SYMPOSIUM M
Developing Nano-Bio Interfaces
March 29 - 31, 2005

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* Invited paper
Design Rules for Biological Adhesives. Deborah Leckband, 1 Chemical and Biomolecular Engineering, University of Illinois, Urbana, Illinois; 2 Chemistry, University of Illinois, Urbana, Illinois.

Biological adhesives are central to tissue organization, and they play a key role in maintaining the structural integrity of all soft tissues. The organization of intercellular adhesive junctions is mediated by a complex array of proteins with different architectures, kinetic properties, and binding energies. This talk focuses on the relationship between the structures of these essential proteins and their functions as both molecular adhesives and structural elements that control the nanoscale organization of cell-cell junctions. In particular, we use direct measurements of the forces between membranes to quantify the impact of adhesion protein architectures on the mechanical properties of intercellular junctions. Many adhesion molecules exhibit modular, multi-domain architectures. With our unique approach, we directly demonstrated that this molecular design generates proteins that form multiple bonds spanning different membrane gaps. These features have a significant impact on not only the organization of intermembrane junctions, but also on their assembly and their stabilization under force. Our investigations are revealing fundamental design rules for these complex molecular machines.

SESSION M1: Control of Protein, Membrane, and Cellular Interfaces

Thursday, March 29, 2005
Room 3009 (Moscone West)

8:30 AM M1.1
Design Rules for Biological Adhesives. Deborah Leckband, 1 Chemical and Biomolecular Engineering, University of Illinois, Urbana, Illinois; 2 Chemistry, University of Illinois, Urbana, Illinois.

9:00 AM M1.2


Nanophase materials are emerging as prime candidates for the next-generation of biomaterials because, depending on the size of their nanoscale features, such materials have been shown to elicit selective, directed responses from cells. Differences in the sizes of nanoscale features (compared to larger scale features) have been attributed to the type and conformation of proteins (such as fibrinogen and vitronectin) adsorbed to the substrate material. Although integrin-mediated pathways are involved in cell adhesion, to date, other underlying protein-related mechanisms occurring at the cell-material interface are not completely understood. For this reason, the current study utilizes nanoparticle-decorated surfaces (prepared as discussed in reference 3) as an interface between cells and materials to investigate how nanoscale surface features modulate protein behavior and, thus, the subsequent adhesion of different mammalian cells. This research aims to develop protein-decorated nanoscale materials by investigating and quantifying protein-material interface interactions. Development of well-characterized nanoscale interfaces could lead to novel biomaterial formulations for prosthetic devices and tissue engineering. 1, 2 D. Ballard, R. H. Doren, R. W. Siegel, and R. Bizios, Journal of Biomedical Materials Research 51, 475 (2000). 2 T. J. Webster, L. S. Schadler, R. W. Siegel, and R. Bizios, Tissue Engineering 7, 291 (2001). 3 Ballard, J.D., L.M. Dell’Acquabellavitis, R. Bizios, and R.W. Siegel, RSS Proceedings, Fall 2004, Symposium AA: Applications of Micron and Nanoscale Materials in Biology and Medicine (2004).

9:15 AM M1.3
Site-Selective and 3-Dimensional Cell Culture on Super-Hydrophobic/Super-Hydrophilic Patterns. Osamu Takei 1, Yuning Wu 1, Masuo Kouno 2, Yasushi Inoue 1, Akira Rico 1, Hirokazu Hanai 3, and Nagahiro Satoh 1, 4.

1 EcTopia Science Institute, Nagoya University, Nagoya, Japan; 2 Department of Biotechnology, Graduate School of Engineering, Nagoya University, Nagoya, Japan; 3 Department of Materials, Physics and Energy Engineering, Graduate School of Engineering, Nagoya University, Nagoya, Japan; 4 Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Nagoya, Japan.

Ultra-water-repellent (UWR) film has a water contact angle of over 150°. Generally, commercial sprays for water repellent treatment have a maximum water contact angle of ca. 130°. In scientific nomenclature, this state is referred to as high water repellency (HWR). What is the difference between UWR and HWR films? Indeed, both prevent water droplets from spreading out on their surface. The difference lies in the dynamic motion of the water droplets. A water droplet on an UWR surface moves readily upon receiving even a minute lateral force due to the surface's extremely low frictional co-efficient and its strong repulsion force. We have been successful to fabricate micron-patterns of super-hydrophobic super-hydrophilic from UWR films through a vacuum ultra violet lithography. We found the super-hydrophobic/super-hydrophilic pattern was useful to novel cell culture, that is, site-selective cell growth and 3-dimensional fabrication of cell. This potential is based on anti-bio-affinity of super-hydrophobic surface and bio-affinity of super-hydrophilic surface. In this presentation, we demonstrate site-selective culture of fibroblast and capillary blood vessel on the pattern. The fibroblast growth was observed on super-hydrophilic surface. The capillary blood vessel growth was also observed in only the super-hydrophilic surface. Furthermore, we found that the cell structure became tubular itself by controlling the wide of line patterns. These results are useful to the field of tissue engineering.

