Biomaterial Interfaces

Room: 4C - Session BI-ThP

Biomaterial Interfaces Poster Session

BI-ThP1 HAp/Chitosan Composites from Electrospinning Technique, *F. Vazquez-Hernandez, S.A. Lopez-Haro,* UPIITA-IPN, Mexico, ***C.O. Mendoza-Barrera***, Universidad Veracruzana, Mexico, *M.A. Melendez-Lira,* CINVESTAV-IPN, Mexico, *M.L. Albor-Aguilera, E. Diaz-Valdez*, FCFM-IPN, Mexico

Human bone is a hidroxyapatite HAp ($HCa_5O_{13}P_3$) and collagen based composite. Actually many methods and techniques have been developed and applied to design advanced materials for bone replacent. An stratergy to biomimic bone tridimentional structure, composition and mechanical properties is mimic it at nanoscale. In other words, selectively incorporate nano particules of apatites into a polymeric matrix by controlling structure and composition of the fibres. In this work we present the preliminary results of HAp/Chitosan composites prepared via electrospinning technique. Chitosan fibers were spun from aqueous solutions (pH= 3) and nano particles of HAp were added prior to the deposition. Prior and after the deposition the compositional and structural characteristics were verified by using scanning electron microscopy SEM, energy dispersive spectroscopy EDS, x-ray diffraction XRD, Raman spectroscopy and Fourier transform infrared spectroscopy FTIR. Energy dispersive spectroscopy and x-ray diffraction confirmed that the mineral deposits were hydroxyapatite and calcium phosphate monobasic MCP ($CaH_4\ O_8\ P_2$), both of them apatites present in bone while Fourier transform infrared studies FTIR showed the characteristic 1220-1020 cm^{-1} chitosan region in agreement with Raman results.

BI-ThP2 TOF-SIMS Imaging Study on Water Soluble and Organic Soluble CdSe/ZnS Core/Shell Quantum Dots, ***T.G. Lee***, *H. Min,* KRISS, Korea, *Y. Kim, S.J. Lim,* POSTECH, Korea, *D.W. Moon,* KRISS, Korea, *S.K. Shin*, POSTECH, Republic of Korea

Water-soluble CdSe/ZnS core/shell quantum dots capped by 3-mercaptopropionic acid(MPA) have been studied by using time-of-flight secondary ion spectroscopy (TOF-SIMS) imaging analysis. TOF-SIMS images provide direct evidence of local chemical information on the quantum dot surfaces. The water-soluble quantum dots can be conjugated with protein, DNA and other biomolecules, and thus be useful to applying to bioimaging and biosensing. These quantum dots were generated by converting original organic soluble ligands to MPA ligands. We characterized both surfaces of water soluble and organic soluble quantum dots and confirmed the successful exchange of ligands by using TOF-SIMS images of ligand molecules and molecular metal adducts.

BI-ThP3 The Effect of TOF-SIMS Ion Sources on the Fragmentation Pattern of Adsorbed Protein Films, ***S. Muramoto,*** University of Washington, *D.J. Graham,* Asemblon, Inc., *R. Michel,* University of Washington, *M.S. Wagner,* Proctor & Gamble Co., *T.G. Lee, D.W. Moon,* Korea Research Institute of Standards and Science, *L.J. Gamble, D.G. Castner*, University of Washington

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is a powerful surface analysis technique for the characterization of organic surfaces due to its high surface sensitivity, molecular specificity, and high mass resolution. However, the fragmentation patterns of positive secondary ions from adsorbed proteins, produced by primary ion bombardment, are complex due to multiple fragments originating from each of the 20 amino acids present in proteins. Therefore, the multivariate analysis technique principal component analysis (PCA) was used to identify fragment peaks that vary significantly between spectra. This study utilized Cs^+, Au^+, Au_3^+, Bi^+, Bi_3^+, Bi_3^{++}, C_{60}^+, and C_{60}^{++} ion sources to generate mass spectra for five single-component proteins (albumin, fibrinogen, lysozyme, collagen and immunoglobulin G) adsorbed onto mica. With the use of PCA, we observed differences in fragmentation patterns among the ion sources for all proteins. However, the differences between ion sources for a given protein were smaller than the differences between different proteins. This allows the type of protein to be identified regardless of the ion source used. For each of the five proteins, the fragmentation patterns generated from Cs^+, Au^+ and Au_3^+ ions were differentiated at the 95% confidence level. For the Bi ion sources, the fragmentation patterns from the Bi^+ ions were differentiated from the fragmentation patterns from the Bi_3^+ and Bi_3^{++} ions at the 95% confidence level, but the fragmentation patterns from the Bi_3^+ and Bi_3^{++} ion sources could not be differentiated. From the PCA loadings it appears that there may be a dependency between the mass of the ion source and the number of carbon atoms in the emitted secondary ions (i.e., the Au ions may produce more smaller fragments than the Cs ions). Also, sampling depth may play a role in the observed differences between monoatomic and the cluster ion beams. The results from this study show how the combination of TOF-SIMS with PCA can be used to identify the influence of primary ion type on secondary ion fragmentation patterns.

BI-ThP4 Synthesis of CdSe Nanoparticles by Nanocaged Protein sHSP 16.5, ***J.-W. Park***, *S.H. Moh, N.H. Kim, K.K. Kim, Y.H. Roh*, Sungkyunkwan University, Korea

Synthesis of semiconductor nanoparticles is one of the interesting research fields in nanotechnology. The semiconductor nanoparticles are applicable to various electronic device, optical devices and bio sensors. Since the energy levels of the semiconductor nanoparticles are quantized depending on their size and shape, therefore it is important to synthesize semiconductor nanoprticles with the same size and shape. Quantum-effect devices were actively investigated to solve these problems. In this study, we performed biomimetic approach to control these factors by using inorganic material (CdSe nanoparticle) and organic nanocaged proteins. Protein cage architectures can be utilized as size- and shape-constrained reaction environments for nanomaterial synthesis. Biomimetic fabrication could be performed in the optimized conditions using small heat shock protein 16.5 (sHSP16.5) mutant and ion complex of cadmium and selenium. A directed nucleation could be achieved through the specific residues on the negatively charged center domain of sHSP16.5. CdSe nanoprticles had the excellent size uniformity in the core of the mineralized sHSP16.5. For 2D array on the Si wafer, we treated chemical such as 3-aminopropyltrethoxysilane (APTES). The mineralized sHSP16.5 has highly binding affinity on the APTES treated Si wafer, which the reason is hydrogen interactions between amine group of the APTES and carboxyl group of sHSP16.5. These results give us to control arrangement of quantum dots on the Si wafer. This biomimetic approach will be possible to achieve advanced floating gate memory devices and single electron transistor in the near future.

