminated thiols. Methyl terminated SAMs on Ag oxidise more slowly than similar monolayers on Au, while the reverse is true for carboxylic acid terminated SAMs, reflecting the strong influence of molecular packing on photo-oxidation kinetics. The kinetics of SAM photo-oxidation have also been studied and quantified by FFM. Samples of carboxylic acid terminated thiols were exposed to UV light for varying periods of time and then immersed in solutions of methyl terminated thiols. Oxidised adsorbates were replaced by solution-phase thiols. For macroscopic samples, the variation in the coefficient of friction determined by FFM as a function of SAM photo-oxidation correlates closely with the variation in the contact angle (ie, as oxidation proceeds the SAMs become increasingly hydrophobic, and exhibit an increasingly small coefficient of friction). Similar types of analysis may be used to quantify rates of reactions in photopatterned materials SAMs. For materials with structures as small as a few tens of nm, fabricated by scanning near-field optical lithography, FFM enables the monitoring of chemical reactivity.

3:40pm **AS+BI-WeA6 Synthesis, Characterization and Modeling of Tethered Poly (N-isopropylacrylamide)**, ***S. Mendez***, *G.P. Lopez,* The University of New Mexico*; H. Yim, M.S. Kent, J.G. Curro,* Sandia National Laboratories*; J.D. McCoy,* New Mexico Tech

Tethered polymers are widely used to control surface properties such as adhesion and wettability. By making thin films out of polymers that are thermo-responsive, we can modulate surface properties with changes in temperature. Specifically, we use poly(N-isopropylacrylamide) (PNIPAM) since this exhibits lower critical solution temperature (LCST) behavior near 32 degrees Celsius in water. At temperatures below the LCST, the polymer is hydrated and swollen, whereas above the LCST, the polymer collapses, and when tethered, the surface becomes more hydrophobic. We report a method of growing PNIPAM from mixed self-assembled monoloyers (SAMs) using atom transfer radical polymerization. The use of two-component SAMs with varying composition permits for the control of polymer surface coverage, and the molecular weight can be controlled by the polymerization time. We have used both surface plasmon resonance and neutron reflectivity techniques to make direct measurements of the polymer brush structure at temperatures above and below the solution LCST. The effects of polymer surface coverage and molecular weight on the polymer structure were investigated. To model the temperature-induced

structural changes of these brushes, we employed self-consistent field (SCF) theory using as input the chi parameter extracted from the experimental polymer solution phase diagram. The brush structure as predicted by SCF theory is in qualitative agreement with experimental data.

4:00pm **AS+BI-WeA7 Reversible Control of Free Energy and Topography of Nanostructured Surfaces**, ***Q. Fu***, *G.V. Rama Rao, S.B. Basame, D.J. Keller, K. Artyushkova, J.E. Fulghum, G.P. Lopez,* The University of New Mexico

We describe a facile method for the formation of dynamic nanostructured surfaces based on the modification of porous anodic aluminum oxide with poly(N-isopropyl acrylamide) (PNIPAAm) via surface-initiated atom transfer radical polymerization. These hybrid membranes were characteriazed by FTIR, TGA, SEM. The dynamic structure of these surfaces was investigated by atomic force microscopy (AFM), which showed dramatic changes in the surface nanostructure above and below the aqueous lower critical solution temperature of PNIPAAm. These changes in surface structure are correlated with changes in the macroscopic wettability of the surfaces, which was probed by water contact angle measurements. Principal component analysis was used to develop a quantitative correlation between AFM image intensity histograms and macroscopic wettability. Such correlations and dynamic nanostructured surfaces may have a variety of uses.

4:20pm **AS+BI-WeA8 Characterization of Bound Nucleotides using XPS and ATR-FTIR**, ***D.B. Barbash***, *J.E. Fulghum, G.P. Lopez, Y. Wu,* The University of New Mexico

In this work we utilize XPS and ATR-FTIR to probe the attachment of nucleotides to self-assembled monolayers. The attachment of DNA onto functional surfaces is utilized in applications ranging from DNA microarray technology to molecular wires. We are utilizing nucleotides as model systems in the development of surface-specific methods for the analysis of bound DNA. The nucleotides or DNA are covalently bound to self-assembled monolayers on glass or gold substrates using a diazotization-based method we recently developed (Dolan, P.L. et al. Nucleic Acids Research 2001, 29, 21e107). Three of the four nucleotides can be attached using this method, and we report unique spectral signatures for the nucleotide bases adenine (dATP), cytosine (dCTP), and guanine (dGTP) based on ATR-FTIR and XPS analyses. Based on these spectral signatures, results of competitive binding experiments will be discussed. XPS is also utilized to characterize the attachment steps and estimate surface coverage. ARXPS results of bound nucleotides will be discussed.

Biomaterial Interfaces
Room 210D - Session BI-WeA
"Passive" and Non-Fouling Surfaces

Moderators: H.J. Griesser, University of South Australia, T.A. Horbett, University of Washington

2:00pm **BI-WeA1 PEG Modified Trichlorosilanes as Protein Repellent Coatings for Oxide Surfaces**, ***R. De Palma***, IMEC, Belgium*; K. Jans,* KULeuven, Belgium*; K. Bonroy, W. Laureyn,* IMEC, Belgium*; G. Maes,* KULeuven, Belgium*; C. Van Hoof,* IMEC, Belgium

The construction of oxide based microelectronic devices interfaced with biological components requires methods for assembling biomolecules on their surfaces in a controlled manner. Examples include biosensors, chip-based diagnostic assays and biomaterials used for implants and tissue engineering. A key issue in the design of analytical devices which contact biomolecules is that non-specific adsorption of biological species, particularly proteins, can limit their performance. Surface-bound poly(ethyleneglycol) is a powerful reagent to construct protein repellent surfaces on various substrates. Most procedures reported to yield PEG layers on oxides require several steps and thus decrease the surface controllability. To overcome the problems encountered during PEG surface modification, we have synthesized novel reagents which combine the silane surface modification properties and the protein resistant properties of short PEG (@<=@ 6) units to generate robust coatings for glass and metal oxides. Tantalum was used as a substrate because of the high chemical stability of its thin passivating oxide and was found to play an important role in the silane SAM formation. The molecular architecture of the deposited silane layers and the PEG chain conformation was studied using contact angle measurements, XPS, AFM, RAIRS, LDI-TOF-MS and ellipsometry. The non-specific adsorption of human serum and its 4 most abundant proteins were elaborated using quartz crystal microbalance with dissipation monitoring (QCM-D) and confocal fluorescence microscopy. The protein repellent properties of the PEG silane SAMs were shown to be strongly correlated to the PEG chain length and their molecular architecture. The correlation between the PEG length and the viscoelastic properties of the adsorbed protein film have led to a better insight into the phenomenon of protein repellence. Future work will involve the deposition of mixed PEG silane SAMs to further improve the protein resistant properties.

2:20pm **BI-WeA2 The Effect of Cloud-Point Grafting of Sulfonated Poly(ethylene glycol) on Albumin Adsorption**, ***L.G. Britcher***, *H.J. Griesser,* University of South Australia, Australia*; Y.H. Kim,* Korea Institute of Science and Technology

Sulfonated poly(ethylene glycol) (PEG-SO@sub 3@) has shown promise as a thromboresistant material, although its activity does reduce when grafted onto a polyurethane surface. It is thought that a synergistic effect exists between the PEG and terminal SO3 groups, improving its blood compatibility@footnote 1@. The flexible hydrophilic PEG chains cause protein rejection by a steric barrier mechanism, while the negative charge of the SO@sub 3@ terminal groups causes electrical repulsion of negatively charged proteins and platelets@footnote 1@ @super ,@@footnote 2@. Though platelet adhesion is decreased on PEG-SO@sub 3@ grafted surfaces compared with PEG grafted surfaces, protein adsorption is not suppressed completely. A negative cilia adsorption model has been proposed for these surfaces, however, it does not explain why albumin adsorption is enhanced. Therefore, further work is required in order to understand what conformation of the PEG-SO@sub 3@ on the surface along with the distribution of SO@sub 3@ groups will lead to improved anti-fouling properties. One method for changing the PEG conformation is to use cloud point conditions for grafting the PEG. Surfaces grafted under these conditions have shown to decrease protein adsorption significantly, as the coating is very dense due to the brush conformation of the PEG@footnote 3@. However, charge repulsion effects may arise with densely packed sulfonated PEGs. In this study, we aim to investigate whether cloud point conditions can be used to graft PEG-SO@sub 3@ onto surfaces and if the coating thus obtained suppresses albumin adsorption. This should lead to further understanding of the protein adsorption model on PEG-SO@sub 3@ surfaces. @FootnoteText@ @footnote 1@ Y. Hann et al., Biomaterials 24, 2213 (2003).@footnote 2@ H. Lee et al., Colloid Surf B: Biointerfaces 18, 355 (2000).@footnote 3@ P Kingshott et al., Biomaterials 23, 2043, (2002).

