Biomaterial Interfaces
Room Exhibit Hall C&D - Session BI-MoP

Biomaterial Interfaces Poster Session

BI-MoP4 Pulsed rf Plasma Polymer Modification of Microfluidic Devices, ***Z. Segu***, Southern Illinois University; *R.B. Timmons,* University of Texas at Arlington; *G.R. Kinsel,* Southern Illinois University

There is a growing interest in the use of miniaturized systems - so-called "lab-on-a-chip" devices - for various analytical applications due to reduced solvent/reagent/sample consumption, shortened analysis time and the applicability of these devices to process / field analysis. One performance requirement for these devices often involves the ability to separate sometimes complex mixtures of analytes prior to detection. In conventional analytical instruments the separation step is most often achieved via gas or liquid chromatography using columns having a broad diversity of stationary phase chemistries. Introduction of similar diversity of chemistries into microfluidic devices can offer similar capabilities for complex analyte mixture separation while retaining the unique capabilities of the miniaturized system. In this research we explore the use of pulsed RF plasma polymer deposition for coating of channels in microfluidic devices. This approach to channel modification is attractive due to the conformal, sterile, pinhole-free, surface coverage of plasma polymer films and the wide variety of surface chemistries and functional group densities that can be achieved using RF plasma polymer deposition. In these initial studies RF plasma deposited microchannel polymer film coatings are investigated as a function of reactor power, monomer flow, monomer pressure, and positioning of the sample in the plasma reactor chamber. Resultant films are characterized by ellipsometry, SEM, FT-IR and XPS to determine various film properties including, film thickness, film uniformity and chemical functionality. These studies demonstrate the pulsed RF plasma polymer deposition can offer an effective means to incorporate a wide variety of chemical functionalities into microfluidic devices.

BI-MoP5 Pulsed RF Plasma-Modified Surfaces for On-Probe Fractionation and MALDI Mass Spectrometric Characterization of Bacterial Proteins, ***G.S. Fernando***, Southern Illinois University Carbondale (SIUC); *L.G. van Waasbergen, R.B. Timmons,* University of Texas at Arlington; *G.R. Kinsel,* Southern Illinois University Carbondale (SIUC)

Mass spectrometric characterization of bacteria is of growing importance, not only for applications in basic research but also as a means for rapid, unambiguous identification of bacterial pathogens. In this study, crude protein mixtures from cyanobacteria Synechocystis sp. strain PCC 6803 are characterized by Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) following fractionation directly on the surface of pulsed RF plasma polymer modified On-Probe Affinity Capture (OPAC) MALDI probes. Pulsed RF plasma polymer deposition allows the incorporation of a wide diversity of surface chemistries and functional group densities directly on the surface of the OPAC MALDI probe, which can be subsequently used to selectively capture subpopulations of a complex protein mixture - for example, as derived from a bacterial source. OPAC protein mixture fractionation can be based on broad chemical properties (e.g. hydrophobic / hydrophilic, acid / base) or on highly bioselective interactions (e.g. metal binding properties, protein-substrate interactions). With careful optimization of the protein mixture deposition and washing procedures, fingerprint MALDI mass spectra of the bacteria proteome can be recorded which contain unique peptide and protein signature ions, not observed in the MALDI mass spectrum of the crude bacterial protein extract. The simplicity, speed and high sensitivity of the OPAC MALDI approach makes it an attractive option for bacterial proteome characterization.

BI-MoP6 Micropatterned Surface Modification of Polydimethylsiloxane via UV-Initiated Graft Polymerization of Acrylates, ***N. Patrito***, *S. Chiang, P.R. Norton,* University of Western Ontario, Canada; *N.O. Petersen,* National Institute for Nanotechnology, Canada

Polydimethylsiloxane (PDMS) is a transparent, elastic polymer that is becoming an increasingly popular substrate for the fabrication of microfluidic devices. The widespread application of PDMS-based microfluidic devices to bioanalytical research has, however, been limited by the material's extreme hydrophobicity and surface inactivity. A desire to improve the wettability and biocompatibility of PDMS has resulted in a large body of research into the surface modification of siloxane polymers. One promising method for the permanent modification of PDMS is the covalent linkage of hydrophilic polymers on its surface via UV-initiated graft polymerization (UV-GP). Combining UV-GP with photolithographic techniques, poly(acrylic acid) and poly(methacrylic acid) patterns are successfully grafted onto PDMS thin films with micron-scale fidelity. Contact angle measurements, AFM imaging, surface roughness analyses, and XPS spectra confirm the presence of the grafted layers and provide insights into their morphology and surface coverage. This report also examines the effects of the graft materials on the adhesion and proliferation of common experimental cell lines, CV-1 and A-431. AFM images illustrate the improved attachment and growth of both cell types on the PAA and PMAA patterned substrates. These observations confirm the utility of UV-GP as a means of improving PDMS biocompatibility. They also demonstrate the amenability of the UV-GP technique to precise patterning, providing researchers with an effective, efficient means of localizing bio-adhesion on a variety of substrates.

BI-MoP7 Formation of Highly Oriented Hydroxyapatite Coating by rf Thermal Plasma Spraying, ***M. Inagaki***, *Y. Yokogawa, T. Kameyama,* National Institute of Advanced Industrial Science and Technology (AIST), Japan

Hydroxyapatite (Ca@sub 10@[PO@sub 4@] @sub 6@ [OH] @sub 2@; HA) has been used for medical applications to promote the osteoconductivity of implanted materials.@footnote 1@ The HA crystal has hexagonal structure with space group of P63/m and has anisotropic properties of matter with respect to the crystallographic axis due to crystal structure of itself. Moreover, the HA crystal has two major surfaces i.e. (100) surface (a-surface) and (001) surface (c-surface) with different properties, such as protein absorption@footnote 2@ and dissolution behavior.@footnote 3@ In this study, highly oriented hydroxyapatite (HA) coatings were successfully obtained on titanium (Ti) substrates through a radio-frequency thermal plasma spraying method. XRD patterns showed that the HA coating layer had an apatite structure with (00l) preferred orientation vertical to the coating's surface. TEM observation showed that 200-800 nm-width prismatic crystals were formed in HA splats and the longitudinal axis of such prismatic crystals oriented vertical to the coating's surface. TEM images also indicate that the interface between prismatic crystals became compacted. SAD pattern show that the longitudinal axis of prismatic crystals corresponds to the (001) axis of HA. Protein absorption behavior of such a crystal oriented surface was also studied. @FootnoteText@ @footnote 1@ L. L. Hench, J. Am. Ceram. Soc., 81, (1998) 1705.@footnote 2@ T. Kawasaki, J. Chromatogr., 544, (1991) 147.@footnote 3@ H. Aoki, Surface Science, 10, (1989) 96.

