Wednesday Evening Poster Sessions, October 27, 1999

Biomaterial Interfaces Group

Room 4C - Session BI-WeP

Poster Session

BI-WeP1 Self-Assembly of Tetraphenylporphyrin Monolayers on Gold Substrates, ***A.L. Bramblett***, *M.S. Boeckl, T. Sasaki, B.D. Ratner, J.W. Rogers, Jr.,* University of Washington

The development of the next generation of medical implants involves attaching appropriate biorecognition molecules in the proper orientation and concentration on the surface of an implant, to prevent the cellular activation that leads to the foreign body response, and implant encapsulation. Porphyrin molecules are ideal for the development of a uniform monolayer, with controlled, optimal spacing of biorecognition groups. Self-assembled porphyrin monolayers on gold surfaces have been demonstrated with three custom synthesized alkylthiol substituted tetraphenylporphyrin molecules. Several techniques including x-ray photoelectron spectroscopy (XPS), ultraviolet/visible absorption spectroscopy (UV/Vis), scanning tunneling microscopy (STM), and grazing-angle infrared spectroscopy (GAIR) have been used to characterize the monolayers. XPS binding energy shifts in the S(2p) spectra reveal that the porphyrins are chemisorbed to the surface through a sulfur-gold bond.@footnote 1@ A red shift without a significant blue shift of the Soret band in the UV/Vis absorption spectra demonstrates that the porphyrin molecules are aligned on the gold surface in a side-by-side orientation.@footnote 2@ GAIR with a polarized light source, indicates that the porphyrin rings are oriented parallel to the gold surface. Round STM features, approximately 2 nm in diameter, correspond closely to the diameter of tetraphenylporphyrin (1.8 nm), and are distributed relatively evenly over the surface. Finally, XPS and UV/Vis coverage calculations show approximately monolayer coverage. Taken together, this data indicates the formation of self-assembled porphyrin monolayers. @FootnoteText@ @footnote 1@ Castner, D.; Hinds, K.; Grainger, D. W. Langmuir 1996, 12, 5083-5086. @footnote 2@ Osuka, A.; Maruyama, K. J. Am. Chem. Soc. 1988, 110, 4454-4456.

BI-WeP2 Protein Nanopatterning on a Gold/Aluminum Nanoarray, ***C.K. Woods***, *Z.-P. Yang, A. Chilkoti,* Duke University

Protein nanopatterning has potential applications in the fabrication of multianalyte, proximal probe biosensors, genomic arrays, as well as modulation of cell-substrate phenomena. We have developed a technique to immobilize proteins on a surface with spatial resolution of around 100 nm. An ultraflat nanoarray of gold and aluminum is created on a silicon wafer by combining nanosphere lithography with "ultraflat template stripping"- a technique for creating ultraflat thin films of metal. The ultraflat gold/aluminum nanoarray is then incubated in a hexadecanethiol (HDT) solution, which forms a hydrophobic, self-assembled monolayer (SAM) on gold but does not adsorb onto the hydrophilic, native oxide layer on aluminum. We hypothesized that protein adsorption on a HDT-functionalized gold/aluminum nanoarray should occur preferentially on the HDT SAM, thereby allowing proteins to be nanopatterned on the 100 nm gold features. Formation of the HDT SAM on gold but not on aluminum was investigated separately on gold and aluminum substrates using contact angle goniometry, ellipsometry and atomic force microscopy (AFM). The model protein, Ribonuclease A, was found to adsorb preferentially to the HDT SAM on gold with a signal to background ratio of about 6. AFM studies of protein adsorption on HDT-functionalized ultraflat gold/aluminum nanoarrays are currently in progress as are experiments on extending this approach using SAMs presenting biological ligands.