BI-ThP5 Visible Light Activated Conjugated Polyelectrolytes as Antimicrobials, ***T Corbitt***, *L.K. Ista, K. Ogawa, S. Chemburu, G.P. Lopez,* University of New Mexico, *K. Schanze,* University of Florida, *D. Whitten*, University of New Mexico

Conjugated polyelectrolytes (CPs) are polymers that contain ionic solubilizing groups. These materials are water soluble and feature strong visible absorption and fluorescence. Recently we have shown that CPs functionalized with cationic groups such as trimethyl ammonium (1) and diazobicyclooctane bis quaternary salt (2) polyphenyleneethynylene display efficient light-activated biocidal activity against a variety of microorganisms including Escherichia coli, Cobetia marina, Pseudomonas aeruginosa and Bacillus anthracis, Sterne spores. When bacteria are mixed with aqueous solutions of the polymers the CPs form a surface coating on the microorganism. Irradiation of the bacteria surface-coated with polymers 1 or 2 leads to efficient deactivation of the bacteria as revealed by culturing and live/dead assays. We have also demonstrated that CPs are effective against bacteria when coated at near monolayer coverage on a surface. An additional format currently under investigation involves polymers with the repeat unit of 1 grown covalently from the surface of silica nanoparticles and microspheres (SGCP). The polymer coatings of the SGCPs are more rough and appear from studies using confocal fluorescence microscopy and electron microscopy to protrude from the surface. We find that the SGCP entrap Cobetia marina; live-dead assays of entrapped bacteria kept in the dark and irradiated with visible light reveal that bacteria entrapped on the surface of a 5 micron SGCP are killed while those kept for a similar period in the dark remain viable. We are currently determining whether the light-activated pathogen killing requires molecular oxygen and, if so, whether singlet oxygen is a key intermediate. Our observation that bacteria are not only killed but also degraded suggests that singlet oxygen may subsequently generate much more powerful reactive oxygen species. Structure-property relationships are being developed to optimize the biocidal effect of specific CPs, with the ultimate objective being to develop a new class of polymer-based materials and coatings that are highly effective antimicrobial agents with broad-spectrum activity. The CPs offer advantages over low molecular weight compounds in that they are relatively stable and easily adapted to form robust coatings. Their ease in processing indicates they can be incorporated into filtration devices, foams, paints and fibers for diverse protection applications.

BI-ThP6 Determination of the Surface pK_d of Carboxylic Acid and Amine-Terminated Alkanethiols Using Surface Plasmon Resonance, *K.P. Fears, **S.E. Creager**, R.A. Latour*, Clemson University

Alkanethiol self-assembled monolayers (SAMs) are widely used as model surfaces because they form very well-characterized monolayers over a broad range of surface chemistries. An important property for SAMs with ionizable functional groups is the surface dissociation constant (pK_d), which defines the charge-density for a given pH solution. Using surface plasmon resonance (SPR) spectroscopy, we developed a method for the direct measurement of the pK_d of COOH and NH_2-SAMs by combining the ability of SPR to detect the changes in mass concentration close to a surface and the shift in ion concentration over the surface as a function of surface charged density. An analytical study was first performed to theoretically predict the general shape of the expected SPR plots by calculating the excess mass of salt ions over the SAMs as a function of the difference between the solution pH and surface pK_d. SPR studies were then conducted to measure the shift in salt concentration as a function of bulk pH, with the resulting data being used to determine the pK_d for HS-$(CH_2)_{11}$-COOH SAMs to be 7.4 ± 0.2 (N=4, mean ± 95% C.I.) and 6.5 ± 0.4 (N=4, mean ± 95% C.I.) for HS-$(CH_2)_{11}$-NH_2 SAMs . These methods present a way to calculate the pK_d for charged SAM surfaces that is non-disruptive and minimally interactive with the surface, thus providing an accurate and direct measure of the pK_d of the surface.

BI-ThP7 Effect of PNIPAAM Chain Length on Thermal Responsive Properties and Cellular Adhesion, ***B.P. Andrzejewski**, J. Fenton, L.K. Ista, G.P. Lopez*, University of New Mexico

Poly(N-isopropyl acrylamide) (PNIPAAm) is one of the most extensively studied responsive materials exhibiting a thermally triggered molecular transition effecting hydration. Our poster will present work on surface grafted PNIPAAm by the controllable reaction of atom transfer radical polymerization (ATRP). We use ATRP to investigate the effects of polymer length on both thermal responsive and biological adhesion properties. X-ray photoelectron spectroscopy (XPS), contact angle measurements, ellipsometric thicknesses and biological attachment studies will be used to probe thermal responsiveness to the polymer chain length. By precisely varying the polymer length, we give insight into the chemical and physical properties of the surface that govern its thermal responsiveness and resulting cellular adhesive properties.