3:00pm **BI-WeA4 Biologically Inspired Peptide-Mimetic Polymers for Prevention of Cell and Protein Fouling**, ***P.B. Messersmith***, *A.E. Barron, J.L. Dalsin, A. Statz, R.J. Meagher,* Northwestern University **INVITED**

The minimization of nonspecific interactions between biomolecules, cells and material surfaces is integral to refining the biological response biomaterials, and therefore is important to the success of numerous emerging healthcare technologies. A primary motivation for this study is the significant need for new nonfouling strategies capable of functioning effectively for long periods of time in-vivo, and which can be readily applied to a variety of material or device surfaces. In this talk, I will describe our ongoing research efforts aimed at developing new macromolecules that meet these criteria. Specifically, we are focusing on two key aspects of biomaterial surface modification related to prevention of protein and cell fouling: 1) the design and synthesis of new polymers capable of minimizing nonspecific protein and cell attachment to biomaterials; and 2) the development of robust and versatile approaches for anchoring these polymers onto biomaterial surfaces. We have synthesized new peptidomimetic polymers designed to be both fouling resistant and adhesive to surfaces. The anchoring component of the polymers is inspired by the adhesive proteins secreted by mussels for attachment to marine surfaces, whereas the nonfouling polymer is either poly(ethylene glycol) (PEG), or a poly(N-substituted glycine) (polypeptoid). Polypeptoids offer the advantages of resistance to enzymatic degradation, low immunogenicity, and with proper design, the ability to prevent protein and cell attachment at surfaces. The synthesis and characterization of these peptidomimetic polymers will be described, along with evidence for their surface immobilization and performance as antifouling coatings.

3:40pm **BI-WeA6 Stable Protein-resistant Surfaces: Covalent Immobilization of Poly(L-Lysine)-g-Poly(Ethylene Glycol) onto Plasma-modified, Aldehyde-activated Substrate Surfaces**, ***T.M. Blättler***, *S. Pasche, M. Textor,* Swiss Federal Institute of Technology, Switzerland*; H.J. Griesser,* University of South Australia, Australia

The fabrication of protein resistant surfaces is of considerable interest for a number of applications. Electrostatically adsorbed PEGylated graft

copolymers, such as poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG), have been very successful in reducing protein adsorption on negatively charged metal oxide surfaces. The drawback is their instability under extreme conditions (extreme pH or high ionic strength). We have overcome this limitation in the present work by covalently immobilizing PLL-g-PEG onto aldehyde plasma modified substrates. PLL-g-PEG was immobilized on silicon wafers in two consecutive steps: First the silicon wafer was coated with a propionaldehyde plasma polymer layer (AHPP); secondly, the PLL-g-PEG was immobilized covalently by reacting part of the amine groups of the PLL backbone with the aldehyde groups present on the plasma-deposited polymer layer (reductive amination). The stability and the protein resistance of different architectures of PLL-g-PEG were quantitatively investigated by XPS, OWLS and ToF-SIMS. Protein resistance of the polymer-modified surfaces was tested against bovine serum albumin (BSA). Adsorption of BSA was below the detection limit (below 2 ng/cm@super 2@), similarly to the electrostatically adsorbed PLL-g-PEG. However, after 24 h exposure of the covalently immobilized PLL-g-PEG to high ionic strength buffer (2400 mM NaCl) no significant change in the protein resistance was observed, while under the same conditions electrostatically adsorbed PLL-g-PEG coatings lost their protein resistant properties. These findings provide good evidence for the covalent nature of the PLL-g-PEG binding to the surface. This work has created a general platform for the covalent immobilization of PLL-g-PEG onto a wide variety of substrates provided that they are compatible with the AHPP coating process.

4:00pm **BI-WeA7 Control of Protein Adsorption Using Poly(propylene sulfide)-block- poly(ethylene glycol) Adlayers: New Potential Candidate for the Modification of Biosensor Chip Surfaces**, ***L. Feller***, *S. Tosatti,* Swiss Federal Institute of Technology (ETHZ), Switzerland; *S. Cerritelli, S. Terrettaz,* Swiss Federal Institute of Technology (EPFL), Switzerland; *M. Textor,* Swiss Federal Institute of Technology (ETHZ), Switzerland; *J.A. Hubbell,* Swiss Federal Institute of Technology (EPFL), Switzerland

Poly(ethylene glycol) (PEG) has been used in numerous biomedically relevant systems to aid in the minimization of protein adsorption and cell adhesion. PEG can be attached to surfaces through a variety of different approaches including silanization, self-assembly of thiols, and plasma polymerization. In our approach a block copolymer containing one (di-block) or two (tri-block) PEG chains separated by a poly(propylene sulfide) (PPS) part was used. Adsorbed to gold surfaces, a stable linkage between the sulfur atoms of the PPS thioether and the metal surface was observed. The hydrophilic PEG part formed a dense layer of biocompatible PEG chains, which is exposed to the aqueous environment. Various architectures of di- and tri-block PPS-PEG copolymers were synthesized, characterized, and deposited on gold substrates. While the PPS part was kept constant (MW 4000), the PEG part was varied between 1100 and 5000 Da molecular weight. Adsorption of the polymer to the gold surface was characterized by ex situ ellipsometry, X-ray photoelectron spectroscopy (XPS), and in situ surface plasmon resonance (SPR). The resistance of the surfaces to protein adsorption was evaluated using SPR. PPS-PEG readily chemisorbed on gold surfaces after a simple dip-and-rinse process in 1mg/ml methanol solution. We compared different architectures of PPS-PEG and correlated the PEG/PPS ratio with adsorbed mass values and resistance to protein (HSA) adsorption with the aim to find the optimum architecture regarding surface adhesion, stability, polymer conformation and protein resistance.

4:20pm **BI-WeA8 Surface Segregation of Pluronic® P104 in Poly(@subL@-lactic acid) Characterized by XPS and ToF-SIMS**, ***J.-X. Yu,*** State University of New York at Buffalo; *C.M. Mahoney,* National Institute of Standards and Technology; *J. Gardella,* State University of New York at Buffalo

This study reports results of the surface and in-depth characterization of two component blend films of poly(L-lactic acid) (PLLA) and Pluronic® surfactant [poly(ethylene oxide) (A) poly(propylene oxide) (B) ABA block copolymer]. The bulk properties of these polymers have been well studied by many groups due to their biomedical applications. Angle dependant X-ray photoelectron spectroscopy (XPS) and Time-of-flight secondary ion mass spectrometer (ToF-SIMS) depth profiling were used for monitoring the surfactant's surface concentration at different sampling depths. We found a critical saturation concentration of the surfactant, a depletion region beneath the topmost surfactant enriched zone, and the existence of the surfactant's segregation in the whole film with different intensities. We conclude that the surfactant's surface segregation increases and then stays stable when increasing its bulk concentration. These results suggest that the selection of the surfactant bulk concentration of the thin film matrices for drugs/proteins delivery should achieve a relatively homogeneous distribution of stabilizer/protein in the PLLA matrix.

4:40pm **BI-WeA9 Heterobifunctional PEG Tethered Chains Surface - Preparation, Physicochemical and Biochemical Properties**, ***Y. Nagasaki***, *K. Uchida,* Tokyo University of Science, Japan; *H. Otsuka, K. Kataoka,* The University of Tokyo, Japan **INVITED**

In the case of microanalysis in a crude sample such as serum, nonspecific adsorption of various proteins and lipids to the surface is an important consideration to achieve specific biosensing with high S/N ratio. In order to avoid the nonspecific adsorption, many types of modification on the sensor surface have been considered. Modification by poly(ethylene glycol) (PEG) tethered chains leads to reduce the nonspecific interaction of biomolecules such as proteins and cells with biomedical devices because PEG is a nontoxic and hydrophilic polymer with low interfacial free energy in water and high-chain mobility inducing excluded volume effects. In this paper, we are focusing on preparation of complete non-fouling surface by mixed PEG tethered chain, which denotes the introduction of short under-brushed PEG layer to the surface pre-modified with comparatively long PEG chain resulted. By using our original heterotelechelic PEG, which means PEG having a functional group at one end and another functional group at the other chain end quantitatively, ligand-installed non-fouling surface was constructed. In the case of dextran gel as a control, non-specific adsorption was avoided to some extent in the case of high molecular weight protein. With decreasing the size of the protein, the non-specific adsorption increased significantly. The conventional PEG tethered chain surface suppressed the non-specific adsorption of the proteins possessing the molecular weight higher than 10kD. However, it is not enough performance for the protein lower than 10kD. In the case of the mixed PEG tethered chain surface, complete non-fouling character was observed. Especially, the mixed PEG tethered chain avoided tetrapeptide (RGDS, MW=450), which is anticipated as ideal biomateials surface.

