Applied Surface Science
Room 2005 - Session AS+BI+NS+NM-MoM

Organic Surface Modification and Nanoscale Chemical Patterning

Moderator: R. Haasch, University of Illinois, Urbana

8:00am **AS+BI+NS+NM-MoM1 Biomolecular Lithography on GaAs Surfaces, *A. Ivanisevic,*** Purdue University **INVITED**

Atomic force microscopy (AFM) was used to fabricate well-defined peptide templates onto GaAs surfaces via Dip-Pen Nanolithography (DPN). DPN is a powerful technique to write specific organic and/or inorganic molecules onto a surface with an AFM tip. In this work, DPN was used to construct arrays of peptides with nanometer features. TAT peptides (e.g. CGISYGRKKRRQRRR) which exhibit rapid uptake in cells, were patterned onto the surface in either contact or tapping mode. Several techniques were used for the characterization of the modified surfaces: X-ray photoelectron spectroscopy (XPS), Fourier Transforms Infrared (FT-IR) spectroscopy and contact angle. Transmission FT-IR provided structural information such as peptide conformation. The complementary analysis confirmed the binding of the peptide onto the substrates and allowed to quantify the density of immobilized peptides on a given surface. Furthermore, the nanoscopic features were successfully used in recognition experiments where an RNA sequence with a loop structure, known for its specific interaction with the peptide, was tested. The results in this report indicate that one can use nanolithographic strategies to pattern GaAs surfaces, and therefore provide a proof-of-concept experiment that can be transferred in complex microfabricated semiconductor architectures.

8:40am **AS+BI+NS+NM-MoM3 The Effect of Ring Substitution Position on the Structural Conformation of Mercaptobenzoic Acid Self-Assembled Monolayers on Au(111), *J.R.I. Lee,*** Lawrence Livermore NAtional Laboratory*; T.M. Willey, J. Nilsson, L.J. Terminello, J.J. De Yoreo, T. van Buuren,* Lawrence Livermore National Laboratory

Mercaptobenzoic acid (MBA) is a viable alternative for preparing SAMs with carboxyl functionality. These molecules, as opposed to carboxyl-terminated alkylthiols, offer an extended pi-bonded system formed by the aryl and carboxyl groups facilitating charge transfer, and these extremely thin SAMs (< 8 Angstroms) have been shown via STM to contain a high degree of structural order. Furthermore, three different isomers of mercaptobenzoic acid may lead to differing surface properties. In this work, the orientation and bonding of self-assembled monolayers of the three positional isomers of mercaptobenzoic acid adsorbed on gold are investigated using near edge X-ray absorption fine structure (NEXAFS) spectroscopy and photoemission spectroscopy (PES). The isomer of MBA and solvent chosen in SAM preparation has considerable bearing upon film morphology. Monomers of 3- and 4-MBA assume an upright orientation on the Au substrates in monolayers prepared using an acetic acid in ethanol solvent. The aryl ring and carboxyl group of these molecules are tilted from the surface normal by a colatitudal angle of ~ 30 degrees. Preparation of 4-MBA SAMs using pure ethanol solvent, a more traditional means of synthesis, had no appreciable effect upon the monomer orientation, but S(2p) PES measurements illustrate that it results in extensive bilayer formation via carboxyl group hydrogen-bonding between 4-MBA monomers. In 2- MBA monolayers prepared using acetic acid/ethanol solvent, the monomers adopt a more prostrate orientation on the Au substrates, in which the aryl ring and carboxyl group of the molecules are tilted ~ 50 degrees from the surface normal. This configuration is consistent with an interaction between both the mercaptan sulfur and carboxyl group of 2-MBA with the underlying substrate. This work was supported by the Division of Chemical Sciences, Office of Basic Energy Science, and performed under the auspices of the U.S. DOE by LLNL under contract No. W-7405-ENG-48.

9:00am **AS+BI+NS+NM-MoM4 XPS Analysis of Arenes and Proteins on Gold Substrates Attached by Electrochemical Reduction of Aryldiazonium Salts, *J.L. Fenton,*** University of New Mexico*; S.M. Dirk, D. Wheeler,* Sandia National Labs*; J.E. Fulghum,* University of New Mexico

The properties of most materials can be altered by the attachment of molecules to their surface. Recent work has shown that diazonium salts can be used to attach aryl compounds to metal and semiconductor surfaces including silver, iron, platinum, silicon, and glassy carbon. Diazonium salts can be electrochemically reduced in acetonitrile leading to the attachment of aryl groups to the surface of a substrate. The attachment of aryl compounds using diazonium salts developed in our work, allows one to attach aryl compounds with different organic moieties onto metal substrates including gold. Aryl organic layers can be made of different thicknesses, and stacked structures can be created by alternating layers of molecules. This work is then expanded upon to attach proteins to metal surfaces. This approach provides a methodology to generate arrayed proteins on electrode arrays without the use of microfluidic methods such as ink jetting etc. The development of protein arrays can often provide high-throughput, quantitative measurement of receptor-ligand complexation giving an understanding of protein function, modification, and regulation. X-ray photoelectron spectroscopy (XPS) is used to deduce the different types of chemistries in single molecule samples, stacked structures, and surface tethered proteins. Angle resolved XPS is used to determine the relative location of each molecule in the stacked structures and possible orientation of proteins as well as to deduce the existence of azo linkages.

9:20am **AS+BI+NS+NM-MoM5 Challenges in the Modification and Characterization of Two- and Three-Dimensional Biointerfaces, *M. Textor,*** ETH Zurich, Switzerland **INVITED**

Surface modifications based on biochemical or biological principles are important tools for the fabrication of biosensor chips, biomedical devices such as implants, and of drug delivery carriers. Moreover, well-designed model biointerfaces have substantially contributed in the last decade to a better insight into fundamental aspects of cell-surface interaction. An overview will be given on tools enabling the surface engineer to tailor the interface of biomaterials, with special emphasis on the approach of eliminating non-specific adsorption and adding to such a silent surface biological functionalities. Preservation of active conformation and optimum presentation (orientation, density) of surface-immobilized moieties are particular challenges in this field. Different approaches to micro- and nanopatterning of surfaces, their specific advantages/disadvantages and applications in biorelated fields are discussed. Special emphasis is placed on methods that combine top-down (e.g., lithography) and bottom-up (self-assembly) approaches. Substantial new insight into the factors that govern cell-surface interactions and cell differentiation has recently be gained by using two-dimensional (2D) patterns. There is, however, an increasing interest to explore (stem) cell development in 3D microwells with well-controlled geometry (shape/size), surface chemistry and mechanical properties (substrate stiffness). The challenges of independently tailoring chemistry and structure/topography are addressed; self-assembly techniques are in this respect particularly useful. Finally, characterization/imaging techniques that allow the monitoring of biointerface reactions in situ, in real time and quantitatively are important tools; selected methods will be presented based on highly surface- and detection-sensitive evanescent-field-based sensing.

10:20am **AS+BI+NS+NM-MoM8 Nanoscale Chemical Patterning and Architectures, *P.F. Nealey,*** University of Wisconsin **INVITED**

Diblock copolymers are self-assembling materials consisting of two polymer chains connected at one end that tend to form ordered nanostructures, including spheres, cylinders, and lamellae, whose shape and dimensions depend on the molecular weight and composition of the polymer. Block copolymer lithography refers to the use of these ordered structures in the form of thin films as templates for patterning through selective etching or deposition. Already block copolymer lithography has been used to pattern dense periodic arrays for the applications such as quantum dots, nanowires, magnetic storage media, increased capacitance gate devices and FLASH memory. One of the current goals in block copolymer nanolithography is to reproduce many of the characteristics of the lithographic process used ubiquitously in nanomanufacturing, including pattern perfection over macroscopic areas, the ability to pattern arbitrary and non-regular geometries, dimensional control of features within exacting tolerances and margins, and registration and overlay. Two strategies will be discussed to integrate self-assembling materials into existing manufacturing practices so as to achieve molecular-level process control and the ability to produce useful architectures: directed assembly of block copolymers on topographically patterned substrates, and directed assembly of block copolymers on chemically nanopatterned substrates.

11:00am **AS+BI+NS+NM-MoM10 Tailoring of Functional Nano-Patterned Surfaces for Biosensing Applications by Combination of Plasma Processes and Electron-Beam Lithography,** *F. Brétagnol, L. Ceriotti, A. Valsesia, T. Sasaki, D. Gilliland, G. Ceccone, P. Colpo,* ***F. Rossi,*** JRC-IHCP-BMS, Italy

Fabrication of micro and nano-patterned surfaces with a well defined geometry and a controlled chemistry is a fundamental step for the development of bioengineered materials Micro-arranged surfaces

containing functionalities such as cell or protein adhesive in a non non-adhesive matrix provide a very useful tool in a large field of applications (tissue engineering, cell behavior investigations , artificial growth of neurons networksâ?¦) . Numerous methods have been successfully developed for the production of such surfaces including conventional photolithography, photochemistry , micro-contact printing , micro fluidic patterning . Nevertheless, the development of surfaces chemically patterned at nano scale is still a challenging issue for the implementation of new generation of miniaturized biochips and for the study of cell surface interactions. In this study, we present an innovative method for the fabrication of chemically nano-patterned surfaces. Maskless lithography based on electron-beam technique was successfully combined with plasma-based processes to create COOH terminated spots over a PEO-like matrix. Spots from micron to submicron size have been created. Quality control of the patterned surface was studied by Atomic Force Microscopy, XPS and ToF-SIMS analysis. Experiments with fluorescent proteins on the patterned surfaces exhibit a preferential adhesion on the active region showing the ability of this technique for the design of biosensing platforms.

11:20am **AS+BI+NS+NM-MoM11 Optically-Stimulated Surface Diffusion Exploited for Directed Self-Assembly on Amorphous Semiconductors**, *E.G. Seebauer,* ***Y. Kondratenko,*** University of Illinois at Urbana-Champaign

Nanoscale device fabrication technologies require toolsets for miniaturization and organization of materials at nanometer dimensions. Current toolsets have developed from two diametrically opposite strategies: top-down and bottom-up. This laboratory is taking a different approach based on a new physical mechanism for photostimulated diffusion discovered here. This new strategy combines attractive features of top-down and bottom-up approaches by exploiting the self-organization capabilities latent in amorphous materials, but in a way that can be controlled by optical or electron beam exposure tools. We have developed a new surface self-assembly method at the 10-200 nm length scale using amorphous semiconducting materials. Patterned optical or electron beam exposure yields a spatially varying surface mass flux that, when performed at an annealing temperature just at the cusp of crystallization, provides the extra nudge to crystallize subcritical nuclei in regions dictated by the light flux. The full-fledged crystallites then grow by surface diffusion and Ostwald ripening until the desired fraction of the film has accreted onto the original nuclei. We have demonstrated this technique with titanium dioxide as the substrate material. This scheme should apply to a wide variety of semiconducting materials on nearly arbitrary substrates to form nanoarrays, nanowalls, and possibly three-dimensional structures. Possible applications include chalcogenide semiconductors for data storage media; nanoparticles arrays for direct use in sensors and solar cells; and semiconductor arrays for indirect use as seed layers for the subsequent deposition of sintered particle films in fabricating advanced ceramics and devices such as rechargeable batteries, solar cells, gas sensors, and photonic band gap materials in solar windowpanes.

11:40am **AS+BI+NS+NM-MoM12 Nanowires and Nanodevices via Assembly of Clusters**, ***S.A. Brown,*** Nano Cluster Devices Ltd, New Zealand

We report the achievement of contacted electronic devices, self-assembled from atomic nanoclusters. Features of this technology, which overcomes the difficulties in the assembly of building blocks inherent to many bottom-up approaches to nanotechnology, include: Interchangeable cluster sources so as to have available a wide variety of cluster materials in a useful size range, allowing exploitation of novel structures and properties. Self-assembly methods which avoid time consuming positioning of building blocks. Use of lithographic processes which are compatible with both cluster deposition technology and standard microelectronics fabrication protocols. Understanding of basic physical assembly processes in order to predict and control device formation, including availability of suitable computer simulations. In this paper we will review the assembly methods developed, which include directed assembly in silicon V-grooves and on polymer-patterned surfaces, as well as stencilling techniques. We will then focus on the application of these techniques to one class of devices which have been realised i.e. cluster-assembled hydrogen sensors. These sensors are realised by the deposition of Pd clusters on a substrate such that one or several conduction paths are formed between a pair of contacts. The sensing principle relies on the expansion of Pd clusters and the resulting change of conductance as a result of their absorption of hydrogen and we have demonstrated very high sensitivities.

Biomaterial Interfaces
Room 2001 - Session BI1-TuM

Microbe-Surface Interactions
Moderator: G.P. Lopez, University of New Mexico

8:00am **BI1-TuM1 Getting to the Root of Bacterial Hair**, ***R.J. Emerson***, *T.A. Camesano, N.A. Burnham,* Worcester Polytechnic Institute

Many bacteria use their extracellular polymers to attach to surfaces, leading to such phenomena as biofouling and biofilm-initiated infections of medical devices. One method of determining the physicochemical and physicomechanical properties of these hairlike layers is through the acquisition and interpretation of AFM force curves. Most previous researchers have defined the point of zero separation as the cell wall, and assumed the constant compliance region of the force curve to be representative of that location. With the data processed in this manner, the force at the "wall" has been used to calculate the equilibrium length of the brush, as well as the grafting density of the polymers at the point of zero separation. Here, we show: 1) that the constant compliance region frequently occurs in the middle of the layers, 2) how the layer thicknesses may be more accurately quantified, 3) a quantitative method of establishing the position of the "roots of the hairs" at the cell wall, i.e., the true zero of the separation axis, and 4) the more appropriate use of the mesh density of the polymer brush, in lieu of the polymer grafting density. For the specific case of Pseudomonas aeruginosa, a commonly found bacterium in both hospital settings and the natural environment, we have identified two discrete layers, of equilibrium thicknesses 160 ± 8 and 1700 ± 400 nm, with respective mesh densities of (1.9 ± 0.1) x 10@super 26@ m@super -3@ and (7 ± 2) x 10@super 22@ m@super -3@. AFM force curves of bacterial exopolymers can now be meaningfully interpreted.

8:20am **BI1-TuM2 Antifouling Surface Treatments for Microfluidic Applications**, ***N.E. Chang***, *M.H. Lean, S.J. Limb,* Palo Alto Research Center, Inc.

To remediate bioparticle losses from adhesion to microfluidic device surfaces, we have implemented polyethylene glycol (PEG)-type coatings by self-assembled monolayer and plasma-polymerizing deposition techniques. A variety of substrates representative of device materials in contact with bioparticle solutions were subjected to static microbial adhesion tests. We saw significant reduction in B. thuringiensis adhesion for both types of PEG coatings and reduction in B. globigii adhesion for the plasma-polymerized coating. Furthermore, we have demonstrated that both PEG-type coatings on MEMS traveling-wave arrays are effective at reducing adhesion to polystyrene beads as well as both Bacillus species. Results from both experiments have provided groundwork for in-situ experiments in flow environments for assessment of bioparticle recovery and adhesion to electrically conductive parallel flow plates. Spectrophotometry is used to gauge bioparticle concentration before and after circulation within the flow chamber. Open circuit voltage is monitored to investigate electrical behavior affected by bioparticle adhesion to the flow plates. Comparison of both measurements for parallel flow plates of different conductivities with and without our plasma-polymerized PEG coating will help corroborate and predict the degree to which bioparticle losses can be minimized.

8:40am **BI1-TuM3 Biofilm Formation on Biomaterials Implant Surfaces**, ***H. Busscher***, University Medical Center Groningen, The Netherlands **INVITED**

Biofilm formation on biomaterials implant surfaces and subsequent infectious complications are a frequent reason for failure of many biomedical devices, such as total hip arthroplasties, vascular catheters and urinary catheters. The development of a biofilm is initiated by the formation of a conditioning film of adsorbed macromolecules, such as proteins, followed by adhesion of microorganisms, where after they grow and anchor through secretion of extracellular polymeric substances. Adhesion of microorganisms is influenced by the physico-chemical properties of the biomaterial surface. Positively charge materials stimulates bacterial adhesion, but prevent growth of adhering bacteria. The use of low surface free energy materials did not always reduce in vitro adhesion of bacteria, but has been found beneficial in in vivo applications where fluctuating shear forces prevail, like on intra-oral devices and urine catheters. Polymer brushes have shown a very high reduction in in vitro adhesion of great variety of microorganisms, and AFM demonstrated weak adhesive forces. However, for clinical application, the long term stability of many types of polymer brushes is still a limiting factor.

9:20am **BI1-TuM5 Minimising of Biofilm Formation by Surfaces Coated with Fish Proteins**, ***P. Kingshott***, University of Aarhus, Denmark; *N. Bernbom,* Danish Institute for Fisheries Research; *L.M. Meyer, S. Xu, F. Besenbacher,* University of Aarhus, Denmark; *L. Gram,* Danish Institute for Fisheries Research

No surface exists that can prevent biofilm formation. Many surface treatments (e.g. use of antimicrobial agents) may help reduce biofilm formation, but the potential exists for developing bacterial resistance, and loss of function by agents getting depleted or covered up by organic matter (e.g. proteins). New surfaces that provide a barrier to bacterial attachment, but are not inhibitory and toxic, are highly desirable. We have recently discovered that attachment of bacteria found in the food industry is reduced by several orders of magnitude by coatings of an extract made from fish, compared to other organic layers (e.g. from meat, broth, milk). The coating does not per se inhibit microbial growth, the effect lasts up to 48 hours and the treatment is non-toxic. The coatings are equally effective against a range of bacteria persistent on food processing equipment including Ps. fluorescens strain AH2, E. coli strain MG1655, Vibrio anguillarum strain 90-11-287, and Aeromonas salmonicida strain Jno 3175/88. The extracts can be coated on metals (stainless steel) or polymers (polystyrene) and still be effective. Our aim is to use surface techniques (XPS, AFM, ToF-SIMS, surface-MALDI) to characterise the fish extract adlayer, and find out which component(s) reduce bacterial adhesion. Initial results demonstrate that the adlayer is protein in nature and that all surfaces adsorb high levels. Surface-MALDI shows that there are proteins of common molecular weight that adsorb to all surfaces tested, and these most likely play a role in the antifouling effect. The surface protein patterns are different to other conditioning media (e.g. TSB, chicken extract). Results will also be presented for the fish extract fractionated by chromatography and coated on surfaces, aimed at identifying more specifically the protein(s) involved. The results are discussed in terms of the potential antifouling mechanisms, such "steric-repulsive" effects or are the proteins antimicrobial.

9:40am **BI1-TuM6 Microfluidic Devices That Capture Bacteria for Growth and Kill Analysis**, ***M. Lochhead***, Accelr8 Technology Corporation

New clinical diagnostic instruments to address critical public health issues depend on a fundamental understanding of bacterial adhesion and growth at solid surfaces and the ability to control these processes. New technologies that decrease the time required for accurate identification and antibiotic susceptibility profiling of pneumonia-causing bacteria in intensive care units are one important example. Current methods require extended bacterial culture time and often force pre-mature clinical empiric antibiotic therapies, reducing positive patient outcomes and contributing to the emergence of resistant strains. Further, susceptibility testing from culture-based methods present a population-derived result and frequently can obscure the effects of minority sub-populations. We have developed a new microfluidics device that quantitatively tracks bacterial real-time growth rate and allows antibiotic susceptibility monitoring, reducing the overall time of a bacteria antibiotic challenge to less than 6 hours. Device performance with several bacterial strains and samples, and polymer coating surface characterization using protein, cell, bacterial and several surface analytical (e.g., XPS) measurements will be described. Application of this tool in the development of a fully integrated microfluidic system for automated bacterial growth and kill analysis will be demonstrated.

10:40am **BI1-TuM9 Vapor Phase Photografting of Antimicrobial Polymer Coatings**, ***T.P. Martin***, *K.K. Gleason,* Massachusetts Institute of Technology

An all-dry vapor phase photografting process is employed to covalently attach antimicrobial polymer coatings to polymeric substrates, including both spun-cast PMMA layers and nylon fabric. Antimicrobial fabrics are of interest in military applications, such as biowarfare protection, self-decontaminating fabrics, undergarments for long term use on deployment, as well as civilian uses such as textiles in hospital environments, including bedding, draping, and scrubs. Antimicrobial coatings are also of interest for use on medical devices. Microbial colonization of medical devices is associated with significant expense and mortality. A permanent, durable non-leaching antimicrobial surface is important for both of these applications. A fabric coating must survive many wash cycles, and a medical device coating must not detach in the body. Existing strategies for imparting antimicrobial properties to surfaces commonly employ an antimicrobial agent, such as silver ions or antibiotic drugs, which leaches out from the bulk material. However, the time of effectiveness will be limited as the agent will eventually be exhausted. Additionally, the use of antibiotic drugs with medical devices has the potential to promote drug

resistant microbes, whereas the class of antimicrobial polymers under examination here has not been shown to do so. In this work, the type-II photoinitiator benzophenone is used in conjunction with methacrylate- or styrene- based monomer containing a tertiary amino group with pKa > 9. The amino group is protonated to the active cationic state at physiological conditions. The coatings were tested according to ASTM E2149-01, and demonstrated good antimicrobial activity against e. coli, with a 99.999% reduction (5 log) in viable bacteria. In addition, the coatings show no zone of inhibition against e. coli, indicating the active polymer is not leaching from the surface. Finally, the active surface is maintained through various durability testing.

11:00am **BI1-TuM10 Biocompatible Ag-doped Carbon Coatings with Biocidal Effect***, **J.L. Endrino,*** Lawrence Berkeley National Laboratory*; M. Allen,* SUNY Upstate Medical University*; R. Escobar Galindo, J.M. Albella,* Instituto de Ciencias de Materiales de Madrid, Spain*; A. Anders,* Lawrence Berkeley National Laboratory

Medical implants can be an appropriate solution to many health problems, however, any time a foreign device is implanted into one's body there is a high risk for infection. For this reason, there is an increasing interest in the development of multifunctional coatings that can provide a highly biocompatible surface while protecting from infection threats. Recent studies have shown the possibility of incorporating antibacterial elements into carbon coatings with the idea of providing medical implants with necessary infection resistance. In general, diamond-like-carbon (DLC) coatings have excellent tissue biocompatibilities and high chemical inertness; consequently they are suitable as a matrix material that can embed different drug release substances. In this interdisciplinary study, Ag/C nanocomposite coatings have been prepared using two different deposition techniques: i) dual cathode pulsed cathodic-arc (PCA) from silver and graphite cathodes and ii) Ag cathodic-arc in reactive methane (CH4) atmosphere. The silver to carbon ratio in the samples was varied from 0 to 0.1 and was controlled by adjusting the relative arc pulse frequencies of the silver and carbon pulsed sources. Chemical composition, microstructure and mechanical properties of the samples were analyzed using glow discharge optical spectroscopy (GDOES), scanning electron microscopy (SEM) and the nanoindentation technique. Morphological examinations of samples deposited on 24 well tissue culture plates confirmed that there were no adverse effects in the production of specific osteoblast proteins on low content silver-doped carbon coatings.

11:20am **BI1-TuM11 Multi-functional Ag/Plasma Polymer Coatings for Antibacterial Biomaterial Surfaces***, **D.J. Balazs,*** *D. Hegemann, M. Heuberger,* EMPA Materials Science and Technology, Switzerland

In the past decades much research pertaining to biomaterials surface modification was centred on modifying chemistry or wettability as a strategy to manage interactions with the surrounding environment. New demands for biomaterials surface enhancements have evolved to include multi-functionality and controlled-release. Low pressure plasma techniques represent a unique opportunity to develop tailor-made surfaces with one-step processing in the framework of an eco-friendly process. Thus, low pressure plasma processing is used to develop multi-functional coatings for smart biomaterials applications. Coatings that combine properties such as enhanced cell growth, improved wound healing, and bacterial infection prevention are the focus of the work presented. The deposition of multi-functional silver/plasma polymer (Ag/pp) nano-composites, consisting of nano-scaled Ag clusters embedded within a plasma-polymer matrix is described. Multi-functional Ag/Acrylic acid coatings are deposited to combine anti-bacterial and enhanced cell adhesion properties for wound healing applications. Likewise, Ag/amino-hydrocarbon coatings can be used to attract proteins important to cell growth and simultaneously prevent bacterial colonization. Data is reported pertaining to bacterial adhesion testing in both Gram negative and positive culture environments. A key aspect to the development of controlled-release biomaterials is the ability to characterize release or adsorption kinetics in a precise manner. In order to achieve this, we are using a novel in situ analysis technique that is equally sensitive as other commonly used in situ methods (ca 1 ng/cm@super 2@), and has the benefit that is significantly cheaper and faster. Using plasma deposition a multi-layer biosensor is built, permitting the quantification of both release and adsorption kinetics of the multi-functional coatings. The kinetics of silver release and protein adsorption to Ag/pp is also described.