BI-ThP8 Universal Route for Synthesis of Protein Resistant Polymer Brushes by Surface-Initiated Atom Transfer Radical Polymerization, ***A. Hucknall**, A.J. Simnick,* Duke University*, B.D. Ratner,* University of Washington*, A. Chilkoti*, Duke University

The ability to resist non-specific protein adsorption is an important enabling technology for the design of biosensors and biomedical implants. We have previously shown that surface-initiated atom transfer radical polymerization (SI-ATRP) of oligoethylene glycol methacrylate (OEGMA) can be used to create exceptionally robust and non-fouling surface coatings. In our previous studies, examples of substrates modified with poly(OEGMA) brushes were limited to materials which support the formation of self-assembled monolayers (SAMs) capable of initiating SI-ATRP, such as gold, silicon and metal oxides. However, the surfaces of many technologically relevant materials, such as plastics, do not support SAM formation. This paper presents a simple method to modify the surface of virtually any material with a robust, non-fouling poly(OEGMA) brush by SI-ATRP. Surface initiator layers capable of supporting SI-ATRP were formed by two routes: (1) plasma polymerization of 2-chloroethyl methacrylate and (2) dip-coating of poly(vinylbenzyl chloride). These layers were then used to initiate SI-ATRP of OEGMA. XPS revealed that the poly(OEGMA) brushes formed by either route were indistinguishable from those formed on alkanethiol SAMs on gold. The ability of the resulting poly(OEGMA) layers to resist non-specific protein adsorption was evaluated by incubating the surfaces in undiluted fetal bovine serum for 12 hours-subsequent XPS analysis showed no detectable protein adsorption. Substrates were also incubated for 12 hours in a solution of human umbilical vein endothelial cells in serum containing media and no cell attachment was observed on the poly(OEGMA) coated substrates.

BI-ThP9 Modeling Force versus Distance Profiles of Terminally Anchored Poly (N-isopropyl acrylamide) with Self-Consistent Field Theory, ***S. Mendez**, B. Andrzejeweski, D.H. Keller, H.E. Canavan, G.P. Lopez, J.G. Curro,* University of New Mexico*, J.D. McCoy*, New Mexico Tech

Tethered polymers are widely used to control surface properties such as wettability or cell adhesion. By making thin films out of polymers that are thermo-responsive, we can modulate surface properties with changes in temperature. Specifically, we use poly(N-isopropyl acrylamide) (PNIPAM) since this exhibits lower critical solution temperature (LCST) behavior near 32°C in water. At temperatures below the LCST, the polymer is hydrated and swollen, whereas above the LCST, the polymer collapses, and when tethered, the surface becomes more hydrophobic. In the past we reported on a method of synthesizing thin films of terminally anchored PNIPAM from self-assembled monoloyers using atom transfer radical polymerization.[1] We used neutron reflectivity techniques to measure the polymer brush structure at temperatures above and below the solution LCST. To model the temperature-induced structural changes of these brushes, we employed self-consistent field (SCF) theory using as input the Flory-Huggins chi parameter extracted from the experimental polymer solution phase diagram.[2] As a continuation of that work, we used the SCF theory to calculate the force between the PNIPAM brush and a test wall as a function of wall separation distance, i.e., we generated force-distance profiles. The parameters that we varied were temperature, polymer surface coverage and molecular weight, and the interaction between the PNIPAM and the test wall. AFM techniques were employed to obtain force-distance profiles of PNIPAM samples. We found that the force-distance profiles predicted by the theory were in qualitative agreement with those from experiment. Our ultimate goal is to employ theoretical predictions to guide future efforts to optimize tethered PNIPAM for cell attachment/detachment applications.

[1] Yim et al, Macromolecules 2006, 39, 3420.
[2] Mendez et al, Macromolecules 2005, 38, 174.

BI-ThP10 Electrochemical Behavior of Electroactive Species in Nucleic Acid Monolayers of Different Chain Length, ***K. Wang**,* Polytechnic University*, M.A. Gaspar,* Columbia University*, R.A. Zangmeister,* National Institute of Standards and Technology*, R. Levicky*, Polytechnic University

Monolayers of immobilized nucleic acids (DNA) are promising experimental models for investigating fundamental properties of charged polymers at solid-liquid interfaces. We have investigated the charging behavior of single-stranded DNA polyelectrolyte brushes. In this study, voltammetric behavior of hexaamineruthenium(III) chloride (RuHex) in end-tethered single-stranded DNA monolayers of different strand lengths is investigated. The surface coverage of non-labeled DNA chains was determined independently with X-ray photoelectron spectroscopy (XPS). Our results show that, for DNA chains varying from 5 to 100 thymine nucleotides, the reduction potential of $RuHex^{3+}$ counterions associated with the DNA monolayer is predominantly a function of chain surface coverage and is rather insensitive to the chain length. However, the total charge passed to reduce the counterions to the 2+ oxidation state is predominantly a function of the total nucleotide number, given by the product of chain surface coverage and chain degree of polymerization. A model is proposed to explain the observed behavior. The dynamic evolution of the reduction peak area and potential are also investigated, providing a picture of the time dependence of the adsorption of $RuHex^{3+}$ into the monolayers. The research provides a method to estimate the chain coverage of non-labeled, end-tethered DNA chains with various chain lengths.

BI-ThP11 Tuning the Zeta Potential of Poly-L-Lysine Substrates for the Selective Immobilization of Nanoparticles and Biomaterials, ***N. Farkas**, J.A. Dagata,* National Institute of Standards and Technology*, K.F. Pirollo, E.H. Chang*, Georgetown University Medical Center

Colloidal systems composed of nanoparticles must be charge stabilized in order to prevent aggregation. In many applications of nanotechnology it is necessary to immobilize nanoparticles intact and dispersed on a substrate so that high-resolution imaging and characterization can be carried out. An essential first step in sample preparation therefore involves appropriately matching the zeta potential of nanoparticles in solution to the zeta potential of the substrate surface and adjusting the pH and ionic strength of the solution environment. Here we report a method for preparing patterned substrates with regions of optimally tuned surface zeta potential by combining fluid scanning probe microscopy and a recently reported surface zeta potential apparatus [P. J. Sides et al., Langmuir 22 (2006) 9765]. Specifically, we vary the zeta potential of a poly-L-lysine substrate over a range of approximately -60 mV $< \zeta <$ + 100 mV by exposure to UV/ozone and control nanoparticle adsorption from effectively zero to full monolayer coverage. Exposure through a mask produces local regions with positive and negative surface charge resulting in selective adsorption of nanoparticles. We demonstrate attachment, followed by particle size distribution, and zeta potential measurements, for hard and soft nanoparticles including 10- to 80-nm diameter gold nanoparticles and 30- to 80-nm diameter liposomes.