Biomaterial Interfaces
Room 210D - Session BI+AS+SE-ThM

Surface Modification of Biomaterials

Moderator: D.G. Castner, University of Washington

8:20am **BI+AS+SE-ThM1 Strategies for the Biofunctionalization of Surfaces using Functional Polymer Layers**, ***J. Ruehe,*** University of Freiburg, Germany **INVITED**

The modification of surfaces with polymers for the promotion of cell outgrowth either in a dense layer or following a distinct pre-determined pattern is a challenging field of research with possible applications in the field of medical implants as well as for specific sensor devices. We present results from various studies in our group that range from the modification of bioimplant surfaces (e.g. glutar aldehyde treated porcine heart valves) with polymer monolayers in order to allow for a re-endothelialization of these devices to the arrangement of neuronal cells on a substrate by depositing synthetic and natural polymers on these chips in the form of a microarray. We will put a strong emphasis on synthetic approaches for establishing a strong, i.e. usually covalent interaction between the polymeric coating and the substrate in order to guarantee a sufficient long-term stability of the layer assemblies. These assemblies may be polymer monolayers as well as networks and we will also report on strategies for the incorporation of biological functions such as cell adhesion motifs or peptides. Finally, approaches for the laterally patterned deposition of these layers will be discussed.

9:00am **BI+AS+SE-ThM3 Interfacial Biomaterials: Guiding Biology on Synthetic Surfaces**, *E.B. Walsh, X. Huang,* Duke University; *M.W. Grinstaff,* Boston University; ***D.J. Kenan,*** Duke University Medical Center **INVITED**

Interfacial biomaterials represent a novel coating technology capable of directing biological processes at the interface between a biologic and a synthetic surface. The approach relies on screening combinatorial libraries to identify unique peptides that adhere to a synthetic target such as a plastic or metal, or to a biological target such as a protein or cell. Next, two or more adhesion peptides are synthetically coupled to create an interfacial biomaterial that mediates the interaction of the protein or cell with the synthetic material. Other interfacial biomaterials may be created by coupling known signaling molecules to peptides that bind synthetic materials. Mixtures of interfacial biomaterials may be applied to a surface to achieve a particular desired biological outcome, such as adhesion of a given cell type to the surface, followed by induction of one or more signal transduction pathways. These interfacial biomaterials are amenable to numerous coating and patterning techniques suggesting their use for diverse applications ranging from biomedical device coatings to anti-infectives to tissue engineering.

9:40am **BI+AS+SE-ThM5 Antibacterial Surfaces of Covalently Immobilized Dendrimers**, *D. Weber, N.R. Choudhury,* University of South Australia; ***H.J. Griesser,*** University of South Australia, Australia

The need to limit bacterial adhesion to surfaces of biomedical implants, contact lenses, and other devices has prompted considerable recent research into antibacterial compounds and coatings. To ensure long-term efficacy and eliminate concerns about potential adverse biological effects on sensitive organs remote from the implant site, release strategies seem less suitable, and the covalent surface immobilization of antibacterial compounds is the approach of choice in our work. However, the question then becomes whether a covalently immobilized antibacterial is still biologically active, and can maintain activity over extended service life spans. In this study we have principally explored the surface immobilization of dendrimers, which have previously been shown to be antibacterially active in solution (eg CZ Chen and SL Cooper, Biomaterials 23 3359 2002). Another approach involves extracts of some Australian plant species, but their chemical characterization and synthesis is less developed. We have immobilized amine-terminated dendrimers onto aldehyde plasma polymer interlayers via reductive amination and characterized the coatings by XPS, ToF-SIMS, and AFM. Using various plasma conditions the surface density of aldehyde groups can be varied. The surface density of immobilized dendrimers is determined from XPS elemental ratios, using the dendrimer-specific N signal. Following surface immobilization, the remaining amine groups are quaternized in order to produce a cationic surface. The distinct signal arising from quaternary N in the XPS N 1s spectrum enables assessment of this reaction. The plasma approach also enables us to apply this coating strategy onto a wide variety of substrates both polymeric and inorganic (ceramic and metallic).

10:00am **BI+AS+SE-ThM6 Biomimetic Vascular Engineering: Exploiting Concepts from Nature to Create New Biomaterial Interfaces**, ***R.E. Marchant,*** Case Western Reserve University **INVITED**

The abundance of complex supramolecular structures in Nature provides lessons in structural hierarchy and functional efficiency that are being explored and exploited in the development of novel biomimetic strategies for creating new biomaterial interfaces for biomedical applications. Mimicking and adapting structural concepts from Nature to create tissue compatible interfaces for biomaterials that incorporate molecular recognition and self-assembly will be the central theme of this presentation. We have developed a biomaterial architecture using "surfactant polymers" that undergo surface and self-induced assembly on hydrophobic surfaces. Our biomimetic designs benefit from understanding the structural and functional properties of the corresponding system in Nature. One example is the external region of a cell membrane, known as the glycocalyx, which is dominated by a complex milieu of glycosylated molecules. The glycosylated molecules direct specific interactions such as cell-cell recognition, and provide an important physical basis for maximizing steric repulsion that prevents undesirable non-specific cell and molecular adhesions. Conversely, understanding the nature of a cellâ?Ts adhesive interactions with the extracellular matrix facilitates design of biomimetic materials with cell adhesion properties. Using these biomimetic concepts, we have designed and studied oligosaccharide and peptide surfactant polymers that provide suppression of non-specific protein interactions and facilitate well-controlled interactions with endothelial cells.

10:40am **BI+AS+SE-ThM8 Stability of Polypeptide Multilayers as Studied by in situ Ellipsometry: Effects of Drying and Post-Buildup Changes in Temperature and pH**, *T.J. Halthur,* YKI AB, Institute for Surface Chemistry, Sweden; *P. Claesson,* KTH, Royal Institute of Technology, Sweden; ***U. Elofsson,*** YKI AB, Institute for Surface Chemistry, Sweden

Polyelectrolyte Multilayers (PEM) of poly(L-glutamic acid) (PGA) and poly(L-lysine) (PLL) with a initial layer of polyethyleneimine (PEI) were built on silica and titanium surfaces using the Layer-by-Layer (LbL) technique. The stability of the film during drying/rewetting, temperature cycles and pH shifts was studied in situ by means of ellipsometry. The filmthickness was found to decrease significantly (approximately 70%) upon drying, but the original film-thickness was regained upon rewetting and the buildup could be continued. The dry thickness was found to be extremely sensitive to ambient humidity, needing several hours to equilibrate. Changes in temperature and pH was also found to influence the multilayer thickness, leading to swelling and de-swelling of as much as 8% and 10-20% respectively. The film does not necessarily regain its original thickness as the pH is shifted back, but instead shows clear signs of hysteresis.

11:00am **BI+AS+SE-ThM9 PCA of TOF-SIMS Spectra from p(AAm-co-EG/AAc) IPNS on Quartz**, ***D.J. Graham,*** University of Washington; *G.M. Harbers, K.E. Healy,* University of California, Berkeley; *D.G. Castner,* University of Washington

PCA of TOF-SIMS has been carried out on many well defined model surfaces in structured experiments. These studies have shown the utility of PCA in extracting information from TOF-SIMS experiments from a wide variety of substrate surfaces. This work reports on the application of PCA to a more complex interpenetrating polymer network system. The goal of this project was to verify each step in the IPN synthesis procedure on a quartz substrate. This system presents a challenge to PCA due to the similarity of the polymers used in the IPN and the addition of a peptide chain. PCA of the entire data set (including all synthesis steps for the IPN) showed that PC1 was able to separate most samples. The PC1 loadings were dominated by the overall differences between the hydrocarbons on the bare quartz and the PEG related peak fragments after the addition of the IPN onto the quartz surface. This is likely due to the high PEG content of the IPN polymers. PCA comparing each successive synthesis step gave further insight into the success of the IPN chemistry. PCA was able to distinguish each surface modification up until the addition of the peptide precursor and peptide. The presence of the peptide was verified in subsequent experiments where it was shown that RGD-peptide modified p(AAm-co-EG/AAc) surfaces supported rat calvarial osteoblast adhesion, proliferation, and matrix mineralization. Consequently,surfaces without the RGD peptide or with a control RGE peptide did not support cell attachment. PCA also gave insight into the uniformity of the surface modifications by way of the scores plots. Increasing scatter was seen in the last few synthesis steps

suggesting that a less uniform surface chemistry was achieved. The trends seen in the PCA of the TOF-SIMS data were consistent with those seen by XPS.