BI-MoP8 Nanoscale Adhesion, Friction and Wear Studies of Biomolecules on Silicon Based Surfaces, ***D.R. Tokachichu***, *B. Bhushan, M.T. Keener, S.C. Lee,* The Ohio State University

Protein layers are deployed over the surfaces of synthetic microdevices like bioimplants and bioMEMS to facilitate biocompatibility with biological tissue. When a biosensor comes in contact with any exterior environment like tissues, or fluids with a variable pH, the biomolecules on the sensor surface may get abraded due to a change in the adhesion between the biomolecules and microdevice surface. Friction and wear properties of biomolecules (e.g. proteins) on silicon based surfaces are important because these devices come across wear and friction when they are introduced into these environments. Changes in adhesion have been studied between streptavidin and a thermally grown silica substrate in phosphate buffered saline (PBS) solution with various pH values as a function of concentration of immobilized biomolecules in solution. Wear and friction properties of streptavidin (protein) biomolecules coated on silica by direct physical adsorption and chemical linker method were studied in PBS using tapping mode AFM at a range of free amplitude voltages.

BI-MoP9 Grafting of PEG-Macromonomers to Plasma Polymers Using Ceric Ion Initiation, ***N.J. Vickers***, University of Sheffield, UK, United Kingdom; *A.G. Shard, S. Mac Neil,* University of Sheffield, UK

Bioadhesion, the adsorption of proteins, cells, or bacteria to a surface can be extremely detrimental to the performance of medical devices. Prevention of non-specific adsorption is therefore a key characteristic for many biomaterial applications and applying a non-fouling surface treatment can improve the performance of some medical devices. Poly(ethylene glycol) [PEG] is currently the most effective chemical modifier at reducing bioadhesion. Plasma polymers provide a thin, conformal base on which to graft. It is proposed that grafting PEG onto plasma polymers will confer non-fouling properties. Ceric ion initiation is commonly employed to graft polymers to natural polysaccharides e.g.

starch. The initiation is thought to proceed through oxidation of hydroxyl groups. We have investigated ceric ion initiated grafting of PEG-macromonomers to several plasma polymers. Radio frequency glow discharge plasma polymerisation of isopropanol was carried out to obtain functional surfaces containing alcohol groups. Octadiene plasma polymer surfaces were made as a control hydrocarbon. Aqueous solutions of PEG-macromonomers and cerium ammonium nitrate were combined and plasma polymer samples introduced. Characterisation was carried out via X-ray photoelectron spectroscopy [XPS]. The influence of reagent concentration, chemical nature of the plasma polymer and grafting time were investigated. The success of ceric initiated grafting was demonstrated by the presence of a chemically shifted peak in the C1s narrow scan at 286.5eV binding energy. The intensity of this peak can be directly correlated with the amount of grafted material. Both time of reaction and concentration of initator have been shown to influence the polymer graft density, whilst the plasma polymer chemistry is of paramount importance. Currently work is underway to investigate the non-fouling properties of the PEG-grafted surfaces using protein adsorption methods.

BI-MoP10 SAM-Modification of Biomaterial Surfaces as an Antimicrobial Therapy, ***R.J. Emerson***, *E.R. Soto-Villatoro, W.G. McGimpsey, T.A. Camesano,* Worcester Polytechnic Institute

Microbial infections of medical implants occur in more than 2 million surgical cases each year in the United States, increasing patient morbidity, mortality, cost and recovery time. While many methods exist to treat these infections, surgical excision of the infected device is the only certain cure. Clinically, it is of interest to determine the factors affecting microbial adhesion, the precursor to infection, and to formulate adhesion-resistant materials that are effective over protracted time periods. Self-assembled monolayers (SAMs) with a variety of terminal groups were developed and screened for antimicrobial activity. SAM molecules were bound to the surface using non-covalent metal-ligand bonds. The terminal groups of the surfaces included alkanethiols (C11 and C12), isophthalic acid (IPA), silver-containing isophthalic acid (IAG), bovine serum albumin (BSA), and tri-ethylene glycol (TEG). This methodology represents an advantage over physisorbed coatings and antimicrobial-impregnated implants, which may only prevent infection for short time periods, if at all. To screen the surfaces, single, viable bacterial cells (Staphylococcus epidermidis, Clinical isolates) were chemisorbed to the silicon cantilever of an atomic force microscope (AFM) probe, and brought into contact with the SAM surfaces. Force profiles were measured for approach and retraction interactions. IPA and IAG coatings showed promising results, since they demonstrated the lowest adhesion forces for the S. epidermidis probe.

BI-MoP11 The Effect of Solid Surface Tension on Biofilm Adhesion, ***R.A. Brizzolara***, *R.M. Lennen,* NSWC, Carderock Division

The effect of material properties on the biofouling of a surface is of great practical importance. Biofouling is a chronic and costly problem in the maritime industry as well as being a significant issue for the medical community. This study investigates the effect of solid surface tension on microbial biofilm adhesion and accumulation. The experimental approach was designed to isolate the effects of solid surface tension on the biofilm from the effects of other material properties such as elastic modulus and surface topography, as well as to isolate biofilm accumulation from biofilm adhesion. Covalently bound monolayers of organosilanes were prepared on native titanium oxide surfaces. The solid surface tension was varied through the choice of the terminal group, using hexadecyltrichlorosilane, 1H,1H,2H,2H-perfluorooctyltrichlorosilane and 3-chloropropyltrichlorosilane precursors. This resulted in surfaces with a wide range of solid surface tensions, while retaining the original topography and modulus. Monolayer deposition was verified using contact angle/solid surface tension measurements and x-ray photoelectron spectroscopy (XPS). Biofilm accumulation and adhesion measurements were performed by growing Pseudomonas fluorescens biofilms under gentle agitation (~120 RPM shaker) and performing a Bradford protein assay before and after exposing the coupons to hydrodynamic shear stresses of 3.7 N m-1 in a turbulent flow cell. It is expected that the results of this study will assist in the design of advanced coatings and surfaces by defining the optimum solid surface tension for reduced biofouling. This work was funded by the NSWC, Carderock Division In-House Laboratory Independent Research (ILIR) program.

BI-MoP13 Controlled Release of Calixarenes from Chitosan Hydrogel Coated Polymeric Surfaces as Antimicrobial Treatment of Staphylococcal Infections, *M. Vinante, C. Pederzolli, L. Pasquardini, L. Lunelli,* ***R. Canteri***, *M. Anderle,* ITC-irst, Italy*; C. Potrich, G. Viero, M. Dalla Serra,* CNR ITC, Italy*; G. Prevost, O. Joubert,* Institut de Bactériologie de la Faculté de Médecine, France

The proliferation of pathogenic microorganisms on biomaterial surfaces is one of the most widespread causes of failure of biomedical devices such as catheters, medical implants, vascular graft and joint prostheses. Staphylococcus species (e.g. aureus and epidermidis) are one of the major pathogens isolated in hospitals and are responsible for numerous nosocomial infections. Common virulence factors of these pathogenic staphylococcal strains are a group of secreted leucotoxins, which belong to the family of @beta@-barrel pore-forming toxins. They form poorly selective holes into the membrane of attacked cells, causing their lysis. We developed a local drug delivery system composed of chitosan hydrogel deposited on polymeric surfaces and loaded with hydrophilic agents, calixarenes. These molecules have been demonstrated to have an inhibitory effect on leucotoxins during pore-formation (reduction of 50% of hemolytic activity of 12 nM HlgA/HlgB on HRBC using 6 µmM of 4-sulfonic-calix(6)arene). Two different strategies of hydrogel formation are evaluated, one based upon ionic interactions between positively charged groups of chitosan chains and negative charges of calixarene molecules; the latter utilizing an homofunctional derivative polyethylene glycole as cross-linking agent (e.g. ButyrALD-PEG-ButyrALD) in order to form a permanent network with enhanced mechanical properties. These two kinds of interactions were also employed to stabilize the hydrogel film to the substrate. Data on the physical-chemical and morphological properties of the chitosan deposited hydrogel and the kinetics of calixarene release will be presented. @FootnoteText@ @footnote 1@Work supported by the P.A.T. Trento- Italy- Fondo Unico (project StaWars).