BI-ThP12 Chemical Characterization of Taq DNA Polymerase Adsorption on Different Surfaces, ***R. Canteri**, R. Dell'Anna, S. Forti, L. Lunelli, L. Pasquardini, L. Vanzetti, M. Anderle, C. Pederzolli*, Fondazione Bruno Kessler-irst - Italy

PCR (polymerase chain reaction) represents the most widely used method for amplification of defined DNA sequences in medical and biological applications. The most recent innovative technologies are based on PCR

reaction miniaturizion. In fact, reductions in reagent consumption lower costs and increase scalability, enabling genome-wide approaches. Due to the increased surface-to-volume ratio of microchip PCR, a crucial role is played by the internal surface. Effects related to the non specific surface adsorption of PCR reagents (e.g. the replicating enzyme DNA polymerase) become significant and may reduce the efficiency of DNA amplification. In this study we investigate the Taq (Thermus aquaticus) DNA polymerase adsorption on different material surfaces, namely silicon (with different deposited oxide layer), pyrex glass, chromium nitride, cyclic olefin co-polymer (COC), polycarbonate (PC), poly(methyl methacrylate) (PMMA), and polydimethysiloxane (PDMS) surface. We carry out analyses via time of flight secondary ion mass spectrometry (ToF-SIMS), providing a physical-chemical surface picture, and via immunofluorescence by using anti-Taq DNA polymerase monoclonal antibody, giving the surface distribution and the amount of the protein. By combining these different techniques a deeper insight into the mechanisms governing the non specific surface adsorption of PCR reagents is possible.[1]

[1]This work was accomplished in the framework of LaTEMAR (Laboratorio di Tecnologie Elettrobiochimiche Miniaturizzate per l'Analisi e la Ricerca - Laboratory of Miniaturized Electrobiochemical Technologies for Analysis and Research), Centre of Excellence funded by MIUR (Italian Ministry for Education, University and Research) grants - FIRB 2003-2004 - for public/private structures involved in research fields characterized by strategic value.

BI-ThP13 Surface Chemical and Geometric Determination of Neuronal Migration on Patterned Surfaces, ***W. Wang***, *A. Natarajan, P. Molnar, S. Lambert, M. Das, M. Stancescu, N. Bhargava, J.J. Hickman*, University of Central Florida

Highly organized neuronal networks exist in the brain and are formed by appropriate neuron migration during the developmental stage. In vitro, engineering the appropriate neuron migration pathways and controlling the destination of single migrating neurons has been a challenge due to the insufficient understanding of integrated physiochemical mechanisms that regulate this process. In this work, we show that with controlled surface chemistry and proper design of pattern geometry of the substrate, single neuron migration pathways and destinations can be controlled. However, more importantly, the mechanism of this migration of how the neuron populations respond to the different surface chemistry and pattern geometry has been investigated by time lapse morphological analysis. We recorded the dynamic neuron migration that occurs during the formation of patterned two-neuron circuits using embryonic hippocampal neurons, where the somal adhesion sites, axon and dendrites outgrowth pathways are precisely determined. The cellular patterns were maintained in a defined serum free culture medium. Substrate surfaces were modified with self-assembled monolayers and patterns formed by laser ablation through a quartz photo mask. The surface chemistry was analyzed utilizing X-ray photoelectron spectroscopy and contact angle measurements. The patterns were visualized by metal deposition and optical profilometry. The neurons were characterized by static and dynamic morphological analysis and immunocytochemistry. Synaptic connections were determined by dual-patch clamp electrophysiology. The neurons were observed to migrate to designed somal adhesion sites by leading edge extension along the designed neurite pathways using a previously unknown process. After soma attachment, axon and dendrite outgrowth then continued along the designed pathways. This result will contribute to the methods of designing neuron network formation in culture, for the study of neuron migration in vitro and sensor design and fabrication.

BI-ThP14 Surface Characterisation and Biological Response of Enzymatically Tailored, Surface-Coupled Polysaccharides Pectic Hairy Regions*, ***G. Ceccone***, *D. Gilliland, I. Liakos, F. Rossi,* EC-JRC-IHCP, Italy, *M. Morra, C. Cassinelli, G. Cascardo,* Nobil-Bio-Ricerche, Italy, *C. Della Volpe,* University of Trento, Italy, *R. Verhoef, H. Schols*, University of Wageningen, The Netherlands

The exploitation of the bio-active properties of polysaccharides covalently linked to materials surfaces is a rapidly growing area of biomaterials surface science. Recent findings on bioactivity of plant carbohydrate polymers are spurring an activity of biomolecular scouting and suggest that pectic polysaccharides are promising flexible molecules for novel bioactive surfaces. In this work we have investigated the properties of surface linked pectic rhamnogalacturonans(RG-I) fractions(MHRs) obtained by commercial enzyme preparations of homogenized vegetable tissue. MHRs were covalently linked to different substrates, namely polystyrene(PS), Titanium(Ti) and polycarbonates(PC) surfaces aminated by glow discharge plasma and analysed by XPS, ToF-SIMS, AFM, and contact angle measurement. Cell adhesion experiments using L-929 fibroblasts and Aortic Smooth Muscle cells(SMC) were performed to evaluate the effect of the MHRs nature on cell adhesion. Moreover, cells growth and specific alkaline phosphatase (ALP) activity of osteoblast-like SaOS2 cells were also measured. Surface analyses of different samples indicate that coupling of MHRs polysaccharides was successful for all substrates. XPS analysis of plasma amminated PS shows significant amount of N (13at%) related to the presence of amino groups. After MHRs coupling, strong increase of O/C ratio is detected, whilst nitrogen signal is still present indicating that the thickness of MHR layer is below the XPS sampling depth(<10nm).ToF-SIMS analysis supports the XPS data: amminated surfaces present CxHyN peaks expected in allylamine-like films, whilst large fragments peaks (m/z>250amu) are observed both on parent and on surface-coupled polysaccharides.AFM force-separation curves show that immobilization of MHRs significantly affects the interfacial forces with the absence of any attractive interaction until repulsive contact is reached. Results of cells experiments reveal that the structure of the immobilized MHRs (long vs short hair) has great influence on adhesion, morphology and cells enzymatic activity. In particular the long-haired MHRs are found less adhesive. Interestingly, specific ALP activity of the modified surfaces is upregulated respect to that of the control, suggesting that MHRs coated surfaces present interfacial properties suitable for osteoblast differentiation.