11:40am **BI1-TuM12 Detection and Mapping of Individual Adhesins on Living Bacteria using Atomic Force Microscopy***, **V. Dupres,*** Université Catholique de Louvain, Belgium*; F.D. Menozzi,* Institut Pasteur de Lille, France*; Y.F. Dufrene,* Université Catholique de Louvain, Belgium

Bacterial pathogens adhere to host cells via the specific interaction between surface proteins, referred to as adhesins, and host surface receptors. Although much progress has been made in the identification and characterization of adhesins borne by pathogenic bacteria, the molecular details underlying such interactions remain largely unknown owing to the lack of appropriate probing techniques. In this work, we used atomic force microscopy (AFM) with tips bearing biologically active molecules to measure the specific binding forces of individual adhesins and to map their distribution on the surface of living bacteria.@footnote 1@ First, we determined the adhesion forces between the heparin-binding haemagglutinin adhesin (HBHA) produced by Mycobacterium tuberculosis, the etiologic agent of tuberculosis, and heparin, used as a model receptor. We obtained a bimodal distribution of the adhesion forces with average forces of 50 pN and 117 pN, which could be attributed to one and two binding events between HBHA and heparin. Both the adhesion frequency and adhesion force increased with contact time, indicating that the HBHA-heparin complex is formed via multiple intermolecular bridges. We then mapped the spatial distribution of single HBHA molecules on the surface of living mycobacteria using heparin-modified tips. Strikingly, adhesion events were observed in about half of the locations and were concentrated into nanodomains over the mycobacterial surface. @FootnoteText@ @footnote 1@ Dupres V., Menozzi F.D., Locht C., Clare B.H., Abbott N.L., Cuenot S., Bompard C., Raze D. and Dufrene Y.F., Nat. Methods, 2, 515-520 (2005).

12:00pm **BI1-TuM13 Dynamic Interactions of the Streptococcal C5a Peptidase with Fibronectin***, **J.R. Hull,*** *G. Tamura, D.G. Castner,* University of Washington

Group B Streptococci (GBS) are a leading cause of sepsis and meningitis in newborns, and an emerging cause of serious bacterial infections in immunocompromised adults and the elderly. The streptococcal C5a peptidase (ScpB) of GBS is found in virtually all clinical isolates of GBS. ScpB inhibits neutrophil chemotaxis by enzymatically cleaving the complement component C5a. ScpB is a known Fibronectin (Fn) adhesin; however, it only binds to immobilized Fn and not soluble Fn. Therefore, it is unknown whether or not ScpB binds to a conformational determinate of Fn or multiple adjacent Fn molecules. For this study, Surface Plasmon Resonance (SPR) was used to investigate the interactions of soluble Fn with Scp bound the sensor surface. Scp was made as a GST fusion and bound to the sensor surface through a self-assembled monolayer of glutathione. It was found that binding of soluble Fn with Scp is significantly lower than the binding of Scp to immobilized Fn (KD ~4.0 nM). Next, immobilized Fn was probed with Scp attached to an AFM tip via the bifunctional crosslinker pyridyldithio poly(ethylene glycol) succinimidylpropionate. Each step of the tip functionalization was verified by X-ray photoelectron spectroscopy, and static secondary ion mass spectrometry. It was found that the interaction force between immobilized Fn and Scp is roughly 100 pN. With this force value, a force map was made showing where the Fn/ScpB interactions occurred.

Biomaterial Interfaces
Room 2014 - Session BI2-TuM
Biodiagnostic Innovation

Moderator: S. Saavedra, University of Arizona

8:00am **BI2-TuM1 The Fabrication and Characterization of Polycrystalline Silicon Active Layer Thin Film Transistor with Vertically Aligned Carbon Nanofiber***, **J.-W. Park,*** *S.-J. Jun,* The University of Tennessee*; A.V. Melechko, T.E. McKnight, M.L. Simpson,* Oak Ridge National Laboratory*; P.D. Rack,* The University of Tennessee

Thin film transistors (TFT) with vertically aligned carbon nanofibers (VACNF) are an attractive electronic switching device for nanoscale electroanalysis and delivering biological material into live cells. In our previous work, we have demonstrated an inverted back-channel-etched amorphous silicon TFT array integrated with VACNFs. To enhance the device performance, we have explored a polycrystalline silicon active and n+ contact layer with a standard top-gate structure. For the current device, source/drain and gate metals and gate dielectric thin films were rf magnetron sputter deposited. The Polycrystalline active layer and n+ contact layers were deposited by low pressure chemical vapor deposition (LPCVD). We have examined

several enhanced crystallization strategies including stress induced crystallization (SIC) using dielectric caps and metal induced crystallization (MIC). The silicon films have been characterized by x-ray diffraction, scanning electron microscopy, and Raman spectroscopy. In this presentation, we will demonstrate the process flow of the staggered structured polycrystalline TFTs and the nanofiber integration scheme. The electrical characteristics of the transistor will be discussed and correlated to the silicon active layer crystallization. Furthermore, we will demonstrate the electrochemical characteristics of the TFT addressed array in various biological electrolyte solutions.

8:20am **BI2-TuM2 Detection of Surface Potential Modulation Induced by Molecular Dipole Moment for Biosensor Platform Development on GaAs**, ***K. Lee***, *H. Park, R. Jean,* Purdue University*; A. Yulius,* Yale University*; A. Ivanisevic, J. Woodall, D.B. Janes,* Purdue University

Ultra-sensitive RNA detectors have been receiving enormous attentions with recent breakthroughs in finding RNA biomarkers for specific diseases. One way to detect a small quantity of specific biomarkers is to utilize well-established biological interactions. Generally, there are two key challenges when using bio-molecules as active sensing components: 1) reliable immobilization of bio-molecules and 2) an efficient sensing mechanism to convert a sensing event into a quantifiable signal. In this study, 1-octadecanethiol (ODT) and TAT peptides are self-assembled onto pre-fabricated GaAs-based sensor platforms, and conductivity modulation due to surface potential changes of ODT- or peptide-modified devices will be investigated. The TAT peptides used in this experiment are known to retain their recognition properties with TAR RNA after being attached on a GaAs surface. The sensor layer structure, which consists of a undoped low-temperature grown GaAs cap (3 nm), a 1e20 cm@super -3@ n-GaAs cap (10 nm), a 5e17 cm@super -3@ n-GaAs channel (50 nm), and a 5e16 cm@super -3@ p-GaAs base (100 nm) from the top, was grown by MBE on semi-insulating GaAs. The first two layers are added to achieve non-alloyed ohmic contacts with low specific contact resistivity. Deposition of Au/Ti injector/collector contacts completes the device fabrication, and the I-V curve displays ohmic characteristics with high current density. Subsequent wet-etching of the top cap layer resulted in ~20x decrease in conductivity, which is explained by surface Fermi level pinning of air-exposed GaAs. ODT-modification increased conductivity by ~30%, whereas no significant change was observed with peptide-modification, which could be attributed to either passivation or molecular dipole effect. The dip-pen nanolithography technique is being investigated to increase the surface coverage of sensing molecules on active device regions, and studies on reactivity of modified devices to TAR RNA is still in progress.

8:40am **BI2-TuM3 Covalent Functionalization of Amorphous Carbon Thin Films: Materials Integration for Real-time Electromechanical Biosensing**, ***B. Sun***, *P. Colavita, H. Kim, M. Marcus, L.M. Smith, R.J. Hamers,* University of Wisconsin at Madison

One of the barriers to real-time biosensing is the need for development of interfaces that are compatible with microelectronics processing methods and that also provide the requisite selectivity and stability when exposed to biological environments. Previous studies have shown that diamond thin films exhibit excellent stability and selectivity. However, diamond deposition temperatures are typically high, limiting the ability to integrate with low-temperature materials. Amorphous carbon films can be deposited to form very thin coatings on a wide variety of materials. Here, we show that varpor-deposited amorphous carbon (a-C) can be covalently modified with functional organic layers in order to covalently tether biomolecules, yielding excellent interfaces with excellent selectivity and stability. We characterized functionalized films by X-ray Photoelectron Spectroscopy (XPS), Infrared Reflection-absorption Spectrocopy (IRRAS), and fluorescence techniques. Surfaces coated with amorphous carbon and covalently modified by single stranded DNA exhibit high biochemical selectivity when exposed to complementary vs. non-complementary sequences. Our results show that covalently modified amorphous carbon films display excellent chemical stability, superior to alternative substrates such as gold, glass, etc. We also demonstrate that the chemical treatments developed here are compatible with metal electrode structures, by integrating amorphous carbon thin films with Quartz Crystal Microbalance (QCM) sensor. We demonstrate real-time DNA detection on the carbon-coated QCM sensor. Moreover, the sensor surface can be regenerated multiple times with no detectable degradation of its performance. Our results demonstrate that amorphous carbon thin film forms a stable bio-interface with excellent microelectronic compatibility, that provides a suitable platform for integrated real-time bio-electrical sensing and existing microelectronic technology.

9:00am **BI2-TuM4 Single Nanopores for the Detection and Characterization of DNA and Anthrax Toxins**, ***J.J. Kasianowicz***, *B. Nablo, M. Misakian, S.E. Henrickson, K. Rubinson,* NIST*; T. Nguyen, R. Gussio,* NCI*; K.M. Halverson, S. Bavari, R.G. Panchal,* USAMRIID*; V.M. Stanford,* NIST **INVITED**

We are using electrophysiology, molecular biology and computer modeling to better understand how biopolymers (e.g., single-stranded DNA and proteins) partition into and transport through nanometer-scale pores that are formed by bacterial toxins. The results provide insight into the mechanism by which these toxins work in-vivo. They are also the physical basis for several potential nanobiotechnology applications including rapid DNA sequencing, sensitive and selective detection of a wide range of target species, high throughput screening of therapeutic agents against several anthrax toxins, and detection of anthrax lethal toxins in blood. Supported in part by NIST, the NIST Advanced Technology Program, the NIST Office of Law Enforcement Standards, and NSF.

9:40am **BI2-TuM6 A Novel High-Throughput DNA Chip Analysis Platform**, ***O. Prucker***, *T. Neumann, G. Dame, T. Brandstetter, J. Ruehe,* University of Freiburg - IMTEK, Germany

One of the key problems in the development of a DNA chip for a given analytical problem is the determination of suitable chip parameters: the correct DNA probe sequences need to be found, different types of buffers are to be tested at different buffer concentrations and the optimum temperatures for, both, hybridization and melting need to be determined. This list is by no means exhaustive but already demonstrates that the development and optimization of a chip requires thousands of experiments and test chips leading to often unacceptable development times and costs. In this contribution we will present a chip analysis platform technique that addresses this problem. The entire system consists of two key features: A total internal reflectance fluorescence (TIRF) readout system in which the fluorescence is excited through the chip itself which acts as a waveguide. The exciting light is such provided through the evanscent wave travelling along the chip surface. This mode of excitation allows for the fluorescence readout in the presence of the analyte solution and a flow cell with heating / cooling devices can be used to follow the hybridization and to reset the chip by melting/washing protocols without the need to take the chip out of the detector. The second key feature of this system is the chip itself. We have developed a polymer chip made from PMMA or cyclic olefin copolymers (COCs) onto which the probes are printed together with a photocrosslinkable polymer. Upon illumination the polymer forms a three dimensional hydrogel that acts as a carrier for the probes. This "skyscraper" approach allows for the deposition of a higher probe concentration per surface area leading to an enhanced chip sensitivity and selectivity. Examples will be given that demonstrate that most of the parameters that are essential for chip performance can be determined on one single chip such that most questions are answered "at the end of the day".

10:40am **BI2-TuM9 Biosensing with Fluidic Force Discrimination and Microbead Labels: Comparison of Sequential and Mixed-Homogeneous Assay Schemes**, ***S.P. Mulvaney***, *K.M. Myers, L.J. Whitman,* Naval Research Laboratory

A significant challenge for all biosensors is to achieve high sensitivity and specificity while minimizing sample preparation requirements, protocol complexity, and assay time. We have achieved multiplexed, femtomolar detection of both DNA and proteins in complex matrices in minutes by labeling conventional assay schemes with micrometer-scale beads, and applying fluidic force discrimination (FFD). In such assays, analytes captured onto a microarray are labeled with microbeads, and nonspecifically bound bead labels are preferentially removed through the application of controlled microfluidic forces. The density of beads that remain bound is proportional to the analyte concentration. Our typical microbead-labeled assay uses a sequential scheme, whereby the biorecognition builds from the capture probe, to the target, to the label probe, and finally the bead label. Alternatively, a two-step mixed-homogeneous assay scheme is possible with microbead labels and FFD. In this scheme, the sample is mixed with all the reagents (including the microbeads) in solution, and the analyte-loaded beads are then allowed to interact with the capture probes on the microarray. In the second step, FFD is applied and the bound labels counted. This scheme holds potential for enhanced target capture via preconcentration, but the large relative size of the microbead label relative to the molecular analytes complicates the biophysics. Our analysis of the sequential vs. mixed-homogeneous assay schemes at high and low analyte concentrations will be discussed. @FootnoteText@ S.P.M. and K.M.M. are employees of Nova Research Inc., Alexandria, Va.

11:00am **BI2-TuM10 HaloTag@super TM@ Technology for Protein Arrays**, ***N. Nath***, *J. Zhu, D. Klaubert, D. Storts, K. Wood,* Promega Corporation

Protein interaction arrays are emerging tools geared toward proteome scale detection of protein-protein, protein-drug or protein-DNA interactions. Wide application of protein array technology however faces significant challenge due to lack of high-throughput method for protein expression and purification. Here we present a new HaloTag@SUPER TM@ technology for rapid and covalent capture of fusion proteins in an oriented fashion directly from complex protein matrices without any prior purification. HaloTag protein is a mutant hydrolase that makes covalent bonds with its chloroalkane substrate. We demonstrate that proteins expressed as HaloTag fusions can be sensitively and irreversibly captured on a PEG based microarray substrate modified with chloroalkane substrate. Because HaloTag is compatible with in-vitro protein expression systems, multiple fusion proteins can be rapidly synthesized (90min) and immobilized in parallel. We also demonstrate that captured fusion proteins are functionally active and are able to interact with their interacting partner. Result indicates that HaloTag technology is perfectly suited for rapid prototyping of protein interaction arrays for large scale study of interaction networks.

11:20am **BI2-TuM11 Partial Hydrolysis of a Polyimide Surface for Biomolecule Immobilization and Biosensing**, ***S. Chakraborty***, *P. Betala, W.J. Buttner, V.H. Perez-Luna, J.R. Stetter,* Illinois Institute of Technology

The unique electrical, thermal, chemical and mechanical properties of polyimide films make them widely used in electronic components. Thus, their surface modification could be of interest in the biosensors field. Here we present a detailed study on the surface modification of polyimide films through partial hydrolysis. The surface modification is explored by wettability measurements and Fourier Transform Infrared (FTIR) Spectroscopy. Strategies to immobilize biomolecules such as antibodies on these surfaces are presented. Immobilization strategies take advantage of the carboxyl groups formed during the hydrolysis procedure. The application of this process to biosensing is illustrated with an impedance based imaging system array that is adapted to the detection of biological entities.

11:40am **BI2-TuM12 Label-Free Biosensing in Array Format Utilizing Surface-Adsorbed Core-Shell Nanoparticle Layers**, ***R. Dahint***, *E. Trileva, H. Acunman, U. Konrad, M. Zimmer,* University of Heidelberg, Germany; *V. Stadler,* German Cancer Research Center, Germany; *M. Himmelhaus,* University of Heidelberg, Germany

A novel method for spatially resolved, label-free biosensing based on core-shell nanoparticle films is presented. For the preparation of the layers, polystyrene particles of 350 nm in diameter are self-assembled on a gold-coated substrate to form a random-close-packed monolayer. Afterwards, the nanoparticle layer is covered with a metal film by first depositing gold colloid of 2-3 nm in size, followed by an electroless plating step. The resulting surface shows a pronounced optical extinction upon reflection of white light with the extinction maximum located in the NIR region of the electromagnetic spectrum. When organic molecules bind to the surface, the peak position of this maximum shows a pronounced red-shift. In case of octadecanethiol adsorption, a peak shift of 55 nm on average has been observed, which is about five times that of established label-free sensing methods based on propagating and localized surface plasmons. By immobilizing a pattern of different peptides on the nanoparticle layers and reacting the surface with specific antibodies it has been demonstrated that biospecific interactions can be label-free detected in array formats with high sensitivity. For the suppression of non-specific adsorption, which may lead to false results in the identification of binding events, the peptides have been embedded into an inert matrix material based on poly(ethylene glycol). In the future we intend to immobilize high-density peptide libraries onto the nanoparticle layers by combinatorial synthesis to facilitate in situ, parallel, time-resolved, and label-free screening of biospecific binding processes.

12:00pm **BI2-TuM13 Towards a Label-Free Biosensor: Defining and Measuring Sensitivity of Nanoscale Plasmonic Systems**, ***G. Nusz***, *A. Curry, A. Wax, A. Chilkoti,* Duke University

A label-free biosensor based on the optical response of the surface plasmon resonance of nanostructures (nanoSPR) is proposed. This system transduces nanoscopic changes in refractive index near the nanostructure surfaces into a macroscopically measureable signal. The sensor is designed such that binding of the target biomolecule to the metal nanostructure effects this refractive index change, generating the signal. Here, we present our work on optimizing the nanostructures to create the most useful biosensing system. We examine possible routes towards optimizing sensor sensitivity with an emphasis towards design considerations such as nanostructure synthesis, detection method, analyte structure and system applicability to biological samples. Specifically, we present our analysis of single-nanoparticle spectroscopy of both chemically synthesized and electron beam lithographically fabricated arrays of single and near-field coupled structures. We define and analyze sensitivity of nanostructures in terms of refractive index sensitivity, electric field enhancement and model binding studies. With these assays, it is possible to form a more complete characterization of nanoparticles in general and to determine of optimal nanoparticle characteristics for biosensors.

Biomaterial Interfaces
Room 2014 - Session BI+MN-TuA

Microfluidics, MEMS, Lab-on-Chip
Moderator: M. Lochhead, Accelr8 Technology Corporation

2:00pm **BI+MN-TuA1 Disposable Molecular Diagnostics: Microfluidic Laboratories for the Field**, ***C.M. Klapperich,*** Boston University **INVITED**
The impact of infectious disease on resource poor areas of the world is devastating. It is unlikely that the financial climate surrounding drug development for diseases prevalent in third world countries will soon change. Our best approach at dealing with some of these diseases will be prevention efforts assisted by the widespread availability of inexpensive and accurate diagnostics. Probes to amplify and identify microbial or viral nucleic acids are available for almost every well known disease vector. Assays for serum antibodies to many organisms are also well documented. Dedicated engineering of test protocols (patient sample preparation, dilutions, washing, blocking, and detection) and devices is required to move these technologies out of the research laboratory and into the field where they can have a more immediate impact on world health. We are focused on designing and prototyping disposable microfluidic platforms to enable molecular diagnostic testing using patient samples in locations far from a full service laboratory. Device design goals are low production costs, long shelf lives and relative ease of use. This talk will cover the formulation, fabrication and testing of microfluidic solid phase extraction columns based on polymer monoliths impregnated with nanoparticulate inclusions for protein and nucleic acid isolation from patient samples. Mammalian and bacterial cell lysis in microfluidic chips will be covered in addition to a discussion of strategies for micro and nanoscale enzyme linked immunosorbent assays. Practical concerns about the direct use of patient samples (blood, urine, saliva, and stool) will also be addressed.

2:40pm **BI+MN-TuA3 Surface Modification of Microfluidic Devices for Biological Applications**, *M. Salim, G.J.S. Fowler, B O'Sullivan, P.C. Wright,* ***S.L. McArthur,*** University of Sheffield, UK
Microfluidic systems are becoming increasingly important for bioanalytical and biochemical research such as proteomics, genomics, clinical diagnostics and drug discovery. Miniaturisation has been applied to bioassays and biological applications such as electrophoresis, DNA sequencing, DNA separation, immunoassays, polymerase chain reaction (PCR), cell counting, enzymatic assays, cell sorting and cell culture onto a chip. In all of these applications the ability of control biomolecule interactions with the device surface is critical. In this study we use ELISA, fluorescence microscopy, XPS and ToF-SIMS to investigate a range of surface modification methods for controlling the non-specific adsorption and immobilisation of biomolecules on glass microfluidic devices and micro-capillaries. The results highlight that protein adsorption occurs rapidly on the devices and that while varying concentration, residences time, flow, pH all influenced the adsorption profiles, none of these were able to completely eliminate protein adsorption. Plasma polymerisation has proved to be a flexible system for controlling the interactions along microfluidic channels. The wide range of monomers available for polymerisation has enabled us to develop low-fouling channels, functionalised substrates for the immobilisation of enzymes and antibodies and spatially control the surface chemistry along a channel length.

3:00pm **BI+MN-TuA4 Study of FET Flow Control and Electrostatic Response of Charged Molecules in Nanofluidic Channels**, *Y. Oh, D.N. Petsev,* University of New Mexico*; C.F. Ivory,* Washington State University*; C.H. Chung,* Sungkyunkwan University, Korea*; S.R.J. Brueck, G.P. Lopez,* ***S.M. Han,*** University of New Mexico
Using scanning laser confocal fluorescence microscopy (SL-CFM) and multiple internal reflection Fourier transform infrared spectroscopy (MIR-FTIRS), we have studied the field-effect-transistor (FET) flow control of charged dye molecules (Alexa 488 and Rhodamine B) in a parallel array (~10@sup 5@) of nanochannels during electroosmosis. For fluidic FET, a DC potential is applied to the gate surrounding an isolated mid-section of the channels. The gate potential controls the surface charge on SiO@sub 2@ channel walls and therefore the @xi@-potential. Depending the polarity and magnitude, the gate potential can accelerate, decelerate, or reverse the flow. We observe that the isolated gate, which is heavily doped with B (~10@sup 19@ cm@sup -3@), shows more pronounced control than applying the bias to the entire substrate. We also demonstrate a pH shift in nanochannels, when bulk electrolyte solutions enter the channels. This outcome illustrates that the solution pH can be further controlled by the gate bias. To improve the controllability of flow and to introduce a pH gradient along the channels for isoelectric focusing, our latest nanochannel device contains multiple gates. A different potential is applied to each gate to differentially control the surface charge on the SiO@sub 2@ channel walls and to create a pH gradient along the channels. Since the nanochannels are integrated into a MIR infrared waveguide, we can also probe the molecular orientation, segregation, and reaction of charged molecules in nanochannels in response to the gate bias. For instance, the xenthene-skeletal C-C vibrational modes at 1617 and 1552 cm@sup -1@ of Rhodamine B shift upon gate biasing, indicating a conformational change. The control of pH gradient as a function of gate bias as well as molecular orientation will be further discussed in this presentation.