BI-MoP14 The Effect of Covalent Tethering on the Function of a Quaternary Ammonium Antimicrobial Compound, *R.A. Brizzolara,* ***D.M. Stamper***, *R.M. Lennen,* NSWC, Carderock Division

In the development of antimicrobial coatings and materials, biocidal molecules can be incorporated into a coating either as free molecules, or by covalently tethering the molecules to the coating or surface. Covalently tethering a biocidal moiety to a coating or surface has the benefit of reducing leaching of the biocide into the environment. However, the effects of covalent tethering on the biocidal activity of the molecule need to be more fully characterized. This paper reports on experiments to determine the effects of surface-immobilization on the activity of an antimicrobial molecule. Monolayers of a covalently bound organosilane containing a quaternary ammonium functional group (QAS) were used as the test platform. QAS monolayers were bound to silica surfaces and characterized by x-ray photoelectron spectroscopy (XPS). Viability of Staphylococcus aureus exposed to QAS-derivatized surfaces was measured by dilution plate counting. Control substrates derivitized with an amino-terminated silane were also included in the analysis. Flat surfaces derivatized with QAS did not possess antimicrobial activity. Results will be presented that differentiate between 1. loss of biocidal activity as a result of covalent tethering of QAS molecules versus 2. insufficient dose of antimicrobial molecules per S. aureus cell, as the cause of lack of antimicrobial activity of QAS-derivatized surfaces. It is expected this work will contribute to the development of materials and coatings with inherent antimicrobial properties and to reduced use of decontaminating solutions containing toxic chemicals. This work was funded by the NSWC, Carderock Division In-House Laboratory Independent Research (ILIR) program.

BI-MoP15 Protein Adsorption on Poly(Ethylene Glycol)-Modified Surfaces under Flow Conditions in Microfluidics Systems, ***C.J. Chun***, *K. Lenghaus,* University of Central Florida*; D.C. Henry,* Clemson University*; L. Riedel, A. Bhalkikar, J.J. Hickman,* University of Central Florida

In the last decade microfabrication technology has been used to create new microfluidics systems, bioanalytical and medical devices. The handling of relatively small amounts of analytes, at significantly lower concentrations, combined with the fact that the surface-to-volume ratio increases in direct proportion to the device size decreasing, could create potential problems in device utilization. The problem being that, the analytes or target molecules may be completely non-specifically adsorbed on the surfaces of the microdevices before they reach the detector. Thus, the basic understanding of an adsorption behavior of biomolecules@footnote 1@ on the surfaces of these systems is critical for their use in microfluidics as well as bioanalytical devices. To investigate the situation we have developed assays@footnote 2@ to evaluate protein

adsorption under flow and static conditions at submonolayer coverages on poly(ethylene glycol) (PEG)-modified surfaces, which are well known to resist protein adsorption.@footnote 3@ In this study, protein adsorption onto PEG-modified microcapillary surfaces, under flow conditions, has been determined at different flow rates as well as various protein concentrations. Alkaline phosphatase and horseradish peroxidase were used to evaluate proteins adsorption behavior, which although low, was still significant. The flow rate was also seen to affect the protein adsorption on the PEG-modified-surfaces. Simulation studies of the protein adsorption behavior are also being used to help in the development of new biocompatible microfluidics systems.@footnote 4@. @FootnoteText@ @footnote 1@K. Nakanishi, T. Sakiyama, and K. Imamura, J.Biosci.Bioeng., 91, 233 (2001). @footnote 2@K. Lenghaus, J.W. Dale, J.C. Henderson, D.C. Henry, E.R. Loghin, and J.J. Hickman, Langmuir, 19, 5971 (2003). @footnote 3@M. Zhang, T. Desai, and M. Ferrari, Biomaterials, 19, 953 (1998). @footnote 4@J. Jenkins, B. Prabhakarpandian, K. Lenghaus, J.J. Hickman, and S. Sundaram, Anal.Biochem., 331, 207 (2004).

BI-MoP16 Materials Characterization for Blood-Flow Dynamics and Platelet-Adhesion Simulation of Hematocompatible Plasma-Polymerized Tetraglyme Surfaces, ***E. Hanley***, *J.L. Shohet, J.L. Lauer, R.M. Albrecht, S. Esnault, J.S. Malter, R.H. Blick, H.S. Kim,* University of Wisconsin-Madison*; U. von Andrian,* Harvard Medical School*; S.B. Shohet,* University of California, San Francisco

The realization of small-scale biomedical devices will be closely related to the non-fouling/biocompatible properties of the exposed surfaces and the uniformity of the surface treatment throughout the device. Thrombus formation and embolization are significant problems for blood-contacting biomedical devices which often begin with platelet adhesion. In this work, we explore plasma polymerization (PP) to improve the hematocompatibility of silicon-based surfaces and the process conditions necessary to develop a uniform PP coating on the lumenal surface of artificial blood vessels. To minimize these effects, plasma-polymerized tetraglyme was deposited on flat Si@sub 3@N@sub 4@ and SiO@sub 2@ samples to produce a PEO-like surface coating. The dynamics of platelets can be modeled using a numerical simulation of adhesive particles interacting with an adhesive surface.@footnote 1@ Experimentally, emitted light from the plasma during the PP process was fed into a monochromator. Coating thickness and chemical composition of the surfaces was measured using ellipsometry and XPS, respectively. Contact-angle measurements were carried out on the PP surfaces. An atomic force microscope was used to determine the surface topology of the coated PP surface. To test platelet adhesion, the PP surfaces were exposed to heparinized human blood. After blood exposure, a scanning electron microscope was utilized to assess the density of adhering platelets on the PP surfaces. The plasma-treated surfaces showed fewer blood adherents than the untreated surfaces. The simulation can include the surface topology as measured by the AFM. By suitably modifying the plasma parameters, the plasma-polymerization treatment can be optimized with the eventual goal of producing biocompatible, small-diameter (< 5 mm ID) artificial blood vessels that contain integrated sensor systems. @FootnoteText@ @footnote 1@M.R. King and D.A. Hammer, Biophys. J. v.81, 799-813 (2001).