11:20am **BI+AS+SE-ThM10 Development of an Antimicrobial Polymer Surface Coating for the Prevention of Staphylococcal Infections**, ***M. Anderle**, L. Pasquardini, L. Lunelli, R. Canteri, P. Villani, C. Pederzolli,* ITC-irst, Italy

The proliferation of pathogenic microorganisms on polymer surfaces is one of the most widespread causes of failure of biomedical devices such as catheters, medical implants, vascular graft and joint prostheses. The inhibition of pathogenesis and subsequent mechanisms of protection are possible by killing bacteria in the first steps of colonization. This work describes a polymeric surface coating with liposomes as method to provide a sustained delivery of antibiotics into the local micro-environment of the implant. In this study liposome formulations composed of Phosphatidylcholine (PC), Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-MethoxyPolyethylene glycol (DSPE-PEG) and cholesterol are utilized. Liposomes, different in size, are attached to an amine activated substrate through the formation of covalent bonds with the distal end of the PEG (Polyethylene glycol) derivative molecules. Data on the surface coating using large unilamellar vesicles (LUV) and multilamellar vesicles (MLV) will be shown. The AFM analysis is performed to study the nanoscale structure of the coated surface while the fluorescence spectroscopy and microscopy are engaged to determine the immobilisation efficacy adding a fluorescent lipid (L-@alpha@-Phosphatidylethanolamine-N-lissamine rhodamine B sulfonyl) to the liposome composition. The MLV coating on polystyrene shows a more uniform distribution with a lipid concentration of about 2x10@super 15@ mol/cm@super 2@. Finally drug (rifampicin) release and bacterial colonisation rates with their correlation will be reported.

11:40am **BI+AS+SE-ThM11 Spectroscopic Characterization of Surface-Immobilized Antibacterial Furanone Coatings**, ***S. Al-Bataineh**, H.J. Griesser,* University of South Australia, Australia*; M. Willcox,* University of New South Wales, Australia*; L.G. Britcher,* University of South Australia, Australia

The colonisation by bacteria of biomedical devices presents a serious concern for human implant surgery. In this study, we explore how bacterial colonisation can be prevented by the appropriate design and fabrication of antibacterial coatings, with a major focus on surface-immobilised furanone molecules. These compounds are produced naturally by the marine algae, Delisea pulchra and are used as defence agents to prevent fouling on their surface@footnote 1@. Several studies have shown that brominated furanones as well as synthetic analogues possess potent antimicrobial activity against bacteria@footnote 2@@super ,@@footnote 3@. The previously used azide protocol was adopted to prepare furanone coatings@footnote 4@. XPS and ToF-SIMS results showed successful surface modifications and furanone immobilisation. Detailed analysis of the C 1s and N 1s XPS spectra using constrained curve fitting showed that they are more complicated than anticipated from the theoretical reaction scheme. In addition, the presence of a Br@super-@ peak partially overlapped with a C-Br peak indicated that furanones are partially degraded on UV illumination. More surface characterisations are needed for full understanding of the chemical reactions that occurred. Seven furanone compounds used in this study were tested for their ability to inhibit biofilm formation and growth of two bacterial strains, Staphylococcus aureus (Saur19) and Pseudomonas aeruginosa (Paur6206). Initial results are promising; detailed investigation of the efficacy of the coatings is ongoing. Furthermore, none of the compounds used in this study showed any cytotoxicity potential at the tested concentrations. @FootnoteText@ @footnote 1@ de Nys R. et al., 1995, 4:259-71.@footnote 2@ Kjelleberg S. et al., Patent No. PCT/AU99/00284, 1999.@footnote 3@ Read R. et al. PCT international application PQ6812, 2001.@footnote 4@ Muir B. et al., Proc. 6th World Biomat. Congr., Hawaii, May 2000, p. 596.

Biomaterial Interfaces
Room 210D - Session BI-ThA

Biosensors and Bio-Diagnostics

Moderator: D. Grainger, Colorado State University

2:00pm **BI-ThA1 Multi-analyte Immunoassays in Packed Microcolumns: Design and Analysis**, ***G.P. Lopez,*** University of New Mexico **INVITED**

This talk will present recent developments at the University of New Mexico in the development of microfluidic based biosensor systems and their use in analysis of biomolecular recognition. The method involves real-time detection of soluble molecules binding to receptor-bearing microspheres, sequestered in affinity column-format inside a microfluidic channel. The packed microcolumn format is (1) well suited for enhancing reaction times of analyte with immobilized receptors, (2) compatible with electro-osmotic pumping, and (3) allows detection of multiple analytes. Identification and quantitation of analytes occurs via direct fluorescence measurements or fluorescence resonance energy transfer (FRET). Several immunoassays have been developed that can potentially detect sub-femtomole quantities of antibody with high signal-to-noise ratio and a large dynamic range spanning nearly four orders of magnitude in analyte concentration in microliter to submicroliter volumes of analyte fluid. Kinetic and equilibrium constants for the reaction of this receptor-ligand pair are obtained through modelling of kinetic responses of the affinity microcolumn and are consistent with those obtained by flow cytometry. Because of the correlation between kinetic and equilibrium data obtained for the microcolumns, quantitative analysis can be done prior to the steady state endpoint of the recognition reaction. This method has the promise of combining the utility of affinity chromatography, with the advantage of direct, quantitative, and real-time analysis and the cost-effectiveness of microanalytical devices. The approach has the potential to be generalized for high sensitivity, high selectivity, rapid detection of a host of bioaffinity assay methods and analyte types.

2:40pm **BI-ThA3 Biosensing Based on Light Absorption of Immobilized Metal Nanostructures**, ***F. Frederix,*** *K. Bonroy, G. Reekmans, C. Van Hoof,* IMEC, Belgium*; G. Maes,* K.U.Leuven, Belgium

The "Transmission Plasmon Biosensor" is a novel, cheap and easy to handle biosensing technique. Surface plasmon resonance sensors are widely used for biosensing. These sensors are highly sensitive to the refraction index at the interface between the metal film deposited upon a prism and a sample upon this metal surface. This principle can also be applied to a dielectric planar surface coated with nanostructures. The plasmon absorption peak position and intensity is highly dependent on the size of these particles and on the close proximity of these particles immobilized onto a surface. This research compromises the synthesis of metal nanoparticles with different sizes and morphologies, which were covalently immobilized on transparent substrates, e.g. glass, quartz and polymers using a molecular glue of silane layers. Particle films were also realized using various evaporation strategies, e.g. thermal evaporation, e-beam evaporation, sputtering and electroless plating. The different strategies were evaluated using TEM, AFM and absorption spectroscopy. The resulting plasmon resonance and interband absorption bands in the visible and UV region were compared. Mixed SAMs were used to couple antibodies to the metal nanoparticle films. The change in absorbance properties of the nanoparticle films upon antibody-antigen binding was monitored in order to obtain quantitative information on the antibody-antigen interaction. Besides the localised plasmon resonance sensing, we observed a novel physical phenomenon namely the interband transition absorption enhanced sensing. Furthermore, the applied technique was identified to be a useful alternative for the most widely used clinical immunosensing technique, i.e. the ELISA technique. This promising alternative was applied onto modified microtitre plates, which allow the implementation into an array technology. The Transmission Plasmon Biosensor fulfils therefore the needs of an ideal, multi-analyte bio(nano)sensor.

3:00pm **BI-ThA4 2-D Array Biosensor using Waveguide Bragg Grating**, ***K.S. Choi,*** *H.J. Lee, C.I. Jung, H.J. Park, N.W. Park,* Chonnam National University, Korea

Bragg grating based planar integrated optical circuit technology is applied to biosensors. Varieties of detection mechanisms such as antigen-antibody are investigated and an appropriate structures of Bragg grating optical sensors circuit is demonstrated. Trenches are fabricated onto a planar waveguide substrate and polymer core material with high refractive index is deposited in these trenches.Bragg gratings are formed onto this polymer core and series of Bragg gratings with different periods are fabricated along these waveguides. These waveguides are repeated in serial such repetition makes 2-D array of bio sensors which might be vey effective in sensing of disease. We will introduce and demonstrate the Bragg grating based silica waveguide sensors in this paper and some theoretical designs will be considered.