BI-WeP3 Osteoblast and Monocyte Response to Nanometre Surface Topography In Vitro, ***P. Hanarp***, Chalmers University of Technology, Sweden*; J. Rice, J.A. Hunt, J.A. Gallagher,* University of Liverpool, UK*; D.S. Sutherland, J. Gold,* Chalmers University of Technology, Sweden

It is well known that cells adhering to surfaces are influenced by micron-sized chemical and topographical features, but very little is known about cell behaviour on surfaces with smaller, nanometre-sized features. We have used a method based on adsorption of colloidal particles to produce surfaces with well-controlled nanotopography. Surfaces of silicon wafers, pre-coated with 30 nm of thermally evaporated Ti, were treated with aluminium chloride hydroxide giving a net positive charge at neutral pH. Then the surfaces were exposed to dilute aqueous solutions of 110 nm polystyrene particles, and the negatively charged particles adsorbed onto the positively charged surfaces randomly by electrostatic interactions. A submonolayer of particles was obtained with coverage controlled either by salt concentration in the colloidal solution for equilibrium adsorptions, or particle concentration and adsorption time in interrupted (non-equilibrium) adsorptions. To produce chemically homogenous surfaces, a film of titanium (82.5nm) was evaporated on top of the particle films. The titanium film was naturally oxidised in air. The response of primary human osteoblasts and monocytes to these surfaces has been investigated. The cells were cultured in contact with the samples, 1) flat titanium oxide, 2) 10% and 3) 20% coverage of 110 nm particles coated in titanium oxide, for 1 and 7 days. They were examined using fluorescent cytoskeletal staining, confocal microscopy and lactate dehydrogenase assays in conjunction with flow cytometry. At each time point, both osteoblasts and monocytes cultured on the flat titanium oxide and the 20% coverage surfaces showed a greater affinity for adherence than at the 10% surfaces. SEM analysis of the samples after cell culture showed that the surfaces are still intact. This work is ongoing, but these preliminary results indicate that osteoblasts and monocytes are influenced by nanotopography in vitro.

BI-WeP4 Gene Expression in Reaction to Micro and Nano-topography, ***R. Hartley***, University of Glasgow, Scotland, UK*; A.S.G. Curtis,* University of Glasgow, Scotland, UK, Scotland

Cellular reaction to surfaces has particular relevance to engineering tissue constructs. Advances in microfabrication enabling production of structures with defined surface topography have facilitated our understanding of cell elongation, orientation and movement. The vastly differing cell morphologies and cytoskeletal arrangement on planar and topographical surfaces necessitate an investigation of adhesion, signal transduction and transcriptional regulation. This work investigates gene transcription in reaction to micro and nano-topography. In this study we used two differing methods Differential Display RT-PCR to assess gene transcription. The first relies on large scale total RNA isolation following in-situ cell lysis or transferral to suspension and subsequent lysis. The second follows the Klebe method of RT-PCR without RNA isolation, where although RT-PCR is generally carried out and optimised for 250 cells, the protocol is suitable for four cells. This method has particular significance for analysis of gene expression on small areas where cell number is limited. Methods: Sub-confluent tendon epitenon were trypsinised and seeded directly onto topographic and control substrata. For large scale total RNA isolation directly in-situ, or in suspension, GITC phenol/chloroform was used and RNA equilibrated using 260/280nm absorbance. The Klebe method used a -70@super o@ C freeze/rapid-thaw in the presence of RNase inhibitor allowing time for cDNA library creation. In each case cDNA libraries were amplified by PCR in the presence of @super 32@P dATP, then denatured and run on a 7M urea, 5% acrylamide electrophoresis gel. Separated isotopically labelled ssDNAs were viewed by autoradiography.Phage display systems were also used.

BI-WeP5 Do Oligoethyleneglycol Terminated Alkanthiols Induce Complement Activation?, ***J.M. Benesch***, *S. Svedhem, S. Svensson, P. Tengvall,* Linköping University, Sweden

oligoethyleneglycol terminated alkanthiols with varying length of the ethylenglycol repeats were self assembled on gold surfaces. In Situ antibody ellipsometry techniques were used to study protein adsorption when the surfaces are incubated in serum or plasma. The results so indicate depositions of serum and also deposits of complement factors. This indicates that oligoethyleneglycol surfaces are not serum and plasma resistent. This behavior is dependent on the presence of calcium and the number of repeats in the ethylenglycol chain.