BI-MoP17 Studies of Protein Interactions with CaP Surfaces Using XPS and ToF-SIMS, *C. Mendoza-Barrera, H.E. Canavan, R. Michel,* ***D.G. Castner***, University of Washington

Proteins directly control the nucleation and growth of biominerals, but the details of molecular recognition at the protein-biomineral interface remain poorly understood. The elucidation of recognition mechanisms at this interface may provide design principles for advanced materials development in bone replacement. In this work, we characterize the interactions of proteins with the principal calcium phosphate components of bone. Using X-ray diffraction (XRD), we characterized the purity and phases of hydroxyapatite (HAp), dibasic calcium phosphate dihydrate (DCPD), dibasic calcium phosphate (DCP), @beta@ tribasic calcium phosphate (@beta@-TCP) and monbasic calcium phosphate (MCP). Next, adsorption isotherms of different proteins (e.g. BSA and fibrinogen) were performed on each calcium phosphate substrate. In this way, the solution concentrations necessary to produce sub-monolayer and monolayer thicknesses of each protei n was determined via X-ray photoelectron spectroscopy (XPS). As the conformation of proteins is greatly influenced by their density on a surface, we next used time-of-flight secondary ion mass spectrometry (ToF-SIMS) to compare the comformation of different protein layers adsorbed on the different calcium phosphate substrates. Finally, we discuss the effect of protein identity, conformation, and the character of the calcium phosphate substrate have on protein adsorption.

BI-MoP18 Syntheses of Polymer-Protein Composites by Plasma-Spinning Deposition, ***R. Ohta***, *N. Saito, O. Takai,* Nagoya University, Japan

Blood and/or tissue contacting biomaterials as catheters, artificial blood vessels and artificial valves are desired to have higher biocompatibilities in the medical field. Therefore, polymer-protein composites, which are expected to have excellent biocompatible surfaces, have been attracting attentions of many researchers. In this research, we aimed to synthesize polymer-protein composites by plasma-spinning deposition (PSD). In the PSD, the polymer composites were synthesized from precursor solutions. The precursor solutions were filled in a metallic capillary tube. Plasma were produced by applying voltages to the capillary tube. The polymer composites were synthesized on substrates from the precursors, which passed through the plasma. Two different processes were examined to synthesize the polymer-protein composites: (i) PSD of the polymer-protein composites from mixed solvents of the precursor polymers and proteins, and (ii) immobilization of proteins to the polymers synthesized by the PSD. Polyethylene terephthalate (PET), polyurethane (PU) and polyacrylonitrile (PAN) were used as precursor polymers. Proteins as fibrinogen, heparin and albumin were contained into the polymer composites. The PSD process was optimized by varying the concentrations of the polymers and proteins in the precursor solutions and by controlling the plasma states during the PSD. The chemical structures of the polymer-protein composites were analyzed by spectroscopic methods as X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy (RS), etc. The surface characteristics of the polymer-protein composites were investigated by scanning electron microscope (SEM), atomic force microscope (AFM), etc.

BI-MoP19 Alginate Adsorption to Charged Surfaces: The Importance of Protein Co-Adsorption, ***N. Chandhok***, *K.T. Queeney,* Smith College

The adhesion of extracellular bioploymers, including polysaccharides and proteins, to solid surfaces is a critical step in biofilm formation. It has been shown previously that alginate, a charged polysaccharide, will adhere to amine-terminated surfaces,@footnote 1@ and that the presence of a protein conditioning film can enhance alginate adsorption.@footnote 2@ We have used surface infrared spectroscopy to examine the adsorption behavior of alginate and find that protein co-adsorption is in fact essential for adhesion of this negatively charged polymer even to positively-charged surfaces. Specifically, the co-adsorption of protein impurities, which are present in quantities less than 50 ng/mL, is seen in all cases of alginate adsorption. When these impurities are filtered out by adsorption to high surface area MgO, no alginate adsorption is detected. Exposure of purified alginate solutions to protein conditioning films prepared from specific, known proteins is used to identify the critical polysaccharide/protein interactions that favor biofilm formation. @FootnoteText@ @footnote 1@R. Dhamodharan and T. J. McCarthy, Macromolecules 1999, 32, 4106-4112.@footnote 2@P. A. Suci and G. G. Geesey, J. Coll. Interface Sci. 1995, 172, 347-357.

BI-MoP21 Prediction of Protein-Surface Interactions by All-Atom Molecular Dynamics Simulations Using Implicit Solvation, ***Y. Sun***, *F. Wang, R.A. Latour,* Clemson University

The orientations and conformations of adsorbed proteins on biomaterials surfaces have profound influences on their bioactivities. However, it's very difficult to resolve the structures of adsorbed proteins experimentally. Empirical force field-based molecular simulation can be used to complement experimental studies to investigate protein adsorption behavior and potentially provide a more detailed understanding of molecular-level interactions. The predictive power of such an approach is largely dependent on the accuracy of the underlying force field used and the adequacy of sampling in the simulation. The objective of this study is thus to develop an empirical force field method with enhanced sampling to enable protein adsorption to be accurately simulated. We are evaluating the use of a generalized Born-based analytical continuum electrostatics (ACE) implicit solvent model for the purpose of enabling protein adsorption to be simulated with solvation effects treated implicitly. To further enhance sampling, replica-exchange molecular dynamics (REMD) is employed in combination with ACE to predict the equilibrium structures of a model protein (lysozyme) on alkanethiol self-assembled monolayer (SAM) surfaces. We have determined that ACE predicts reasonable energy-distance relationships of mid-chain peptide residues on functionalized SAM surfaces; it also predicts reasonable and stable trajectories of native lysozyme structure and significant surface-induced conformational changes

of lysozyme on SAM surfaces. Qualitative agreement between model predictions and experimental observations has been established, and further studies for model validation are planned.

BI-MoP22 Role of the Chemical and Morphological Surface Properties in Platelet Binding and Protein Adsorption to Biomaterial Surfaces, *S. Forti, M. Vinante, L. Pasquardini, L. Lunelli, L. Vanzetti, R. Canteri, C. Pederzolli,* ***M. Anderle,*** ITC-irst, Italy*; S. Pascale,* Sorin Biomedica Cardio S.p.A, Saluggia (VC), Italy*; G. Rossetti,* S. Chiara Hospital, Italy

Many existing medical implants are associated with poor interfacial biocompatibility. An example is represented by the cardiovascular devices; their implantation induces a complex blood-material interaction, often leading to thrombus formation. This work describes the characterization of the protein layer and the process of platelets adhesion on four different materials (Sorin pyrolytic carbon (PyC), two different types of polystyrene and titanium alloy) after contact with human platelet poor plasma and platelet rich plasma in static conditions. Total protein quantification on eluted samples revealed that PyC adsorbed the lowest amount of plasma proteins. Using immunofluorescence microscopy specific proteins promoting platelets adhesion were characterized on PyC and titanium alloy: fibronectin was found to be present at very low levels on both surfaces while fibrinogen and von Willebrand factor adhered to PyC in a higher proportion. Adherent platelets and shape categories distribution were quantified using scanning electron microscopy (SEM) and atomic force microscopy (AFM). PyC induced less adhesion with mostly weak activated platelets, however aggregates may be present. Titanium alloy promoted a higher adhesion, with more active platelets but less cohesive. Polystyrene materials were almost covered by spread platelets. Material surface properties were evaluated by contact angle, electron spectroscopy for chemical analysis (ESCA), secondary ion mass spectrometry (SIMS) and atomic force microscopy (AFM). The final goal will be to correlate the biological response with surface morphological and physico-chemical properties of the materials.@footnote 1@ @FootnoteText@ @footnote 1@Supported by the Provincia Autonoma di Trento, post-doc project Emosurf and by National Department of Health.