3:20pm **BI-ThA5 Biological Sensors Based on Brownian Relaxation of Magnetic Nanoparticles**, *S.-H. Chung, A. Hoffmann,* ***S.D. Bader,*** *L. Chen, C. Liu, B. Kay, L. Makowski,* Argonne National Laboratory

We present a biological sensing platform that is based on a modification of the dynamic magnetic properties of ferromagnetic nanoparticles suspended in a liquid. For a narrow size range the ac magnetic susceptibility of the ferromagnetic nanoparticles is dominated by Brownian relaxation. By coating the nanoparticles with a suitable ligand the Brownian relaxation and thus the ac magnetic susceptibility can be modified through the binding to the corresponding bio-receptor. The size of the particles has to be large enough to avoid superparamagnetism and at the same time small enough to have a homogeneous single domain magnetization. We demonstrate a proof-of-principle of this concept by using avidin-coated Fe@sub 3@O@sub 4@ particles that are ~10 nm in diameter, which were investigated before and after binding to biotinylated S-protein and bacteriophage particles. The ac susceptibility measurements show that the magnetic relaxation occurs via a Brownian mechanism; the frequency shift for the peak in the imaginary part of the susceptibility after binding to the target indicates the increase of the hydrodynamic radius. We are currently developing magnetic phage viruses in order to further improve this bio-sensing platform. @FootnoteText@ * Supported by DOE, BES under contract W-31-109-ENG-38, and DARPA under contract 8C67400.

3:40pm **BI-ThA6 G-Protein Coupled Receptor Biosensors - New Opportunities and Applications**, ***E.J. McMurchie,*** *W.R. Leifert,* CSIRO Health Sciences and Nutrition, Australia*; L. Wieczorek, B. Raguse,* CSIRO Telecommunications and Industrial Physics, Australia **INVITED**

Future diagnostic and biosensor platforms will require development of cell-free, high-throughput, microarray formats with bioengineered sensors mimicking the specific interactions between ligand and cell membrane receptors. For bio-diagnostic technologies, G-protein coupled receptors (GPCRs) are likely to have application as biosensors reporting on ligands influencing physiological and pathophysiological functions. GPCRs are a large and ubiquitous class of membrane-associated receptors activated by a wide range of extracellular ligands, (biogenic amines, amino acids, ions, peptides, and bioactive lipids) which act as hormones, neurotransmitters, chemokines etc. Signalling through these receptors regulates responses such as neurotransmission, chemotaxis, inflammation, cell proliferation, muscle contractility, and visual and chemosensory perception. GPCRs signal to numerous down stream cellular effectors via a set of heterotrimeric G-proteins through GTP dependant processes. GPCRs are the target for >50% of current therapeutic drugs with drug discovery programs relying on high throughput screening technologies. The future development of microarray technologies for GPCRs is relevant for the development of highly specific ligands in drug discovery and for utilising GPCRs as potential biosensors. Present assays for ligand screening against GPCRs can be classified into two major categories; whole cell assays with cell-associated, down-stream signalling systems for detecting activated receptors, and homogeneous, cell-free assays consisting of membrane fragments containing (usually cloned) GPCRs. For the latter, some form of signalling/reporting system must be added if functional assays, as opposed to ligand binding, are to be used. Our strategic objective is the construction of a cell-free system to enable reconstitution and nanoconstruction onto appropriate surfaces for future adaptation to microarray formats suitable for high throughput, multiplex screening.

4:20pm **BI-ThA8 Detection of Human Immunodeficiency Virus-1 Using Micro-Cantilever Deflection Biosensors**, *Y. Lam,* ***N. Abu-Lail,*** *M. Alam, S. Zauscher,* Duke University

Having a simple, efficient, and sensitive technique for the diagnosis of human immunodeficiency virus-1 (HIV-1) is extremely important due to the increasing trend in HIV-1 cases, and the current lack of a rapid and simple method to detect the disease. We show that surface modified micro-cantilevers, decorated with monoclonal antibodies (mAb) A32, deflect upon specific binding of mAb A32 to HIV-1 envelope glycoprotein gp120 (HIV-1 Env gp120). This deflection of the micro-cantilever is a direct result of the surface stress induced by molecular recognition mediated protein binding. The specific binding between the two proteins was confirmed

through force spectroscopy measurements between mAb 17b tethered to a surface and HIV-1 Env gp120 immobilized on the cantilever; HIV-1 gp120 will only bind mAb 17b if the former has bound to mAb A32. Our results show that micro-cantilever deflection can effectively be used for the sensitive detection of molecular recognition events, encouraging the further development of this technique as a rapid response biosensor for disease diagnosis. Work on determining the detection limits of the micro-cantilever deflection method and its extension to whole virus detection is in progress.

4:40pm **BI-ThA9 Realization and Characterization of Porous Gold for Increased Protein Coverage for Biosensor Applications**, ***K. Bonroy***, *J.-M. Friedt, F. Frederix, R. De Palma,* IMEC, Belgium*; M. Sàra,* Center for NanoBiotechnology, Austria*; B. Goddeeris,* KULeuven, Belgium*; G. Borghs,* IMEC, Belgium*; P. Declerck,* KULeuven, Belgium

In recent years, there has been an increasing need for the detection of biochemical substances with low molecular weight. Biosensors could be an alternative to conventional analytical methods for monitoring these substances. However, generally applied biosensor systems are often not sensitive enough for direct detection of these compounds. Therefore, our research focuses on the development of biosensors with improved transducer capabilities and biological interfaces. We chose gold surfaces in combination with SAMs of thiols as platform for the immobilization of biomolecules because of its compatibility with existing biosensors. In previous research, the use of SAMs of thiols on flat gold surfaces showed several advantages concerning specificity and reproducibility for final biosensor applications. However, the main disadvantage of this approach is the 2D aspect of these layers compared to 3D surfaces (e.g. polymers). 3D surfaces, such as porous gold, would allow for the immobilization of a large number of molecules per surface area, facilitating higher biosensor responses. The presented research describes the analysis of the different parameters, which define the electrochemical growth of porous gold, starting from flat gold. QCM-D technique was used for online monitoring of the porous gold deposition. The resulting surfaces were characterized using SEM, cyclic voltammetry and contact angle measurements. Applied potentials of -0.5V were found to be the most adequate conditions to grow porous gold, resulting in a 16x increase in surface area. In addition, we evaluated the immobilization degree of S-layer and IgG proteins on these porous surfaces. The optimized deposition conditions for realizing porous gold substrates, lead to 3x increase of S-layer adsorption and 5x increase of anti-IgG recognition using QCM-D as biological transducer. We can conclude that the high specific area of the porous gold amplifies the final sensitivity of the original flat surface device.

Biomaterial Interfaces
Room 210D - Session BI+MN-FrM

Bio-MEMS and Microfluidics
Moderator: J.J. Hickman, Clemson University

10:00am **BI+MN-FrM6 Creating Protein and Vesicle Arrays Using Designated Surface Chemistry in Combination with a Novel Microfluidic Pattering Device**, *B. Niederberger, M. Dusseiller, D. Falconnet, B. Städler, G.L. Zhen, F. Rossetti,* ***J. Vörös,*** ETH Zurich, Switzerland

Protein microarrays play a key-role in drug discovery, drug development and diagnostics by providing a highly sensitive, parallel analysis of the proteome of complex samples. The methods (e.g. spotting) that are currently available for the creation of DNA microarrays can not be directly used for proteins because they are subject to the loss of function upon contact with an ambient environment. In this work, we present a novel way to create arrays of different proteins or vesicles using a microfluidic device. The concept relies on a designated surface chemistry, which allows activation for subsequent binding events, in combination with crossing microfluidic channels for the local functionalization by separated laminar streams. Besides its simplicity and cost efficiency, this concept has the major advantage that it keeps the proteins in a hydrated environment throughout the experiment. The working principle of this arrayer is to activate spots by an activation stream and to do subsequent functionalization by reagent streams flowing perpendicular to the first stream. The surface pattern was provided by a MAPL-chip (Molecular Assembly Patterning by Lift-off) which consists of well defined areas (i.e. spots) of biotin or NTA functionalized PLL-g-PEG surrounded by a resistant surface of unfunctionalized PLL-g-PEG. The PDMS flow cell was fabricated by soft lithography and sealed to the sample surface by pressure. The position and the width of the streams containing the analytes could be adjusted using different flow rates in the microchannels. Fluorescent microscopy was used to monitor in situ the creation of a microarray consisting of alternating spots of streptavidin labeled with two different fluorophores. The concept was further extended to create heterogeneous arrays of his-tagged proteins and vesicles. This novel technique enables the creation of protein (including membrane-protein) microarrays in normal research labs in a simple and cost efficient way.