BI-WeP6 Hybridization of DNA Monolayers on Gold Observed In Situ with Surface Plasmon Resonance, ***G.B. Saupe***, *M.J. Tarlov,* National Institute of Standards and Technology

The hybridization of end-tethered single-stranded DNA (ssDNA) probes on gold surfaces with ssDNA targets in salt solutions was monitored with surface plasmon resonance (SPR). SPR is a sensitive technique for measurement of angstrom-level changes resulting from surface DNA hybridization reactions. Surfaces derivatized with DNA are of interest for a variety of applications including genetic diagnostics, forensics, and infectious disease detection. In this study we have used a model system of thiol derivatized ssDNA probes self assembled on gold. The surfaces were subsequently treated with mercaptohexanol to passivate regions of unreacted gold and eliminate non-specific binding of the DNA. The effects of target ssDNA length, base pair mismatches, and the locations of the matching sequence within the target were evaluated in high salt conditions (1M NaCl). Probe coverages, hybridization efficiencies, and times needed for hybridization were measured. Recent data for the hybridization of mixed target solutions, where single basepair mismatched targets compete

with exact sequence matched targets to hybridize a single probe, will be discussed.

BI-WeP7 Multivariate Comparison of Dodecanethiol Self-Assembled Monolayers Prepared by Microcontact Printing and Solution Assembly, ***D.J. Graham***, *D.N. Price, S.L. Golledge, M.D. Garrison, T.C. McDevitt, B.D. Ratner,* University of Washington

In this study we show how exploring the entire TOF-SIMS spectra using multivariate statistics can enhance the analysis of a surface and bring to light details that are not obvious from univariate analysis. We have applied a PCA analysis to TOF-SIMS spectra of SAMs of dodecanethiol on gold prepared by solution assembly (assembly time: t =2sec,1min,5min,15min,30 min,1hr,24hr,6d) and microcontact printing (stamping concentration: c =0.001,1,5,10,50,100,200,1000mMol). PCA models from the TOF-SIMS negative and positive data were constructed. ESCA composition scans were also taken of all surfaces. Both univariate and multivariate comparisons were made between the solution assembled and stamped samples. The SIMS ratio sum[I(MolecularIons)]/sum[I(C-C3hydrocarbons)] and the ESCA C/Au ratio were used to compare the samples. Both methods found good correlation between samples in which a stamping concentration of 10-50 mMol produced SAMs similar to fully solution-assembled SAMs. This data is consistent with results from a similar stamping study using STM and contact angles by Larsen et. al.@footnote 1@ PCA analysis provided deeper insight into the two sets of samples. The scores from the first principal component (PC1) showed the similarities between the samples prepared by the two methods, though there was more scatter in the stamped samples. The second principal component (PC2) showed that some differences exist between the two preparations. Loadings from the second principal component showed that the differences in the samples were due mainly to the presence of oxidized species and silicone contaminants. Only trace amounts of Si (<1%) were seen in the ESCA spectra on some samples. The increase in oxidized species in the stamped samples may be due to the stamping procedure that was carried out in air. These differences may not be detected by other methods or by univariate analysis. @FootnoteText@@footnote 1@N. B. Larsen, H. Biebuyck, E. Delamarche, and B. Michel, J. Am. Chem. Soc. 119, 3017-3026 (1997).

BI-WeP8 Observation of Metal Clusters on Pancreatic Cells by Lateral Force Microscopy, ***A. Cricenti***, Consiglio Nazionale Delle Ricerche, ITALY*; R. Generosi, S. Cotesta, M. Girasole, P. Perfetti,* Consiglio Nazionale delle Ricerche, Italy*; A. Congiu-Castellano,* Universita' di Roma La Sapienza, Italy

The interaction between pancreatic cells and metal ions have been studied at membrane level by Atomic/Lateral Force Microscopy in the repulsive regime of contact mode. The atomic force microscope can give both topographic and chemical (in lateral friction mode) informations on the cellular membrane of cells. Morphological characteristics of non infected cells and metal infected cells were easily imaged from fixed and dried cell preparations. Upon Cd2 and Zn ions uptake, the pancreatic cells don't change their morphology but the lateral friction images localized several metal clusters on the cellular membrane.