*This work is carried out within the EU STREP Project # 517036 (PECTICOAT).

BI-ThP15 Optically Responsive Nano-Composite Layers for Quantitative Label-Free Detection of Biospecific Interactions, *P. Buecker, E. Trileva, M. Himmelhaus,* ***R. Dahint***, University of Heidelberg, Germany

In a recent paper,[1] we presented a novel way of preparing densely packed, metal coated nanoparticle films for the label-free detection of binding events. The layer system is composed of dielectric nanoparticles, which are adsorbed onto a plain gold surface and subsequently metallized by deposition of gold colloid prior to electroless plating. Upon reflection of white light, the layers exhibit pronounced extinction peaks which shift to higher wavelengths when molecules adsorb onto the surface. For the same concentration and incubation time of octadecanethiol, an about fivefold higher red-shift of the extinction maximum was observed than reported for conventional surface plasmon resonance (SPR).[2] However, as no quantitative information existed on the amount of adsorbed molecules, which may be different for our nanoparticle layers and the plain gold surfaces used in standard SPR, no clear decision could be made regarding their sensitivity towards adsorbate layer thickness or mass density. Thus, the goal of the present study was to accurately determine the mass sensitivity of the nano-composite films in order to facilitate quantitative studies of binding events. For this purpose, self-assembled monolayers of simple and ethylene glycol terminated alkanethiols with various chain lengths were prepared on the nanoparticle coated substrates. The measured red-shift of the extinction spectrum upon molecule adsorption was related to the thickness and mass density of the films as determined by X-ray photoelectron spectroscopy. Special attention was paid to the question whether sensitivity decreases with increasing film thickness, as this could limit the use of the nanoparticle layers for biosensing applications, which often involve the detection of high molecular weight molecules. Experiments on antigen/antibody interactions show that the sensitivity factors determined for thin organic films can also be used to quantify the amount of surface bound protein in immunoreactions.

[1] R. Dahint, E. Trileva, H. Acunman, U. Konrad, M. Zimmer, V. Stadler, M. Himmelhaus, Biosensors & Bioelectronics, in press.

[2] L. S. Jung, C. T. Campbell, J. Phys. Chem. B 2000, 104, 11168-11178.

BI-ThP16 Synthesis Biocompatible Gold Nanorods, *S. Reed,* ***B. Ayres***, Portland State University

The ability to tune the optical properties of metal nanoparticles by changing their size and shape make them an ideal and diverse tool for biomedical applications. Challenges remain to utilizing nanomaterials for in vivo medical applications. By selecting benign compounds as synthons for nanoparticles it is predicted that toxicity can be greatly reduced. Furthermore, the resulting synthetic waste can be minimized and the process made more environmentally friendly and safe. We report nanoparticle-liposome composite materials that are stable, water soluble, and anticipated to be benign. Specifically, soy lecithin has recently been used to synthesize particles with these characteristics. These lipids are a cheap, readily available and non-toxic ligand for the synthesis of gold particles. Soy lipids form liposomes that function as nanoreactors in which particles form. We aim to change the shape and size of particles by manipulating these nanoreactors allowing for tuning of their optical properties. The optical applications of gold nanoparticles are of particular interest. Design of particles with a particular shape and size are desirable for use in vivo. Rod shaped nanoparticles absorb near infrared light that penetrates into deep tissue and presents a unique possibility to locate and treat maladies non-invasively. Using soy lecithin as a ligand, particles of a uniform size distribution can be created with reproducible results. Soy components have also shown promise for shape control of particles. Using these naturally occurring ligands, a series of gold nanoparticles have been synthesized and characterized. The resulting nanoparticles are stable for long periods with little aggregation. UV-Visible spectroscopy and transmission electron microscopy have been used to characterize size and

shape of the resulting nanoparticles. It is believed a plethora of components contained in soy could also play a role in particle synthesis. We have isolated a small number of these compounds and identified them using NMR and mass spectrometry. Active components have been identified which contain linoleate tails. Ethyl linoleate has been positively identified and its ability to effect the shape of the gold nanoparticles is under study. Synthetic ethyl linoleate is being used in parallel with ethyl linoleate recovered from the soy lecithin to reveal its role in shape control of the nanoparticles as well as the rate of reaction in particle synthesis.

BI-ThP17 Surface Plasmon Resonance Microscopy Combined with a Novel Microfluidic System for High-Throughput Immunoassays*, J. Liu, M.A. Eddings, B.K. Gale,* ***J.S. Shumaker-Parry***, University of Utah

Surface plasmon resonance (SPR) microscopy provides quantitative, real-time information about adsorption and desorption on an SPR-active sensor surface with high spatial resolution. Label-free, high-throughput analysis of biomolecule interactions is made possible by combining patterned biomolecule immobilization with SPR microscopy. Typically, the biomolecules are immobilized ex situ using a pin-based microspotting device with many parameters that must be well-controlled in order to create an active and reliable sensor surface. We demonstrate the combination of a high-throughput microfluidic device with SPR microscopy for quantitative, in situ antibody immobilization. The microfluidic device provides 48 separate flow channels that can be used simultaneously for antibody immobilization and subsequent antibody-antigen interaction analysis. Because the biomolecules can be immobilized in situ, exposure to harsh environments can be avoided, a major benefit for protein immobilization. In addition, the biomolecule immobilization process can be monitored in real time by SPR microscopy and characterized quantitatively. Applications in immunoassay development for studying patient immunogenic response to antibody-based drugs will be described.

BI-ThP18 Characterization of Plasma Polymerized Immunosensor by XPS, SPR and ToF-SIMS*,* ***E.N. Newman****, F. Cheng, L.J. Gamble, K. Bomsztyk, D.G. Castner*, University of Washington

Surface treatment of polypropylene with plasma polymerized acrylic acid (PPAA) has been used to fabricate an immunosensor. This study examines both the amount and the bioactivity of the immobilized antibody. A comparison of PPAA - based strategy to the traditional coupling chemistry onto self-assembled monolayers was done using X-ray photoelectron spectroscopy (XPS) and surface plasmon resonance (SPR). XPS results for the PPAA surfaces indicate that 1) PPAA can be deposited onto various substrates (e.g., polypropylene, Si wafer and gold), 2) ~ 50% of the low-power, deposited PPAA film dissolves after soaking overnight in water, and 3) the N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide / N-hydroxysuccinimide method activates carboxyl groups at PPAA surface and allows proteins to be coupled to it. SPR provides a quantitative comparison of the amount of anti-ferritin immobilized by two strategies and the amount of ferritin the immobilized anti-ferritin binds. In addition, time-of-flight secondary ion mass spectrometry (ToF-SIMS) is used to investigate the structure of the PPAA surface as well as the structure of the immobilized anti-ferritin. Our results suggest that the PPAA - based strategy improves the biological activity and stability of the immobilized antibodies.