3:20pm **BI+MN-TuA5 Soft-state Biologic ASICs and nSERS for Quantitative Medicine**, ***L.P. Lee,*** University of California, Berkeley **INVITED**
In order to create high-content Integrated Quantitative Molecular Diagnostics (iQMD) chip, Biologic Application Specific Integrated Circuits (BioASICs) and quantum nanoplasmonic probes such as nanostructured surface enhanced Raman scattering (nSERS) substrates are developed. Soft-state BioASICs are created by connecting existing and novel microfluidic circuits for high-content experimental biology in new ways. We are creating a library of these "building blocks" to develop multifunctional biological microprocessors. To build a solid foundation of future high-speed micro- and nanofluidic bioprocessors for experimental systems biology and biomarker discovery, we have developed design rules and critical modules of BioASICs such as single cell analysis chip, integrated multiple patch-clamp array, dynamic cell culture array, on-chip cell lysing device, sample preparation chip, cell separation device, high-density single cell analysis chip, molecular harvesting device, cell-cell communication array, animals on a chip, and nanofluidic SERS substrate. For nanoscale spectroscopic molecular imaging and photothermal therapeutic applications, nanocrescent SERS probes are developed. The formation of asymmetric nanophotonic crescent structure is accomplished by the interfacing both bottom-up and top-down methods, which allows to create effective local field enhancement structures, batch nanofabrication, and precise controls of hot spot coupling distance for in-vivo molecular imaging. Gold-based nanocrescents have structures with a sub-10 nm sharp edge, which can enhance local electromagnetic field at the edge area. The advanced nanocrescent SERS probes can be applied for sensitive molecular detection and electron transfers of biomolecules. The functional BioASICs and quantum nanoplasmonics have a potential to impact on systems biology and quantitative medicine.

4:00pm **BI+MN-TuA7 Challenges in Microfluidic Technology Development and Commercialization**, ***A. Chow,*** Caliper Life Sciences
Research activities in microfluidics have exploded in the past decade and a half from just in a handful of laboratories to becoming an important research area in most major universities and research institutes in the world. Commercialization of microfluidics technologies, on the other hand, has been much less ubiquitous. In this presentation, the successes and key challenges in product development and commercialization will be examined, focusing on analyzing the role of scientific and engineering innovations that may be required to bring new microfluidics products and applications to the market.

4:20pm **BI+MN-TuA8 Biomolecule Assembly and Functionality in Completely Packaged Microfluidic Devices**, ***X. Luo,*** *J.J. Park, H. Yi, R. Ghodssi,* University of Maryland*; G.W. Rubloff,* University of Maryland, US
We demonstrate in situ biomolecule assembly at readily addressable sites in a completely packaged bioMEMS device. Chitosan's pH responsive properties for site-selective electrodeposition and its amine functionality for biomolecular conjugation allow this aminopolysaccharide to be employed as the platform for electric signal-guided assembly of biomolecules onto conductive inorganic surfaces from aqueous environment, preserving the activity of biomolecules being assembled. Our completely packaged microfluidic device features consistently leak-free sealing, fluidic inputs/outputs for solution transport, electrical ports to guide the assembly onto selective sites, and simple in situ and ex situ examination of the assembly procedures within the channel for 4 different cases. (1)We electrodeposited fluorescently labeled chitosan at specific electrodes to directly demonstrate the potential of biomolecule assembly inside a microfluidic channel. (2)We assembled fluorescent marker molecules (fluorescein and green fluorescent protein (GFP)) onto an electrodeposited chitosan scaffold to illustrate the in situ biomolecule assembly at readily addressible sites, with GFP's biofunctionality retained through the assembly process. (3)We covalently assembled probe single-stranded DNA onto an electrodeposited chitosan scaffold, then exposed

the scaffold to mismatching and matching fluorescently labeled target DNA to show the ability to assemble probe species and to detect sequence-specific hybridization through sequences of reactions. (4)We compared fluorescence images to post-process profilometer measurements, confirming the relation between the active chitosan sites and the observed fluorescence. These results demonstrate the first signal-directed chitosan-mediated in situ biomolecule assembly in the microfluidic environment after complete packaging of the bioMEMS device, preserving the biological activities of assembled biomolecules.

4:40pm **BI+MN-TuA9 A Bio-MEMS Device for Modeling the Reflex-arc**, ***K.A. Wilson***, *M. Das, J.W. Rumsey, P. Molnar, J.J. Hickman,* University of Central Florida

The reflex-arc is the most basic functional unit of the animal nervous system and consists of three elements: a muscle fiber, a motoneuron (MN), and a dorsal root ganglion cell (DRG). These elements form a circuit that serves to control functions such as breathing, digestion, and involuntary muscle contraction. Due to the importance of this biological circuit it is of great interest in the study of neurodegenerative disease (ND), traumatic spinal cord injury (SCI), and bioprosthetics/biorobotics. However, due to the complex interactions of the cell types involved, novel strategies are required to study this circuit in vitro. Our objective is to develop a MEMS device for recreating the spinal reflex-arc as a model system for studying ND, SCI, and bioprosthetics/biorobotics. As a first step we have demonstrated the ability to culture the various elements of the reflex-arc in a defined serum-free culture system. We have also developed a MEMS platform for integrating these elements into a format that allows stringent control of physiological parameters as well as selective stimulation of the individual elements of the reflex-arc. Furthermore we are now able to measure contractile forces generated by the muscle component of the circuit using this system. Ultimately this device will allow the controlled interrogation of the reflex-arc for basic science and therapeutic research applications.

Biomaterial Interfaces
Room 2001 - Session BI-TuA

Cells at Surfaces

Moderator: K.E. Healy, UC Berkeley

2:00pm **BI-TuA1 Substrate Rigidity Regulates the Formation and Maintenance of Tissues**, *W. Guo,* University of Massachusetts Medical School*; M.T. Frey,* ***N.A. Burnham,*** Worcester Polytechnic Institute*; Y. Wang,* University of Massachusetts Medical School

PLEASE NOTE: YOU MUST IDENTIFY A DIFFERENT PRESENTER FOR THIS ABSTRACT. YOU MAY PRESENT ONE (1) PAPER ONLY AT THE CONFERENCEThe ability of cells to form tissues represents one of the most fundamental issues in biology. However, it is unclear what triggers cells to adhere to one another in tissues or to migrate once a piece of tissue is planted on culture surfaces. Using substrates of identical chemical composition but different flexibility, we show that this process can be controlled by substrate rigidity. The moduli of the polyacrylamide substrates were determined through analysis of atomic-force-microscopy (AFM) force curves to be a few kilopascals, the exact value depending on substrate preparation. The novel aspects of the acquisition and treatment of the force-curve data were 1) the use of log-log plots to observe where on the curve the tip was indenting the sample like a punch, sphere, or cone, and 2) the necessary metrology to bring the typical uncalibrated AFM relative uncertainty of a few hundred percent down to 13%. These methodologies can help researchers more properly interpret their results. @FootnoteText@ Biophysical Journal 90, 2213-20 (2006).

2:20pm **BI-TuA2 Compartmentalized Bioreactor: In Vitro Model for Osteogenesis and Breast Cancer Metastasis**, ***D. Ravi***, *L.A. Shuman, A.M. Mastro, C.V. Gay, E.A. Vogler,* Pennsylvania State University

An advanced bioreactor that permits long-term study (up to 10 months) of cell/protein mediated processes such as bone accretion and cancer metastasis was developed and tested. The bioreactor based on the principle of simultaneous-cell-growth-and-dialysis, separates a cell growth chamber from a media reservoir by a dialysis membrane, compartmentalizing cell growth and cell nutrition functions. Extraordinarily stable culture conditions afforded by the reactor sustained mouse calvarial osteoblasts (MC3T3-E1, ATCC CRL-2593) for periods up to 10 months without the need for sub-culture. Months-long culture resulted in the formation of macroscopic (mm scale) sheets of bone over the inner surface of the dialysis membrane. Development of three-dimensional, tissue-like biosynthetic bone in the reactor was followed by light microscopy, scanning electron and transmission electron microscopy. Markers of osteoblast differentiation (alkaline phosphatase) and mineralization (Von Kossa Assay, SEM-EDS, XRD) were used to analyze the progression of isolated osteoblast inoculum to highly collagenous mineralizing tissue. In the second part of the study, the biosynthetic bone tissue was challenged by co-culture with GFP-expressing metastatic breast cancer cells (MDA-MB-231). Effect of the cancer cells on the morphology and organization of bone tissue was followed by confocal, scanning and transmission electron microscopy. Co-culture with breast cancer cells resulted in migration of cancer cells through the osteoblast tissue, disruption of the collagenous matrix, apoptosis and increased production of inflammatory cytokines (IL-2, IL-6). Compartmentalized bioreactor permits development of mineralizing 3-D collagenous bone tissue from isolated osteoblast inoculum over extended time periods up to 10 months and is an ideal in vitro vehicle for studying long-term cell-cell interactions involved in osteogenesis and osteopathology that are inaccessible to conventional cell culture techniques.

2:40pm **BI-TuA3 Bio-Microactuator using Cultured Cardiomyocytes**, ***Y. Tanaka***, The University of Tokyo, Japan*; K. Morishima,* Kanagawa Academy of Science and Technology, Japan*; T. Shimizu, M. Yamato, A. Kikuchi, T. Okano,* Tokyo Women's Medical University, Japan*; T. Kitamori,* The University of Tokyo, Japan **INVITED**

Integration of chemical systems onto a microchip, sometimes referred to as micro total analysis systems (Âμ-TAS) or labs-on-a-chip, are currently a major interest due to their desirable characteristics, including reductions in reagent consumption, space requirements and analysis times. New concepts in integrated chemistry aims to create both a new academic field and a new associated (bio)chemical industry exploiting advantages of micron dimensions. As living cellular systems often exhibit complex reaction sequences and unique reagents, harnessing cell-based reactions by incorporating cells into Âμ-TAS systems is now frequently reported. However, to date, only cellular biochemical functions have been used to enhance microchip functions. Here, we have utilized cellular mechanical functions to produce more efficient biochemical processes. Our concept utilizes cardiomyocytes as microactuators, using the cellâ?Ts endogenous ability to transform chemical energy into mechanical energy. To demonstrate the concept of the cardiomyocyte bio-microactuator, we firstly actuated micropillars made of flexible polymer using cardiomyocytes.@footnote 1@ Then, we created a pump on-chip using a cardiomyocyte sheet@footnote 2@ as a prototype bio-microactuator.@footnote 3@. Our demonstrated pump could be used as a drug-delivery implant device responding body conditions. Also, that could be applied as a sophisticated in vitro micro model for research of circulatory system for revealing the mechanism of the circulatory illnesses. We anticipate our demonstrated device to be applied in various fields especially for medical usages. @FootnoteText@ @footnote 1@Y. Tanaka, K. Morishima, T. Shimizu, A. Kikuchi, M. Yamato, T. Okano, T. Kitamori, Lab Chip (2006) 6, 230 - 235. @footnote 2@T. Shimizu, M. Yamato, Y. Isoi, T. Akutsu, T. Setomaru, K. Abe, A. Kikuchi, M. Umezu, T. Okano, Circ. Res. (2002) 90, e40-e48. @footnote 3@Y. Tanaka, K. Morishima, T. Shimizu, A. Kikuchi, M. Yamato, T. Okano, T. Kitamori, Lab Chip (2006) 6, 362 - 368.

3:20pm **BI-TuA5 Integrin-Ligand Affinity Affects Neuron Outgrowth**, *Z. Zhang,* University of Delaware*; J. Zheng,* Alfred I. duPont Hospital for Children*; Y. Leng,* University of Delaware*; K.W. Dabney, J.L. Twiss,* Alfred I. duPont Hospital for Children*;* ***T.P. Beebe Jr,*** University of Delaware

The role of ligand-receptor affinity on the outgrowth of neuronal processes was studied directly on postnatal day-1 primary cultures of living neurons by atomic force microscopy (AFM). To accomplish this, micropatterned test substrates were created and analyzed using contact angle measurement, AFM, X-ray photoelectron spectroscopy (XPS), and time-of-flight secondary ion mass spectrometry (TOF-SIMS) at each step of the multistep surface functionalization process, for both substrates and AFM tips. The affinity force between an individual fibronectin molecule (Fn) and an individual growth cone integrin receptor was found to be 106 ± 8 pN, while that between the GRGDSY peptide and an individual integrin receptor was 170 ± 10 pN when measured under the same physiological conditions. In a conventional outgrowth assay on the same substrates, although both the Fn- and peptide-modified substrates supported significantly greater neurite outgrowth than controls, and outgrowth on both substrates was inhibited by the addition of soluble RGD peptide, ~30% longer neurite extension was observed on Fn-modified substrates than on GRGDSY-modified substrates. This implies that integrin-ligand affinity plays an important role in neurite

outgrowth in vitro, and that adhesivity represents a balance between the formation and breakage of cell-substratum contacts. Neurite outgrowth involves the formation of new attachments at the front of the advancing growth cone, and the breakage of previous attachments now at the rear or the advancing growth cone. The higher binding affinity between a neurite's integrin receptors and the GRGDSY ligand implies a reduced rate of ligand-receptor breakage.

3:40pm **BI-TuA6 Electronically Controlled Biointerface for Neuron Growth**, ***M. Gabi***, ETH Zurich, Switzerland; *P. Schulte, A. Offenhäusser,* Research Centre Jülich, Germany; *J. Vörös,* ETH Zurich, Switzerland

Experimental investigation of the neuronal network information processing is important for understanding how the brain performs functions such as memory and learning. The first research step is to develop novel ways for the assembly of neural networks with controlled topology. The guided growth of neurons is one basic requirement to build such defined neural networks. A variety of different surface patterning techniques have been used to achieve controlled growth, including microcontact printing, photolithograpy, ink-jet printing and topographical control, but none of these methods has been capable of controlling the "wiring" of the neurons so far. We have developed electrically responsive "smart" surfaces for controlling the growth of neurons and neurites on custom made indium-tin-oxide (ITO) microelectrodes. The substrate has a suitable microstructure to mechanically guide the out-growth of neurites from the landing spots where the soma of the neuron is located. At the same time poly(ethylene glycol) grafted polyelectrolytes are used to provide an appropriate biointerface on the connecting ITO wires between the cells. The key feature of this chemistry is that initially it inhibits the cell-attachment and neurite outgrowth but it can be switched electrically to a cell-adhesive, IKVAV peptide presenting biointerface that promotes the outgrowth of the neurites. The possibility of guiding neuron growth with the help of electronically controlled biointerfaces is an important step towards building neural networks with controlled topology. The performance of such basic networks and network elements will be characterized using a double patch-clamp setup.

4:00pm **BI-TuA7 Surface Chemical Gradients to Optimise Substrata for Self-Renewal of ES Cells**, ***R.D. Short***, University of Sheffield, UK; *P. Murray,* University of Liverpool, UK; *K. Parry,* Plasso Technology; *D. Edgar, R.S. McGreal,* University of Liverpool, UK

Various reports detail how the culture conditions for mouse ES (mES) and human (hES) may be manipulated to maintain these cells in an undifferentiated state.@footnote 1,2@ Significant differences between mES and hES cells have been commented upon, as well as common mechanisms in maintaining self-renewal. It has been recently shown@footnote 3@ that the self-renewal of mES and hES cells can be promoted by restricting the degree to which these cells spread. This result implies that the self-renewal mES and hES cells can occur when their spreading is restricted by culture on weakly adhesive substrates. Herein, we show how using surface chemical gradients, of varying carboxylic acid density,@footnote 4@ an optimal chemistry is readily identified whereby cells can be maintained in compact small colonies, retaining cell-cell contact, without loss of the key ES cell markers, alkaline phosphatase and Oct-4. Our preliminary results are strongly suggestive that the capacity of ES cells for self-renewal may be maintained by surface chemistry alone. If true, this has the important implication that geometric control (ie control over cells spreading) is an important factor in the maintenance of self-renewal. Surface chemical gradients are an ideal tool for rapid (high throughput) screening. @FootnoteText@ @footnote 1@A.G. Smith, et al, Nature (1988) 336, 688-690 @footnote 2@T. Burdon et al. Trends Cell Biol.,(2002) 12, 432-38 @footnote 3@Murray P. et al., in preparation @footnote 4@J. D. Whittle, R. D. Short et al. Chem. Comm., (2003) 14; 1766.

4:20pm **BI-TuA8 Tunable Biomimetic Artificial Extracellular Matrix Coatings**, ***E.F. Irwin***, *K. Saha, K.E. Healy,* UC Berkeley

In this work we have designed an artificial extracellular matrix that can be utilized for both in vitro cell culture and biomaterial coatings. In our system, we can modulate both ligand density and the mechanical properties of the coating. The base coating is a greater than 100 +AEA-micron+AEA- thick acrylamide (AAm) gel in which the crosslinker density is varied in order to modulate stiffness. Next, this surface is modified with a 4 nm thick poly(ethylene glycol) (pEG) based interpenetrating gel which prevents non-specific protein and cell attachment. Finally, the surface is functionalized with RGD peptides from bone sialoprotein via a 3400MW pEG spacer arm, where the surface density can be controlled by varying input peptide concentration. The mechanical properties of these coatings were measured using force-mode atomic force microscopy (AFM) and analyzed with a Hertzian mechanics model. According to the model, the Young's modulus varied linearly in the range of crosslinker used (0.3-0.03 wt percent) from 1.16 +AEAAKwBA- 0.32 kPa to 9.03 +AEAAKwBA- 1.02 kPa. The immune response to these coatings of varying ligand density and mechanical stiffness is currently being assayed by culturing THP-1 cells, a monocytic leukemia cell line, on our system. It is anticipated that softer surfaces will exhibit a reduced immune response, as macrophages will not be able to spread and activate as readily. Preliminary experiments indicate that the softer surfaces allow less THP-1 cell attachment and spreading. In summary, we have designed a tunable artificial extracellular matrix coating to modulate cell behavior.

4:40pm **BI-TuA9 Plasma Polymer Gradients and Their Use for Cellular Guidance**, *M. Zelzer, R. Majani,* University of Nottingham, UK; *J.W. Bradley,* The University of Liverpool, UK; *F.R.A.J. Rose, M.C. Davies,* ***M.R. Alexander***, University of Nottingham, UK

Plasma polymers have recently been shown to be useful in 3D tissue engineering to encourage cell ingress within porous PLA scaffolds and channels intended for applications in bioreactors.@footnote 1,2,3@ To investigate the mechanism of cell ingress we carried out experiments to form surface chemical gradients on planar substrates by diffusion through defined apertures. Consecutive depositions of plasma polymerised allyl amine and hexane on glass has been used to obtain significant variation of water contact angle from 30 to 90 deg, and corresponded to ppHex overlayer thicknesses of of 0 to 10 nm (XPS). AFM was used to identify island deposition on ppAAm at the transition zone from ppHex to ppAAm. This was supported by Tougaard background analysis of the XPS substrate signals which importantly is also applicable to non-planar substrates. It is proposed that the gradual transition may play a role in forming protein adsorption gradients to induce haptotactic cell movement. We present evidence of cellular guidance on such gradients and propose a mechanism based on model protein adsorption studies using quartz crystal microbalance invoking to Vroman effect. This mechanism relies on a ready displacement of albumin by heavier adhesive proteins (e.g. fibronectin) from the hydrophilic ppAAm surface compared with strong albumin adsorption to the hydrophobic ppHex surface. @FootnoteText@ @footnote 1@ Barry, J,et al. Using Plasma Deposits to Promote Cell Population of the Porous Interior of Three-Dimensional Poly(D,L-Lactic Acid) Tissue-Engineering Scaffolds. Advanced Functional Materials 15, 1134-1140 (2005).@footnote 2@ Barry, Jet al. Using a core-sheath distribution of surface chemistry through 3D tissue engineering scaffolds to control cell ingress. Advanced Materials (2006 in press).@footnote 3@ Dehili, C, et al. Comparison of primary rat hepatocyte attachment to collagen and plasma polymerised allylamine on glass. Plasmas Processes and Polymers (2006 in press).

Biomaterial Interfaces

Room 3rd Floor Lobby - Session BI-TuP

Biomaterial Interfaces Poster Session

BI-TuP1 Effect of Cross-linking Ultra-high Molecular Weight Polyethylene on Surface Molecular Orientation and Wear Characteristics, *S. Sambasivan,* ***D.A. Fischer****, S.M. Hsu,* National Institute of Standards and Technology

Cross-linked ultra-high molecular weight polyethylene (UHMWPE) has become the dominant material used in human artificial joints due to substantial increase in wear resistance and ability to form conformal contacts. Wear debris resulting from wear has been identified as a major contributor to the eventual loosening of acetabular prostheses and failure of implants. Cross-linking of UHMWPE by gamma irradiation has been developed but the detailed mechanism of how cross-linking increases wear resistance is not understood. Wear of the UHMWPE surface layer changes the hardness and brittleness of the surface, and this, in turn, affects the wear resistance. This study uses a soft x-ray spectroscopic technique Near edge X-ray absorption fine structure (NEXAFS) to examine the degree of molecular orientation of the worn UHMWPE surface layer (up to 10nm). NEXAFS measurements at Carbon-K edge of worn UHMWPE samples which were subjected to gamma, ethylene-oxide (EtO), and electron beam sterilization techniques was conducted. Results conclusively suggest that cross-linking resists orientation when the samples were mechanically pulled or worn in a knee simulator. The molecular orientation in the C-C alkyl chains showed a monotonic decrease with increase in gamma radiation dosage levels suggesting highly crosslinked samples resists molecular orientation. EtO sterilized samples showed more C-C chain orientation compared hence less resistance to wear as compared to electron beam radiated samples. Direct comparison of surface molecular orientation of C-C alkyl chains of UHMWPE may offer explanation which of the cross-linking methods are more effective to produce a wear resistant artificial joints.

BI-TuP2 Biosurfaces Generated Using AFM-Based Nanolithography and Surface Activation Chemistry, ***J.N. Ngunjiri****, W. Serem, J.C. Garno,* Louisiana State University

The immobilization of biological ligands in precisely defined locations on surfaces is a critical technology for the integration of biological molecules into miniature bioelectronics and sensing devices. The selectivity of protein adsorption with designed surfaces is compared at the nanoscale using in situ atomic force microscopy (AFM). The high-resolution capability of AFM characterization is combined with nanografting for investigations of protein binding on chemically activated self-assembled monolayers (SAM). Using a computer program, the AFM tip is translated at designated speed, direction, and force to enable fabrication of arrays of SAM nanopatterns with well-defined shapes and sizes. Nanografting provides superb control of parameters of ligand density, pattern spacing and the size of array elements. Nanostructures ranging in size from 10-100 nm are inscribed within a resistive matrix SAM (such as methyl or hydroxyl) which imparts selectivity for protein adsorption. The terminal moieties of carboxylate-terminated SAMs can be reacted with coupling agents such as N-ethyl-N'(dimethylaminoporpyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) to establish covalent coupling of proteins to arrays of SAM nanopatterns. The activity of the immobilized proteins for binding immunoglobulin G (IgG) and peptides can then be investigated by viewing successive changes in the height and morphology of nanostructures during in situ AFM experiments. The resistive matrix SAM surrounding the nanopatterned proteins selectively defines regions for viewing adsorption of proteins with exquisite detail. In situ AFM images of nanoengineered surfaces will be presented which provide direct views of the progression of biomolecular reactions on surfaces.