10:20am **BI+MN-FrM7 Polymeric Materials for DNA Sensing and Integration into Microfluidic Channels**, ***R.A. Zangmeister***, *M.J. Tarlov,* National Institute of Standards and Technology

Advances in microchip technology coupled with innovative bioassays are advancing the field of biosensing in microfluidics. We have previously reported a method for immobilizing single-stranded DNA (ss-DNA) probe molecules in polyacrylamide hydrogels within plastic microfluidic channels, creating a sensing matrix for target oligos. Spatially defined plugs are formed by photopolymerization of a solution containing 19:1 polyacrylamide/bisacrylamide and ss-DNA modified at the 5' end with an acrylic acid group. Low concentrations of ss-DNA targets can be electrophoresed into the hydrogels where complementary strands are captured by hybridization and are detected. We are interested in identifying and characterizing other polymeric materials that can be used as DNA sensing matrices for use in microchannel devices. Our goal is to identify polymeric materials that can be patterned within a microchannel, either by photochemical or electrochemical means, and that possess surface chemical groups that can be used to chemically graft probe oligos, or potentially other biological probe molecules, onto the surface. One such candidate that we are currently investigating is poly(3-aminophenol). Our strategy is to pattern a poly(3-aminophenol) thin film, modify it with probe oligos, and demonstrate a hybridization based DNA assay on that surface for use in a microfluidic format. We are able to selectively deposit poly(3-aminophenol) thin films onto gold electrodes under potential control. Surface pendant amine groups, as evidenced in infrared studies, allow for linkage of probe oligos to the polymer surface. Polymer deposition conditions, characterization, modification with probe oligos, and success of target hybridization detection will be discussed.

10:40am **BI+MN-FrM8 Cell Biology On a Chip: Elastomeric Microfluidic Platforms for Cell Culture Applications**, ***A. Folch,*** University of Washington
INVITED

The ability to culture cells in vitro has revolutionized hypothesis testing in basic cell and molecular biology research and has become a standard methodology in drug screening and toxicology assays. However, the traditional cell culture methodology - consisting essentially of the immersion of a large population of cells in a homogeneous fluid medium - has become increasingly limiting, both from a fundamental point of view (cells in vivo are surrounded by complex spatiotemporal microenvironments) and from a practical perspective (scaling up the number of fluid handling steps and cell manipulations for high-throughput studies in vitro is prohibitively expensive). The recent advances by our laboratory to address both limitations will be presented, including a microfluidic long-term cell culture platform that features cellular micropatterns and focal delivery of soluble factors to single cells. We are also developing elastomeric sensors and actuators for single-cell probing and manipulation by inexperienced users. These inexpensive technologies allow us to test novel hypotheses concerning neuromuscular development, chemotaxis, and neuronal axon guidance.

11:20am **BI+MN-FrM10 Ultrasensitive MALDI MS Analysis of Peptides Separated in an RF Plasma Polymer Modified Microfluidic Device**, ***G.R. Kinsel***, *X. Li,* University of Texas at Arlington

Rapid, information rich analysis of complex biological samples, such as the proteome of a given cellular system, represents a significant challenge for modern bioanalytical devices. A prototype open-channel microfluidic device under development in laboratory integrates an array of technologies available and/or developed in our laboratory to achieve efficient separation and ultrasensitive detection the components of peptide/protein mixtures. Specifically, separation of peptide mixtures is achieved through electroosmotic flow of the sample through 100 micron open-channels imprinted into a PMMA wafer. Modulation of the separation characteristics is achieved by either using the channels as formed or following coating of the channels by pulsed RF plasma polymerization of thin films having various chemical properties. Changes in peptide retention characteristics have been observed to correlate with changes in the column coating chemistry. Separation of simple mixtures can be achieved in minutes using this device. Following separation of the peptides, MALDI mass spectra of the isolated compounds is achieved by rastering the desorption / ionization laser down the open channel. This approach clearly allows the unambiguous assignment of the peptide molecular weight. In addition, because of the confinement of the sample to extremely small volumes, and the consequent high surface concentrations, extremely low limits of detection have been obtained for the separated peptides e.g. an LOD of 1.6 attomole of the peptide casomorphin has been observed. The coupling of this microfluidic device with MALDI mass spectrometry clearly holds enormous promise for substantially lowering the limits of detection and the requisite analysis time, while providing maximum information content for components in complex peptide / protein mixtures.

11:40am **BI+MN-FrM11 Measurement and Analysis of Changes in EOF with Protein Adsorption using the Dynamic Current Monitoring Method.**, ***K. Lenghaus***, Clemson University*; M.J. Tarlov, L. Locascio,* NIST*; J. Jenkins, S. Sundaram, S. Krishnamoorthy,* CFD Research Corporation*; J.J. Hickman,* Clemson University

The high surface to area ratio of MEMS devices places certain constraints upon their operation. One of these is that conventional, pressure driven flow is a relatively inefficient means of moving liquids through microfluidic channels, owing to the large backpressure encountered. The parabolic flow profile of pressure driven flow can also be undesirable in certain applications, especially in regards to sample separation and delivering analytes to detectors. Electro-osmotic flow (EOF), providing that conditions are conducive to its operation, can thus be a preferable option, since it doesnâ?Tt have the same problems with high backpressures, and its top hat flow profile, as shown by capillary electrophoresis, is well suited to separations and analysis. However EOF is sensitive to the type and density of electrical charges at the wall, and the adsorption of molecules or biomolecular species can substantially alter the EOF characteristics of the system. Using the dynamic current monitoring method, the change in EOF with protein exposure was tracked on the timescale of minutes, and the effect of changing the driving voltage, buffer composition, capillary surface and other parameters was obtained. Building on our previous protein adsorption work, we show that under some circumstances changes in EOF with exposure to different proteins can be extremely rapid. Whether or not desorption and recovery of the original EOF characteristics occurs depends on the specific protein/surface combination, as does the final EOF reached. The rates of adsorption and desorption were also determined using finite element analysis methods, compared with those obtained under pressure driven flow conditions, and a hypothesis of the method of interaction has been postulated.

Biomaterial Interfaces
Room 210D - Session BI-FrM

"Active" - Dynamic Biointerfaces
Moderator: S. Zauscher, Duke University

8:20am **BI-FrM1 The Effect of Cell Detachment Method on the Identity and Quantity of Residual ECM Proteins Retained at Surfaces**, ***H.E. Canavan***, *X. Cheng, B.D. Ratner, D.G. Castner,* University of Washington

Treatment of tissue culture polystyrene (TCPS) with poly(n-isopropylacrylamide) (pNIPAM) has been developed as a technique for the harvest of intact cell monolayers. Although low-temperature liftoff from pNIPAM is known to be less damaging to cells than traditional cell removal methods, little is known about the effects these techniques have on the underlying Extracellular Matrix (ECM). Recently, we demonstrated that although immunoassay of ECM components show that low-temperature liftoff removes the majority of the ECM concurrently with the cells, XPS and SIMS results reveal that some protein does remain at the pNIPAM surface. In this work, we further examine the effect that low-temperature liftoff from pNIPAM and traditional cell removal methods have on the ECM. Using XPS, we compare the relative amount of ECM remaining at culture surfaces after cell removal by the different methods. Using SIMS, MALDI, and immunostaining, we identify the individual proteins left behind. Finally, LDH assay is used to ascertain the viability of the residual ECM left behind by each cell removal method. We find that in addition to its dramatic effects on cell viability and morphology, trypsin removes much of the underlying ECM and often adsorbs to the surface itself, drastically reducing the adhesion of new cells. Although mechanical dissociation of the cell layer is less damaging to the underlying ECM, harvest via this method results in incomplete cell layers with partially damaged appearance. Of these techniques, only low-temperature liftoff from the pNIPAM surface harvests a complete cell monolayer while leaving behind ECM proteins capable of promoting new cell adhesion.

8:40am **BI-FrM2 Thermally Responsive Surfaces Formed by Plasma Polymerisation of N-Isopropyl Acrylamide**, ***N.A. Bullett***, University of Sheffield, UK, United Kingdom*; R.A. Talib, S.L. McArthur, R.D. Short, A.G. Shard,* University of Sheffield, UK

Temperature responsive surface coatings show great potential for a number of novel applications, such as 'smart' cell culture substrates for the control of cell attachment and detachment. Surface grafted N-isopropyl acrylamide (NIPAAm) has been shown to be suitable for this purpose, and the possibility of producing these coatings by deposition from plasma has been recently demonstrated by Pan et al.@footnote 1@ We find that, although it is possible to produce surfaces that demonstrate a temperature-dependent transition (as determined by contact angle goniometry and ellipsometry), there is a significant risk of delamination or solubilisation of the plasma polymers when they are in contact with water. In this work we demonstrate the importance of substrate temperature and discharge power on the properties of plasma polymerized NIPAAm. X-ray photoelectron spectroscopy (XPS) analyses were used to examine the composition of the deposited films, demonstrating that the high power deposits contain less characteristic functional groups (notably amides), indicating greater fragmentation of the monomer, and a high degree of cross-linking, whilst the low power deposit closely resembles conventionally polymerised NIPAAm. However, the very low power plasma polymers were shown, by XPS analysis and ellipsometry, to be unstable to rinsing with distilled water. The effect of substrate temperature during deposition was also investigated. It was found that higher substrate temperatures produced a more stable film at lower plasma powers. Useful films are therefore only produced within a narrow parameter space. The effect of using pulsed discharges and co-monomers was also investigated. We demonstrate that these plasma polymers can be patterned and used for cellular co-culture. @FootnoteText@ @footnote 1@ Pan YV, Wesley RA, Luginbuhl R, Denton DD, Ratner BD; Biomacromolecules 2001, 2, 32-36.