BI-ThP19 Label-free Plasmonic Detection of Biomolecular Binding by a Single Gold Nanorod*,* ***G.J. Nusz****, S.M. Marinakos, A.C. Curry,* Duke University*, A.B. Dahlin, F. Hook,* Lund University, Sweden*, A. Wax, A. Chilkoti*, Duke University

We report the use of isolated gold nanorods as plasmonic transducers to detect the binding of streptavidin to biotin-conjugated nanorods on a surface in real time by tracking the wavelength shift of their resonant scattering spectrum using a darkfield microspectroscopy system. The limit-of-detection of streptavidin binding by a single biotinylated nanorod is 1 nM. An analytical model is presented that provides a rational framework from which optimal nanoparticle geometries can be predetermined for a specified detection experiment. In addition, the model provides a method for quantifying the number of molecules bound to the nanorod surface based on the resonant wavelength shift. Signal saturation occurs upon the binding of approximately 700 streptavidin molecules to the surface of gold nanorods that are immobilized on a glass substrate, with a detection limit of approximately 40 protein molecules per nanorod. The limits of molecular detection that can be theoretically achieved by a single nanorod are discussed as well as the prospects of detection of single receptor-analyte binding events in real-time.

BI-ThP20 Preparation of High Resolution SPR Imaging Microarray Using Polymeric Micropatterns*, J. Jung,* Seoul National University, Korea*, J. Yuk, K. Ha,* Kangwon National University, Korea*,* ***J. Hyun***, Seoul National University, Korea

In this paper, we demonstrate a simple method to fabricate SPR imaging microarrays using polymer micropatterns. The use of thick polymeric micropatterns in imaging SPR microarray passivates the region by removing SPR signals completely or by saturating the SPR signal far beyond the detection range in SPR imaging. Two schemes to create polymeric micropatterns on the surface were demonstrated by micropatterning a thick insulating layer before depositing a metal layer or after depositing a metal layer. A biotin-streptavidin system was successfully performed to verify the systematic binding of biomolecules and the adsorption of cell culture media on the microarrays was quantitatively characterized. This SPR microarray can be applied in a variety of areas including protein adsorption, cell research, diagnosis of diseases, and more.

BI-ThP21 Development of Metal Polymer Based Hybrid Micro Channel Network in bio-MEMS*, M. Dhayal, R.R. Pandey, S.C. Jain,* ***K.K. Saini***, National Physics Laboratory, India

In this paper development of cost effective polymeric material based micro devices using soft lithography techniques had been discussed. This includes polymer based micro fluidic devices for bioengineering applications to study the self-assembly of bio-molecules in bio-MEMS. We had investigated the effects of diffusing transition metals into soft polymer based micro channel network (MCN) to control the surface charge and chemistry. These inorganic coatings and metal particle diffused into the MCN has been derivatised with various organic functionalities. This process can lead to novel characteristics of these devices for different bioengineering applications including bio-molecules separation and controlled electro osmotic mobility.

BI-ThP22 Fabrication of Micron-sized Retroreflectors*,* ***T. Sherlock****, S.M. Kemper, P. Ruchhoeft,* University of Houston*, R.L. Atmar,* Baylor College of Medicine*, R.C. Willson*, University of Houston

We have fabricated micron-sized retroreflectors (structures that return incident light directly back to the source) and have shown that they are extremely bright and detectable over a large range of angles when inspected with a simple optical microscope. These retroreflectors are part of an ultra-sensitive detector platform for sensing small quantities of virus particles, bacteria, DNA, RNA, or any variety of molecules of interest. In particular, we are targeting our first generation sensor to detect Norwalk virus particles. In this proposed system, the base of the retroreflector is decorated with antibodies to the virus, and, if present, the virus particles are captured by this surface. After capture, gold nanoparticles, coated with a secondary antibody, are introduced into the system, attach to the virus, and drastically reduce the retroreflector brightness with a specific, well-understood spectral signature. If no virus is present, the reflectivity is unaffected. We have measured the reflectivity versus 40nm diameter gold nanoparticle surface density and have found a 40% reduction in signal for 100 nanoparticles per square micron when illuminated with broad-band light. The base of the retroreflector is about 4 square microns in size, yielding a detection sensitivity of hundreds of particles. Further optimization of particle size and illumination wavelength is expected to increase this sensitivity substantially. Retroreflectors are fabricated by coating a silicon wafer with 2.5 microns of polyimide and 200nm of resist. A lithography step is used to generate the retroreflector pattern as openings in the resist and a 50nm thick nickel coating is deposited using thermal evaporation. After a lift-off step, which leaves behind only the nickel that coated the base of the resist openings, the patterns are transferred into the polyimide in an O_2/CF_4 reactive ion etch, leaving the retroreflecting structure with very straight relatively smooth walls. Next, gold is evaporated to coat the base of the structure and a directional evaporation step is used to cover all but the sensor base with aluminum. The gold is selectively functionalized with amine-reactive thiol molecules which serve as a platform for attaching antibodies, oligonucleotides, or other detector molecules.