10:00 AM M1.4

We describe the formation of alternative patterned synapses in living T cells that are produced through substrate-imposed constraints on molecular motion in the supported membrane. Substrates displaying various configurations of 100 nm-wide chrome lines were fabricated by electron-beam lithography. Supported membranes, containing GPI-linked PMHC and ICAM1, were assembled on these prefabricated substrates by vesicle fusion. The chromosome retains barriers that restrict the motion of lipidic and proteins with an otherwise fluid and homogeneous supported membrane. Proteins on the T cell become subject to these restrictions as a result of interactions with their cognate ligands in the supported membrane. Spatial patterns within fully formed synapses are drives entirely by the geometry. The patterns differ from the wild type concerning the c-SMAC and p-SMAC as a result of the mechanisms by which the T cell drives protein rearrangements and the different geometric constraints frustrate these processes.

10:30 AM M1.5
Building with, Manipulating, and Interrogating Biomolecules at the Surface at Nanoscale Dimensions. Ashutosh Chilkoti, Biomedical Engineering, Duke University, Durham, North Carolina.

I will describe the work of my laboratory, along with that of collaborators at Duke University in two areas of Nanobiotechnology. In the first area, primarily in collaboration with Stefan Zauscher at Duke University, we have developed new methods to pattern proteins, biomolecules and synthetic polymers at the nanoscale by new variants of dip-pen nanolithography (DPN). Two examples of collaborative research with the Zauscher group will be presented; in the first example, I will describe a versatile method to pattern biomolecules by constructing nanoscale patterns of covalently immobilized biotin. The nanoscale patterns of biotin are then incubated with streptavidin, and the streptavidin patterns provide a versatile and flexible nanoscale template to immobilize diverse biomolecules by incubating their biotinylated conjugates from solution, driven by the tetramer architecture of streptavidin and the high affinity of the biotin-streptavidin interaction. I will present results that demonstrate the fabrication of stimulus-responsive nanoscale arrays of a genetically engineered polypeptide by DPN that enable a few hundred-protein molecules be captured from solution, displayed in a functionally active conformation at the surface, and then reversibly desorbed from the surface. Finally, I will describe a recent example from my laboratory, in which we have harnessed the catalytic power of enzymes to carry out enzymatic transformations at the nanoscale on a surface without the constraint of tethering the enzyme to the AFM tip. In a second area of research in my laboratory, work will be presented on exploiting the particle surface plasmon resonance (SPR) behavior of gold nanostructures for label-free protein detection. In our implementation of chip-based nanosensor SPR, we have chemisorbed chemically synthesized gold nanoparticles onto glass slides, functionalized the surface of the immobilized gold nanoparticles with biological ligands, and shown that transduction of a nanoparticle-decorated surface is capable of being detected by the shift in the extinction spectrum of individual nanoparticles. This assay is analogous to conventional, planar SPR with the added advantage of being performed in widely available, low-cost UV-visible spectrophotometers and its facile extension to array-based assays. The extension of this detection modality using gold nanorods and the increased sensitivity afforded by these anisotropic particles will also be discussed, as well as alternative methods to directly fabricate metal nanostructures on optically transparent materials by nanolithography.

11:00 AM M1.6
Protein Adhesion to Bare and Polymer-coated Nanostructures. Maureen Dyer, 1 Kristy M. Ainslie 1, Gaurav Sharma 2, Craig Grimes 3, 4 and Michael V. Pishko 1, 4.

1 Chemical Engineering, University of Illinois, Urbana-Champaign, Urbana, Illinois; 2 Chemical and BioEngineering, University of Illinois, Urbana-Champaign, Urbana, Illinois; 3 Department of Sustainable Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, Virginia; 4 Virginia–Virginia Tech Transportation Institute, Christiansburg, Virginia.
For an implanted sensor to be viable, it must be able to resolve. This method allows obtaining patterns as well as culture of the surface. The adsorbed protein was high in pCP.