BI-MoP23 Adsorption of Human Serum Albumin on Carbon Nitride Films Studied with in-situ Ellipsometry, ***T. Berlind,*** *M. Poksinski, L. Hultman, P. Tengvall, H. Arwin,* Linköping University, Sweden

Carbon based materials have received considerable attention during the last decades due to their interesting tribological, electronic and optical properties. So far not much effort has been put into the investigation of the use of these materials in biotechnology. The objective with this study is to investigate the interaction of carbon and carbon nitride surfaces with proteins. Carbon nitride (CN@sub x@) and amorphous carbon (a-C) thin films were deposited on silicon substrates by reactive sputtering. By changing sputtering parameters the microstructure can be controlled and amorphous, graphitic and fullerene-like films were grown to a thickness of 200 nm. Prior to protein adsorption, the three structures of CN@sub x@ films and the a-C films were optically characterized with spectroscopic ellipsometry in the wavelength range 350-1700 nm and with infrared ellipsometry in the range 2-30 µm to determine their complex-valued refractive index N=n+ik. Contact angles for the films were measured with water. The films were exposed to human serum albumin and the adsorption was monitored in-situ using dynamic ellipsometry. From the ellipsometric data the adsorbed amount of proteins was quantified in terms of surface mass density using de Feijters model. The protein layer index was described with a Cauchy model. The results indicate larger adsorption of protein onto the amorphous films compared to the films with more ordered structure.

BI-MoP24 Infrared Spectra of Serum Albumin Immobilized in Porous Alumina, ***L.G. Castro,*** *S. Sarkar, D.W. Thompson, J.A. Woollam,* University of Nebraska-Lincoln

While most studies of protein-surface interactions rely on chemistry to obtain specific information about what proteins are present, infrared absorption spectra also contain protein-specific features. Reliable measurement of these spectra could, for example, help identify nonspecific binding. Here porous alumina was used as a capture matrix to increase the detectability of protein infrared spectra. Layers of porous alumina were fabricated electrochemically and fully characterized using visible and mid-infrared (mid-IR) spectroscopic ellipsometry (SE). Pore sizes and center-to-center spacings were engineered to efficiently capture human serum albumin (HSA). The layers were exposed to solutions of HSA in an acetate buffer. The incorporation of the proteins into the matrix was monitored by multiwavelength visible SE. The samples were characterized before and after protein attachment with mid-IR SE. A methodology was developed to obtain the infrared dielectric function @epsilon@ of the adsorbed proteins. Full optical modelling was essential to separate the protein peak signatures from those of the alumina. Strategies to improve capture efficiency and reduce uncertainty of the @epsilon@ spectrum are discussed.

BI-MoP25 Vacuum UV to Mid-Infrared Optical Study of Immunoglobulin G Attachment to Chemical Modifications of Chitosan, ***W.H. Nosal,*** *S. Sarkar, D.W. Thompson, A. Subramanian, J.A. Woollam,* University of Nebraska-Lincoln

Optical constants of spin-cast chitosan films were determined from vacuum-ultraviolet (VUV) to mid-infrared (130 nm to 30 microns). Both pure and chemically modified chitosan films were studied using spectroscopic ellipsometry (SE). Chemical modification by attachment to the amine group in chitosan was performed using 1,2 Epoxy-3-phenoxy-propane, commonly known as glycidyl phenyl ether (GPE), to produce a hydrophobic surface. A similar modification with succinic anhydride yields a hydrophilic surface. Quantitative lineshape analysis of the optical constant spectra was performed using Lorentzian and Gaussian line-shapes, including anisotropic response due to molecular-bond orientations in-plane and out-of-plane. Dynamic in situ visible SE has been used to study the attachment of immunoglobulin G to each modified surface type (hydrophobic/hydrophilic), followed by VUV to mid-infrared ex situ SE optical analysis. Research supported by U. S. Army contract # W911NF-04-2-0011, and the College of Engineering, University of Nebraska.

BI-MoP26 Effects of Fluorescent Dyes on the Structure of Lipid Membranes, ***J.J. Heetderks,*** *P.S. Weiss,* Penn State University

Cell membranes are complex, dynamic mixtures of lipids, proteins, and cholesterol; their precise mode of molecular organization is unknown. Transient associations of molecules form â?olipid raftsâ? in active cells that may affect membrane-associated protein activity. One model to study the lipid component of these molecular interactions is the giant unilamellar vesicle (GUV). The lipids, without contribution from membrane proteins, cytoskeletal structures, cholesterol, or outside forces, form domains in GUVs when the conditions are within an appropriate range of the multi-dimensional phase diagram of lipid composition and temperature. Fluorescently labeled phospholipids and lipid analogues are used at low concentrations to visualize the vesicles and domains, and are found to influence measured membrane properties, even at concentrations below those typically used in structural studies. Through basic membrane organization measurements, we determine the effects on the vesicle properties for which the labeling is responsible. We incorporate varying amounts of fluorescent dyes into 2-phase vesicles and find clear divisions of gel and fluid domains at room temperature. The temperature is then slowly raised while monitoring the membrane domains until the domains melt into one homogeneous phase. Preliminary results show that the incorporation of several common fluorescent labels in the membrane cause measurable changes in the demixing temperatures of the two-phase vesicles at less than one percent dye concentration.

BI-MoP27 Cell Adhesion of Plasma Polymerized Allylamine Coating on Polymeric Substrates, ***S.R. Kim,*** Chungju National University, South Korea

RF plasma enhanced chemical deposition was used to get biocompatible coating on polymeric substrates with various processing conditions, such as input power, monomer/oxygen feed ratio, modulated frequency and duty cycle. Allylamine was used as feed monomer and oxygen was used as mixing gas. Input power was varied from 50 to 300 Watt. Deposition rate was 16 nm/min. Chemical bonding information of deposited film by FTIR-ATR showed peak broadening compared to the allylamine monomer and C=N stretching peak near 2200 cm@super -1@ appeared for polymerized coating. Contact angle was changed from 88° for untreated polystyrene to 30° for treated polystyrene without oxygen input. Contact angle of plasma polymerized allylamine coating obtained with oxygen input was 8°. Untreated polystyrene film had rough surfaces and the average roughness was 10.91 nm, however, the plasma coating deposited polystyrene with introduction of oxygen, the roughness decreased to 3.91 nm and it decreased to 1.98 nm without oxygen input. ESCA showed N/C ratio of 13.4, 4.36 for samples prepared with oxygen input and without oxygen, respectively. Fibroblast cell was used and MTT assay was done. Cell viability 4 days after seeding was decreased as amount of oxygen increased.