BI-WeP9 Microfabricated Cantilever Force Sensor for Measurement of Cell Locomotory Forces, *C.D.W. Wilkinson, A.S.G. Curtis,* ***B.W. Leslie***, University of Glasgow, Scotland

Microfabricated cantilever force sensors are potentially capable of measuring attoNewton (10E-18 N) forces. Here, we propose a design for such a force sensor capable of measuring forces involved in cell locomotion. The cell guidance structures and cantilevers were fabricated as one integrated unit using dry-etching of polysilicon. Substrate used was a 300 µm thick (100) oriented silicon wafer coated on both sides with a 50 nm thick layer of silicon nitride. Wafers were cut into 25 mm squares. A 10 µm thick layer of isotropic polysilicon was deposited on one side of the wafer square. After patterning, the polysilicon layer was dry-etched to a depth of 10 µm to form cell guidance structures and a row of fifteen cantilevers along two opposing edges of the wafer square. Controlled etching of the silicon nitride layer ensured that cantilevers were released from the substrate while cell guidance structures remained in place. Cell guidance structures are designed to steer the cells toward the free end of the cantilever where deflection is largest for a given force. Cantilever dimensions (length=900 µm, height=10 µm, thickness=1 µm) combined with optical lever sensing of cantilever deflection are theoretically capable of measuring forces as small as 3 pN.

BI-WeP10 Surface Characterization of Microfluidic Devices, ***H. Canavan***, *M.S. Ravenscroft, D. Ramaker,* George Washington University*; M.J. Tarlov,* National Institute of Standards and Technology*; J.J. Hickman,* George Washington University

Interest in the interactions of biomolecules with surfaces stems from various sources, including biocorrosion of ship hulls, the rejection of transplant materials in the human body, and biological fluids interactions with MEMS devices. A variety of chemical and physical factors affect biological fluid behavior in microchannels used in lab-on-a-chip devices. We report an investigation of the effect of polymer surface condition on microfluidic properties. The primary goal of this work is to correlate the electrophoretic flow properties through microfluidic channels of different polymer substrates with the surface condition of the polymers. While polymer substrates hold great promise for biological microfluidic applications, polymer surfaces are often poorly defined which can lead to irreproducible microfluidic behavior. To circumvent this problem, we are developing various protocols to control the surface functionality of polymers. Oxygen plasma treatment has been used to introduce oxygen functionalities that can then serve as active sites for covalent attachment of organosilane monolayers. XPS, SIMS, and contact angle measurements have been used to characterize as-is, oxygen plasma treated, and organosilane-modified surfaces of polystyrene, polymethylmethacrylate, and various co-polyesters. We will report on these measurements and preliminary fluid flow experiments.

BI-WeP11 Time-of-Flight SIMS Analysis of Micropatterned Biomaterial Arrays, ***S.L. Golledge***, *M.D. Garrison, B.D. Ratner,* University of Washington

Micropatterned arrays of thin films, self-assembled monolayers and biomolecules are a critical enabling technology for the development of miniaturized biosensors and next generation biomaterials. Accurate characterization of the locally defined surface functional states is therefore crucial to the successful implementation of these novel approaches to surface engineering. We employed Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS), in both spectroscopic (static) and imaging modes to define and identify the spatial presentation of (1) bioactive polymeric materials prepared via radio-frequency glow-discharge (RFGD), (2) non-fouling organic thin-films patterned through microcontact printing (uCP), and (3) direct uCP matrix proteins important in biological wound healing. TOF-SIMS allowed characterization of both the bioactive species and the engineered reference regions, in addition to assuring that unwanted contaminant species were not present. Future prospects for TOF-SIMS analysis of micropatterned arrays will be proposed.

BI-WeP12 Atomic Force Microscope and Surface Plasmon Resonance Investigation of Polymer Blends of Poly(Lauryl Methacrylate) and 2-Methacryloyloxyethyl Phosphorylcholine-co-Lauryl Methacrylate, ***S. Clarke***, *M.C. Davies,* University of Nottingham, U.K.*; V. O'Byrne,* Biocompatibles Ltd, U.K.*; C.J. Roberts,* University of Nottingham, U.K.*; J. Russell,* Biocompatibles Ltd, U.K.*; S.J.B. Tendler, P.M. Williams,* University of Nottingham, U.K.