"write" or "position" (pCP)

the modified alginic acids will be discussed.

the first step of the host response. A modified ELISA monitored tissue-material surface. Further, the microarchitecture of the wafers were used as model surfaces for developing the hyperbranching state University, University Park, Pennsylvania; Electrical stanlp materials and light; it does not necessitate mass transport or study, we immobilized alginic acid on amino-functionalized stainless steel surface through amide bonding between carboxylic group of the nickel coating. Blood compatibility through the prevention of platelet adhesion and some kinds of blood proteins adsorption. However, the mechanism of protein adsorption on a non-nanostructured surface is still unknown in detail, and some protein adsorbs on that surface. In order to make it possible that the alginic acid-immobilized surface prevents more kind of protein or leads to the selective adsorption of protein, the detailed adsorption mechanism should be clear. In this study, we immobilized alginic acid on amine-functionalized stainless steel surface through amide bonding between carboxylic group of alginic acid and amino group of the surface. The adsorbed protein was characterized and quantified by means of Fourier transform infrared reflection absorption spectroscopy (FT-IRRAS). The protein adsorption property on alginic acid-immobilized surface in phosphate buffered protein solutions with various pH and ionic strength was examined. This result showed that surface chemistries of alginic acid layers could accommodate the interaction between the biological proteins and synthetic materials. The detailed protein adsorption property on the modified alginic acids will be discussed.

An implanted sensor would aid in the treatment of the 5.3 million people worldwide who suffer from Type 1 or insulin-dependent diabetes. For an implanted sensor to be viable, it must be able to retain its accuracy and detection ability in vivo for extended periods of time. Introduction of a foreign material into the body generally induces a host response, beginning with protein adhesion to the implanted surface. The host response to an optimum sensor implanted in subcutaneous tissue was observed. A non-specific feature of the interaction between the sensor and the host, as a result in sensor failure due to isolation of the sensor from the environment it is intended to monitor. Studies have indicated that microporous materials are capable of maintaining vascular growth near the tissue-material interface. Therefore, the microstructure of the individual features, rather than the material comprising the features, appears to affect the host response. Taken together, these results suggest that nanofabricated surfaces could prevent host response induction while maintaining vascularization necessary for analyte detection by the sensor. Our initial studies have focused on preventing the first step of the host response. A modified ELISA monitored protein adhesion to a non-biological material that could be used as a casing for a biosensor or other implant. The materials used were composites of Ni-Co-Fe and nanowire arrays of the same composition. Protein adhesion was reduced on the nanowire arrays compared to the composite wafers. Reduction was most evident when the nanostructurized nanowires were placed in a magnetic field. To enhance resistance to protein adhesion, the possibility of coating the wafers with hyperbranched polymers, such as poly(ethylene glycol) was investigated using an in situ synthesis and modifications. Silicon wafers were used as model surfaces for developing the hyperbranching procedure. Poly(siloxane) and PEG were grafted in layers, ranging from one PAAm layer to a total of six alternating PAAm and PEG layers. Protein adsorption on wafers at each stage of polymer hyperbranching was lower than that on bare silicon wafers. By hyperbranching polymers on the nanowires, we hope to combine these protein resistant properties to further reduce protein adhesion. Decreasing protein adhesion would delay the host response to the coated implant, thereby increasing its efficacy and lifetime.

11:15 AM M1.7

Chemically Modified Alginic Acid Layers for Control of Protein Adsorption. Tonomiho Yoshida1, Kazuki Tsuru1,2, Satoshi Hayakawa1 and Akiyoshi Osada1,2; 1Faculty of Engineering, Okayama University, Okayama-shi, Okayama, Japan; 2Research Center for Biomedical Engineering, Okayama University, Okayama-shi, Okayama, Japan.

Polysaccharide coating is commonly known as cell- or protein-resistant coating. We have also reported that the chemical immobilization of alginic acid, natural polysaccharide, on stainless steel leads to blood compatibility through the prevention of platelet adhesion and some kinds of blood proteins adsorption. However, the mechanism of non-specificity of alginic acid layer on the surface is still unknown in detail, and some protein adsorbs on that surface.

To enhance resistance to protein adhesion, the possibility of coating the wafers with hyperbranched polymers, such as poly(ethylene glycol) was investigated using an in situ synthesis and modifications. Silicon wafers were used as model surfaces for developing the hyperbranching procedure. Poly(siloxane) and PEG were grafted in layers, ranging from one PAAm layer to a total of six alternating PAAm and PEG layers. Protein adsorption on wafers at each stage of polymer hyperbranching was lower than that on bare silicon wafers. By hyperbranching polymers on the nanowires, we hope to combine these protein resistant properties to further reduce protein adhesion. Decreasing protein adhesion would delay the host response to the coated implant, thereby increasing its efficacy and lifetime.