BI-MoP28 Controlled Passive Transport through a Cellular Mimetic Membrane Consisting of a Stochastic Array of SiO2-coated Vertically Aligned Carbon Nanofibers, ***J.D. Fowlkes***, The University of Tennessee, Knoxville, US; *B.L. Fletcher, E.D. Hullander, M.L. Simpson, A.V. Melechko,* The University of Tennessee, Knoxville; *M.J. Doktycz,* Oak Ridge National Laboratory

A cell mimic device has been fabricated for the purpose of mimicking and interfacing to biological processes at the molecular scale. The design of the device addresses the challenge of fabricating and filling small physical volume enclosed membrane structures. The device has the additional advantage of being totally synthetic. The feasibility of using vertically aligned carbon nanofibers (VACNF) as the semi-permeable membrane component of the cell mimic device has been successfully verified by demonstrating the controlled and size-selective transport of nanoscale species by the VACNF membrane. To date, the controlled delivery and containment of picoliter volumes to individual cells has been achieved and the efficacy of the semi-permeable membrane component of the cell mimic to mediate passive diffusion transport has been evaluated. We report here the identification of discrete regimes of membrane transport behavior based on the ability to tailor nanoscale aspects of the membrane pore by controlled oxide deposition. VACNF based membrane elements that are stochastically prepared exhibit a strong statistical nature on the nanoscale. Stochastic features are preferable over patterned ones in terms of reduced fabrication complexity but exhibit statistical deviations that lead to properties that are difficult to predict. We have created a Monte Carlo based simulation to replicate and simulate the stochastic nature of the VACNF membrane and the passive diffusion through the membrane, respectively. The simulation was found to correlate strongly with experimental results. These predictive capabilities help facilitate device design and reduce the number of experimental characterizations. Further, the results reported here implicate stochastic, statistical nanoscale structures as realistic components in integrated devices with stringent requirements on discrete and reproducible behavior.

BI-MoP29 Calcium Ion Free Supported Lipid Bilayer Formation by Giant Vesicle Fusions, ***Y.-H. Kim***, *Md. Rahman,* The Graduate University for Advanced Studies, Japan; *R. Tero, T. Urisu,* Institute for Molecular Science , NINS, Japan

Supported membranes are the lipid bilayers supported on the solid substrate, which are commonly used as a model membrane for the in vitro study of the fundamental properties of biological membranes and various biotechnological applications. We have investigated the effect of the electrostatic attractive force between vesicles and the surface on the supported lipid bilayer formation using atomic force microscopy (AFM) and fluorescence microscopy. In most of the lipid bilayer formation by vesicle fusion, Ca@super 2+@ are added to the vesicle suspensions to induce the rupture of the vesicle. The lipid bilayer formation under the Ca@super 2+@ free is required in such cases of the study of Ca@super 2+@ effects on the membrane surface reactions. When the bare SiO@sub 2@ surface was incubated in the suspension of the negatively charged giant vesicle without Ca@super 2+@, extremely low surface coverage of lipid bilayer was observed due to the surface-vesicle charge repulsion. while, high coverage of lipid bilayer was observed when Ca@super 2+@(final concentration 5 mM) was added before incubation. The remarkable difference in the coverage of the lipid bilayer on the SiO@sub 2@ surface according to the existence of calcium ion can be explained in terms of the adsorption of giant vesicles. As to the formation of the lipid bilayer, the adsorption is the initial step, and therefore a stable and strong adsorption is necessary to form high coverage of the lipid bilayer. In case of the positively charged surface modified by Aminoproplydimethylethoxysilane, the high surface coverage of the lipid bilayer was obtained without adding Ca@super 2+@. The strong electrostatic attractive force between vesicles and the surface of opposite charge enhances the stable adsorption of negatively charged giant vesicles, which promotes the lipid bilayer formation. The control of the surface and the vesicle charge is an important factor to make a supported lipid bilayer without calcium ion.

BI-MoP30 ToF-SIMS Chemical Imaging Analysis of Micropatterned Streptavidin and Cells without Labeling, ***T.G. Lee***, Korea Research Institute of Standards and Science (KRISS), Korea; *H.K. Shon,* Korea Research Institute of Standards and Science (KRISS); *K.-B. Lee,* Korea Advanced Institute Science and Technology (KAIST); *J. Kim,* Sungkyunkwan University, Korea; *J.C. Lee,* Samsung Advanced Institute of Technology (SAIT), Korea; *I.S. Choi,* Korea Advanced Institute Science and Technology (KAIST); *D.W. Moon,* Korea Research Institute of Standards and Science (KRISS)

In this work, three different analysis ion beams (Ga@super +@, Au@super +@ and Au@sub 3@@super +@) were used to obtain label-free time-of-flight secondary ion mass spectrometry (ToF-SIMS) chemical images of microcontact printed streptavidin. The image of total ions obtained by an Au@sub 3@@super +@ primary ion beam corresponded well to the real image of micropatterned streptavidin, whereas the total-ions image by Ga@super +@ or Au@super +@ primary ion beams did not. A principal component analysis (PCA) of ToF-SIMS data was initially performed to identify characteristic secondary ions of streptavidin. Chemical images of each characteristic ion were reconstructed from raw data and used for the 2nd PCA run, which resulted in a contrasted, and corrected, image of micropatterned streptavidin by Ga@super +@ and Au@super +@ ion beams. This suggests that ToF-SIMS imaging along with multivariate data analysis would be an effectual method of obtaining label-free chemical images of patterned proteins or biomolecules. Label-free chemical images of micropatterned A431 cells were obtained by using the same procedure.

BI-MoP31 Effects of Surface Topography, Chemistry and Wettability on Osteoblast Cell Adhesion and Mineralization on Sol-Gel-Derived Titanium Alloy, ***M.C. Advincula***, *E.T. Ada, F.G. Rahemtulla,* University of Alabama at Birmingham; *R.C. Advincula,* University of Houston; *S.L. Bellis, J.E. Lemons,* University of Alabama at Birmingham

The biological events occurring at the bone-implant interface are influenced by the topography, chemistry and wettability of the implant surface.The surface properties of titanium alloy Ti6Al4V prepared by surface sol-gel processing (SSP) were investigated systematically using x-ray photoelectron spectroscopy, scanning electron microscopy, atomic force microscopy and contact angle metrology. Biocompatibility of the oxide was assessed by evaluating MC3T3 osteoblastic cell adhesion to the substrate, as well as by matrix mineralization. The sol-gel coated surface had predominantly a TiO@sub 2@ composition with abundant hydroxyl OH-groups, and was highly wettable, with increased roughness and porosity. Significantly more cells adhered to the sol-gel, as compared with passivated surfaces, at 1 and 24 hours following cell seeding, and a markedly greater number of bone nodules were observed on sol gel coatings. Favorable cellular responses were attributed to the rougher porous surface, hydrophilicity and increased hydroxyl group content of the sol gel, properties which, in turn, are known to regulate the adsorption of pro-adhesive serum proteins onto material surfaces.Collectively our results show that surface properties of titanium alloy can be modified by SSP to further enhance the bioactivity of this biomaterial.