9:00am **BI-FrM3 Using Enzymes to Switch Surface Properties**, ***R.V. Ulijn***, University of Manchester, UK, United Kingdom*; M.R. Alexander, F.J.M. Rutten,* University of Nottingham, UK*; J.E. Gough, F. Carabine, J.L. Rutherford,* University of Manchester, UK

We study synthetic surfaces that change their properties upon biochemical stimuli (i.e. an enzymatic reactions). Surfaces that can be tuned (switched) to either bind or resist (biological) molecules or cells are desirable for a number of applications in the biomedical sciences. Enzymes are ideal tools for such surface engineering because they are highly selective and truly compatible with biology. We demonstrated that a peptide-hydrolyzing enzyme (chymotrypsin) could be employed to significantly alter the wettability of modified glass surfaces by hydrolysis of surface bound di-peptides. Here, we describe the use of XPS and ToF-SIMS analysis to characterise the changes in surface chemistry achieved using this approach. The methodology was applied in switching the attachment of cells to surfaces. A number of surfaces have been identified that are â?~stickyâ?T (promoting cell adhesion) or â?~non-stickyâ?T to certain cells. By using our biochemically responsive surfaces we demonstrate for the first time the possibility of switching between these two surface properties and therefore to switch cell adhesion in real time.

9:20am **BI-FrM4 Conformational Mechanics of Surface Grafted Stimulus-Responsive Polypeptides**, ***A. Valiaev***, *D.W. Lim, N. Abu-Lail, A. Chilkoti, S. Zauscher,* Duke University

Stimulus-responsive macromolecules have attracted significant research interest due to their potential applications in drug delivery, molecular motors, and nanoscale actuation devices. ELPs are stimuli-responsive biomacromolecules that undergo an inverse phase transition triggered by changes in solvent temperature, pH, or ionic strength. Associated with this transition is a significant conformational collapse and change in surface energy. To date, most research on ELPs has focused on the investigation of bulk properties and their aggregation behavior in solution. Our research is the first, we believe, to provide detailed insight into the mechanisms of elasticity and conformational mechanics of ELPs immobilized as ensembles on surfaces and on the single molecule level. First we performed single-molecule force spectroscopic experiments on elastin-like polypeptides (ELPs) that shed light on their time-dependent structural changes, physico-chemical and mechanical properties. We also present results obtained with a quartz crystal microbalance (QCM), cantilever deflection measurements, and adhesion force spectroscopy on surface immobilized ELP ensembles. QCM enabled us to study the effective mass change and the change in the mechanical dissipation behavior when ELPs collapse and swell as waters of hydrophobic hydration are released or consumed by the surface grafted protein. Experiments with micro-cantilevers decorated on one side with covalently grafted ELPs showed that a phase transition induces a considerable surface stress, leading to significant cantilever bending. Our approach promises to yield simple and sensitive bioanalytical devices, because cantilever bending can be easily measured. Lastly, adhesion force spectroscopy enabled us to quantify surface energetic changes associated with the phase transition behavior of surface grafted ELP ensembles.

9:40am **BI-FrM5 pH Induced Conformational Behaviour of Polyelectrolytes in Bulk Solution and Grafted to Surfaces: Neutron Reflectometry and Fluorescence Studies**, ***M. Geoghegan***, Univ. of Sheffield, UK, United Kingdom*; L. Ruiz-Perez, A.J. Parnell, J.R. Howse, A.J. Pryke, C.J. Crook, P. Topham, S.J. Martin, A.J. Ryan, R.A.L. Jones,* Univ. of Sheffield, UK*; A. Menelle,* Lab. Lön Brillouin, France*; J.R.P. Webster,* Rutherford Appleton Lab, UK*; I. Soutar, L. Swanson,* Univ. of Sheffield, UK

We present neutron reflectometry results on (deuterated) water-swollen poly[diethylamino)ethyl methacrylate] (PDEAMA, a polybase) and polymethacrylic acid (PMAA) brushes grafted from silicon substrates using atom transfer radical polymerisation. The PDEAMA data are presently the more comprehensive and reveal that the expanded brush (low pH) is some 6 to 10 times thicker than the collapsed brush at high pH. Brush data will be compared with bulk solution data of the collapse transition of PMAA in water measured by a variety of fluorescence techniques: steady state energy transfer, lifetime, and time-resolved fluorescence anisotropy measurements, which enable a correlation of this single polymer in solution collapse transition with the confinement-influenced transition which occurs when the polymers are tethered to a surface.

Plasma Science and Technology
Room 213C - Session PS+BI-FrM

Plasmas in Bioscience
Moderator: K. Seaward, Agilent

8:20am **PS+BI-FrM1 X-Ray and Neutron Reflectivity Studies of Plasma Polymer Coatings**, *S.K. Øiseth,* Unaffiliated*; P.G. Hartley,* ***K.M. McLean***, CSIRO Molecular Science, Australia*; A Nelson, M James,* Bragg Institute, Australia

Plasma polymer coatings adhere to and contour the surfaces of most organic and inorganic materials, and are attractive as surface chemical modification systems, since they offer both robustness and inherent surface chemical functionality for further surface chemical derivatisation. A

variety of different techniques have been used to characterise the physico-chemical properties of surfaces of plasma polymer films. In many cases, however, it is also desirable to probe the internal structure of both modified and unmodified plasma polymer coatings in order to optimise their properties for a given application.. Reflectometry techniques are now becoming increasingly important in the characterization of nano-scale structured interfaces. X-ray reflectivity in particular is ideally suited to the study of the internal properties of layered film structures on surfaces, yielding data concerning sub surface structure and material properties. Neutron reflectivity meanwhile offers the ability to characterise surface layers in aqueous environments. In this study heptylamine and allylamine plasma polymer coatings were prepared on silicon wafers, and analysed using X-ray reflectometry before and after further surface modification procedures (e.g. adsorption of protein species from solution). Surface chemistry of the coatings was characterised using X-ray photoelectron spectroscopy (XPS). Atomic force microscopy (AFM) was also used to characterise both roughness and local film thickness at step-edges on the films. The excellent reflectivity data obtained demonstrate the suitability of plasma polymers for reflectivity studies (primarily due to their low surface roughness). Preliminary experiments on plasma polymer layers using neutron reflectivity are also described, which highlight the effect of surface hydration on layer properties.

8:40am **PS+BI-FrM2 Angle Resolved XPS Characterisation of Plasma Polymerised Chemical Gradients**, *K.L. Parry,* Plasso Technology Ltd.*; A.G. Shard,* University of Sheffield, United Kingdom, UK*; R.G. White,* Thermo Electron Corporation, UK*; J.D. Whittle,* Plasso Technology Ltd.*;* ***A. Wright****,* Thermo Electron Corporation, United Kingdom, UK

Well-defined chemical gradients are potentially important materials in a wide range of research activities. The surfaces of such materials can be derivatised with differing functional groups to provide spatially resolved surface chemical properties. Such surfaces can, for example, be used to immobilise biomolecules, which may become a route to producing novel biosensors. This study is concerned with chemical gradients within thin layers of plasma co-polymers deposited onto glass substrates. Continuous gradients of chemistry (hydrocarbon to either acid or amine) are produced by carefully programming the composition of the plasma monomer mixture while varying the area of the substrate exposed to the plasma. It will be shown that spatially resolved XPS is an ideal tool to determine the composition of the near surface region as a function of distance along the chemical gradient. Parallel angle resolved XPS provides additional information about the uniformity of the layer with depth. Such measurements can show, for example, surface enrichment of one of the functional groups in the co-polymer layer or variations in thickness along the chemical gradient. It is therefore possible to construct chemical state and thickness line scans or maps from the same ARXPS data set. Such data provide valuable information regarding the plasma co-polymer deposition process.