BI-ThP23 Patterning Live Bacterial Cells for Biological Applications*, Z.Y. Suo,* ***R. Avci****, P. Rugheimer, X.H. Yang, Y. Idzerda, D.W. Pascual*, Montana State University

The immobilization of live bacterial cells in a controlled fashion in well-defined patterns will have many applications in biosensors, and in biomedical and fundamental biological studies. The surface antigens, fimbriae and flagella of Salmonella typhimurium and Escherichia coli and corresponding antibodies were used to demonstrate the immobilization of live bacteria in well-defined patterns. The leashing of live bacterial cells was achieved on antibody-modified substrates of gold, silicon and glass. The tendency of bacterial cells to remain adhered (leashed) only to the antibody-modified areas was used to fabricate microarray patterns whose

size can be controlled down to a micron scale. Patterns are generated with either a focused ion beam milling system or a microplotter. Cells patterned in this way retain their viability for at least six hours in a PBS buffer solution and are capable of regeneration if incubated in a growth medium. These microarray patterns can serve as prototype sensors which are able to capture targeted pathogens including bacteria, virus and proteins. For example, we have already demonstrated the use of such microarrays as a bacterial sorting system, in which a pre-targeted bacterial strain is captured and isolated from a mixed culture of microorganisms. The technique offers a reliable approach for fundamental microbiological research on the behavior of bacteria in an immobilized mode, as microorganisms respond to environ¬mental changes. For example, we observed that individual S. typhimurium cells gradually adjust their orientation from a "lying down" to a "standing up" position during regeneration, presumably trying to leave their position in search of more food. In such a struggle, immobilized cells produce a larger number of flagella as compared with planktonic cells, as confirmed by SEM and AFM studies.

BI-ThP24 A Bio-MEMS Device for Measuring Contractile Forces of Cultured Myotubes on Microfabricated Cantilevers, ***K.A. Wilson****, M. Das, P. Molnar,* University of Central Florida*, K.J. Wahl, R.J. Colton,* U.S. Naval Research Laboratory*, J.J. Hickman*, University of Central Florida

The boom in the semiconductor manufacturing industry of the past three decades has yielded a vast array of techniques for fabricating devices with micro to nano-scale features. Concomitantly, advances in biotechnology have opened new avenues for the application of these technologies in the form of gene and protein arrays, lab-on-a-chip devices and biological micro-electromechanical systems (Bio-MEMS). To date, application of these technologies has largely focused on the study of biomolecules and single cells or cell types. However, these technologies also hold great promise for the study of complex cellular and tissue interactions that are of critical importance when developing new drug therapies for disease and catastrophic injury. A tissue type of broad interest with regard to drug development and basic cell biology is skeletal muscle, which is affected by a variety of pathological conditions such as Parkinson's, ALS, and muscular dystrophy. For this reason we have developed a Bio-MEMS device based on microfabricated silicon cantilevers for the controlled, real-time interrogation of embryonic rat myotubes as a high-throughput test bed for drug discovery and basic science. The cantilevers were fabricated using standard photolithographic and micromachining techniques. The surfaces of the cantilevers were then modified using an amine-terminated alkylsilane SAM (DETA) to improve cellular adhesion, growth and differentiation. Dissociated embryonic rat myocytes were cultured for 7-10 days in a defined serum-free medium until contractile myotubes had formed. Monitoring and interrogation of the myotubes was accomplished using an AFM detection system of our own design, which consisted of a microscope, photodiode laser, position sensitive detector, field stimulation chamber, and a computer with data acquisition and analysis software. This simple system allows the real-time, high-throughput analysis of the physiological properties of the contracting myotubes. With this system we have shown the ability to selectively control the frequency and magnitude of myotube contraction as well as induce and observe physiological phenomena such as tetanus and fatigue. Contraction forces were calculated using a modified Stoney's equation for bending of a cantilever due to thin film stress. Ongoing work will allow the selective patterning and co-culture of neuronal cell types with myotubes for studying the neuromuscular junction and in vitro biological circuits.

BI-ThP25 Surface Modification and Photolithographic Patterning of Microelectrode Arrays for Cell-Based Biosensor Applications, ***A. Natarajan****, N. Bhargava, P. Molnar, M. Das, J.J. Hickman*, University of Central Florida

A major research area in the field of cell-based biosensors and pharmaceutical testing is the development of functional cell-based networks and their integration with silicon-based platforms. The development of a hybrid cell-electrode system could also aid in understanding neuronal circuits, cardiac physiology and function, and the interactions between these cells. Using surface chemistry, we have developed a technique to first modify the surface of commercially available microelectrode arrays and glass using self-assembled monolayers (SAM). This is done using a cell-adhesive SAM like trimethoxysilylpropyldiethylene-triamine (DETA). Patterns are then made on the microelectrode arrays using a photolithography based method with a quartz mask that defines and guides neuron attachment and development. The patterned surface is then backfilled with an appropriate cell-repulsive SAM like perfluoroalkyltrichlorosilane (13F). The surfaces have been characterized by both X-ray Photoelectron Spectroscopy (XPS) and contact angle measurements. Dissociated Embryonic hippocampal cells, in a serum-free medium, were cultured on these patterned microelectrode arrays in order to create neuronal networks with directed synaptic connectivity. The cells were characterized by morphological analysis as well as immunocytochemistry. The functionality of these networks was further studied using long term recording of the electrical activity of these cells in the presence and absence of toxins. We will report on the characterization of these devices as well as the methods developed for toxin detection and elucidation using these devices. We have also developed a technique to look at myocardial tissue function by manipulating surface chemistry in order to pattern and guide the growth of actively beating monolayers of neonatal rat cardiomyocytes on glass. These devices have also been characterized for their response to toxins and its effect on cardiac physiology. These hybrid systems are being used to further study basic neuronal networks and cardiac physiology properties like functional reentry. More importantly the devices are being applied to study toxic effects in pharmacological evaluation and to study disease models like Arrhythmia.