BI-MoP33 Electrical Monitoring of Cell Interaction on a Microelectronic Interface, *H.D. Wanzenboeck,* ***K. Dominizi***, *P. Hagl, E. Bertagnolli,* Vienna University of Technology, Austria; *E. Bogner, F. Gabor, M. Wirth,* University Vienna, Austria

*****PLEASE NOTE: YOU MUST IDENTIFY A DIFFERENT PRESENTER FOR THIS ABSTRACT. YOU MAY ONLY PRESENT ONE (1) PAPER AT THE CONFERENCE*****The electrical measurement of tissue properties and of cell signals has gained increased interest for ceel-based biosensor applications in medicine, pharmacology and biology. Yet, the interaction of living cells on solid sensor surfaces has not been thoroughly investigated. For application mainly microelectronic sensors are attractive due to the small size and the low cost in mass production. The objective of this work was to investigate the interaction of living human cells with microelectronic surfaces. We have performed a comprehensive study of the cells behaviour on semiconductors, metals and dielectric materials commonly used in microelectronics. Human ephitelal cells (Caco-2) were grown in-vitro on the surface of the microelectronic substrates. In a second step we have systematically varied the geometry of the surface by etching trenches with a width from 2 up to 60 µm and a depth of 2 to 30 µm into a biocompatible substrate. The growth of ephitelial cells on flat and on ridged surfaces was compared. The response of the cell behaviour on the varying surface was investigated by optical, electronoptical, enzymatic and biochemical methods. The effects of surface alterations on the proliferation rate, the cell adhesion, the cell coverage and the differentiation of cells was investigated. Finally, a microelectrode structure with microelectrodes (2x2 µm2) smaller than a single cell was implemented on the previously

investigated surfaces. The electrical properties of the cell layer and of single cells could be evaluated by impedance spectroscopy. Differences of the impedance were monitored during progressing aging of cells. The interpretation of the interrelated analysis results shines a new light on the interaction of living cells with sensor interfaces. The feasibility to identify changes of the cell-surface interaction by electrical measurements was successfully demonstrated.

BI-MoP35 Fabrication of a Polyallylamine-Based, Label-Free Piezoelectric Biosensor Platform, ***A. Papadopoulou-Bouraoui***, *F. Bretagnol, M. Lejeune, A. Valsesia, J. Barrero-Moreno, D. Gilliland, G. Ceccone, F. Rossi,* European Commission-Joint Research Centre, Italy

The Quartz-Crystal Microbalance (QCM) is a sensitive acoustic technique allowing the measurement of resonance frequency changes occurring due to changes in the total oscillating mass of the crystal. It operates as a highly sensitive weighing device. The aim of the present study was to use active amino groups provided through plasma deposition of an allylamine polymer film (PALL) towards further antibody immobilization. Two antibody immobilization routes were investigated. The first involved the use of glutaraldehyde as a crosslinker, followed by protein A (PA) or protein G (PG) for optimal antibody orientation and consecutively highly sensitive antibody-antigen interaction. The second involved the use nanometer-sized gold (20 nm) particles in combination with PA or PG. PALL films were deposited in a capacitively coupled plasma reactor onto the SiO2 covered surface of the quartz crystal and were completely characterized before antibody immobilisation. The active amino groups were quantified through evaluation of the X-Ray Photoelectron Spectroscopy (XPS) C1S high-resolution spectra of the trifluoromethylbenzaldehyde derivative. Evaluation of the stability and reactivity of all main intermediate steps was performed using the QCM in combination with other surface characterization techniques such as the Ellipsometry, Atomic Force Microscopy (AFM), Time-of-Flight Secondary Ion Mass Spectrometry and XPS. Results from this work show the possibility of producing simple, direct piezoelectric immunoprobes through appropriate antibody orientation, using PA or PG, or surface increase, using GNP, without the need for labeled compounds. The combination of surface analytical, optical and mass balance techniques is confirming the effectiveness of these immunosensor fabrication strategies. This fully characterized biosensor platform has been tested for the detection of a food allergen, ovalbumin.

BI-MoP36 Formation of Carbohydrate Microarrays with the MAPL Technique for the Detection of Specific Interactions, ***K. Barth***, *G. Coullerez, M. Textor,* ETH Zurich, Switzerland

Carbohydrates play an important role in many biological processes, like cell-cell and cell-pathogen recognition.@footnote 1@ Because of missing analytic tools there is until now little known about the role of carbohydrates in these processes. Therefore it exists a need for methods that allow high throughput screening of these specific interactions.@footnote 2@ We have newly developed a method to covalently graft mono-, di- and trimannosides to the polycationic copolymer poly(L-lysine)-graft-poly(ethylene glycol) (PLL-[g]-PEG). With this system it is possible to tailor the density and distribution of the immobilized mannosides on the polymer backbone. While spontaneously adsorbed on negatively charged oxides surfaces (Nb @sub 2@O@sub 5@, TiO@sub 2@) the copolymers show specific lectin and bacteria recognition. Furthermore we could demonstrate the dependence of the carbohydrate surface density for the interactions between the mannoside and the multivalent model systems Concanavalin A (Con A) or Escherichia coli (E. coli). This was done with methods like Optical Waveguide Lightmode Spectroscopy where no additional labeling is required. In order to develop arrays, we propose herein to combine this chemical approach with the patterning method MAPL (Molecular assembly pattering by lift-off) developed by Falconnet et al.@footnote 3@ This technique combines photolithography and the attribute of functionalized PLL-[g]-PEG to form uniform layers on many metal oxide surfaces. We are able to control the pattern geometry and size as well as the surface density of the mannosides in the adhesive patterns. Fluorescent labeled Con A and E. coli can be easily detected and is proofing the high specificity of the developed system with a non fouling background. @FootnoteText@ @footnote 1@ Sharon et al., Sci. Am. 1993, 268, 82.@footnote 2@ Seeberger et al., ChemBioChem 2004, 5, 1375.@footnote 3@ Falconnet et al., Nano Letters, 2004, 4, 1909.

BI-MoP37 Combinatorial Characterization of Geometric Effects on the Optical Properties of Gold Nanostructures for Biosensors Optimization, ***G. Nusz***, *A. Curry, A. Wax, A. Chilkoti,* Duke University

Optimizing the performance of nanoparticle optical biosensors requires design of nanostructures that exhibit the greatest change in their extinction spectrum upon receptor-analyte binding. Previous studies have suggested that highly anisotropic nanostructures that exhibit geometric asperities are likely to provide enhanced sensitivity compared to isotropic particles. Unfortunately, the optical sensitivities for nanostructures with complex shapes must be determined experimentally because current theoretical modeling and computer simulation methods for complex geometries are computationally intensive and time-consuming. Thus, a combinatorial experimental approach that allows the rapid and high-throughput optical characterization of many structures of different size and shapes is desirable to rapidly optimize the design of such nanobiosensors. As proof-of-principle of this high-throughput optimization approach, arrays of nanostructures with varying geometries with minimal dimensions of 60 nm were fabricated on glass substrates by electron beam lithography. Scattering spectra were collected with a grating spectrometer simultaneously from several nanostructure configurations on a customized Zeiss Axiovert 200 under darkfield illumination. The characterization of the geometric dependence of the optical properties of the gold nanostructures could be experimentally determined in single snapshot mode at the individual nanoparticle level using this set-up. Studies on the optical sensitivity of these nanostructures in response to perturbation of their local refractive index are currently in progress.