9:00am **PS+BI-FrM3 Surface Characterization of Plasma Processed Bio-Functional Micro-Patterned Polymeric Surfaces**, ***A. Valsesia****, M. Manso, M. Kormunda, P. Colpo, D. Gilliland, G. Ceccone, F. Rossi,* EU-JRC-IHCP, Italy

The functionalization of the material surfaces is one of the major requirements for the control of the biological response and for the improvement of the biocompatibility. Among the functionalization techniques, PE-CVD is of high importance since the control of the film properties is achieved by an accurate modulation of the plasma processing parameters. PE-CVD allows the synthesis of a wide spectrum of bio-functional polymers: acid/base fouling surfaces (PAA, PAL) and super-hydrophilic anti-fouling surfaces (PEG, PEG-like coatings). Moreover the combination of plasma deposition and plasma etching techniques allows the formation of micro and nano-patterned surfaces with contrasted functionalities. In this work we have studied the plasma deposition of PAA (COOH functional), PAL (NH2 functional) and PEG-like (anti-fouling) layers. The chemical surface characterization of the films has been performed by XPS and TOF-SIMS and the surface free energies components have been calculated by Contact Angle in static and dynamic mode. QCM provided the evaluation of the mechanical stability of the samples in buffer solutions as well as the calculation of the bio-activity of the surfaces in proteins absorption experiments. The surface topography of the samples has been investigated by AFM. The micro-patterned surfaces have been characterized by TOF-SIMS and XPS in imaging mode, revealing the capability of the plasma processing techniques to produce chemically contrasted micrometric motives. The bio-response (protein absorption and cell adhesion) of the micro-patterned samples is under study.

9:20am **PS+BI-FrM4 Plasma Sterilisation and De-pyrogenation of Surfaces: Review and Analysis of Mechanisms**, *F. Rossi, R. De Mitri, M. Hasiwa,* European Commission Joint Research Centre, Italy*; S. Bobin, R. Eloy,* Biomatech, France*; T. Hartung,* European Commission Joint Research Centre, Italy*;* ***P. Colpo****,* EU-JRC-IHCP, Italy

Mechanisms of plasma sterilisation are reviewed and analysed in terms of radiation induced desorption, UV radiation effects and etching. Different plasma discharges are analysed with Optical Emission Spectroscopy in order to find optimum conditions of UV emission and radicals production. Those effects are compared and related to effective sterilisation and depyrogenation rates obtained from the literature and experimentally on Bacillus subtilis and LPS. It is shown that UV emission is the major contribution for sterilisation and chemical etching for depyrogenation. SEM analysis of spores at different times of treatment show the degradation of the outer shell, as well as size and coverage reduction as the treatment duration increases. Effects of plasma on pyrogen is illustrated by AFM and ToF SIMS. A strategy for optimised sterilisation and depyrogenation treatment is proposed.

9:40am **PS+BI-FrM5 Plasma Processes for Micro- and Nano-Patterning Biomedical Polymers**, ***P. Favia****,* University of Bari, Italy **INVITED**

Low pressure plasma processes can tune chemical composition, surface energy and topography of most substrates of biomedical interest in a well controlled way. Among the wide range of plasma-modification procedures, micro- and nano-patterning plasma-deposition processes of thin coatings are among the most interesting and novel technologies aimed to drive the behaviour of cells on surfaces. Two kind of plasma procedures will be described in this contribution, and some interesting in vitro tests will be discussed. The first one consists in the deposition of "cell-repulsive" coatings through physical masks to produce patterned surfaces; here "cell-adhesive" domains are alternated to non-adhesive ones. The second process involves the deposition of teflon-like coatings of CF@sub x@ chemical composition, including randomly distributed surface features with nanometric dimensions, which are peculiar of certain deposition conditions in modulated regime. Acknowledgments This research has been developed in the framework of the MIUR-FIRB RBNE01458S_006, COFIN '99 "Biomaterials with micro- and nano- structured surfaces" and 'NANOMED' QLKE-CT-2000 projects, whose financial contribution is gratefully acknowledged.

10:20am **PS+BI-FrM7 Novel Plasma Modification of Microfluidic Devices for Control of Electroosmotic Flow**, ***E.R. Fisher****, C.S. Henry, M.A. Boggs, I.T. Martin, Y. Liu, C.D. Garcia,* Colorado State University

Microchip capillary electrophoresis (CE) is a widely used separation technique that combines the efficiency of CE with the portability of a microchip. Poly(dimethylsiloxane), PDMS, is often used to fabricate these microfluidic devices because it is inexpensive, has good optical properties, and the fabrication of complicated channel geometries is straightforward. Separations that occur in PDMS are based on the electroosmotic flow (EOF) within the channel. This, in turn, depends on the density of negatively charged groups on the PDMS surface, which is sensitive to both the pH of the solution and the sealing method (air plasma treatment, methanol). An additional issue is the hydrophobicity of the PDMS, which leads to the adsorption of hydrophobic analytes such as proteins during separations. The goal of this work is to treat PDMS with both non-depositing and depositing plasmas and fully characterize the altered surface chemistry, and its effects on EOF and separations. We have used depositing plasma systems to alter preassembled PDMS microchips, yielding novel surface chemistries. Plasma treated PDMS has been characterized using various surface analysis techniques, including contact angle measurements and XPS. XPS mapping shows that fluorocarbon (FC) plasma treatments permeate the channel via the reservoirs, not through the porous PDMS. Consequently, the reservoirs and channel are selectively coated with a FC film, resulting in reduced EOF. Conversely, plasma deposition of a hydrophilic hydrocarbon film yielded an increase in EOF. Selected coatings are stable over multiple EOF measurements. Separations conducted with treated chips evaluate biomolecule fouling characteristics. This is the first report of the modification of these devices via depositing plasma systems; plasma treatment of PDMS microchips has essentially been limited to O@sub 2@ or air plasmas to oxidize or cure the PDMS, for the enhancement of adhesion of PDMS to PDMS/glass.

10:40am **PS+BI-FrM8 Thiol-Based Plasma Polymer Coatings as Platforms for Biosciences Applications**, ***P.G. Hartley***, *S.K. Øiseth, T.R. Gengenbach, G. Johnson, K.M. McLean,* CSIRO Molecular Science, Australia

Radio frequency glow discharge plasma polymer coatings form robust thin films which contour and adhere strongly to the surfaces of polymeric and other materials. Their ability to modify surface properties, for example, to enhance biocompatibility or to introduce defined chemical functionalities at interfaces for the subsequent coupling of bioactive molecules have seen their widespread application in the field of biomaterials research. We report on the development of sulphur containing plasma polymers using ethanethiol as the feed monomer. In order to ascertain the influence of deposition conditions on the properties of the films, a range of protocols were employed. The films were characterised by X-ray photoelectron spectroscopy (XPS); atomic force microscopy; streaming potential and contact angle measurements. Since XPS data are not sufficiently specific to distinguish between similar carbon-sulphur functional groups (e.g. thiol vs. sulphides), the nature and density of the surface functionalities were quantified by using a thiol specific maleimide containing probe. The stability of the films was tested by assessing coating thickness and chemistry before and after autoclaving. The effects of ageing in air, particularly with respect to the chemical structure were monitored over several months. The incorporation of sulphur functionalities provide reproducible supports for the subsequent grafting of proteins and for the adhesion of gold nanoparticles. The coatings were also shown to act as supporters of cell attachment and growth.

11:00am **PS+BI-FrM9 The Low Damage Surface Modification of the Self-assembled monolayer by the N@sub 2@ Neutral Beam Irradiation**, ***Y. Ishikawa***, Tohoku University, Japan*; T. Ishida,* National institute of Advanced Industrial Science and Technology, Japan*; S. Samukawa,* Tohoku University, Japan

For the realization of future organic molecular devices, controlling surface property of molecular film, such as the electric properties of organic molecule, is quite important. To improve surface property of molecular film, fine surface modification method is highly expected. Thus, we propose the method for controlling the surface properties of organic films by applying plasma process. For this purpose, we used our nearly developed the neutral beam system.@footnote 1@ The system could prevent the charged particles and ultraviolet photons, and only the neutral particles were irradiated to the substrates. In this study, we irradiated the N@sub 2@ neutral beam to the robust self-assembled monolayers (SAMs) made from terphenyls@footnote 2@ on the gold substrate as the first attempt for neutral beam system to the organic molecular thin films. Energy of the N@sub 2@ neutral beams are at the highest 10 eV. We compared the X-ray photoelectron spectra of terphenyl SAMs before and after the N@sub 2@ beam irradiation. Then the C-N bonds were generated by the beam irradiation with maintaining the surfer molecular structure. This result indicates that the surface of the terphenyl SAMs would be replaced from carbon or hydrogen to nitrogen, and we can expect that the electric properties of the organic materials would be drastically changed by this method. @FootnoteText@ @footnote 1@ S. Samukawa, K. Sakamoto, and K. Ichki: Jpn. J. Appl. Phys., Part 2 40, L779 (2001)@footnote 2@ T. Ishida, M. Sano, H. Fukushima, M. Ishida, and S. Sasaki: Langmuir, 18, 10496 (2002).

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