BI-TuP3 QCM-D and AFM Characterization of the Adsorption and Functionality of Laminin in Large Area Nanopatterns for the Study of Neural Stem Cells, ***J. Malmstrom****,* University of Aarhus, Denmark*; H. Agheli, E.M. Larsson,* Chalmers University of Technology, Sweden*; M. Textor,* ETH Zurich, Switzerland*; D.S. Sutherland,* University of Aarhus, Denmark

A number of reports have made use of chemical or topographic surface structures to pattern assemblies of neural cells in an effort to control their growth and development. Here a nanostructured surface is used to immobilize Laminin at discrete locations on a solid substrate in well-defined nanometer scale patterns in order to investigate the response of neural stem cells (AHP - adult hippocampal progenitor). Nanostructured quartz crystal oscillators and silicon wafer chips with ~120nm diameter regions of alkanethiol modified gold on a silicon oxide background are produced by colloidal lithography. Immobilization of protein resistant layers (PLL-g-PEG) specifically to the silicon oxide parts of the surface allows the generation of nanoscale patches of protein by the non-specific adsorption to the hydrophobically modified gold. The adsorption of Laminin onto homogenous and nanostructured surfaces is studied with parallel sets of samples using the Quartz Crystal Microbalance with dissipation (QCM-D) and Atomic Force Microscopy (AFM). Viscoelastic modeling of the QCM-D data, supported by surface plasmon resonance (SPR) data, on homogenous surfaces indicate a thick hydrated Laminin layer (71±2 nm, >95% water) with high antibody binding capacity (~3 polyclonal anti-Laminin IgG's binding per surface bound Laminin). QCM-D data indicates a higher surface density of Laminin binding for the structured surfaces than the homogeneous surfaces and a greater IgG binding capacity. A new approach to analyze AFM height histograms is used to quantify protein and monoclonal/polyclonal anti-Laminin antibody binding to the nanoscale patches showing

BI-TuP4 Investigating Model Peptides on Surfaces using XPS, SIMS and NEXAFS, ***J.S. Apte****, L.J. Gamble, D.G. Castner,* University of Washington

The purpose of this investigation is to study protein-surface interactions at a fundamental level using model peptides to examine individual adsorption phenomena that comprise the collective interactions of larger proteins with surfaces. The model peptides used were made up of leucine (L) and lysine (K) amino acids arranged in specific sequences to reliably create @alpha@-helix or @beta@-sheet secondary structures. These peptides were adsorbed onto self-assembled monolayer (SAM) surfaces that have well-defined, extensively studied characteristics. The terminal functional groups of the SAMs used in these studies are a methyl group, a carboxylic acid group, an alcohol group and an amine group. Studies were also done on a plasma-deposited fluoropolymer for comparison. Initial adsorption isotherm studies with the 14-mer LK @alpha@-helix peptide showed that the adsorption behavior varied greatly on the different SAM surfaces. Percent nitrogen measured with XPS was used to determine the amount of adsorbed peptide. It was found on methyl- and COOH-terminated SAMs that adsorption from PBS formed patches of peptide with bare spots. There was no adsorption on OH-terminated SAMs and uniform adsorption on fluoropolymer. It was also found that the concentration required to achieve a partial peptide layer with some bare spots on methyl SAMs was 100-fold higher that required for COOH SAMs. Adsorption was investigated for three different buffer salt concentrations to observe the influence of ionic strength on adsorption. On methyl SAMs, it was found that no peptide adsorption was detectable from deionized water, even at peptide concentrations of 1 mg/mL. In contrast a nitrogen concentration of about 5 atomic percent was detected when adsorbed from PBS. Angle-dependent XPS and NEXAFS studies were used to probe the organization of the adsorbed peptides.

BI-TuP5 Characterization of Nitrilotriacetic Acid-Terminated Self-Assembled Monolayers for Orientation-Controlled Immobilization of Proteins, ***F. Cheng****, L.J. Gamble, D. Graham, D.G. Castner,* University of Washington

In recent studies of orientation and conformation in adsorbed protein films, it is essential step to immobilize proteins onto surfaces in a controlled and well-defined manner. The oriented immobilization of genetically engineered histidine-tagged proteins onto nitrilotriacetic acid (NTA)-terminated self-assembled monolayers (SAMs) on gold has been a widely used model system. X-ray photoelectron spectroscopy (XPS), angle-resolved XPS (ARXPS) and surface plasmon resonance (SPR) have been used to characterize monolayers generated via self-assembly of the NTA thiol onto gold and backfill of methyl-terminated thiol (mercaptoundecane, MUD) into the loosely-packed NTA SAMs. In pure NTA SAMs, self-assembly conditions can significantly affect lateral packing density and nickel-activation efficiency. In mixed NTA SAMs, the correlation between the MUD backfill time and surface compositions indicates a two-step backfill process: 1) at short backfill times (@footnote 1@1h), MUD binds to unoccupied gold sites in NTA monolayer; 2) at long backfill times (@footnote 2@1h), MUD displaces NTA from the gold surface. ARXPS analysis shows that the MUD backfill method can optimize the vertical distribution of NTA endgroup and enrich NTA endgroup in the outmost layer of the films. The binding constants of oligo (histidine) onto the NTA surfaces were determined by SPR.

BI-TuP6 Interface Biology at Structured Surfaces of Titanium Dental Implants, ***H.P. Wiesmann***, *L. Lammers, F. Abusua, U. Joos,* University M@um u@nster, Germany

In the present investigation three comparable threaded dental titanium implants were evaluated for the bone cell reactions at the surface in cell culture and after insertion in the mandible of minipigs. Implants with comparable size and geometry but various surface structures were used; TBS surfaces, sandblasted - acid-etched (SLA) surfaces, and microgrooved surfaces. Osteoblast-like cells were seeded under defined culture conditions on the implants and cell reactions were investigated during a 14-day culture period. For the in vivo experiments implants were placed in the mandible of 10 minipigs. Light- and electron microscopy as well as energy dispersive x-ray analysis were used. Cultured osteoblasts attached to all tested surfaces. TBS and microgrooved surfaces showed significantly more attached cells after 1 day. Proliferation of cells was best on microgrooved surfaces followed by TBS- and SLA-surfaces. After 14 days a high expression of osteocalcin, osteonectin, fibronectin, collagen type I, and osteopontin of osteoblasts cultured on microgrooved surfaces was demonstrated, whereas a lower level was present on SLA surfaces. In vivo, the most prominent difference at the interface between the implant systems was the extent of titanium wear. Number and size of titanium particles in the vicinity of the implantation bed were high around TBS-implants and low around microgrooved implants. SLA-implants showed many but small titanium particles in the interface region. In conclusion, grooved surfaces offer under in vitro conditions a better cell attachment and proliferation as well as a higher expression of typical bone related matrix proteins than the other surfaces studied.

BI-TuP9 Proteins Patterning on Plasma PEG Surfaces by Microcontact Printing, ***A. Ruiz***, *L. Ceriotti, F. Brétagnol, D. Gilliland, H. Rauscher, P. Colpo, F. Rossi,* European Commission, Institute for Health and Consumer Protection, Italy

Arrays of surface-bound biomolecules are significantly needed in a variety of applications, such as diagnostic immunoassays, DNA microarrays, cell culturing or biosensing. Among all known techniques, soft lithography is a printing processes used for the fabrication of micro and nano structures based on a physical contact between a stamp and a substrate. The most representative technique of this technology is the microcontact printing. This technique is based on the use of PDMS stamps with micropatterned relief inked by biomolecules that are transferred to a substrate. Surfaces with biomolecules localised in a well arrangement can be used for cell growth studies and for improving the understanding of their interactions with different cell types. In this framework, we have used microcontact printing for patterning PEG surfaces with Poly-L-Lysine and BSA. We used the fact that PEG is known to be antifouling, i.e. protein repellent, in solution but accept protein adhesion when printed in dried conditions, as previously suggested by Delamarche.@footnote 1@ The proteins stamping is done in dried conditions and the stability of the protein patterns when put in solution has been proven. Characterisation by Fluorescence Microscopy, ToF-SIMS and Ellipsometry showed well-defined stable motifs even after 24h in water. The PLL microstamped PEG surfaces were then incubated with a solution of L929 fibroblasts. It was clearly observed how the cells adhered and grew in the regions patterned with PLL. Controlled patterning of biomolecules on an antifouling substrate like PEG has been achieved by microcontact printing technique, which also offers the advantage of being a simple, convenient, inexpensive and accessible method. @FootnoteText@ @footnote 1@ E. Delamarche, C. Donzel, F.S. Kamounah, H. Wolf, M. Geissler, R. Stutz, P. Schmidt-Winkel, B. Michel, H.J. Mathieu, K. Schaumburg. Langmuir 19 (2003) 8749 - 8758.

BI-TuP10 Optical Waveguide Spectroscopy of DNA Molecules Adsorbed on an Amino-Terminated Surface, ***H. Mori***, *M.A. Bratescu, N. Saito, O. Takai,* Nagoya University, Japan

Deoxyribonucleic acid (DNA) chip has recently emerged as a powerful tool for genetic research. The optical properties of DNA have received considerable attention in the past few years, motivated by the detection of the adsorption processes in a DNA chip. Our work is focused on the adsorption processes study of the DNA molecule, on an amino-terminated self assembling monolayer (SAM) surface. The investigation method is the absorption of the evanescent light by the DNA molecules lying on the surface, in a depth of hundred nanometers. DNA absorption of the evanescent light was performed by using a slab optical waveguide (SOWG), made from quartz, in the UV region, around 260 nm. The average number of total reflections on SOWG surfaces, controlled by the incident light angle was established in order to obtain a maximum absorbance signal. A comparative analysis of the DNA molecules adsorption was investigated on SOWG surface covered with an amino-terminated SAM and on the clean quartz surface of SOWG. The amino-terminated SAM was obtained by dipping the freshly clean SOWG into 1 wt % solution of (3-aminopropyl) trimethoxysilane in toluene for 3 hours at 60°C. Different DNA molecules are investigated: Oligo-DNA (5@super '@-AAAAAAAA-3@super '@), Oligo-DNA (5@super '@-AAATAAAA-3@super '@), Oligo-DNA (5@super '@-TTTTTTTT-3@super '@) and Oligo-DNA (5@super '@-carboxydecyl-TTTTTTTT-3@super '@). The concentration of DNA solution in distilled water was in the range from 25 @micro@M to 200 @micro@M. The time dependence of the absorption spectra shows an increased DNA UV absorbance on SOWG surface covered with amino-terminated SAM as compared with clean SOWG surface.

BI-TuP11 The Influence of Material Surface Properties on the Polymerase Activity in Microchip-Based PCR, ***S. Forti***, *R. Canteri, R. Dell'Anna,* ITC-irst, Italy; *C. Della Volpe,* University of Trento, Italy; *L. Lunelli, L. Pasquardini, L. Vanzetti, C. Pederzolli, M. Anderle,* ITC-irst, Italy

PCR (polymerase chain reaction) represents the most widely used method for amplification of defined DNA sequences for medical and biological applications. As a result of the increasing demand for high performance PCR devices with high sample throughput, but low reagent consumption, PCR has recently become the focus of investigation for miniaturization in order to enhance its efficiency. However, in order to perform PCR in microchips, because of the increased surface-to-volume ratio upon miniaturization, special attention must be paid to the internal surface, which comes into contact with the PCR-reaction mixture. Effects related to the non specific surface adsorption of PCR reagents (e.g. the replicating enzyme DNA polymerase) may become significant and reduce the efficiency of DNA amplification in microchip PCR. Therefore, effects of silicon (with different deposited oxide layer), glass, chromium and titanium nitride surfaces on Taq (Thermus aquaticus) DNA polymerase adsorption will be investigated by immunofluorescence, using anti-Taq DNA polymerase monoclonal antibody. Surface distribution as well as protein amount will be determined and a correlation with surface morphological and physico-chemical properties, as determined by atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), time of flight secondary ion mass spectrometry (ToF-SIMS) and contact angle (CA) analysis, will be presented.@footnote 1@ @FootnoteText@ @footnote 1@This work was accomplished in the framework of LaTEMAR (Laboratorio di Tecnologie Elettrobiochimiche Miniaturizzate per l'Analisi e la Ricerca - Laboratory of Miniaturized Electrobiochemical Technologies for Analysis and Research), Centre of Excellence funded by MIUR (Italian Ministry for Education, University and Research) grants - FIRB 2003-2004 - for public/private structures involved in research fields characterized by strategic value.

BI-TuP12 Gold Nanoparticle Arrays as a Template for Designing Biosurfaces, ***J Hyun***, *J. Jung, K. Na,* Seoul National University, Korea

We demonstrate a new, reliable and simple method for fabricating micropatterned nanoparticle arrays that can serve as templates for the surface-initiated polymerization of polymeric brushes, which can be used for the control of bilogical adsorption. As a proof of concept we micropatterned Au nanoparticles (Au-NPs, ~10 nm) onto glass, silicon, polystyrene, and gold surfaces by a simple three-step process: 1) ÂμCP of soluble polymer, 2) incubation with a solution of Au-NPs, and 3) lift-off of the template in a mixture of ethanol and deionized water. Forty-micrometer wide features were successfully fabricated without any significant defects or nonspecific adsorption on the background. To demonstrate the utility of these Au-NP templates, we subsequently polymerized N-isopropylacrylamide (NIPAAm) by surface-initiated polymerization, using a surface-bound initiator.

BI-TuP13 Surface Characterization of Plasma-Polymerized Cyclohexane Thin Film used as Non-Biofouling Surface for Proteins, *C. Choi, S. Yeo,* Sungkyunkwan University, Korea; *H.K. Shon, J.W. Kim,* Korea Research Institute of Standards and Science (KRISS); *Y. Park, D.W. Moon,* Korea Research Institute of Standards and Science (KRISS), Korea; *D. Jung,* Sungkyunkwan University, Korea; ***T.G. Lee***, Korea Research Institute of Standards and Science (KRISS), Korea

In this work, plasma-polymerized cyclohexane (PPCHex) thin film, which can be used as a non-biofouling surface for proteins, was characterized by using time-of-flight secondary ion mass spectrometry (TOF-SIMS) along with a principal component analysis (PCA), X-ray photoelectron spectroscopy and Fourier transform infrared spectroscopy. The PPCHex thin film was deposited on a glass surface by using the inductively coupled plasma chemical vapor deposition method and cyclohexane as a precursor.

The non-biofouling property of the PPCHex surface was controlled in a reproducible manner as a function of sample bias plasma power and was correlated with the surface chemical composition and hydrophobicity of each surface. In addition, a PCA of the TOF-SIMS data provided insight in a systematic manner into the surface chemical compositions and molecular cross-linking on plasma-polymerized thin films as a function of sample bias plasma power.

BI-TuP14 Organophosphonate Functionalized Silicon Nanowires for DNA Hybridization Studies, ***D. Pedone***, *A. Cattani-Scholz,* Technical University Munich (TUM), Germany*; M. Dubey, J. Schwartz,* Princeton University*; G. Abstreiter, M. Tornow,* Technical University Munich (TUM), Germany

Semiconductor nanowire field effect devices have great appeal for label-free sensing applications due to their sensitivity to surface potential changes that may originate from charged adsorbates. In addition to requiring high sensitivity, suitable passivation and functionalization of the semiconductor surface is obligatory. We have fabricated both individual and sets of parallel, eventually freely suspended, silicon nanowires from Silicon-on-Insulator substrates using standard nanopatterning (electron beam lithography, reactive ion etching) and surface micromachining techniques (sacrificial oxide etching). The wires of length 2 µm were typically 100 nm wide and high, and consisted of boron p-type 10@super 18@ cm@super -3@ implantation doped Si. Subsequent to nanofabrication, the devices were bio-functionalized in a 3-step sequence: First, a hydroxyalkylphosphonate monolayer was covalently attached to the native oxide of the Si wire, affording stable and dense passivation in aqueous solutions. Then, bifunctional linker groups were bound to this surface, and in the final step thiol-terminated DNA oligonucleotides were allowed to react with a maleimide moiety of the linker. In initial experiments we investigated DNA hybridization on such functionalized nanowires using a difference resistance setup, where subtracting the reference signal from a second wire could be used to exclude most unspecific effects. A net change in surface potential of the order of -2.5 mV could be detected upon addition of complementary DNA.

BI-TuP15 Dimensional Analysis of Living Cells in Liquid by Scanning Probe Microscopy, ***Y.-J. Kim***, Myongji University, Korea*; H.-D. Kim, K.-H. Lee,* Seoul National University, Korea*; J. Kim, Y.J. Choi, Y.-S. Kim, C.J. Kang,* Myongji University, Korea

Recent advances in atomic force microscopy (AFM) allow us to determine fine structures of biological materials even under physiological liquids. Divers cancer tissues are well established to have their own structural identity defined as fractal dimension when they are growing in vivo. The dimensionality of these tissues is thought to reflect their invasiveness and malignancy. However, the fractional dimension of living cells has not been elucidated and may be largely attributed to the technical limitations of conventional imaging tools such as optical microscopy and surface electron microscopy. In this work, we have identified fractional dimension of breast cancer cells (MCF7) and normal breast epithelial cells (MCF10A) derived from the same origin. Using AFM technique, high-resolution surface images of living cells were obtained from both MCF7 cells and MCF10A cells under physiological conditions. AFM images of cells showed finer structure of cell boundary compared with SEM images after fixation. Box-counting analysis of its boundary have defined fractal dimension of each cell line. These results suggest that AFM imaging is a feasible tool for analyzing surface structures of living cells with high resolution, and could provide new insights into cell surface structure.

BI-TuP17 Synthesis and Optical Properties of Shape-Controlled Europium-Doped Gadolinium Oxide Nanocrystals, ***S.Y. Seo***, University of Florida

Shape-controlled luminescent oxide nanocrystals have been synthesized by a high temperature solution phase growth method. Thermal decomposition of metal precursors in the presence of trioctylphosphine oxide (TOPO), hexadecanediol, oleic acid, and benzyl ether resulted in highly crystalline, monodisperse oxide nanocrystals . This route provides crystalline nanoparticles with shapes of square plates or spheres dependent upon the growth variables, such as the choice of metal precursor and capping molecules. Upon evaporation of the hexane solvent, the plate-type Gd@sub 2@O@sub 3@ nanocrystals self-assembled into stacked plates lying on their edge. Alternatively, the reaction of Gd(acac)@sub 3@. with hexadecanediol resulted in spherical Gd@sub 2@O@sub 3@ nanocrystals with a diameter of 2 nm, showing that the type of metal precursors plays an important role in control of the shape of nanocrystals. The effects on the crystal growth of other reaction parameters, including reaction temperature and different combinations of organic solvents, will be discussed. The effects of the shape and size of Gd@sub 2@O@sub 3@:Eu nanocrystals on luminescent properties will also be reported.

BI-TuP18 Gradient Functionalized Plasma Polymer Films for on Probe Affinity Capture (OPAC) MALDI MS, ***C.J. Mathai***, *M.Z. Segu, R.G. Kinsel,* Southern Illinois University

Surface modification of the sample targets used for Matrix Assisted Laser Desorption / Ionization (MALDI) Mass Spectrometry (MS) is receiving considerable attention because these devices allow for rapid, on-probe fractionation of complex protein mixtures. This on-MALDI-probe fractionation of proteins/peptides is now being explored as a novel approach for disease diagnosis. Our research has focused on the use of rf pulsed plasma technique for incorporation of the surface chemical functionality on the MALDI target. In the present study a new rf plasma reactor design is described which allows for rapid deposition of discreet, or continuous, gradients of chemical functionality directly on a MALDI target. The reactor system has a cylindrical chamber with two adjustable electrodes kept parallel to each other. One of the electrodes is powered by 13.56 MHz rf generated by a function generator and amplified by an rf power amplifier. A matching network is used to couple the rf power to the electrode. For pulsed operational mode, a signal generator is employed to pulse the function generator. By varying the pulsing parameters, plasma on and off times can be varied to achieve maximum control on the film chemistry. Patterned and gradient plasma polymer films are obtained by mounting MALDI probes on an automated XY stage with specially designed masks. The complete coating system including the XY stage is fully controlled by using LabView software. Using this system, plasma polymer thin films having gradient hydrophobicity or acidity are depositied directly on the MALDI probe as a series of discreet spots. All surface chemical modifications are fully characterized by FT-IR and contact angel measurement. Subsequently, these gradient surface modified MALDI probes are used to perform on-probe fractionation of various mixtures of peptides and proteins with a goal of identifying optimal conditions for fractionation and analysis of the peptide/protein mixture constituents.

BI-TuP19 Metal Ion Impregnated PMMA as On-Probe Affinity Capture MALDI (OPAC-MALDI) Probes for the Fractionation of Protein Mixtures, ***G.S. Fernando***, *G.R. Kinsel,* Southern Illinois University

MALDI Mass Spectrometry (MS) is currently broadly used in the structural characterization and identification of proteins and other biological molecules. Fractionation of proteins into groups according to their physical or chemical properties is usually an important first step before MALDI MS analysis. Conventional methods typically involve external sample manipulation which limits the overall throughput of the analysis. Incorporation of these fractionation steps directly on a MALDI target plate has given rise to various novel protein chip technologies. In the present studies, the use of a metal impregnated, commercially available, comparatively inexpensive and easily fabricated, poly (methyl methacrylate) (PMMA) surface for the separation of metal binding proteins is explored. Identification of metal binding proteins is expected to facilitate the evaluation of the function of metal ions in protein folding, assembly, stability, conformational change, and catalysis. To produce the chemically modified MALDI target, PMMA is first hydrolyzed with 3M NaOH overnight to obtain carboxylic acid functional groups on the surface. After thorough washing with MilliQ water, the carboxyl groups are further reacted with metal ions, such as Cu2+, Zn2+ or Ni2+, by incubating the PMMA probes in a metal salt solution overnight. All surface modification steps are characterized by XPS, FTIR, AFM, MALDI MS and contact angle measurements and compared with unmodified PMMA. These metal incorporated PMMA MALDI targets are shown to selectively bind phosphorylated proteins, histidine rich proteins and proteins with terminal arginine residues in a series of control studies. Subsequently, the PMMA targets are further evaluated for their ability to fractionate complex mixtures of proteins derived from bacterial sources.

BI-TuP20 Preparation of Biodegradable Double-layered Microshells using Surfactant-free Emulsion Method, ***F. Nagata***, *T. Miyajima,* National Institute of Advanced Industrial Science and Technology (AIST), Japan

Double-layered microshells comprised of poly(lactic acid) (PLA) internal wall and calcium phosphate external wall were synthesized by a improvement surfactant-free emulsion method. PLA is one of the most commonly used hydrophobic and biodegradable polyesters which can encapsulate various poorly water-soluble drugs. PLA-based microcapsules have been investigated for biomedical applications such as drug carriers for targeted delivery. In this study, we demonstrate a method for direct synthesis of double layered microshells composed of PLA and calcium

phosphate using organic/inorganic interaction on the interface between oil and water phases. The microshells were prepared as follows. The organic phase composed of PLA (Mw = 20,000) and dichloromethane was poured in calcium acetate aqueous solution. The mixture was stirred at 300 rpm to yield emulsion at room temperature, then the beaker filled with the emulsion was set in an ice bath and was allowed to cool down for 30 min with stirring. Diammonium hydrogenphosphate aqueous solution was slowly added into the emulsion to precipitate calcium phosphate. After adding diammonium hydrogenphosphate aqueous solution, the beaker was taken out from an ice bath and allowed to return room temperature. The obetained microshells had a wide distribution ranging from 50 to 300µm and their wall thickness was 1 to 10µm. The internal wall of the microshells was smooth, contrary to this, the external wall have rough morphology, which indicate that the wall would have multiple layers. The external wall was identified as Dicalcium Phosphate Dihydrate (DCPD) by XRD. On the interface of the oil-in-water emulsion, DCPD would be precipitated on the nucleation sites of dissolved PLA in the oil droplets, which formed calcium phosphate microshell structure. After the precipitated DCPD stabilized the oil droplets, dichloromethane was volatilized and PLA would be deposited on the internal wall of DCPD microshells.