In order to design new synthetic polymers for use in medical devices it is necessary to characterize the surface of the material to understand the interactions that occur when exposed to biological environments.@footnote 1@ Incorporation of phosphorylcholine (PC) into polymers has been shown to improve biocompatibility by suppressing unfavourable responses that occur on contact with tissue and bodily fluids.@footnote 2,3@ Polymers containing PC are currently in use as coatings for medical devices such as stents, catheters, or to fabricate contact lenses. As an alternative to synthesizing new PC-containing polymers, polymer blends offer the opportunity to investigate the surface properties of PC in new materials. Here, polymer blends of 2-methacryloyloxyethyl phosphorylcholine-co-lauryl methacrylate (PmMl@sub 6@) and poly(lauryl methacrylate) (PLMA) have been produced with varying ratios of the two components. The surface of the blends when coated onto silver has been characterised using X-ray photoelectron spectroscopy (XPS), tapping mode atomic force microscopy (TMAFM), and surface plasmon resonance (SPR). Analysis has revealed that the blends formed by the two polymers are immiscible and exhibit surface-segregation with nanometre-sized domains being formed throughout the range of the blends. The Pmml@sub 6@ is preferentially expressed at the surface of the blends leading to enhanced protein-resistant properties. @FootnoteText@ @footnote 1@ Davies et al, Chapter 4 in Biocompatibility:Assessment of Materials and Devices for Medical Applications. Braybrook, J (Editor) J. Wiley and Sons, 1997 @footnote 2@ Campbell et al (1994) Am. Soc. Artificial Organs J. 40 3 M853-M857 @footnote 3@ Ishihara et al (1991) J. Biomed. Mat. Res. 25 1397-1407.

BI-WeP13 Thermally Responsive Ultrathin Coatings by RF-Plasma Deposition, ***Y.V. Pan***, *R.A. Wesley, R. Luginbuhl, R.M. Overney, D.D. Denton, B.D. Ratner,* University of Washington

Poly(n-isopropyl acrylamide) (pNIPAM) shows a lower critical solution temperature (LCST) of 31@super o@C in aqueous environment. At temperatures lower than the LCST, the polymer chains are well hydrated and fully extended. The polymer chains dehydrate and take on a more compact configuration above the LCST. This interesting phase transition behavior has been observed here with pNIPAM chains grafted onto solid surfaces by plasma deposition. In this presentation, thermally responsive coatings are prepared f rom glow discharges of NIPAM vapor. The synthesis and spectroscopic characterization (XPS, SIMS) of plasma polymerized NIPAM (ppNIPAM) will be presented. The ppNIPAM coatings obtained showed a remarkable retention of the monomer structure, and a phase tr a nsition at 31@super o@C in both water and air. The phase transition was measured by a novel AFM method. The water phase transition was surprising because of the expectation that the plasma environment would destroy the specific NIPAM structure associat ed with t he thermal responsiveness. However, the transition in air was even more unexpected and suggests that adsorbed water on the AFM tip and at the polymer surface is sufficient to produce the phase change, even when the bulk of the polymer is unhydrated. Plasma polymerization of NIPAM represents a one-step method to fabricate thermally responsive coatings without specially prepared substrates and functionalized polymers.

BI-WeP14 Design of a Minimal Peptide for Adsorption to Hydroxyapatite and Cell Binding via an RGD Sequence, ***M. Gilbert***, *C.M. Giachelli, P.S. Stayton,* University of Washington

In the natural remodeling of bone, the proteins that comprise the extracellular matrix play keys roles in the signaling of bone cells. These ECM proteins contain amino acid sequences such as RGD which are important for the adhesion of bone cells to the protein coated bone surface as well as the transmission of signals via outside-in integrin pathways. Many of the ECM proteins also contain stretches of acidic amino acid repeats, gamma carboxyglutamic acid residues, or high degrees of sulfation, glycosolation, or phosphorylation which aid in the adsorption of the protein to the mineral surface of bone. Despite the large body of knowledge on bone biology, most modern bone implant designs do not employ any control over the degree or specificity of protein adsorption to the surface of the implant which can result in lack of integration of the implant or encapsulation of the implant. However, by understanding how the biomineralization proteins bind to mineral surfaces and transmit signals to bone cells, minimal peptides can be designed which contain the features of strong mineral adsorption as well as integrin mediated cell adhesion and intracellular signaling to improve implant integration. A minimal peptide based on the mineral binding motif of salivary statherin combined with an RGD sequence was designed to bind and orient on hydroxyapatite surfaces. This fusion peptide (called N15-RGD) is capable of binding with high affinity to hydroxyapatite with the same Langmuir parameters as just N15 as well as maintaining alpha helical content in solution. N15-RGD, while immobilized on HAP, is also capable of binding cells specifically and in a dose dependent manner via the RGD sequence. The main integrin responsible for the binding of the cells to the RGD sequence is the @alpha@@sub v@@beta@@sub 3@ integrin. The N15-RGD peptide is thus oriented in such a manner that the RGD cell signaling sequence is still solution accessible to mediate integrin binding events.