Authors Index

Bold page numbers indicate the presenter

— A —

Albor-Aguilera, M.L.: BI-ThP1, 1
Anderle, M.: BI-ThP12, 2
Andrzejeweski, B.: BI-ThP9, 2
Andrzejewski, B.P.: BI-ThP7, **2**
Atmar, R.L.: BI-ThP22, 4
Avci, R.: BI-ThP23, **4**
Ayres, B.: BI-ThP16, **3**

— B —

Bhargava, N.: BI-ThP13, 3; BI-ThP25, 5
Bomsztyk, K.: BI-ThP18, 4
Buecker, P.: BI-ThP15, 3

— C —

Canavan, H.E.: BI-ThP9, 2
Canteri, R.: BI-ThP12, **2**
Cascardo, G.: BI-ThP14, 3
Cassinelli, C.: BI-ThP14, 3
Castner, D.G.: BI-ThP18, 4; BI-ThP3, 1
Ceccone, G.: BI-ThP14, **3**
Chang, E.H.: BI-ThP11, 2
Chemburu, S.: BI-ThP5, 1
Cheng, F.: BI-ThP18, 4
Chilkoti, A.: BI-ThP19, 4; BI-ThP8, 2
Colton, R.J.: BI-ThP24, 5
Corbitt, T: BI-ThP5, **1**
Creager, S.E.: BI-ThP6, **2**
Curro, J.G.: BI-ThP9, 2
Curry, A.C.: BI-ThP19, 4

— D —

Dagata, J.A.: BI-ThP11, 2
Dahint, R.: BI-ThP15, **3**
Dahlin, A.B.: BI-ThP19, 4
Das, M.: BI-ThP13, 3; BI-ThP24, 5; BI-ThP25, 5
Della Volpe, C.: BI-ThP14, 3
Dell'Anna, R.: BI-ThP12, 2
Dhayal, M.: BI-ThP21, 4
Diaz-Valdez, E.: BI-ThP1, 1

— E —

Eddings, M.A.: BI-ThP17, 4

— F —

Farkas, N.: BI-ThP11, **2**
Fears, K.P.: BI-ThP6, 2
Fenton, J.: BI-ThP7, 2
Forti, S.: BI-ThP12, 2

— G —

Gale, B.K.: BI-ThP17, 4
Gamble, L.J.: BI-ThP18, 4; BI-ThP3, 1
Gaspar, M.A.: BI-ThP10, 2
Gilliland, D.: BI-ThP14, 3
Graham, D.J.: BI-ThP3, 1

— H —

Ha, K.: BI-ThP20, 4
Hickman, J.J.: BI-ThP13, 3; BI-ThP24, 5; BI-ThP25, 5
Himmelhaus, M.: BI-ThP15, 3
Hook, F.: BI-ThP19, 4
Hucknall, A.: BI-ThP8, **2**
Hyun, J.: BI-ThP20, **4**

— I —

Idzerda, Y.: BI-ThP23, 4
Ista, L.K.: BI-ThP5, 1; BI-ThP7, 2

— J —

Jain, S.C.: BI-ThP21, 4
Jung, J.: BI-ThP20, 4

— K —

Keller, D.H.: BI-ThP9, 2
Kemper, S.M.: BI-ThP22, 4
Kim, K.K.: BI-ThP4, 1
Kim, N.H.: BI-ThP4, 1
Kim, Y.: BI-ThP2, 1

— L —

Lambert, S.: BI-ThP13, 3
Latour, R.A.: BI-ThP6, 2
Lee, T.G.: BI-ThP2, **1**; BI-ThP3, 1
Levicky, R.: BI-ThP10, 2
Liakos, I.: BI-ThP14, 3
Lim, S.J.: BI-ThP2, 1
Liu, J.: BI-ThP17, 4
Lopez, G.P.: BI-ThP5, 1; BI-ThP7, 2; BI-ThP9, 2
Lopez-Haro, S.A.: BI-ThP1, 1
Lunelli, L.: BI-ThP12, 2

— M —

Marinakos, S.M.: BI-ThP19, 4
McCoy, J.D.: BI-ThP9, 2
Melendez-Lira, M.A.: BI-ThP1, 1
Mendez, S.: BI-ThP9, **2**
Mendoza-Barrera, C.O.: BI-ThP1, **1**
Michel, R.: BI-ThP3, 1
Min, H.: BI-ThP2, 1
Moh, S.H.: BI-ThP4, 1
Molnar, P.: BI-ThP13, 3; BI-ThP24, 5; BI-ThP25, 5
Moon, D.W.: BI-ThP2, 1; BI-ThP3, 1
Morra, M.: BI-ThP14, 3
Muramoto, S.: BI-ThP3, **1**

— N —

Natarajan, A.: BI-ThP13, 3; BI-ThP25, **5**
Newman, E.N.: BI-ThP18, **4**
Nusz, G.J.: BI-ThP19, **4**

— O —

Ogawa, K.: BI-ThP5, 1

— P —

Pandey, R.R.: BI-ThP21, 4
Park, J.-W.: BI-ThP4, **1**
Pascual, D.W.: BI-ThP23, 4
Pasquardini, L.: BI-ThP12, 2
Pederzolli, C.: BI-ThP12, 2
Pirollo, K.F.: BI-ThP11, 2

— R —

Ratner, B.D.: BI-ThP8, 2
Reed, S.: BI-ThP16, 3
Roh, Y.H.: BI-ThP4, 1
Rossi, F.: BI-ThP14, 3
Ruchhoeft, P.: BI-ThP22, 4
Rugheimer, P.: BI-ThP23, 4

— S —

Saini, K.K.: BI-ThP21, **4**
Schanze, K.: BI-ThP5, 1
Schols, H.: BI-ThP14, 3
Sherlock, T.: BI-ThP22, **4**
Shin, S.K.: BI-ThP2, 1
Shumaker-Parry, J.S.: BI-ThP17, **4**
Simnick, A.J.: BI-ThP8, 2
Stancescu, M.: BI-ThP13, 3
Suo, Z.Y.: BI-ThP23, 4

— T —

Trileva, E.: BI-ThP15, 3

— V —

Vanzetti, L.: BI-ThP12, 2
Vazquez-Hernandez, F.: BI-ThP1, 1
Verhoef, R.: BI-ThP14, 3

— W —

Wagner, M.S.: BI-ThP3, 1
Wahl, K.J.: BI-ThP24, 5
Wang, K.: BI-ThP10, **2**
Wang, W.: BI-ThP13, **3**
Wax, A.: BI-ThP19, 4
Whitten, D.: BI-ThP5, 1
Willson, R.C.: BI-ThP22, 4
Wilson, K.A.: BI-ThP24, **5**

— Y —

Yang, X.H.: BI-ThP23, 4
Yuk, J.: BI-ThP20, 4

— Z —

Zangmeister, R.A.: BI-ThP10, 2