BI-TuP21 Control of Cell Shape, Cytoskeleton Structure, and Cell Migration Activity by using a Micro 3D Patterned Film, ***H. Sunami***, Hokkaido University, Japan; *E. Ito,* CREST, Japan Science and Technology Corporation (JST); *M. Tanaka, S. Yamamoto, M. Shimomura,* Hokkaido University, Japan

Recently we found that endothelial cells can proliferate rapidly on a micro 3D patterned film (honeycomb film). The cell shape and cytoskeleton structure on the honeycomb films were clearly different from those on a flat film. In order to elucidate the effect of honeycomb films as a 3D scaffold for cell culture, it is needed that the 3D observation of cell behaviors such as the morphological change, expression of cytoskeleton, expression of contact points on extracellular adhesion molecules, and migration on the honeycomb films during cell culture. In this research, effects of 3D honeycomb pattern on above cell behaviors were observed. The honeycomb films were fabricated by applying a moist air to a spread polymer solution containing an biodegradable polymer (poly(@epsilon@-caprolactone)(PCL)) and an amphiphilic polymer. The regular honeycomb structures with pore diameter of 10 µm and a wall thickness of 10 - 12 µm were prepared. The porcine vascular endothelial cells were cultured on the honeycomb films for 24 h at 37 °C. The cells were slender on the flat film, while the cells were spread widely on the honeycomb film. The cell migration activity on the honeycomb films was much lower than that on the flat film. The results of confocal laser scanning microscopic observation of CFSE-stained cytoplasm, the 3D shape of the cells on the honeycomb film was different from that on the flat film. Although the cells top-surfaces on both the honeycomb films and the flat films were flat equally, the bottom surface of cell body on the honeycomb films flagged approximately 1 - 3 µm along the honeycomb pores. The focal contact points (vinculin clusters) and actin cytoskeletons expressed strongly along the pores, too. These results showed the 3D structure of the honeycomb film effects on the cell shape, migration, and cytoskeletons. We will also report the relation between the 3D structure of honeycomb film and the good cell proliferation on the honeycomb film.

BI-TuP22 Nano-Sphere Lithography Based Chemical Nano-Patterns for Biosensor Design, ***A. Valsesia***, *P. Colpo, P. Lisboa, F. Rossi,* European Commission-JRC-IHCP, Italy

Patterning bio-molecules on biosensor platform surfaces is a cornerstone fabrication step for many applications ranging from medical diagnostics, environmental monitoring, food safety, and security applications to more fundamental themes such as cell-surface interactions. Many works are performed worldwide to develop advanced platforms with controlled surface chemistry and well defined nano-patterns. The goal is to be able to immobilize the biomolecules in an active state, avoiding non specific adsorption, in this manner the sensitivity and the specificity of detection is enhanced by surface densification of the recognition agents. An important consequence is the reduction of analyte volume needed for the detection. Among the different nanopatterning techniques nano-sphere lithography is a very flexible technique to produce nano-structured and chemically nano-patterned surfaces. Moreover this technique presents the advantage to be inexpensive and enable to produce nano-topography over large area surfaces. In this work, we present the fabrication strategy and the surface characterization of different types of nano-structures. In particular Poly Acrylic acid (carboxylic functional) nano-domes in anti-fouling matrix have been fabricated by combining colloidal lithography and Plasma Enhanced Chemical Vapor Deposition and carboxylic terminated nano-spots in an anti-fouling matrix have been produced by combining nano-sphere lithography and Self Assembled Molecular Monolayers on gold. We show that these chemical nano-patterns are able to immobilize proteins selectively in the carboxylic functional nano-domains, leaving the anti-fouling matrix clear. Moreover immunoassay experiments were set-up showing that nano-patterned surface constrains the immobilization of the antibodies in a biological reactive configuration, thus significantly improving the device performances as compared to more conventional non-patterned surfaces.

Applied Surface Science
Room 2005 - Session AS-WeM

Molecular Ion Sources and Characterization of Biomaterials

Moderator: J. Shallenberger, Evans Analytical Group

8:00am **AS-WeM1 Recent Advancements in Polymeric Depth Profiling with an SF@sub 5@@super +@ Cluster Primary Ion Source**, ***C.M. Mahoney,*** National Institute of Standards and Technology **INVITED**

The utility of cluster Secondary Ion Mass Spectrometry (SIMS) for depth profiling applications in polymeric materials has been repeatedly demonstrated in the past decade, where it has been used to obtain molecular and fragment information as a function of depth in several polymer systems.@footnote 1,2,3,4@ In addition to homopolymers, polymeric blends, multilayers and copolymer systems have been successfully characterized as a function of depth.@footnote 2,4@ Finally, the capability to monitor the in-depth distribution of small molecules embedded in organic and polymeric matrices has been realized.@footnote 1,5@ This talk will describe the ongoing research efforts at NIST to further develop cluster SIMS as a tool for polymer surface and in-depth characterization. Results indicate that SF@sub 5@@super +@ is a promising tool for polymer depth profiling, and with the right set of conditions, including temperature, sample rotation and selection of analysis source, one can obtain optimal results. @FootnoteText@ @footnote 1@ Mahoney, C.M.; Roberson, S.V.; Gillen, G. Anal. Chem. 2004, 76, 3199-3207.@footnote 2@ Mahoney, C.M.; Yu, J.X.; Gardella, J.A. Jr. Anal. Chem. 2005, 77 (11), 3570-3578.@footnote 3@ Wagner, M.S. Surf. Interface Anal. 2005, 37(1), 42-70.@footnote 4@ Wagner, M.S. Anal. Chem. 2005. 77(3), 911-922.@footnote 5@ Chen, J.; Winograd, N. Anal. Chem. 2005, 77(11), 3651-3659.

8:40am **AS-WeM3 TOF-SIMS Analysis of C60 Sputtered Organic Thin Films**, ***S.R. Bryan***, *J. Moulder, G.L. Fisher,* Physical Electronics*; N. Sanada,* ULVAC-PHI

Sputtering of organic materials using a C60 ion beam has been demonstrated to produce significantly less accumulated damage compared to sputtering with atomic ion beams. This has opened up the possibility of sputtering organic materials while maintaining the organic structural integrity at the bottom of the crater. We have studied C60 sputtering for several years using XPS. This has lead to a better understanding of the optimum sputting conditions. In this presentation we will present new data on TOF-SIMS analysis of C60 sputtered organic films. By combining the quantification and atomic bonding information from XPS with the longer range structural information from TOF-SIMS, a better understanding of the C60 sputtering process will emerge.

9:00am **AS-WeM4 XPS and QCM Studies of PMMA and Teflon AF1600 Films Bombarded by 1-20 keV C@sub 60@@super +@ Ions**, *I.L. Bolotin, S.H. Tetzler,* ***L. Hanley,*** University of Illinois at Chicago

keV C@sub 60@ ions are widely used as projectiles in secondary ion mass spectrometry of polymeric materials. Evidence exists that the mechanism of sputtering by C@sub 60@ ions allows their use for damage-free depth profiling. Surface analysis studies are presented to probe absolute sputtering yields and surface modification of two polymer films by C@sub 60@ ions. Polymethylmethacrylate (PMMA) and Teflon AF1600 spin-casting films studied during different bombardment doses of C@sub 60@ ions with energies of 1-20 keV by quartz-crystal microbalance (QCM) and X-ray photoelectron spectroscopy (XPS) . Measurements for the total sputter yield of films are acquired using a QCM. Mass-lost rate data show that each 20 keV C@sub 60@ cluster leads to emission ~10@super 6@ amu of polymer, indicating that the non-overlapping crater regime exists for doses of 10 keV. Chemical modification is also probed by XPS of the target surface before and after ion bombardment. Both polymers display little to no damage to their film structure at ion fluences below ~10@super 13@ ion/cm@super 2@. Changes in C 1s XPS spectra during higher fluence bombardment can be explained predominantly by differential charging effects. However, ion fluences >10@super 15@ ion/cm@super 2@ modify the film composition to a carbon-rich material with various degradation products.

9:40am **AS-WeM6 Applications of a Bismuth-Cluster Ion Gun in Organic and Inorganic Surface Analysis**, ***F. Kollmer***, *R. Moellers, T. Grehl, D. Rading, E. Niehuis,* ION-TOF GmbH, Germany

In recent years primary ion clusters are increasingly applied for the analysis of organic surfaces by TOF-SIMS. Since they increase the secondary ion formation efficiency by orders of magnitude cluster sources are replacing the mono-atomic primary ion sources more and more. As primary ion species a large variety of different clusters as Au@sub n@, Bi@sub n@, C@sub n@, C@sub 60@, SF@sub 5@ ,...are applied. Possible drawbacks of cluster sources are the applicable cluster currents (measurement time) and the achievable performance with respect to lateral resolution and mass resolution. Recent advances in molecular surface analysis were made by the application of cluster liquid metal ion sources (LMIG) operated with Au or Bi. These sources combine the fundamental benefits of cluster ion bombardment with a high brightness source capable to achieve a high performance with respect to lateral resolution and mass resolution. In particular the Bi source shows interesting aspects as a large variety of emitted singly and doubly charged ions, high cluster currents, and the ability to operate at very low emission currents. At low emission currents the energy spread of the emitted species is reduced which improves the achievable lateral resolution. In this contribution we address fundamental capabilities of the Bi cluster source as the composition of the emitted primary ion beam, the energy spread of the ions, the stability of the source and the influence of the emission current on these. We applied Bi cluster ions to organic surfaces for micro area analysis and imaging with high lateral resolution. We also found new interesting applications of Bi clusters for inorganic surface analysis and dual-beam depth-profiling.

10:40am **AS-WeM9 Utilization of Polymers in Ocular Science Applications**, ***R.M. Braun,*** Bausch & Lomb **INVITED**

The healthcare industry, as a whole, has seen the rapid incorporation of polymeric materials into a variety of devices in recent years. This progression along the development trail has led to a multitude of life-changing products that range from convenience items like liquid bandages to prostheses that repair damaged joints and teeth. Moreover, we can not forget the life-saving products like stents and heart valves, nor the pharmaceutical products that rely on polymers to regulate dosing within a given application. Clearly, the use of polymers within biomaterial applications has become widespread and will likely continue to expand as time progresses. Although advancements in the ocular sciences industry are not often highlighted in the same light as those noted above, contributions toward improving patient health and the quality of life through materials development are abundant. These facts have recently come to light with the aging population associated with the baby-boomer generation. The products and applications within this industry include contact lenses and associated comfort solutions, replacement lenses for cataract issues and pharmaceutical products that treat back of the eye diseases that can lead to blindness. While these industry sectors are too large to discuss completely, this talk will highlight a few biomaterial related systems associated with the human eye and touch on some of the challenges associated with surface analytical measurements.

11:20am **AS-WeM11 Multi-Technique Characterization of a Drug Delivery System to Obtain 3-D Chemical Information**, ***A.M. Belu,*** Medtronic, Inc.*; C.M. Mahoney,* National Institute of Standards and Technology*; K. Wormuth,* SurModics, Inc.

Medical devices are increasingly being designed to incorporate drug delivery. For example, drugs are currently incorporated into catheters to reduce microbial infection, placed on stents to prevent restinosis of the artery, and incorporated into pacing lead tips to prevent inflammation of heart tissue. As more drug delivery systems are being created, it is important to characterize their properties, and relate the properties to how the system will function in the human body. This study focuses on the characterization of a rapamycin/PLGA delivery system on a stent. The goal is to understand the lateral and depth distribution of the drug in the polymer film. The information can then be used to predict the release profile of the drug in vivo. Several different formulations of rapamycin/PLGA were studied (5, 25, 50% drug, and 25% with a capcoat). The surface composition of the films were characterized and quantified by ESCA and TOF-SIMS. Information on drug concentration from the surface towards the bulk of the film was determined by depth profiling by SIMS with a cluster ion source. To determine the distribution of drug/polymer species in the bulk of the film in the x-, y-, and z-direction, confocal Raman was used. In the films with high concentration of drug, individual clusters of drug on the order of a few microns were resolved. The data from the multiple methods of characterization will be compiled to allow a fuller understanding of the delivery systems. The strengths and weaknesses of each of the characterization techniques will be compared.

11:40am **AS-WeM12 Combining Fluidics, Surface Chemistries and Direct Mass Spectrometric Detection to Address Protein Analyte Quantitation from Complex Samples for the Purpose of Diagnostic Assays**, ***L.O. Lomas,*** *E. Fung, E. Boschetti,* Ciphergen

The development of analyte assays for the purpose of diagnostic tests is driven by multiple factors, including sample availability, required throughput and quantitative reproducibility. Although antibody-based assays have dominated in the detection of peptide/protein analytes and serve well in terms of throughput and quantitative reproducibility, mass spectrometry is becoming more main-stream due to the added information provided in terms of precise analyte conformation by mass and/or secondary structure fragmentation. Laser Desorption/ionization mass spectrometry (MS) is particularly well suited for both peptide and protein characterization, however, absolute quantitation has been elusive due to complexities associated with integrating sample processing and final analyte detection. To resolve these issues, we have integrated unique solid-phase extractions chemistries directly on MS probes that allow us to quantitatively extract the protein analytes of interest from a complex sample in a defined and controlled process. The resulting analyte arrays minimize the sample requirements and allow for high-throughput processing using standard sample fluidic systems. To exemplify this process, we describe the development of a seven protein-marker panel that may aid in the stratification of women with a pelvic mass. The specific MS-based analyte array assays permit the discrimination of post-translationally modified forms of the markers and by using reference standards, we are able to achieve absolute quantitation with high reproducibility (CV<10%). The integrated assay platform includes an automated liquid handler, analyte-specific arrays, and a MS reader. Measurements of the seven markers provide a measure of the risk that a pelvic mass is malignant. Women at low risk for ovarian cancer can be further evaluated by their general gynecologist, while women at high risk for ovarian cancer should be evaluated by a specialist gynecologic oncologist.

12:00pm **AS-WeM13 The Surface Characterisation of Arrayed Biomaterial Systems**, ***A.J. Urquhart,*** University of Nottingham, UK*; D.G. Anderson,* Massachusetts Institute of Technology*; M.R. Alexander,* University of Nottingham, UK*; R. Langer,* Massachusetts Institute of Technology*; M.C. Davies,* University of Nottingham, UK

In recent years the increase in interest in combinatorial materials science, via high throughput synthetic techniques, has attracted considerable interest mainly in the facilitation of rapid discovery and the optimisation of functional polymeric biomaterials.@footnote 1@ Critical to the selection of a biomaterial to a specific clinical application is the relationship between polymer interfacial chemistry and biological response. However, there has been to date few reports addressing the challenge of studying the interfacial chemistry of high throughput arrays with high spatial resolution. Here we report, for the first time, on the surface characterisation of a novel polymer array, comprising of 572 polymer species (each polymer spot being approximately 300 µm in diameter) and fabricated by the Anderson et al. method,@footnote 1@ using X-ray photoelectron spectroscopy (XPS), time of flight secondary ion mass spectroscopy (ToF-SIMS), water contact angle (WCA) and atomic force microscopy (AFM). In order to maximise the information obtained from the large data sets principal component analysis was applied to observe trends between polymer composition and stem cell adhesion/proliferation on the arrays. @FootnoteText@ @footnote 1@ D. G. Anderson, S. Levenberg and R. Langer, Nature Biotechnology, 2004, 22, 863.

Biomaterial Interfaces
Room 2014 - Session BI-WeM

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

Moderator: A. Chilkoti, Duke University

8:00am **BI-WeM1 Switchable BioInterfaces with Nanoscale Control**, ***J. Vörös,*** Lab of Biosensors and Bioelectronics, Switzerland*; C.S. Tang,* Swiss Federal Labs for Materials Testing and Res.*; C. Huwiler, B. Stadler, T. Blattler, M. Halter, M. Bally, M. Gabi, D. Grieshaber, O. Guillaume-Gentil,* Lab of Biosensors and Bioelectronics, Switzerland **INVITED**

The success of biomaterials critically depends on the ability to interact with the biological environment. The bioresponse is often determined by the properties of the biointerface which requires precise control on the micron- and nanometer scale. This presentation will highlight examples on how current state-of-the-art surface modification methods, such as self-assembled monolayers and poly(ethylene glycol) grafted polyelectrolytes can be used to tailor biointerfaces. For both molecular systems, functional groups are directly introduced into the molecule. Such functional groups are shown to have three advantages: a) they enable production of well-defined and stable surfaces; b) they can be functionalized with biologically relevant reactive groups such as capture probes, antibodies, peptides, drugs or growth factors; and c) they can be combined with (nano)lithography techniques producing patterns with different surface chemistries on the submicron scale.@footnote 1@ Engineered surfaces with such control are ideal platforms for sensing applications (e.g. for microarrays, or for bioaffinity sensors) and at the same time they can also be applied to drug delivery systems and biomaterials, to control specific and non-specific biomolecule - surface interactions. Recently, we have put a lot of efforts into achieving not only spatial but also a dynamic control over the properties of biointerfaces. Surfaces that change upon external stimuli provide us with new research tools for studying complex biological systems and to overcome difficulties in producing heterogeneous microarrays of fragile biomolecules. Highlights for the use of novel, electronically@footnote 2@ - or photo-active@footnote 3@ surfaces for applications in biosensing and local drug delivery will be presented. @FootnoteText@ @footnote 1@ J. Voros et al; MRS Bulletin, 30(3):202-206, 2005.@footnote 2@ C.S. Tang et al; Analytical Chemistry, 78:711-717, 2006.@footnote 3@ B. Stadler et al; Langmuir, 20: 11348-11354, 2004.

8:40am **BI-WeM3 Investigation and Quantification of Immobilized Protein in Crosslinked Bilayers**, ***R. Michel,*** University of Washington*; V. Subramaniam,* University of Arizona*; S.L. McArthur,* University of Sheffield, UK*; E. Ross, S. Saavedra,* University of Arizona*; D.G. Castner,* University of Washington

Supported lipid bilayers are commonly used as model systems for cell studies. More recently, these layers, which are inherently unstable under ambient or ultra-high vacuum conditions, have been stabilized via cross-linking. These crosslinked bilayers have potential applications as biomedical coatings and biosensors. The detection and investigation of intercalating biomolecules is a first step towards creating artificial cell-like surfaces, and proteins are ideal model systems to study functionality in the bilayer. Recently it was shown that a common membrane protein, rhodopsin, could be reconstituted into these lipid bilayers and retain its photoactivity after cross-linking of the lipid bilayers. Secondary Ion Mass Spectrometry (SIMS) and X-Ray Photoelectron Spectroscopy (XPS) are surface sensitive techniques that yield information on the top few nanometers. We used these ultrahigh vacuum techniques to structurally characterize the lipid bilayers of redox and UV polymerized supported lipid membranes composed of 1,2-bis[10-(2',4'-hexadienoloxy)decanoyl]-sn-glycero-3-phosphocholine (bis-Sorb PC). UV-polymerized bis-Sorb PC bilayers with reconstituted rhodopsin were quantified with XPS. Angle resolved XPS revealed the protein to be located within the bilayer, and not adsorbed on top of it. SIMS was used to investigate the similarities and differences of distinct fragments from the phosphocholine lipids and the protein amino acids.

9:00am **BI-WeM4 Proteo-Phospholipid Assemblies on Nanotextured Surfaces**, ***G.P. Lopez,*** University of New Mexico

This talk will describe our recent efforts to develop methods for incorporation of transmembrane proteins, peptides and receptors in new types of lipid bilayer assemblies supported on nanotextured substrates. Substrates investigated include those fabricated by synthetic (i.e., bottom-up) methods based, for example, on molecular self-assembly and reductive (top-down) nanolithographic methods. Several methods for incorporating tethered receptors, transmembrane proteins and peptides into phospholipids architectures will be presented and methods for characterizing these assemblies will be discussed. The potential for such supported and suspended bilayer assemblies in biotechnological applications will also be suggested.

9:20am **BI-WeM5 Transcending the Time-Domain in Protein Adsorption Simulations**, ***R.A. Latour,*** *Y. Sun,* Clemson University

Although very important, the ability to predict and control the bioactive state of adsorbed or tethered proteins remains a major challenge in the field of biomaterials. While molecular modeling methods have great potential to help understand protein-surface interactions, methods must be specifically developed for this application. Two of the most important problems that must be addressed are the force field problem and the

sampling problem. The force field problem relates to the design of the energy function and its parameters to ensure that atoms of a given system interact with one another in a realistic manner during a simulation. The sampling problem relates to the need to sample molecular events over timeframes that extend far beyond those that are capable of being reached using standard molecular dynamics methods. The objective of this research is to develop computational methods to address both of these issues, with a focus on the application of an advanced sampling method called replica-exchange molecular dynamics (REMD). An REMD simulation transcends the time-domain problem by enabling a Boltzmann-weighted ensemble of states to be generated, thus providing simulation results that should be directly comparable to experimental results for an equilibrated system. In this study, REMD simulations have been conducted to simulate lysozyme adsorption on a hydrophobic surface using a modified CHARMM force field with implicit solvation. The REMD simulations predict five different predominant configurations of lysozyme when adsorbed on this surface. Matched experimental studies are currently being conducted to enable the accuracy of the simulation results to be assessed.

9:40am **BI-WeM6 Biofunctionalizing Nitride Surfaces without Silanes***, **R. Stine**,* Naval Research Laboratory, US*; K.M. McCoy, S.P. Mulvaney, L.J. Whitman,* Naval Research Laboratory

Silicon nitride is widely used as a coating in the microelectronics industry because of its ability to resist penetration by contaminants such as water, oxygen, and ionic species. This property also makes silicon nitride a common terminal passivation layer for chip-based biosensors and bioMEMS devices, all of which come into contact with aqueous saline solutions. Current methods for biofunctionalizing silicon nitride rely almost exclusively on silane-based films, both for direct functionalization and as bifunctional linkers. However, even under stringent controls, the chemistry of silane films on silicon nitride surfaces is notoriously inconsistent and suffers from degradation over time when used in aqueous environments. We have developed an alternate, silane-free, method for functionalizing silicon nitride surfaces. The native oxide is first stripped via HF solution, and then treated with a plasma that makes the surface reactive to aldehydes. Using a bifunctional aldehyde coupler, we then adsorb a robust NeutrAvidin layer that can be used to immobilize any biotinylated biomolecule and has excellent nonfouling properties. We will describe the surface chemistry and compare our approach with silane-based methods as analyzed by XPS using fluorinated benzaldehyde. We will also show that this chemistry can be successfully applied to GaN surfaces, and used for both immunoassays and DNA hybridization assays in a range of sample matrices.

10:40am **BI-WeM9 Characterization of Surface-Immobilized Layers of Intact Liposomes by Atomic Force Microscopy Force Measurements and Quartz Crystal Microbalance***, **H. Brochu**, P. Vermette,* Université de Sherbrooke, Canada

The scientific literature is abundant on the development, characterization and validation of liposome suspensions, particularly in the biomedical fields. However, much less is known on the surface immobilization of layers of intact liposomes, which can find applications in several fields including drug-partitioning chromatography, cell structure mimicking, and localised drug delivery. In fact, surface-bound liposomes have been validated as localized drug delivery systems in some applications. Although some papers are available on the characterization of surface-immobilized layers of intact liposomes, many physicochemical properties of these complex thin layers still need to be elucidated. Briefly, these surfaces are made using radiofrequency glow discharge deposition to coat a surface with a thin polymeric film bearing amine groups with the subsequent covalent attachment, using carbodiimide chemistry, of NHS-poly(ethylene glycol)-biotin (NHS-PEG-biotin) low-fouling polymer prepared under cloud point conditions. Next, NeutrAvidin (NA) molecules are docked on the PEG layers bearing biotin followed by the attachment of biotinylated liposomes, which anchor to the NA. X-ray photoelectron spectroscopy is used to follow-up the chemical surface modification steps. This talk will present surface characterization of layers of intact liposomes by AFM force measurements and QCM analysis with dissipation monitoring with the aim to develop a model of the mechanical properties of these thin layers. With AFM force measurements, estimation of the Young modulus of these well hydrated layers is obtained using the Hertz model. QCM measurements allowing dissipation monitoring provide some information on the mechanical properties of these surface-bound liposomes. QCM is also used to study the dynamics of protein adsorption on these surface-bound liposomes. Also, kinetics of the sequential release of more than one molecules from these layers of intact liposomes is investigated.