11:30 AM M1.8

Photocatalytic Patterning of Biomolecules. Jane P. Bearinger1, Amy L. Hidding5, Kwang Jen J. Wu2, Julie Hamilton1, Nan Shen1, Allen T. Christian1 and Jeffrey A. Hubbell5; 1CMS/MTP, LNL, Livermore, California; 2EPFL, Lausanne, Switzerland.

We have developed a novel method for patterning surface chemistry: Photocatalytic Lithography. This technique relies on parasitic stamp materials and light; it does not necessitate mass transport or specified substrates, and the wavelength of light should not limit feature resolution. We have demonstrated the utility of this technique through the patterning of single cells. Ellipsometry and gold substrates were modified with non-fouling coatings. Next, photocatalyst was selectively positioned on top of the coatings. Controlled patterning and removal of the non-fouling coatings then was achieved by local oxidation via activation of the photocatalyst with 660nm red LED light for about 5 seconds. Freshly patterned and bare regions of the substrates then were modified with adhesive chemistry. Materials and substrates were characterized by microdroplet experiments and Visible Absorption Spectroscopy (UV-VIS), Time-of-flight Secondary Ion Mass Spectrometry (ToF-SIMS) and atomic force microscopy (AFM). The surfaces subsequently were exposed to S1J fibroblast cells or fluorescently-tagged proteins. FITC-NeutrAvidin. Adsorption of the single cells and proteins was limited to areas of the adsorption chemistry, as confirmed through optical microscopy or fluorescence. Initial data suggests that Photocatalytic Lithography may overcome resolution limitations inherent to traditional photolithography and allow rapid lithographic processing with inexpensive optic systems and substrates. The technique also avoids pinholes that may form when patterning via mass transport bonding of chemistry to a surface. Furthermore, Photocatalytic Lithography is not substrate or chemistry dependent, and it is applicable to the study of biological functions interacting with synthetic materials. Presently, we are pursuing patterning at the nanoscale (smaller than the wavelength of light) via patterning of individual bacterium on substrates. We plan to array individual rod shaped bacteria (approximately 1 micron x 100 nanometers) in a high throughput manner and analyze bacterial expression as a function of constraining size, as well as culture media conditions.
Membrane proteins are diverse and highly versatile components of biological systems, having functions ranging from pores and pumps to sensors and energy transducers and more. Because of their high level of functional complexity, size, and order, membrane proteins are challenging targets for engineers with the possibilities of engineering devices functionalized by membrane proteins are very attractive. These proteins are naturally housed in lipid bilayers, amphiphilic membranes which orient the proteins and organize the membrane subcellular membranes. We wish to create and study artificial biomimetic membranes with the ultimate goal of incorporating membrane proteins therein, extending their functional lifetimes beyond hours and allowing their properties to be more fully exploited. Our initial work has centered on the creation, study, and characterization of the biomimetic polymer membranes. We have included a number of proteins, including mechano-sensitive channels, bacterial toxins, pore proteins, and, short peptides into polymer membranes. We present measurements demonstrating the functional incorporation of these proteins as well as significant increases in membrane lifetime as compared to conventional lipid bilayer systems. We have formed these hybrid polymer membranes on conventional Teflon supports as well as microfabricated substrates. We have also begun to work with biomimetic membranes formed from block copolypeptides, which have different properties and have intriguing potential as regards their synthesis, mechanical properties, and biological compatibility. We are developing a new family of active materials which derive their functional properties from membrane proteins. The development of this protein/polymer system should enable the creation of a number of devices, as well as use in the same processes that can be used for engineering other membrane proteins. This implies that once the learning curve necessary for successful production, insertion, and operation of a particular protein in an engineered environment has been successfully mastered, repeating the process will be somewhat easier for other proteins, facilitating a "plug-and-play" approach to membrane protein engineering.

2:00 PM M2.2
Development and Characterization of Planar Biomimetic Membranes with Well-Defined Polymer Tethers. Lisa Y. Hwang and Curtila W. Frank; Chemical Engineering, Stanford University, Stanford, California.