BI-MoP38 Sensitivity Enhanced Biosensor by Prussian Blue Modified Electrode, ***I.J. Yi***, *J.H. Kim, C.J. Kang, Y.S. Kim,* Myongji University, Korea

We propose a sensitivity enhanced biosensor by Prussian blue (PB) modified indium tin oxide (ITO) electrode. A PB film plays a significant role of electron-transfer. Capillary electrophoresis (CE) and amperometric method were adapted to our work. Microchip was fabricated with polydimethylsiloxane (PDMS) to form microchannels and ITO patterned glass in inexpensive and simple method. The PB film was electrodeposited on the working electrode of various conditions to obtain stable PB film. Atomic force microscopy (AFM) was used to observe the changes of PB film surface. Calibrated PB film deposition voltage and time were obtained by AFM topography which shows PB surface characteristics. The optimized thickness of stable PB film was obtained when deposition voltage was about 0.1 V for 3 min. We detected various concentrations of neurotransmitters (dopamine and catechol) and hydrogen peroxide which is for detecting glucose respectively. It is observed that there was 20 times higher peak current for PB/ITO electrode than that for previously reported ECDs using bare-ITO electrode.@footnote 1@ Results are indicating rapid separation and detection of the analytes. The measured peaks of dopamine, catechol and hydrogen peroxide were proportional to their concentrations. When PB-modified electrode was used, the sensitivity was improved compared with bare-ITO electrode. It is believed that the PB film can be a viable candidate for a disposable and sensitive biosensor. @FootnoteText@ @footnote 1@Ju-Ho Kim, C. J. Kang, and Yong-Sang Kim, "A Development of a Microfabricated Disposable Microchip with a Capillary Electrophoresis and Integrated Three-Electrode Electrochemical Detection", Biosensors & Bioelectronics, vol.20, no.11, p.2314-2317, (2005).

BI-MoP39 Organosilane SAMs as a Platform to Tune the Immunosensor Performances, ***R. De Palma***, IMEC vzw, Belgium*; S. Peeters,* KULeuven, Belgium*; K. Jans, K. Bonroy,* IMEC vzw, Belgium*; S. Cappelle,* Cytec Surface Specialities, Belgium*; G. Reekmans, W. Laureyn, G. Borghs, C. Van Hoof,* IMEC vzw, Belgium*; G. Maes,* KULeuven, Belgium

A central requirement in the modification of immunosensor interfaces with biological receptors is to tether the biomolecule of interest covalently and in a well-controlled geometry. A key issue in the design of these sensors involves the development of a sensitive, specific, reproducible and tunable biological interface. Self-assembly of silanes is commonly used as an effective surface modification tool in micro-array applications. However, most silanes used for micro-array are optimized towards DNA applications. Using quartz crystal microbalance (QCM-D), we have shown that the use of these silanes for immunosensing leads to inadequate characteristics, i.e. low sensitivity and specificity. Here we report on the enhanced immunosensing performances of novel preactivated silane SAMs. These preactivated functions allow for the direct coupling of receptors, thereby increasing the amount of immobilized antibodies. Using these preactivated silanes, the antibody immobilization was found to be reproducible, straightforward and controllable and the activity of the immobilized

receptors was retained. Due to the molecular architecture of these silanes, the sensitivity, detection limit and specificity increased significantly. The degree of non-specific adsorption could be tuned by using appropriate blocking agents. Furthermore, a synthesis route was developed to create PEG-modified preactivated silanes. Deposition of these silanes in mixed silane SAMs allows for the creation of tunable immunosensing platforms. The silane-based interfaces were also compared to the well-known system of mixed thiol SAMs. The molecular architecture of all deposited silane SAMs was studied using contact angle, XPS, cyclic voltammetry, AFM and RAIRS. A strong correlation was observed between the layer characteristics and their immunosensing properties. Our approach based on preactivated silane SAMs guarantees a tunable and versatile platform for surface engineering in biosensing and micro-arrays.

BI-MoP40 In Situ Optical Characterization of an Electrodeposited Biopolymer Film, ***S.B. Beatty***, *J.J. Park, E.C. Dreyer, G.W. Rubloff,* University of Maryland

We have previously demonstrated spatially selective, voltage-programmable deposition of the polysaccharide chitosan onto patterned electrodes in low pH solution. We have previously shown that chitosan, an amine rich biopolymer, serves as a useful platform for coupling and conjugation of biomolecules as well as a platform for bioassays and enzymatic catalysis. We have developed an optical reflectivity technique for real-time, in-situ monitoring of the electrodeposition process, demonstrated in a combinatorial deposition cell in concert with real-time electrical (current, voltage) and environmental (pH, temperature) measurements. A beam splitter separates a HeNe laser beam into a reference beam and an incident beam onto the electrodeposition surface in solution. A chopper separates the reflected beam from the surface and the reference beam in time, while both impinge on the same reversed bias Si photodetector. The resulting signal is analyzed to extract the ratio of reflected to reference beam intensities, averaged over an appropriate portion of the chopper period, providing a real-time measure of the reflectivity. Chitosan deposition on the Au electrode decreases reflectivity by about 16% for 1 micron chitosan thickness, with noise levels suggesting a thickness sensitivity of 60nm. Film growth rates vary with current density as seen in reflectivity and confirmed by ex-situ post-process characterization using profilometry and AFM. The reflectivity indicates three stages in chitosan film growth. We plan to extend the technique for use in a confocal optical microscope so that dynamic behavior at active bioreaction sites in a microfluidic network can be monitored.

BI-MoP41 Nano-mechanical and Chemical Mapping Showing Remineralization of Incipient Carious Lesions in Human Dental Enamel, ***M.E. Dickinson,*** Hysitron, Inc.*; A.B. Mann,* Rutgers, The State University of New Jersey

Human dental enamel consists of hydroxyapatite crystals arranged in a complex nano-scale prism structure. This architecture can be altered by chemical variations originating from dietary components and their interactions within the oral cavity. Temporary localized fluctuations of pH on the enamel surface create a flux of minerals into and out of the enamel which normally remains at an overall equilibrium. However, at continually low pH a net loss of mineral from the enamel results, causing the formation of an incipient carious lesion. The lesion - a stage prior to caries formation, is reversible and with specialized care such as remineralization treatments, can become arrested. Many commercial remineralization treatments use topical solutions containing fluoride and calcium phosphates to aid in caries prevention. However, the effect of these treatments at different stages of lesion progression is poorly understood. This investigation uses nanoindentation and TOF-SIMS to create high resolution mechanical and chemical maps of the lesion cross-section at different stages of demineralization. These lesions were analyzed before and after treatment with a commercial remineralization solution to study the effectiveness of remineralization at different stages of lesion progression. The results showed that the treatment acted to remineralize the lesion body for all lesions. However, the most demineralized lesions which had a different structure (no detectable surface zone), resulted in a different, possibly fluoroapatite based material being deposited at the base of the lesion with mechanical properties much higher than that of enamel. The chemical data correlated with the mechanical data to relate the structural integrity of the enamel with the change in hardness and reduced elastic modulus. Visualizing this dependence of lesion structure for efficient remineralization allows further understanding into the effectiveness of commercial dental treatments.

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