11:20am **BI-WeM11 The Clinical Performance of Biomimetic Interfaces: the Long and Winding Road***, **K.E. Healy**,* University of California at Berkeley **INVITED**

For nearly two decades, biomimetic or bio-inspired interfaces have been designed to control both the adsorption of macromolecules and cell fate in the peri-implant region. Although successful performance of biomimetic interfaces has been frequent in the biotechnology and biosensor arena, translation of in vitro efforts into the clinical domain have largely failed. Lack of success can be attributed to complex in vivo microenvironments in the peri-implant region that encompass hypoxia, degradative molecules, and fibrin clot formation that can mask biomimetic surface engineering strategies. These microenvironments are not recapitulated using in vitro models, which leads to the poor efficiency of translational research. This lecture will emphasize the universal nature of biomimetic modification strategies and characterization modalities in the context of surface-mediated photoinitiated polymerization to create nanoscale polymer coatings that control the presentation of ligands for cell adhesion and subsequently cell fate determination. The limitations of this biointerface design approach for in vivo applications and current strategies for clinical success will be addressed.

12:00pm **BI-WeM13 Bioarrays with 10,000 Functionalized Spots per Square cm***, **M.R. Linford**, M.C. Aplund, F. Zhang, G. Saini,* Brigham Young University

We describe here a method for very rapidly patterning surfaces to make bioarrays. In this method, a silicon surface is first coated with a monolayer to make it hydrophobic. A microlens array is then positioned over the surface and a single shot (4 ns) from a YAG laser is directed through it. The microlens array focuses the laser pulse into a myriad of tiny spots on the surface that cause removal of the monolayer, leaving ca. 20 micrometer hydrophilic spots. The density of these hydrophilic spots is 10,000 per square cm, i.e., the center-to-center distance between the spots is 100 micrometers. It is then shown by fluorescence and time-of-flight secondary ion mass spectrometry (ToF-SIMS) that polylysine will preferentially adsorb onto the hydrophilic spots. Bioconjugate chemistry from this polylysine base, e.g., the use of phenylenediisothiocyanate as a crosslinker, then allows amine-terminated oligonucleotides and proteins to be attached at the functionalized spots. This bioconjugate chemistry is confirmed on larger, planar surfaces by wetting, optical ellipsometry, X-ray photoelectron spectroscopy, ToF-SIMS, and fluorescence.

Applied Surface Science
Room 2005 - Session AS+BI-WeA

Imaging and Characterization of Biological Materials

Moderator: L.J. Gamble, University of Washington

2:00pm **AS+BI-WeA1 Imaging Biomolecules at Surfaces**, ***R.M.A. Heeren,*** *E.R. Amstalden, A.F.M. Altelaar, M. Froesch, L.A McDonnell,* FOM-Institute for Atomic and Molecular Physics, The Netherlands **INVITED**

Mass spectrometry is one of the technologies that enable the investigation of the spatial organization of biomolecules at complex surfaces. The potential of imaging MS as a biomedical imaging technique is evident. Direct biomarker visualization on tissue is only one of a few key applications. Imaging mass spectrometry is currently undergoing rapid developments in areas spanning the entire technology chain required to generate a mass resolved chemical image. New detection technology and novel imaging approaches improve speed, sensitivity and spatial resolution of imaging MS. In this contribution we will discuss several technological and methodological aspects of imaging mass spectrometry using a set of selected applications in biomedical imaging. Various approaches exist that use different incarnations of imaging mass spectrometry ranging from protein profiling to high resolution imaging using MALDI and SIMS. In our studies, a novel stigmatic or microscope mode imaging MS strategy is employed that allows for the rapid generation of high resolution, large field-of-view mass resolved images of cells and tissue. This mass microscope is combined with tissue digestion strategies that aid in the identification of larger proteins found in tissue section. One of these strategies involves the so-called molecular scanner, a technique where proteins are electro-blotted from the tissue through a membrane containing immobilized proteolytic enzymes. While the proteins pass through the membrane they are digested into smaller proteolytic peptides that are subsequently captured on a PVDF membrane. This technology enhances the detection sensitivity as multiple peptides are generated from a single protein molecule. The advantages of this high resolution imaging approach, using the molecular scanner will be demonstrated on cervical tissue sections obtained in the framework of a biomarker discovery study for cervical cancer.

2:40pm **AS+BI-WeA3 Acquisition of Chemical Information from Cell Samples Using TOF-SIMS Imaging**, *D. Breitenstein,* TASCON GmbH, Germany*; C. Rommel, J. Wegener,* University of Münster, Germany*; R. Moellers, E. Niehuis,* IONTOF GmbH, Germany*;* ***B. Hagenhoff,*** TASCON GmbH, Germany

Our ongoing studies focus on the mass spectrometric imaging for cells, an emerging area in TOF-SIMS research. In a joint research effort we want to elucidate the transport mechanism for drugs through the blood-liquor barrier. Being a vacuum technique TOF-SIMS faces some challenges for the analysis of epithelial cells. A standard approach to maintain the cellular integrity of cells during the experiments is freeze fracturing or cryomicrotomy. However, these techniques increase the experimental effort significantly. As we were not interested in the lateral distribution of elemental species like Na of K but small and middle sized molecules we concentrated on different fixation techniques instead. Mainly, paraformaldehyd and glutardialdehyd were used. In order to visualize the three-dimensional molecular structure of the cells C@sub 60@ sputtering was combined with imaging using Bi@sub 3@ primary ions. In order to compare the results with a standard analytical technique in biochemistry, additionally cells were treated with fluorescent dyes. The intact fluorophores could be detected successfully in the TOF-SIMS images. The obtained mass resolved images for the fluorescent dyes were compared successfully with the widely accepted technique of confocal laser scanning microscopy (CLSM), proving the validity of the chosen mass spectrometric approach. This work was supported by the German Federal Ministry for Education and Research (Grand: 0312002B).

3:00pm **AS+BI-WeA4 Complementary Application of SIMS and CARS for Biochemical Imaging of Cells and Tissues**, ***D.W. Moon,*** Korea Research Institute of Standards and Science, Korea*; T.G. Lee, E.S. Lee, J.Y. Lee,* Korea Research Institute of Standards and Science*; J.W. Shim,* AmorePacific Corporation*; J.W. Kim, K.W. Kim,* Seoul National University, Korea

There have been significant progresses in analysis of biomolecules on surfaces using surface analysis tools such as XPS, SIMS, SPR, and FT-IR. Major demands of biosurface analysis come from DNA chips, protein chips, and surface modification for tissue engineering. However, cell physiologists and medical and pharmaceutical scientists prefer in-vitro analyses of biomolecules in live single cells and tissues in spite of technical difficulties to biochemical assays. In this presentation, we report our recent studies on 2D or 3D label-free biochemical imaging of various cells and tissues such as liver, skin, retina, and hair based on complementary use of coherent anti-Stokes Raman scattering (CARS) and SIMS. TOF-SIMS measurements are based on cluster ion bombardment such as Au3, Bi3, and C60 and CARS measurements are optimized for the C-H vibration of biomolecules in live cells and tissues. CARS showed clear C-H chemical bond specific images with 300 nm spatial resolution and 1 μm depth resolution down to 100 μm depth, revealing detailed tissue structures in the sub-cellular level without any damage problems. SIMS showed much more surface sensitive and specific biomolecular mass images with some depth profiling capabilities. The present status and the future prospect of complementary use of CARS and SIMS with sensitivity and selectivity improvement based on a broad band spectrum and cluster bombardment, respectively will be discussed for practical applications in disease diagnostics and cell and tissue based drug screening.

3:20pm **AS+BI-WeA5 Desorption Electrospray Ionization: Fundamentals and Applications in Surface Analysis and Biological Imaging**, ***R.G. Cooks,*** *Z. Takats, J.M. Wiseman, D. Ifa, N. Talaty, A. Jackson, A. Venter,* Purdue University **INVITED**

This talk concerns ambient ionization using desorption electrospray ionization (DESI) and the related methods. These procedures allow direct analysis of biological samples, including proteins and lipids, on surfaces or in tissue without sample preparation. Biological fluids can also be examined directly, or by adsorption on a matrix like paper. DESI is suitable for characterization of both large and small molecules and it combines features of electrospray ionization (ESI) with those of the family of desorption ionization (DI) methods. It allows organic molecules present on surfaces to be analyzed by mass spectrometry without requiring that the sample be introduced into the vacuum system of the mass spectrometer. DESI has high sensitivity, is virtually instantaneous in response time, and there is little or no sample preparation. The sample is sprayed with charged microdroplets of water or a simple organic solvent. The sample remains fully accessible to observation as well as additional physical and chemical processing during the analysis. Applications to metabolomics, to high throughput analysis of pharmaceutical preparations, to drugs and drug metabolites in blood, serum and other biological fluids, are described. Tissue imaging is demonstrated with lipids being used as biomarkers to search for disease and in-vivo sampling of living tissue surfaces is described. Laser doppler anemometry is used to characterize the DESI mechanism. It is shown that at least two major processes are involved. One involves transfer of molecules from the surface to the droplet projectiles, the other involves proton or other charge transfer from the slow-moving projectile to the sample molecule. Variations on the DESI method in which reactive compounds are included in the spray solvent allow recognition of specific functional groups.

4:00pm **AS+BI-WeA7 Characterization of Bacterial Spores using Nano-Secondary Ion Mass Spectrometry (NanoSIMS)**, ***S. Ghosal,*** *S.J. Fallon,* Lawrence Livermore National Laboratory*; T. Leighton, K. Wheeler,* Children's Hospital Oakland Research Institute*; I.D. Hutcheon, P.K. Weber,* Lawrence Livermore National Laboratory

Bacterial spores are elementally zoned at the nanometer scale. This zonation may be controlled by spore physiology, physical factors, and elemental diffusion. Here we present a recently developed nanometer-scale secondary ion mass spectrometry (NanoSIMS) technique that allows the direct visualization and quantification of elemental concentration gradients within spores. By using NanoSIMS depth profile analysis together with sample preparation techniques such as focused ion beam (FIB) sectioning, we are able to probe the three dimensional elemental distribution within individual Bacillus thuringiensis israelensis (Bti) spores with nanometer scale resolution (~10 nm depth and 50 nm lateral). Our results show the expected distributions for physiologically controlled elements (Ca, P) and provide baseline data other elements (e.g., Li, F, Cl, S). We also demonstrate cation and anion mobility in spores under hydrous conditions. Our results suggest a permeation mechanism by which elements diffuse into and out of the spore along hydration pathways on rather short time scales. Additional studies are in progress to define the rates and mechanisms controlling ion mobility in spores. @FootnoteText@ @footnote@This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract W-7405-Eng-4.

4:20pm **AS+BI-WeA8 Vacuum Ultraviolet Postionization for Mass Spectrometry of Small Molecule Analytes in Bacterial Biofilms**, *L. Hanley,* ***P.D. Edirisinghe****, M. Zhou,* University of Illinois at Chicago*; K.A. Skinner-Nemec, C.S. Giometti,* Argonne National Laboratory*; J.F. Moore,* MassThink*; J.E. Hunt, W.F. Calaway, M.J. Pellin,* Argonne National Laboratory

Mass spectrometric analysis and imaging of intact microbial biofilms are difficult with established methods. A new experimental strategy is discussed for analyzing small molecule analytes within intact biofioms: laser desorption followed by postionization with 7.87 eV radiation of molecular analytes whose ionization potentials have been lowered by chemical derivatization with an aromatic tag.@footnote 1@,@footnote 2@ Postionization mass spectrometry with derivatization is developed on small peptides with aromatic or native tags such as a tryptophan residue. The new method is then applied to the detection of a quorum sensing peptide in a Bacillus subtilis bacterial biofilm. Finally, detection of an antibiotic is demonstrated by direct 7.87 eV postionization, without derivatization. These mass spectrometric methods show promise for the study of antibiotic resistance in microbial biofilms as well as other studies of small molecule analytes within complex biological matrices. @FootnoteText@ @footnote 1@P.D. Edirisinghe et al., Anal. Chem. 76 (2004) 4267.@footnote 2@L. Hanley et al., Appl. Surf. Sci. (2006), in press.

4:40pm **AS+BI-WeA9 Chemical and Biological Differentiation of Human Breast Cancer Cell Types Using Time-of-Flight Secondary Ion Mass Spectrometry**, *K. Kulp, E. Berman, M. Knize, J. Felton, E. Nelson, J. Montgomery,* Lawrence Livermore National Laboratory*; L. Wu, D. Shattuck,* University of California, Davis*;* ***K. Wu****,* Lawrence Livermore National Laboratory

We use time-of-flight secondary ion mass spectrometry (TOF-SIMS) to image and classify individual cells on the basis of their characteristic mass spectra. Using statistical data reduction on the large data sets generated during TOF-SIMS analysis, similar biological materials can be differentiated on the basis of a combination of small changes in protein expression, metabolic activity and cell structure. We apply this technique to image and differentiate three carcinoma-derived human breast cancer cell lines (MCF-7, T47D, and MDA-MB-231). In homogenized cells, we show the ability to differentiate the cell types as well as cellular compartments (cytosol, nuclear, and membrane). These studies illustrate the capacity of TOF-SIMS to characterize individual cells by chemical composition, which could ultimately be applied to detect and identify single aberrant cells within a normal cell population. Ultimately, we anticipate characterizing rare chemical changes that may provide clues to single cell progression within carcinogenic and metastatic pathways.

Biomaterial Interfaces
Room 2014 - Session BI-WeA

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

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

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

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

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

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

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

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

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

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

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

Chronically implanted neural electrodes lose the ability to record or stimulate neural activity with time,@footnote 1@ generally within a few weeks.@footnote 2@ While there are numerous potential causes of this problem, most involve an unfavorable reaction or interaction at the electrode surface.@footnote 2@ Thus, the electrode surface is of paramount importance@footnote 3@ in improving the functionality and longevity of the electrode. Our laboratory has created a platinum (Pt) electrode surface modification platform that will allow for the incorporation of a biologic at the surface, with the goal of improving electrode performance. Thus far, our data demonstrates successful use of plasma deposition of acrylic acid on Pt to generate carboxyl functional groups. ESCA analysis of these samples consistently shows carboxyl groups to comprise around 17% of the surface carbon content. Soaking samples in H@sub 2@O at room temperature or 37° or under acidic or basic conditions does not significantly decrease carboxyl group content. EDC-activation of these carboxyl groups leads to the successful attachment of poly(vinyl alcohol) (PVA) to the Pt substrate in a concentration and temperature-dependent manner as verified by ESCA and TFAA+ESCA; this PVA layer will function as a hydratable and potentially soft intermediary layer between the Pt and the biologic. The hydroxyl groups of PVA will be activated using CDI chemistry to allow for the covalent attachment of a protein through its lysine residues; we plan to attach fibronectin. Impedance spectroscopy has been performed on bare Pt and Pt with attached PVA and preliminary results show the impedance did not increase significantly with the attachment of the PVA. @FootnoteText@ @footnote 1@ Cui X. J. Biomed. Mater. Res., 56, 261-272 (2001).@footnote 2@ Cui X. Biomat., 24, 777-787 (2003).@footnote 3@ Huber M. J. Biomed. Mater. Res., 41, 278-288 (1998).

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

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

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

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

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

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

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

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

Biomaterial Interfaces
Room 2014 - Session BI-ThM

Plasmonic Methods and Sub-micron Structures for Biology and Medicine

Moderator: S. Zauscher, Duke University

8:00am **BI-ThM1 Light Exposure Affects the Adsorption of Colloidal Gold onto Monolayers of 3-Mercaptopropyltrymethoxysilane**, *K. Pandya, O. Ogunsanwo,* ***V.H. Perez-Luna,*** Illinois Institute of Technology

Light exposure affects the capability of thiol-terminated monolayers to adsorb colloidal gold particles. This effect was determined by forming monolayers of 3-mercaptopropyltrimethoxysilane (MPTMS) on silica surfaces in the presence of white light, red light (635 nm) and under dark conditions. Surface characterization by means of wettability, ellipsometry and FTIR spectroscopy was carried out to determine differences in surface properties for the different preparation methods. Monolayers prepared under different conditions of light exposure (white light, red light and absence of light) were exposed to colloidal gold solutions in order to study the adsorption of gold nanoparticles. The adsorbed colloidal gold monolayers were characterized by optical absorption spectroscopy. In spite of the strong affinity of the thiol group for gold, samples that were exposed to white light did not adsorb gold nanoparticles to a significant extent. In contrast, monolayers prepared using red light or under dark conditions were capable of adsorbing gold nanoparticles at levels comparable to amine-functionalized surfaces. These findings may offer some insights as to differing literature reports on adsorption of colloidal gold to thiol-functionalized surfaces. The thiol groups may undergo oxidation in the presence of oxygen and white light to from sulfonate groups that do not promote adsorption of gold nanoparticles. Hence MPTMS monolayers exposed to light may not present sufficient thiol groups for subsequent attachment of gold. Surface analysis of samples exposed to white and red light and those kept in the dark verify this hypothesis.

8:20am **BI-ThM2 Label Free Nanoscale Optical Biosensors**, ***A. Chilkoti,*** Duke University **INVITED**

I will describe the use of nanoparticles for the fabrication of label-free, surface optical biosensors by exploiting the localized surface plasmon effect exhibited by individual nanoparticles of gold and silver. We have immobilized gold nanoparticles onto glass slides, functionalized the surface of the gold nanoparticles with biological ligands, and shown that these nanoparticle decorated surfaces enable ligand binding to be detected by the shift in the extinction spectrum of individual nanoparticles. This assay is analogous to conventional, planar SPR with the added advantage of being performed in widely available, low-cost UV-visible spectrophotometers and its facile extension to array based sensors. The extension of this detection modality using gold nanorods and the increased sensitivity afforded by these anisotropic particles will also be discussed. Finally, recent results on reducing the size of the optical transducer to the ultimate limit of a single nanoparticle through the use of dark-field microscopy will be discussed, which opens up the intriguing possibility of obtaining single molecule sensitivity.

9:00am **BI-ThM4 Non-Cross-Linking Aggregation of DNA-Fnctionalized Nanoparticles for Single-Base Substitution Assay**, ***M. Maeda,*** RIKEN, Japan **INVITED**

For the detection of DNA single-base substitutions, gold nanoparticles (GNPs) has been attracting considerable interests, because GNP aggregation accompanied by the surface plasmon shift can be clearly recognized with the naked eye. The method is in general a sandwich assay in which a target DNA molecule cross-links two DNA-functionalized GNPs by hybridization. In contrast, we recently discovered that GNPs also aggregate with a non-cross-linking (NCL) manner; formation of fully complementary duplexes on GNP surfaces induces the aggregation at relatively high salt concentration. Interestingly, the NCL aggregation exhibits extraordinary selectivity against terminal mismatches; single-base mismatches at the free ends of the duplexes make very stable dispersions (e.g., no aggregation). Unlike conventional hybridization-based assays, this system can detect the terminal mismatches without precise temperature control. Because of this surprising selectivity for terminal mismatches, rapid and reliable SNPs typing after single-base primer extension is possible without time-consuming analysis such as mass spectrometry. NCL interaction between fully-matched DNA duplex on GNPs (FM-Au) and duplex on a gold substrate was studied using SPR imaging. A significant increase in intensity was observed where the substrate-anchored DNA probe hybridized with its fully-matched target showing a blunt end. In contrast, no change in intensity was observed with the one-base mismatched target. The FM-Au specifically discriminates the terminal base-pairing at the sensor surface.

9:40am **BI-ThM6 Nano-Crescents as Tunable Plasmon-Based Sensing Platforms**, *R. Bukasov, M. Hukill,* ***J.S. Shumaker-Parry,*** University of Utah

Crescent-shaped gold nanostructures exhibit localized plasmon resonances that are tunable from the visible to the infrared. A combination of polymer nanosphere templating, metal film deposition and ion beam etching produces a large number of crescents with defined and uniform size, shape, and orientation on a substrate. Ensemble measurements show the crescents exhibit multipolar plasmon resonances that are tuned from 600 to 2800 nm by controlling the crescents' structural properties. In addition to the template size, the diameter to thickness aspect ratio and the distance between the crescent tips also influence the crescents' optical properties. The crescents' plasmon resonances exhibit a strong dependence on the local dielectric environment, with sensitivity factors of up to 800 nm/refractive index unit, depending on the crescent size and the plasmon resonance wavelength. The high sensitivity, the tunability of the crescents' optical properties over a wide wavelength range, and the bare crescent surface readily available for functionalization with receptor molecules make the crescents strong candidates for development as sensors.

10:00am **BI-ThM7 Plasmonic Coupling in Biomolecule Linked Nanoparticle Assemblies**, ***A.A. Lazarides,*** Duke University, US; *D.S. Sebba, E.R. Irish,* Duke University

Metal nanoparticle assemblies support delocalized plasmon resonances that are highly sensitive to interparticle spacing, when particles are positioned within the near fields of their neighbors. We have observed near field coupling in biomolecule-linked core/satellite structures and have observed, further, controllable plasmon band modulation when the assemblies are linked by reconfigurable tethers. The nature of the modulation depends upon both the strength of the plasmon coupling and the composition of the component particles. Accurate electrodynamic calculations confirm the observed relationships between assembly structure, composition, and optical properties. Approximate, analytic, dipole coupling models provide insight into the material-dependence of the observed band modulation phenomena.

10:20am **BI-ThM8 Semiconductor Quantum Dots for Bioimaging: Bandgap Engineering and Surface Engineering**, ***A.M. Smith,*** *S. Nie,* Georgia Institute of Technology and Emory University **INVITED**

The development of high-sensitivity and high-specificity probes beyond the intrinsic limitations of organic dyes and fluorescent proteins is of considerable interest to many areas of research, ranging from single-molecule biophysics to in-vivo medical imaging. Recent advances have shown that nanometer-sized semiconductor particles can be covalently linked with bioaffinity molecules such as peptides, antibodies, nucleic acids, and small-molecule inhibitors for use as fluorescent probes. In comparison with organic fluorophores, quantum dots (QDs) exhibit unique optical and electronic properties such as size- and composition-tunable fluorescence emission, large absorption coefficients, and significantly improved brightness and photostability. Despite their relatively large sizes (2-8 nm), bioconjugated QD probes behave like fluorescent proteins (4-6 nm), and do not suffer from serious kinetics or steric-hindrance problems. In this mesoscopic size range, QDs also have high surface area-to-volume ratios that can allow multivalent functionalization with many diagnostic (e.g., radioisotopic or magnetic) and therapeutic (e.g., anticancer) agents. We present recent developments in bioconjugated QD probes and their applications in ultrasensitive molecular and cellular imaging. We have generated new classes of QDs with tunable near-infrared emission (700-900 nm) for high-sensitivity imaging deep within living animals and in highly autofluorescent fixed tissue specimens. These QDs are brighter and have narrower emission bandwidths than comparable QDs reported in the literature, approaching the spectral properties of commonly used visible QDs. Using polymeric encapsulation, we have also engineered the surfaces of these and other QDs for ultra-high stability under a variety of harsh conditions, such as high salt buffers, acidic solutions, and oxidizing environments that would normally quench the QD fluorescence and potentially degrade the semiconductor core.