BI-WeP15 Conformational Studies of Human Salivary Histatin-5 Bound to Hydroxyapatite Surfaces and Lipid Bilayers, ***M. Cotten***, *J.L. Dindot, P.S. Stayton, G.P. Drobny,* University of Washington

Histatin-5 (hsn5) is a human salivary polypeptide found in the acquired enamel pellicle. The protein is histidine-rich and basic (DSHAKRHHGYKRKFHEKHHSHRGY), and possesses at least two important functions: control of HAP crystal growth and antimicrobial activity. Previous studies have characterized functionally important regions of the peptide sequence as well as some secondary conformation analysis in solution. Very little is known about specific hsn5/HAP and hsn5/lipid bilayer interactions and the conformation of the HAP-bound peptide. This knowledge is nevertheless necessary to better understand molecular recognition and structure-functions relationships. Our primary goals have been to characterize the conformation of hsn5 both free and bound to HAP crystals and lipid bilayers. Moreover, we have been interested in identifying interactions between the peptide and HAP by using solid state NMR techniques. Solid state NMR experiments that measure internuclear distances to sub-Angstrom accuracy have been performed to determine distances between two 13C carbonyl labels of adjacent amino acids and thereby constrain the conformation of the peptide bound to HAP. In addition, hisn5 with a 15N amide label has been incorporated into hydrated oriented lipid bilayers and used to determine the orientation of the peptide with respect to the bilayer.

BI-WeP16 Determination of Statherin N-Terminal Peptide Conformation on Hydroxyapatite Crystals, ***W.J. Shaw***, *J.R. Long, J.L. Dindot,* University of Washington*; A.A. Campbell,* Battelle, PNNL*; P.S. Stayton, G.P. Drobny,* University of Washington

The interactions between proteins and inorganic crystals play an important role in the development and growth of hard tissues such as bone and teeth. Although many of these proteins have been studied in the liquid state, there is little information describing molecular recognition at the protein-crystal interface. Here we have used 13C solid state NMR (SSNMR) techniques to investigate the conformation of an N-terminal peptide of salivary statherin both free and adsorbed on hydroxyapatite (HAP) crystals. The torsional angle phi was determined at three positions along the backbone of the N-terminal 15 amino acid peptide fragment (DpSpSEEKFLRRIGRFG) by measuring 13C-13C distances between carbonyls in adjacent amino acids using the Dipolar Recoupling with a Windowless Sequence (DRAWS) technique. The peptides adsorbed to the HAP surface have an average phi of -85 degrees at the N-terminus (SS), -60 degrees in the middle (FL) and -72 degrees at the C-terminus (IG). The SS position corresponds to an angle typically associated with random coil peptides. The FL and IG positions correlate with angles known to be alpha-helical. These angles are approximately the same in the lyopholized peptides implying that secondary structure content of the peptide is retained upon adsorption to the crystal surface.