Nanometer-scale Science and Technology
Room 2016 - Session NS+BI-ThM

Biological and Molecular Applications of Nanoscale Structures

Moderator: H.G. Craighead, Cornell University

8:00am **NS+BI-ThM1 A Nanometer-scale Gene Chip: Detecting Single Molecules of DNA using a Silicon Nanopore**, ***G.L. Timp***, Beckman Institute **INVITED**

We describe a prospective strategy for reading the encyclopedic information encoded in the genome: using a nanopore in a membrane formed from an MOS-capacitor to sense the charge distribution in a single molecule of DNA. In principle, as DNA permeates the capacitor-membrane through the pore, the electrostatic charge distribution polarizes the capacitor and induces a voltage on the electrodes that can be measured. The sub-nanometer precision available through silicon nanotechnology facilitates the fabrication of this nanometer-scale gene chip, and molecular dynamics provides us with a means to design it and analyze the experimental outcomes. Double-stranded DNA is a highly charged, unusually stiff polymer. And so, the nano-electromechanics of the molecule profoundly affect the design of this detector. Consequently, we have explored the electromechanical properties of DNA using an electric field to force single molecules through synthetic nanopores in ultra-thin silicon membranes. At low electric fields E =@0.5nm, while double-stranded DNA only permeates pores with a radius @>=@1.5nm because the diameter of the double helix is about 2nm. For pores

8:40am **NS+BI-ThM3 Single DNA Molecules Stretched in Electrospun Polymer Fibers**, ***L.M. Bellan***, *J.D. Cross, E.A. Strychalski, J.M. Moran-Mirabal, H.G. Craighead,* Cornell University

We have deposited electrospun polyethylene oxide (PEO) fibers containing isolated single stretched DNA molecules. The ability to stretch single molecules of DNA is desirable for single-molecule sequencing techniques and also allows the study of the behavior of DNA molecules undergoing various forces. Electrospinning is a popular technique for quickly and easily depositing micro- and nanoscale diameter fibers from a variety of materials, and has recently been used in several studies as a method for assembling nanoscale particles and molecules. In the present study, a dilute concentration of fluorescently labeled lambda phage DNA molecules was added to the water solvent used to mix the PEO electrospinning solution. The solution was used to produce isolated nanofibers using the scanned electrospinning technique. The DNA molecules were stretched in-flight in the electrospinning jet and remained stretched when the fluid jet solidified into fibers. These fibers were deposited on coverslips and imaged using fluorescence microscopy. The embedded DNA molecules were seen as single lines of fluorescence ranging from under 3 microns to 19 microns, which is the extended length of the lambda DNA molecule at the base pair to dye labeling ratio used. The variation in length is thought to be due to variations in the electrospinning jet fluid dynamics. By tuning the process parameters we were able to obtain a distribution of stretched lengths with a mode of ~7 microns. We also observed chain scission in some cases. Given the long relaxation time of DNA in the polymer solution and the high strain rates present in electrospinning jets, both stretching and sporadic chain scission are expected. Current work is focused on mechanical manipulation of the resulting fibers and DNA molecules embedded therein.

9:00am **NS+BI-ThM4 Polypyrrole Based Nano-Electrode Arrays Produced by Colloidal Lithography**, ***P. Lisboa***, *A. Valsesia, P. Colpo, F. Rossi,* European Commission, Institute of Health and Consumer Protection, Italy

The implementation of sensor platforms providing high sensitivity of detection is a crucial step for the design of the new analytical device generation for biosensor developments. Electrochemical sensors have shown a high potential for this challenge. A drastic increase of sensitivity of bio-event detection@footnote 1@ has been proven by designing platform with active/non-actives region at nanoscale. Besides, the electrochemical sensitivity can be as well enhanced by using nano-electrode arrays that increase mass transport rate.@footnote 2@ Polypyrrole (PPy) is a good candidate to fulfil these requirements. Its preferential material for bio-analytical electrochemistry based sensor thanks to its excellent biocompatibility and higher conductivity, together with the possibility of being functionalised with biological relevant functional groups.@footnote 3@ In this work PPy nano pillars were fabricated by electrochemically growing PPy in a nano-template of gold nano-seeds produced by colloidal lithography. Atomic force Microscopy and Scanning Kelvin Microscopy demonstrated that PPy grown only inside the conductive gold seeds, creating conductive nano pillars surrounded by an insulating material. The nano-structured surfaces were studied by Cyclic Voltammetry using hexacyanoferrate and the typical sigmoidal shape voltammogram of nanoelectrodes was obtained.@footnote 2@ The nanoelectrode character of the surfaces is a promising feature to improve the already high sensitivity of biosensors based in nanostructured surfaces. The functionalisation of the nano pillars with bio-active chemical functions gives the possibility of protein immobilization in specific nano-areas which is promising for the production of an array of nano-sensors. @FootnoteText@ @footnote 1@A. Valsesia, P. Colpo, P. Lisboa, M. Lejeune, T. Meziani, F. Rossi, Langmuir 2006, 22, 1763.@footnote 2@D.Arrigan, The Analyst, 2004, 129, 1157.@footnote 3@P. Lisboa, D. Gilliland, G. Ceccone, A. Valsesia, F. Rossi, Applied Surface Science 2005, In Press.

9:20am **NS+BI-ThM5 Biofunctionalized Nanoshells for Biological Detection**, ***J.E. Van Nostrand***, Air Force Research Lab*; C.S. Levin,* Rice University*; J.M. Slocik,* Air Force Research Lab*; J.D. Hartgerink, N.J. Halas,* Rice University*; M.P. Kadakia,* Wright State University*; R.R. Naik,* Air Force Research Lab

The ability to detect chemical and biological agents is arguably one of the highest priority technical challenges today. The capability to obtain specific information at and near single-molecule resolution is the ultimate goal in chemical detection. Recent advances in surface-enhanced spectroscopy have demonstrated that Surface Enhanced Raman Scattering (SERS) displays spectrum enhancements of several order of magnitude when molecules are absorbed onto metallic surfaces. Metallic nanostructures because of their plasmonic properties are attractive SERS substrates, in particular, nanoshells and nanorods. Combining the specificity of biomolecular recognition with these nanostructures might lead to increased sensitivity and selectivity. Localization of biological recognition motifs to the surface of these nanostructures will lead to large signal enhancements when bound to its target. Nanoshells will be functionalized with capture elements (peptide ligands and antibodies) and these biofunctionalized nanoshells will be tested for their ability to detect micoroganisms using SERS.

9:40am **NS+BI-ThM6 Characterization of Silver Nanoparticles Films for the Development of TPB Biosensors**, ***J. Wolstenholme***, Thermo Electron Corporation, UK*; K. Bonroy, G. Borghs, F. Frederix,* IMEC, Belgium*; R.G. White,* Thermo Electron Corporation, UK

Metal nanoparticle films have been subject of much research, primarily due to their interesting optical and electronic properties and due to their high surface-to-volume ratio. According to numerous studies, nanoparticle films are promising as precursors for metallic films, as catalysts and especially as sensing substrates for the development of novel biosensors such as the Transmission Plasmon Biosensor (TPB). This type of biosensor is based upon the optical properties of silver and gold nanoparticles which are used to sense the specific target molecules in a complex matrix. Hereby, the nanoparticles are to be functionalized with self-assembled monolayers (SAMs) of thiols or disulfide molecules. These monolayers form a covalent bond with the gold or silver surface and can have appropriate functional groups to allow the attachment of specific bioreceptors. For surface plasmon based biosensors such as TPB, gold nanoparticles are most frequently used due to their chemical stability and their relatively simple preparation. Nevertheless, various studies and models predict a much higher sensitivity using silver nanoparticles as TPB sensing substrate. However, at this moment the drawbacks for using silver films as sensing substrates are their instability due to the formation of an oxide layer. The latter will negatively influence the formation of well-organized SAMs of thiols and the subsequent functionalization of the particles with specific bioreceptors. In this paper, we describe the FTIR, the XPS and angle resolved XPS characterization of the multilayered TPB sensing substrates, comprising quartz, silanes, silver nanoparticles and thiol molecules. In this study, we compared the thickness of the silane layer, the oxidation ratio of the silver films and the density of the thiol SAM for both silver nanoparticle films and continuous silver films. Our study indicates that the deposition of SAMs decreases the formation of oxides on the silver nanoparticle films.

10:40am **NS+BI-ThM9 Surface Nanostructuring using Colloidal Particles for Improved Biocompatibility**, ***C.J. Nonckreman***, *P.G. Rouxhet, Ch.C. Dupont-Gillain,* Université Catholique de Louvain, Belgium

Nanostructured surfaces offer new perspectives in different fields of application, including the design of biomaterials (implants, catheters, blood bags). The aim of this work is to create model surfaces presenting bimodal roughness characteristics (scales of 500 nm and 50 nm) using colloidal

lithography. An appropriately designed nanoroughness is expected to modulate the effect of the surface chemical composition for controlling the interactions of cells and tissues with materials. Colloidal lithography was performed using adsorption of cationic polymers and adhesion of negatively charged colloidal particles. Polyallylamine hydrochloride was used to confer a positive charge to a glass substrate. On this conditioned surface, a layer of colloids (polystyrene latex) was formed owing to electrostatic attraction. Sequential steps of polycation adsorption and particle adhesion were applied on the substrate, which was then analyzed by scanning electron microscopy. Adjustment of conditions for incubation solutions (concentration, pH and ionic strength), rinsing and drying were tested in order to produce a high surface coverage with colloids and to minimize their aggregation. Thereby, a range of surface structures was obtained: layer of particles with a diameter of 470 nm, layer of particles with a diameter of 65 nm, bimodal roughness made by particles with a diameter of 65 nm on the top of particles with a diameter of 470 nm. The obtained surfaces are conditioned by adsorption of compounds which make them protein repellent, in particular Pluronic F68, a block copolymer of polypropylene oxide and polyethylene oxide. The surfaces finally obtained are being tested with respect to plasma protein adsorption, in particular competitive adsorption of fibrinogen and albumin, and to biocompatibility.

Plasma Science and Technology
Room 2009 - Session PS1+BI-ThM

Plasmas in Bioscience

Moderator: S.G. Walton, US Naval Research Laboratory

8:00am **PS1+BI-ThM1 Study of Plasma Modified PTFE for Biological Applications : Relationship between Non Fouling Properties - Plasma Treatment - Surface Composition and Surface Roughness**, ***N. Vandencasteele*** [1], Université Libre de Bruxelles, Belgium; *B. Nisol,* Unviersité Libre de Bruxelles, Belgium; *P. Viville, R. Lazzaroni,* Université de Mons-Hainaut, Belgium; *D.G. Castner,* University of Washington; *F. Reniers,* Unviersité Libre de Bruxelles, Belgium

Polytetrafluoroethylene was treated by oxygen or nitrogen RF low pressure plasmas. The modified samples were characterized by XPS for surface composition, contact angle for surface energy and atomic force microscopy for surface roughness. The adsorption of bovine serum albumine (BSA) was used as a probe for the (non)fouling properties and potential biological applications. Evidence for BSA adsorption was determined by the appearance or the increase of the N 1s XPS peak. PTFE modified by Nitrogen plasma shows a strong decrease of the contact angle that has previously been correlated to an increase of the nitrogen surface concentration due to grafting and to a decrease of the fluorine concentration.@footnote 1@ Further exposure to BSA leads to an increase of the N 1s signal, and to a concomitant decrease of the F 1s peak, indicating that some protein was adsorbed onto the plasma modified surface. The exposure of PTFE to an oxygen plasma leads to virtually no grafting. XPS results show that there is less than 1% of oxygen on the surface after the treatment. A strong increase in the chamber pressure was observed during the treatment, and optical emission spectrometry reveals the presence of CO, CO@sub 2@ and F in the gas phase, indicating a strong etching of the surface. Depending on the plasma power, water contact angles as high as 170 deg. could be obtained, indicating a super-hydrophobic behaviour, and new surface structures were observed by AFM. At high power, a strong increase in roughness is evidenced, together with the formation of a regular structure. According to the Cassie Baxter model, this increase of roughness is responsible for the super hydrophobic behaviour. Lower amounts of BSA adsorption were detected on high power oxygen plasma-modified PTFE samples compared to nitrogen plasma-modified PTFE samples. @FootnoteText@ @footnote 1@ N. Vandencasteele, D.H. Fairbrother, F. Reniers. Plasma processes and polymers 2, 493-500, (2005).

8:20am **PS1+BI-ThM2 Patterning of Plasma Polymers for Bioarrays**, ***G. Mishra***, *S.L. McArthur,* University of Sheffield, UK

Protein arrays are solid-phase ligand binding assay systems. They require the immobilisation of proteins on a range of surfaces which include glass, Si wafers and a range of polymers. The assays are highly parallel (multiplexed) and often miniaturised (microarrays, protein chips). Their advantages include being rapid and automatable, capable of high sensitivity, economical on reagents, and giving an abundance of data for a single experiment. Plasma polymerisation presents a versatile approach to surface modification of these devices. The range of monomers available for plasma polymerisation makes this approach even more suitable for use in systems where multiple coatings with specific properties are required for a single device. This project investigates the use of plasma polymerisation to produce arrays with a range of chemical functionalities. The ability to spatially define reactive regions is integral to the project. The challenge lies in simultaneously obtaining high spatial and chemical resolution. In this study we use a range of patterning techniques including photolithography and physical masks and compare the resultant pattern resolution and chemical functionality using XPS, ToF-SIMS and AFM. The results highlight the complexities introduced by the gas phase deposition process and the undercutting that can occur with the physical masks. The issue of compatibility of reactive plasma polymers with the photolithographic process is an important aspect under scrutiny. Our results suggest that complex multilayer layered plasma coatings can be produced without compromising chemical properties of deposits.

8:40am **PS1+BI-ThM3 Plasma Polymerized Thin Films for Tailored Interaction with Human Blood and Cells**, ***C. Oehr,*** Fraunhofer Institute for Interfacial Engineering and Biotechnology, Germany **INVITED**

Plasma Polymerization is used since for more than four decades to develop thin films for different kinds of applications. At least since the seventies of the last century application of these films is mentioned in the fields of medicine and pharmacy. Due to the fact that polymers are used to design low-weight devices and to realize different geometries very easily, the films are mainly deposited onto polymeric substrates. It is a characteristic property of plasma polymerized films to show strong adhesion onto this materials due to the creation of chemical bonding between film and substrate. Such thin layers with good adhesion, a defined amount of chemical functionalities and stability to sterilization processes are generated and fulfill the needs for medical application. The interaction of biological systems with materials can be divided in three subsystems. First, the interaction with bio-molecules. Here the binding of molecules with specific activities on one hand and the minimizing of unspecific protein adsorption on the other hand can be influenced by thin plasma polymers deposited on medical devices. Second, the interaction of bacteria can be modulated via depositing of thin films with bacteriostatic or bacteriocidic properties on devices. Third, the interaction of mammalian cells can also be influenced to enhance the cell growth and proliferation for the development of test kits or implants. In the talk examples of the first and the third category will be given. Beside the preparation of the mentioned films also the analytic tools necessary for film development and control of its properties are stressed in this contribution. A correlation between physico-chemical properties of the applied plasma polymerized films and the biological requirements will be given.

9:20am **PS1+BI-ThM5 RF(13.56MHz) Glow Discharges fed with Acrylic Acid and Allylamine vapours to obtain Functional Coatings for Biomedical Applications**, *E. Sardella,* ***P. Favia***, *L. Petrone, M. Nardulli,* University of Bari, Italy; *R. Gristina,* IMIP-CNR, Italy; *R. d'Agostino,* University of Bari, Italy

Today, a broad range of plasma processes is used to control cell adhesion and growth on biomaterials for tissue engineering and manufacturing of biomedical devices. N-@footnote 1@ and O-@footnote 2@ groups are considered very attractive because they are able to improve cell adhesion and function as "anchor" sites for biomolecules immobilization.@footnote 3@ In this work plasma deposition of acrylic acid (AA), allylamine (AAm) vapours and their plasma co-polymerization have been carried out to deposit functional coatings for several kind of biomedical applications. In particular AA/AAm co-polymerisation can provide different "anchor" sites usable for grafting biomolecules with different activities (ex. antibacterial vs. eukaryotic cell-adhesiveness) on the same substrate. Moreover, these coatings can exhibit zwitterionic characteristics in water and they may find utility in the separation of proteins from solution. A correlation between surface diagnostic analyses (XPS, WCA, FTIR) and plasma phase characterizations (AOES) allowed to provide a correlation between relative density trends of the emitting species in the plasma and the chemical-physical properties of the modified substrate. A titration with water solutions at different pH allowed picking out acid/base properties of the films that can render them very attractive both as supports for cell adhesion and as "smart"materials in drug delivery approaches. In vitro cell culturing of 3T3 fibroblast cell line, were performed to assess the ability of such kind of coatings to influence cell adhesion and growth. Acknowledgements MIUR-FIRB RBNE012B2K is gratefully acknowledged for the financial support. @FootnoteText@ @footnote 1@A. Harsch et al.

[1] **PSTD Coburn-Winters Student Award Finalist**

Journal of neuroscience methods, 2000;98:135-144@footnote 2@Detomaso et al. Biomaterials 2005; 26-18: 3831-3841@footnote 3@D.A. Puleo et al. Biomaterials, 2002;23:2079-2087.

10:20am **PS1+BI-ThM8 Fabrication of High Density and High-Aspect Silicon Nano-column Using Neutral Beam Etching and Ferritin Iron Core Mask**, ***S. Saito***, *T. Kubota,* Tohoku University, Japan*; T. Matsui,* Matsushita Electric Industrial Co., Ltd., Japan*; Y. Uraoka, T. Fuyuki,* Nara Institute of Science and Technology, Japan*; I. Yamashita,* Matsushita Electric Industrial Co., Ltd. and Nara Institute of Science and Technology, Japan*; S. Samukawa,* Tohoku University, Japan

Semiconductor devices has been getting smaller and following Moore's Law. The design rule, the smallest line width, of these devices will be less than 50 nm nanometers within the next decade. Conventional optical lithography process has a theoretical limit to draw patterns smaller than the light wavelength and finer processing techniques are now being intensely surveyed but no methods meet the requests for mass nano-structure production. In order to breakthrough this limit, we have already proposed a new method to fabricate 7 nm nano-dots using the ferritin iron-core as an etching mask and Cl neutral beam for Si etching processes. The ferritin is one of the proteins and has a spherical protein shell with a cavity of 7 nm diameter. It can biomineralize iron as hydrated iron oxide in the cavity and store it in vivo. The Cl neutral beam could realize high etching anisotropy and high etching selectivity to ferritin iron-core without any radiation damages. In this study, we also tried to fabricate higher density and high-aspect Si nanocolumn structure using high-density array of ferritin iron core. The ferritin array could be made just over thin SiO@sub 2@ film (~3nm thick) on Si substrate. In this condition, however, the diameter of Si nanocolumn was enlarged to 14 nm and the etching profile had a slight taper because of extremely low SiO@sub 2@ etching rate using Cl neutral beam. To overcome the problem, we tried two-step neutral beam etching process. For quickly etching the surface SiO@sub 2@, F neutral beam was used. After that, the bulk Si was etched with high anisotropy using Cl neutral beam. As a result, for the first time, the diameter of Si nanocolumn could be shrunk with keeping highly anisotropic etching profile even at narrow space of less than 6nm. This study was supported by Leading Project of Ministry of Education, Culture, Sports, Science and Technology.

10:40am **PS1+BI-ThM9 The Influence of Bond-Coating on Plasma Sprayed Alumina-Titania, Doped with Biologically Derived Hydroxyapatite, on Stainless Steel**, ***S. Salman***, *B. Cal, O. Gunduz,* Marmara University, Turkey*; S. Agathopoulos,* Ioannina University, Greece*; F.N. Oktar,* Marmara University, Turkey

The influence of bond-coating on the quality of thin coatings (~100 µm) of alumina-titania (60%-40%), doped with 5% and 10% bovine hydroxyapatite, plasma-spayed on stainless steel (316), was experimentally investigated by measuring the tensile strength and the ratio of adhesive/cohesive strength of the coatings. The bond-coating layer was alumina-titania (60%-40%). The experimental results (mainly the values of the ratio adhesive/cohesive strength and the microstructure of the coating layers) and their discussion in the light of earlier similar studies show that bond-coating process can result in coating composite structures of high quality, which is of high importance for devices used in biomedicine.

Biomaterial Interfaces
Room 2014 - Session BI+AS-ThA

Biomolecule-Surface Characterization I
Moderator: J.S. Shumaker-Parry, University of Utah

2:00pm **BI+AS-ThA1 Nanostructured Titanium Surfaces for Bone Biotemplating Applications**, ***K.C. Popat***, *T. Desai,* University of California, San Francisco

A major goal in orthopaedic biomaterials research is to design implant surfaces which will enhance osseointegration in vivo. Several micro as well as nanoscale architectures have been shown to significantly affect the functionality of bone cell i.e. osteoblasts. In this work nanotubular titania surfaces fabricated by a simple anodization process were used as templates for culturing osteoblasts. The size of nanotubes can be controlled by varying the voltage and the time of anodization. Marrow stromal cells were isolated from rat and were seeded on nanotubular titania surfaces along with control surfaces. Cell adhesion, proliferation and viability were investigated for up to 7 days of initial culture culture. The cells were then provided with differentiation media to induce matrix production. The alkaline phosphatase activity and matrix production were quantified using a colorimetric assay and X-ray photoelectron spectroscopy (XPS) for up to 4 weeks of culture (3 weeks after providing differentiation media). Further, scanning electron microscopy (SEM) was used to investigate osteoblast morphology on these nanotubular surfaces.

2:20pm **BI+AS-ThA2 Nonlinear Optical Studies of Transmembrane Polypeptide Incorporation into Supported Bilayer Membranes**, ***D. Levy***, *K.A. Briggman,* National Institute of Standards and Technology

The structure and organization of proteins in biological membranes play critical roles in cellular functions related to recognition and signal transduction. Supported bilayer membranes (SBMs) have been developed as model membrane systems for the characterization of biomolecular interactions at cell surfaces. In the present study, the nonlinear interface-specific technique of vibrational sum-frequency spectroscopy (VSFS) is used to characterize the thermal phase transition for both single and binary component lipid layers in SBMs. The incorporation of alpha-helical transmembrane polypeptides into SBMs has also been characterized by VSFS and infrared spectroscopy to determine the insertion kinetics, structure and orientation of the polypeptides into the various SBMs.

2:40pm **BI+AS-ThA3 Analysis of In-Vitro Biomineralization Processes Quantitatively by Quartz Crystal Microbalance (QCM)- and Transmission Electron Microscopic (TEM)- Explorations**, ***U. Plate***, *Ch. Mentrup, H.J. Hoehling,* Universitaet Muenster, Germany

The primary crystallites of different developing hard tissues describe an apatitic structure with crystal lattice fluctuations representing an intermediate state between amorphous and fully crystalline. Some non-collagenous proteins (NCPs) bound immobilized at the surface of collagen type I are implicated in the initiation and regulation of crystal formation and growth. In the investigations we have induced synthetic biomineralization processes. Collagenous matrices were reconstituted in-vitro and Phosvitin, a Phosphoprotein from an eggshell, were used (concentration 6 mg/ml in 0,3M Na@sub 2@CO@sub 3@). Phosvitin was cross-linked to collagen type I fibrils (concentration 0,25 mg/ml in 0,05M HAc) with Divinylsulfon (DVS). Dynamic in-vitro biomineralization processes at this matrices, pure collagen and Collagen-DVS-Phosvitin, were induced by contacting their surfaces with defined inorganic Ca- and PO@sub 4@-solutions. Qualitative and quantitative measurements were achieved in an in-vitro model for Quartz Crystal Microbalance (QCM)- and Transmission Electron Microscopic (TEM)- explorations. To locate the organic matrix for QCM- and TEM- measurements statistically, the Au-surfaces on the quartz- and TEM-grids were functionalised with thiols containing chain-length carboxylic-acid groups, in the experiences 10 µmM carboxylic-acid thiols with a mercaptopropyl group. This carboxylic-acid groups form self assembling monolayers (SAMs) and are utilized for the modification of an Au-surface to introduce carboxylic groups on it. Then the carboxylic groups are converted to amines of biomaterials. To induce biomineralisation processes 2,2 mM CaCl@sub 2@- and 1,3 mM K@sub 2@HPO@sub 4@-solutions were pumped along the matrices in defined time intervals with a peristaltic pump. The experiments were carried out in a chamber under native conditions (T = 37°C, pump velocity of the Ca-phosphate solutions of 1,62 ml/min, comparable to the fluid flux of blood in capillaries.).

3:00pm **BI+AS-ThA4 Study of the Interfacial Water Structure on Sulfobetaine-Terminated Thiolate Self-Assembled Monolayers**, ***M.J. Stein***, *B.D. Ratner,* University of Washington

The foreign body response to prosthetic devices limits the extended use of virtually all medical implants and biosensors. Non-specific protein adsorption is believed to be a key determinant of this response. To circumvent or control these reactions, our initial study utilized a zwitterionic sulfobetaine thiol, structurally similar to taurine (HS(CH@sub 2@)@sub 11@N(CH@sub 3@)@sub 2@@super +@CH@sub 2@CH@sub 2@CH@sub 2@SO@sub 3@@super -@), and diluted it with hydrophobic and hydrophilic thiols to determine whether the nonfouling ability of the sulfobetaine self-assembled monolayers (SAMs) could be enhanced by either an improved packing of its bulky headgroup or through an increase in the internal hydrophilicity of the thiol monolayer. In our current study, we hypothesized that the diluted groups that were previously shown to be the most nonfouling would exhibit more structured water (~3200 cm@super -1@) versus free water (~3400 cm@super -1@)) . For this study, attenuated total reflectance (ATR) was utilized to characterize changes in the water peak signal intensity through a full time-series of dilutions at multiple temperatures. Initial results have shown that a trend is present that mirrors the earlier protein adsorption results and that this trend follows a time-dependant pattern.

3:20pm **BI+AS-ThA5 Multi-Technique Characterization of Lipid/PEG Interactions and Oligonucleotide Microarrays**, ***H.J. Griesser***, University of South Australia, Australia*; K. Vasilev, B. Thierry, K. Bremmell, S. Griesser, P.-C. Nguyen,* University of South Australia*; P. Hale, P. Pigram,* LaTrobe University, Australia **INVITED**

This contribution will discuss two recent studies utilizing multi-technique characterization of surfaces by both vacuum spectroscopic methods and in contact with aqueous solutions. The first study aimed to investigate why PEG graft surfaces have produced excellent protein resistance in vitro but disappointing outcomes in vivo. Our hypothesis was that a possible reason involves attractive interfacial interactions with lipids that then provide a platform for subsequent protein adsorption. Using three different proein-resistant PEG coatings it was indeed found that two of them gave measurable lipid adsorption. Using lipid molecules that were neutral, positively charged, or negatively charged, and aqueous media of various ionic strengths, we explored the possible role of electrostatic interactions. Interaction force measurements using the AFM colloid probe method showed purely repulsive steric forces on approach, but on retraction adhesive forces were observed in some cases. A key issue is to differentiate between interfacial forces that emanate from the substrate and 'shine through' the PEG graft layers, and forces associated with the PEG layer itself. The second study involves the fabrication of micro-patterned surfaces and their use for oligonucleotide and protein microarrays. Using a mask with circular holes we plasma polymerize arrays of dots consisting of thin layers of plasma polymers that carry reactive groups (aldehyde, amine, or epoxy) suitable for covalent immobilization of end-functionalized oligonucleotides or proteins. XPS imaging using a Kratos Ultra unit with a DLD clearly showed the arrays of dots on perfluoropolymer substrate and the immobilization of biomolecules. An IonTOF ToF-SIMS unit with a Bi3+ beam was used to analyze immobilized oligonucleotides. Clearly identifiable peaks were observed with masses up to 2,500 Da and higher, from fragments as large as containing five nucleotides. Different oligonucleotides could be distinguished by the distinct fragmentation patterns.

4:00pm **BI+AS-ThA7 Effects of Annealing and Sample Processing Methods on Surface Molecular Orientation of Ultra-high Molecular Weight Polyethylene**, ***S. Sambasivan***, *D.A. Fischer, M.C. Shen, J.A. Tesk, S.M. Hsu,* National Institute of Standards and Technology

Ultra-high molecular weight polyethylene (UHMWPE) has remained the dominant polymer in artificial joints due to its outstanding wear resistance properties. It has been have demonstrated in the past that the molding and annealing the ultra-high molecular weight polyethylene (UHMWPE) at a safe elevated temperature resulted in increased mechanical strength. Also, cross-linking of UHMWPE has been shown to reduce wear significantly. This novel study utilizes resonant absorption of linearly polarized soft x-rays at a synchrotron beamline to characterize the molecular orientation of the UHMWPE surface layer (top 10 nm) which is understood to be a precursor to wear. Carbon-K-edge x-ray absorption measurements were done on the UHMWPE samples, which were annealed in nitrogen atmosphere. Effects of annealing and cross-linking on the wear characteristics were also examined. It was found that the degree of orientation after annealing the

sample at 130°C in nitrogen, the average molecular orientation in UHMWPE decreased significantly (about 80% reduction) compared to the un-annealed UHMWPE. These studies show a promising new insight into how UHMWPE wears and will aid in the development of new materials for artificial joints. In addition to the annealing and cross-linking studies, it was observed that routine surface preparation methods such as molding, polishing and microtoming also induced surface molecular orientation to various degrees.

4:20pm **BI+AS-ThA8 NanoTribological Studies on the Mechanisms of O-Linked Glycosylated Proteins in the Boundary-Lubrication of Articular Cartilage**, *S. Zauscher, N.I. Abu-Lail,* ***D. Chang****, F. Guilak,* Duke University*; G. Jay,* Brown University

The diarthroidal (synovial) joints of the body enable locomotion and activity while withstanding millions of loading cycles, which may be several times body weight. Recent macroscopic tribological experiments and biochemical analyses suggest that heavily glycosylated proteoglycans, encoded by gene proteoglycan 4 (PRG4) and expressed by synoviocytes in synovial fluid as lubricin and by chondrocytes on the superficial zone of articular cartilage as surface zone protein (SZP), provide boundary lubrication in cartilage in the absence of interstitial fluid pressurization. We will present results from nanotribo-mechanical measurements on model surfaces and cartilage, combined with other surface specific physicochemical measurements that shed new light on the mechanisms by which lubricin/SZP provides lubrication and wear protection in diarthroidal joints. Our results suggest that the role of effective boundary lubricants in mediating friction in articular joints is largely one of wear protection of surface asperities, maintaining the surfaces in a nonadhesive mode, and causing shear dissipation in the biopolymeric boundary lubricant layer, even at the cost of attaining "high" coefficients of friction (COF ~ 0.15). Lubricin's ability to form intermolecular disulfide bonds appears to be critical for its ability to develop large steric repulsion forces. Our results contribute significantly to the understanding of the conformation and physico-chemical function of mucinous glycoproteins on biological interfaces.

Biomaterial Interfaces
Room 2014 - Session BI-FrM

Biomolecular Surface Characterization II

Moderator: P. Kingshott, The Interdisclinary Nanoscience Centre (iNANO), Denmark

8:00am **BI-FrM1 Probing Relations Between Molecular Orientation and Electron Transfer in Immobilized Metalloprotein Films Using Frequency-Domain, Planar Waveguide Spectroelectrochemistry**, ***S. Saavedra***, *Z. Oraci, A.F. Runge, W.J. Doherty, S.B. Mendes,* University of Arizona **INVITED**

This talk will describe efforts to develop a better understanding of the relationship between structure of a protein film immobilized on an electrode surface and its electrochemical activity, which is a prerequisite to design of protein-based molecular devices in which vectorial, heterogeneous electron transfer is required for efficient operation. The relationship between molecular orientation and electron transfer in immobilized films of redox-active proteins is being investigated using planar waveguide spectroelectrochemistry. This approach has been used to determine the porphyrin tilt angle distribution in a cytochrome c submonolayer adsorbed to an indium-tin oxide (ITO) electrode. However, only about half of the film is electroactive, which makes to difficult to correlate the broad orientation distribution (measured spectroscopically on the entire film) with the electron transfer rate (measured electrochemically on the electroactive portion of the film). To address this problem, a novel form of electroreflectance spectroscopy, potential-modulated, attenuated total reflection spectroscopy (PM-ATR), has been developed. In PM-ATR, the waveguide output is monitored while an ac potential modulation is simultaneously applied to the planar waveguide electrode. Changes in the absorbance as a function of the light polarization, modulation frequency, and amplitude provide information about electron transfer rates, electro-optical switching rates, and molecular orientation. For cytochrome c films on ITO, the electron transfer rate measured using TM polarized light was four-fold greater than that measured using TE polarized light, which is consistent with a shorter tunneling distance for molecules adsorbed in a vertical orientation (probed with TM) vs. molecules adsorbed in a horizontal orientation (probed with TE). These data are the first to correlate a distribution of molecular orientations with a distribution of electron transfer rates in a redox-active molecular film.

8:40am **BI-FrM3 Volumetric Interpretation of Protein Adsorption: Partition Coefficients, Interphase Volumes, and Free Energies of Adsorption to Hydrophobic Surfaces**, *E. Vogler,* ***H. Noh***, Penn State University

Interpretive mass-balance equations were derived from a model premised on the idea that protein reversibly partitions from bulk solution into a three-dimensional (3D) interphase volume separating the physical adsorbent surface from bulk solution. Theory was shown to both anticipate and accommodate adsorption of all proteins to the two test surfaces, suggesting that the underlying model was descriptive of the essential physical chemistry of protein adsorption. Application of mass balance equations to experimental data permitted quantification of partition coefficients P, interphase volumes V,@footnote 1@ and the number of hypothetical layers M occupied by protein adsorbed within V.@footnote 2@ Partition coefficients measure the equilibrium ratio of interphase and bulk-solution-phase w/v (mg/mL) concentrations W@footnote 3@ and W,@footnote 4@ respectively, such that P-W@footnote 5@/W.@footnote 6@ Proteins studied were found to be weak biosurfactants with 45< P <520 and commensurately low apparent free-energy-of-adsorption, (-6RT<@footnote 7@G@footnote 8@=-RTlnP<-4RT). These measurements corroborate independent estimates obtained from interfacial energetics of adsorption (tensiometry) and are in agreement with thermochemical measurements for related proteins by hydrophobic-interaction chromatography. Proteins with molecular weight MW<100kDa were found to occupy a single layer at surface saturation whereas the larger proteins IgG and Fib required two layers. @FootnoteText@ @footnote 1@sub I@footnote 2@sub I@footnote 3@sub I@footnote 4@sub B@footnote 5@sub I@footnote 6@sub B@footnote 7@DELTA@footnote 8@sub ads.

9:00am **BI-FrM4 Adsorption-Induced Changes in Protein Bioactivity Correlated with Adsorbed Protein Orientation and Conformation**, ***K.P. Fears***, *R.A. Latour,* Clemson University

It is well accepted that an irreversibly bound adsorbed protein layer forms on biomaterial surfaces very rapidly after they come in contact with bodily fluids. The structure and bioactivity of this adsorbed protein layer are recognized to be critical factors that influence subsequent cellular responses; however, little is known about the actual molecular mechanisms involved. The bioactivity of an adsorbed protein could be inhibited due to adsorption-induced conformational changes in the proteinâ?Ts structure or due to orientational effects that result from the active site being sterically blocked by the surface. A set of experimental methods have been developed using alkanethiol self-assembled monolayers (SAMs), with different surface chemistries, along with surface plasmon resonance (SPR) spectroscopy to measure the adsorption-induced changes in protein bioactivity using enzymes with well known molecular structures. The secondary structure of the adsorbed protein layers was determined using circular dichroism (CD) and compared to the native structure of the proteins. Molecular models of the proteins showing the location of hydrophobic and charged residues, as well as the active site residues, were analyzed along with the data collected on adsorbed protein bioactivity and secondary structure to identify the most likely cause for the measured changes in bioactivity as a function of surface chemistry.

9:20am **BI-FrM5 SIMS Detection of Peptides on Alkanethiol Self-Assembled Monolayers**, ***Z. Zhu***, *O. Hadjar, P. Wang, J. Laskin,* Pacific Northwest National Laboratory

Alkanethiol self-assembled monolayers (SAM) on Au substrate provide a very ordered and controllable surface which is friendly to bio-molecules. Numerous protein or DNA molecules have been immobilized on alkanethiol SAMs to fabricate bio-active surfaces which can be used for bio-analysis or some other applications. A number of techniques, such as fluorescence, X-ray photoelectron spectroscopy (XPS), reflection IR, surface plasmon resonance, have been applied to detect these bio-active molecules immobilized on alkanethiol SAM surfaces. During the last several years, secondary ion mass spectrometry (SIMS) detection of bio-active molecules has been of great interest, and a number of efforts have been reported. Although several papers have addressed on SIMS detection of bio-active molecules on alkanethiol SAM surfaces, the detection limit of this technique is not clear. Since static SIMS is a very surface-specific technique, sample preparation plays a very important role in such research. In this work, the electrospray technique was used to prepare samples due to its effectiveness, easy control and simple operation. Sub-monolayer of three peptides (Bradykinin, MW=1060.5; Gramicidin S, MW=1141.6; Substance P, MW=1347.7) were prepared on three alkanethiol SAMs (-S(CH2)11CH3, -S(CH2)10CO2H, -S(CH2)2(CF2)9CF3) and detection limits were tested. 15 keV Ga+ ions were used as the primary ion source. It has been found that the detection limit of peptide molecules can be as low as 0.0001 monolayer or even lower. Our results show that SIMS is a very promising technique to characterize low-mass protein molecules on alkanethiol SAM surfaces.

9:40am **BI-FrM6 Temperature Stability of Protein Monolayers Studied by Ellipsometry in the Infrared, Visible and Ultraviolet Spectral Regions**, ***H. Arwin***, Linköping University, Sweden*; D.W. Thompson, J.A. Woollam,* University of Nebraska, Lincoln

Future devices based on bionanotechnology may contain protein layers and temperature stability will be an issue. One way to monitor temperature induced structural changes in protein molecules adsorbed in monolayers on solid substrates is to analyze the complex-valued refractive index N=n+ik as determined by spectroscopic ellipsometry. In the infrared spectral (IR) region ellipsometry provides a quantification of the amide band parameters of the adsorbed protein molecules and in the visible (VIS) and ultraviolet (UV) regions the layer thickness (surface mass density) can be determined as well as the electronic contributions to N. Earlier studies on protein multilayers (human serum albumin and its antibody) show that heating above 100 °C causes structural changes observed as changes of the amide bands. Heating to 200 °C causes layer degradation seen as irreversible changes in n and k and also a substantial decrease in surface mass density. The objective is here to present methodology and results from pilot studies of effects of heating monolayers of proteins adsorbed on gold substrates. The surface mass density and N=n+ik are determined with spectroscopic ellipsometry (UV-VIS-IR) equipped with a heat stage (20 - 300 °C). A model refractive index function is applied to the optical properties of the protein layer and changes in the model parameters are monitored at elevated temperatures with special emphasis on the amide I band around 1640 cm@super -1@, the amide II band around 1520 cm@super -1@ and the amide A band in the 2800 - 3300 cm@super -1@ region.

10:00am **BI-FrM7 Fibronectin Adsorption onto Tantalum-Oxide Examined by QCM-D and Elipsometry: The Influence of Nano-Roughness**, ***M.B. Hovgaard,*** University of Aarhus, Denmark, DK*; K. Rechendorff, F. Besenbacher, M. Foss,* University of Aarhus, Denmark

The adsorption of fibronectin onto tantalum-oxide is investigated probing the effects of a nano-rough, stochastic morphology on the structure and functionality of an adsorbed protein layer. Nano-rough substrates were produced by e-gun evaporation of tantalum onto standard QCM-D substrates at oblique incidence deposition, allowing the production of self-affine surfaces@footnote 1@ with ~ 4 nm RMS roughness as characterized by AFM. A comparative study of the protein adsorption onto flat versus nano-rough substrates was conducted using Ellipsometry and QCM-D. A characterization of the adsorbed layers was facilitated by variation of the protein concentration and monitoring of the specific versus non-specific antibody adsorption along with utilizing the different sensitivity of the two techniques.This including a direct comparison between the dry(Ellipsometry) and hydrated(QCM-D) surface densities further extended by the viscoelastic properties(QCM-D) of the adsorbed layers. Results show a significant influence of the nano-roughness on saturation coverage and the viscoelastic properties of the resulting protein layer. Compared to the flat morphology, the saturated protein films on the rough substrates show higher surface densities (area-effect) and non-trivial effects such as a more rigid packing (lower QCM-D dissipation) and decreased specific antibody adsorption, both indicating a changed protein configuration. @FootnoteText@ @footnote 1@ K. Rechendorff, M.B.Hovgaard, M.Foss, F.Besenbacher Appl. Phys. Lett. 87, 073105 (2005).

10:20am **BI-FrM8 Electrodeposition of Chitosan for BioMEMS: Real-Time, In-Vitro, and Post-Process Characterization**, ***S.L. Beatty****, E.C. Dreyer, X. Luo, G. Rubloff,* University of Maryland

Chitosan has been shown to serve as a robust and reproducible scaffold for biological functionalization in microfluidic channels, using chitosan electrodeposition at specific bioMEMS sites. To better understand the materials and process science of chitosan electrodeposition onto metal electrodes, we have used both in vitro characterization techniques and post-deposition measurements of air-dried films. Real-time current, voltage, and optical reflectivity show film growth nonlinearities expected for electrical vs. optical property sensing. Raman spectroscopy of films air-dried after electrodeposition reveals the presence of primary amine groups active in biofunctionalization. AFM images of the air-dried films reveal variable and rough morphology not directly correlated to deposition conditions, while hydration increased surface homogeneity. Fluorescence microscopy using fluorescently decorated chitosan shows both film growth and its spatial distribution across the deposition electrode, enabling a comparison of in-vitro and dry morphology of the chitosan film. While the in-vitro images show fairly smooth distribution of chitosan, the air-dried films are much rougher, indicating nonuniform and unpredictable collapse of the film's structure during drying. Thus, in vitro measurements of the film deposition and structure are essential to exploit the potential of chitosan as a platform for biotechnology applications.

10:40am **BI-FrM9 Biomolecule Adsorption Studies at Interfaces using Sum Frequency Generation Vibrational Spectroscopy, Quartz Crystal Microbalance, Dielectric Spectroscopy, and Conducting-Probe Atomic Force Microscopy**, ***O. Mermut****, R.L. York, D.C. Phillips, J.Y. Park,* UC, Berkeley and Lawrence Berkeley National Lab*; K.R. McCrea, R.S. Ward,* Polymer Technology Group*; G.A. Somorjai,* UC, Berkeley and Lawrence Berkeley National Lab

A combination of different surface-specific techniques (probing macroscopic and molecular-scale properties) was employed to examine the adsorption behavior of biomolecules onto surfaces of variable chemistry and hydrophobicity. Various short-chain model peptides, composed to two types of amino acids (hydrophilic and hydrophobic) were synthesized to investigate the effect of the amino acid side-chain chemistry on adsorption at the water/polystyrene and the water/silica interface. Specifically, these peptides contain hydrophobic (X) and charged (Y) amino acids with sequence: Ac-XYYXXYXXYYXXYX-NH@sub 2@ (designed to yield an alpha-helical peptide) or Ac-XYXYXYX-NH@sub 2@ (designed to yield a beta-strand peptide). Sum Frequency Generation (SFG), a non-linear optical spectroscopic tool, provided molecular-level information regarding the orientation of the adsorbed biomolecules. Specifically, the hydrophobic amino acid residues preferentially order on hydrophobic polystyrene/water interfaces. This ordering is strongly dependent on chain length and sequence. Quartz Crystal Microbalance (QCM) allowed quantitative in situ determination of the adsorbed mass of material, which is influenced by the surface hydrophobicity. Impedance measurements were obtained using Dielectric Spectroscopy to investigate perturbations of the electrical double layer in the presence of adsorbed biomaterial. Lastly, we measured the morphological properties (topography, aggregation), mechanical properties (adhesion and friction force), and electrical transport properties (conductance) of adsorbed peptide at these various liquid-solid interfaces using a Conducting Probe-Atomic Force Microscopy (CP-AFM). Using this combinatorial approach of techniques, we will discuss how molecular level information correlates with macromolecular properties.

11:00am **BI-FrM10 Low-Fouling Amine-Terminated Poly(ethylene Glycol) Thin Layers and Effect of Immobilization Conditions on Their Mechanical and Physicochemical Properties**, ***Y. Martin****, P. Vermette,* Université de Sherbrooke, Canada

The physicochemical and mechanical properties of amine-terminated covalently-bound poly(ethylene glycol) (PEG) layers are dependent on fabrication methods. Particularly, the use of a theta solvent yields dense bound PEG layers with properties not well described by traditional models. The polymer concentration is also known to be important for the layer properties. In this study, NHS-PEG-tBoc molecules of molecular weight 3,400 were immobilized on plasma-generated primary amine-containing surfaces at different concentrations and using different solvents including theta solvents. Light diffraction techniques were used in an attempt to understand the influence of polymer aggregation kinetics in a theta solvent on the final properties of the fabricated PEG layers. The polymer layer properties were characterized using X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM) in force mode, quartz crystal microbalance (QCM) and fluorescence microscopy. Results show that polymer concentration in solution is an important indicator of final layer properties, and that the use of a theta solvent induces complex aggregation phenomena in solution, dependant on PEG concentration, yielding layers with unique properties such as greatly variable low-fouling potential or water trapping attributes. The PEG layers fabricated through the process described in this article are also shown to be chemically reactive, paving the way for the immobilization of bio-active molecules.

11:20am **BI-FrM11 Single Molecule Force Spectroscopy on 5'-Methyl thioadenosine/S-Adenosylhomocysteine Nucleosidase (MTAN) from Escherichia Coli by Atomic Force Microscopy**, ***B.I. Kim****, J.O. Holmes, K.A. Cornell,* Boise State University*; P. Deschatelets,* Potentia Pharmaceuticals Inc.

5'-Methyl Thioadenosine/S-Adenosylhomocysteine Nucleosidase (MTAN) is a dual substrate specific enzyme that catabolizes 5'Methyl thioadenosine (MTA) or S-Adenosylhomocysteine (SAH) to form adenine and 5'-methylthioribose or S-ribosylhomocysteine, respectively, in many pathogenic microbes. MTAN is an ideal target for new antibiotic development because it has no corresponding human equivalent and recognizes a substrate that is not present in mammalian cells. Based on this interest, the binding mechanisms of various transition state analogues that inhibit the expression of MTAN were studied using the single molecule force-spectroscopy technique. Force-distance curves were measured as a function of separation distance between a probing molecule and the MTAN molecule under buffer conditions using atomic force microscopy (AFM). Various probing molecules were covalently linked via a flexible spacer polyethylene glycol (PEG) to the tip of an AFM. The force-distance curve exhibits an unbinding event in the retracting curve with a certain unbinding force. Single molecular binding strength, rate constant, and structural data for the binding pocket were extracted from several hundred force-distance curves and were analyzed statistically for each transition state analogue. The statistical values were compared with the equilibrium dissociation constants previously obtained by other groups. Single molecule force spectroscopy provides a new insight into the specific binding mechanism between the inhibitors and a MTAN molecule at the single molecular level.

11:40am **BI-FrM12 Single-Molecule Force Spectroscopy of Stimulus-Responsive Polypeptides**, ***S. Zauscher****, A. Valiaev, A. Chilkoti, T. Oas,* Duke University

Stimulus-responsive elastin-like polypeptides (ELPs) experience a large entropic collapse when exposed to an environmental stimulus, such as an increase in temperature. While interfacial applications of ELPs have been prototypically demonstrated, a systematic investigation of the phase transition behavior at the solid-liquid interface and on the single-molecule level is lacking. We will present results from single molecule force-spectroscopy (SMFS) measurements probing the force-extension and conformational behavior of ELPs, below and above their transition temperature. We show that ELPs are well described by a random coil polymer model, suggesting the absence of significant secondary structure.

Furthermore, we show that single molecule force spectroscopy is able to differentiate different ELP constructs by distinguishing differences in the hydrophobic hydration of side chains. This suggests that SMFS has potential diagnostic abilities for studying the hydration behavior of proteins. We also noticed that some ELP force-extension curves showed temperature independent deviations that could not be described by polymer elasticity models developed for random polymers. We argue that the observed deviations arise from the force-induced cis-trans isomerization of prolines, which are repeated every fifth residue in the main chain of ELPs. We present evidence for this mechanism by Monte Carlo simulations of the force-extension curves using an elastically coupled two-level system. Furthermore, we show results from control experiments with poly-L-proline that demonstrate the similarity of the conformational transition between poly-L-proline and ELPs. We believe that our work is the first demonstration of force induced cis-trans isomerization in proline containing polypeptides. Our results suggest that SMFS could be used to assay proline cis-trans isomerization in proteins and may thus have significant potential diagnostic utility.

Author Index

Bold page numbers indicate presenter

Author Index

Author Index