BI-WeP17 Tethering Phospholipid Bilayers to Porous Substrates, Progress Towards Biosensor Development, ***S.D. Ogier***, *S.D. Evans, R.E. Miles,* Leeds University, UK

This poster concerns work currently being undertaken at Leeds University on the development of a biosensor based on ion channel conductivity. Our approach aims to span a lipid bilayer containing ligand gated ion channels across a hole micro-machined in a solid support. Electrodes either side of this hole allow the electrical properties of the bilayer to be monitored. The device employs a 0.1mm hole micro-machined in a solid support with a gold surface patterned around it. This enables us to modify the surface that the bilayer has to sit upon in order to create the conditions necessary for bilayer formation over the hole. Micro-machining of substrates has been used in a few cases to create architectures to suspend lipid bilayers and most of these use the solvent spreading method of Montal et al. or Langmuir Blodgett transfer. Although these techniques will be suitable for bilayer formation on the device, it is our aim to self-assemble the bilayer over the hole. This could be a very useful method of formation since it is a simple technique, however it does require the hole to have a similar diameter to the vesicle. The bilayer's electrical resistance is measured by applying a small (20mV) potential difference between two silver/silver chloride electrodes, one either side of the hole, and monitoring the current flowing between them. Although we have not yet achieved self-assembly over the holes, as they are too large, a bilayer formed using a solvent spreading method produced an electrical seal in the 10GOhm range.

BI-WeP18 Genetic Diagnostics Using SIMS Detection of Unlabeled DNA, ***T.J. Whitaker***, *K.F. Willey,* Atom Sciences, Inc.

We are developing a new DNA chip technology that uses secondary ion mass spectrometry (SIMS) to determine genetic diagnostic information from unlabeled DNA. Genosensor chips, or DNA chips, typically contain arrays of single-stranded oligodeoxynucleotide (ODN) probes. Each probe has a known sequence and is attached at a specific site on the chip. The sequence of an unknown target DNA is determined by binding single strands of the target to the probes. This binding, or hybridization, ideally will occur only where the target sequence is complimentary to the probe sequence. Evidence of the binding is generally obtained by detecting fluorescent or radioactive labels attached to the target DNA. The new technology uses peptide nucleic acids (PNA) instead of ODNs for the probes. PNA is a DNA analog in which the phosphate and deoxyribose are replaced with polyamides. PNA binds to DNA with the same A-T, G-C rules as DNA/DNA hybrids but has slightly higher melting temperatures, offering the potential for greater discrimination between single-base mismatches. As opposed to DNA, which contains one phosphate along the backbone at each base, PNA contains no phosphorus. We have exploited this difference by using a simple SIMS analysis to detect molecular fragments containing phosphorus. The existence of phosphorus positively identifies the hybridization of unlabeled DNA to PNA probes. This method and a

simplified, relatively inexpensive SIMS instrument are currently being developed under funding from the National Institutes of Health.@footnote 1@ @FootnoteText@ @footnote 1@This work is supported by the National Institutes of Health under contract 2 R44 HG01596-02. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

BI-WeP19 TOF-SIMS Analysis of the Candida Albicans Cell Surface, ***H. Shi***, *B.J. Tyler,* Montana State University

The most common cause of failure for medical devices is infection, and the pathogenic yeast Candida albicans is the third leading cause of these infections. Adhesion is the first step in establishing an infection, the initial adhesion of microorganisms to synthetic polymer surfaces involves physicochemical interactions between molecules present at the polymer surface and those present on the cell surface. These interactions are not well defined, and insight into this area could lead to better material construction to effectively control the infection. The objective of this thesis is to study the initial adhesion event of C. albicans grown in glucose-based medium and galactose-based medium to Fluorinated Ethylene Propylene(FEP) and use Secondary Ion Mass Spectrometry(SIMS) to study the surface structural differences between these two cell surfaces with an attempt to correlate surface functionalities to the adhesion results. A freeze-drier with ultimate vacuum less than 1.00x10-9torr was constructed "in house" to freeze-dry cells for SIMS analysis. A filtration method was used to prepare a smooth layer of cells to be freeze-dried. Scanning Electron Microscopy showed that the freeze-dried cells appeared intact. High resolution SIMS spectra were obtained from these freeze-dried cell surfaces and Linear Discriminant Function Analysis combined with Principle Components Analysis were used to analyze the SIMS spectra. The results showed that the surfaces of glucose-grown cells contained more hydrophobic amino acid residues relative to those of galactose-grown cells. These hydrophobic amino acid residues probably promoted the adherence of glucose-grown cells to FEP surface. The results obtained in this study suggest that hydrophobic interactions are important in the initial attachment of C. albicans to FEP surface. The SIMS spectra presented in this study were the first report of the freeze-dried C. albicans.

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