Polymer-tethered lipid bilayers have become widely studied as model biological membranes for both fundamental physical research and in the development of applications such as biosensors. In these biomimetic systems, the water-soluble polymer layer separates the membrane from the surface of the substrate. This prevents non-physiological interactions between the incorporated biophysical components and the substrate. Additionally, the polymer network allows the mechanical properties of the system to be carefully adjusted. The anchoring of the system to the substrate provides mechanical stability, yet can be tailored to accommodate the required mobility of the membrane components. We report on the development and characterization of lipid bilayers tethered to the surface by polymer chains. This supramolecular assembly starts with a mixture of lipopolymers and free lipids oriented at the air-water interface in a Langmuir film. The lipopolymer/free lipid monolayer is then transferred to a benzophenone-modified substrate by Langmuir-Blodgett deposition. The well-known light-induced reaction between the benzophenone moieties and C-H bonds covalently attaches the lipopolymer to the substrate. Then, the dry lipid polymer/lipid monolayer is subjected to vesicle fusion to complete the bilayer. We have chosen a glyco-lipopolymer as the polymer support for this study. The glyco-based polymer backbone closely mimics the native cellular environment of a glycolipid, and the lipid-like groups on the lipopolymer integrate into the phospholipid bilayer forming covalent points of attachment between the bilayer and polymer support. In this polymer, the lipid analogue is di(2-acetamido)alanine and the hydrophilic backbone monomer is D-glucose-2-propionate. Impacts of the lipopolymer/lipid composition and polymer conformation are systematically compared using atomic force microscopy to monitor the monolayer and bilayer homogeneity and roughness, and fluorescence microscopy to monitor the bilayer lateral mobility, and contact angle goniometry to monitor the wetting behavior of the monolayer. Further implications of the system mechanical properties on the incorporation of biological macromolecules via vesicle fusion are discussed.

2:15 PM M2.3
Investigation of Tethered Phospholipid Vesicle Assemblies Using Quartz Crystal Microbalance with Dissipation and Fluorescence Microscopy. Anjali R. Patel and Curtis W. Frank; Chemical Engineering, Stanford University, Stanford, California.

The realization of a robust, fluid, defect-free cell membrane model, able to house a variety of functional integral proteins, has been elusive despite abundant attention by numerous groups. We have constructed a tethered phospholipid vesicle assembly via a biotin-streptavidin linkage that has the potential to overcome the major limitations of other model membrane environments: protein-substrate interactions in the bilayer supported bilayers, and deforming, defected bilayers present in many polymer-supported bilayer membranes. Such an assembly also allows the use of powerful surface science techniques to record molecular recognition events between membrane proteins and substrates. Using quartz crystal microbalance with dissipation measurement (QCM-D) and fluorescence microscopy, we demonstrate the construction of a molecular assembly in which liposomes are specifically tethered to a planar supported lipid bilayer. We are able to monitor the step-by-step formation of the assembly begining by being able to measure differences in frequency and dissipation data corresponding to differently-sized vesicles. During the course of this investigation, we have discovered a biotin-mediated membrane-membrane interaction and have also been able to measure water content in a streptavidin monolayer.

2:30 PM M2.4
Model Membrane Assemblies on Self-Assembled Fulleren Surfaces. Gabriel A. Montano, Hsing-Lin Wang and Andrew P. Shreve; Bioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico.

Development of molecular architectures that integrate optically or electronically active components with biomolecular assemblies have important implications for material science, biophysics and biotechnology. We report on such architectures that employ fullerenes and phospholipids, Self-assembled monolayers (SAMs) of C60 and partially hydrolyzed polycyclodextrin-C60 (PCS-C60) were created using silane chemistry. The hydrophobic and hydrophilic nature of the C60 and PCS-C60, respectively, was used to drive the formation of model membrane assemblies consisting of either phospholipids monolayers or bilayers. Also, patterned surfaces of C60 and PCS-C60 were created and explored for membrane formation. Assembly and fluidity of the membrane assemblies was assessed using spectroscopic, imaging and fluorescence recovery after photobleaching methods. The results presented show progress in the creation of photoactive biomimetic assemblies appropriate for complex applications.

2:45 PM M2.5
Development of Novel Materials for an Isoprenylcysteine Methyltransferase-Based Supported Membrane Sensor. David H. Thompson, Department of Chemistry, Purdue University, West Lafayette, Indiana.

Our laboratory is currently developing methods for casting an active form of isoprenylcysteine methyltransferase (Ictm) within a stabilized supported membrane architecture as a potential drug screening tool for inhibiting Ras-based oncogenesis. The lateral diffusion dynamics of bipolar lipids such as 2,2-di-O-decyl-3,3-di-O-(eicosanyl)-O-(eicosanyl)-1,1-diphosphocholine (C20BAS) using pulsed-field gradient NMR (PFG-NMR) have been determined to enable structure-property correlations for this class of lipids. Pure C20BAS vesicles display diffusion coefficients (D) that range from 1.1 x 10-5 cm2/s at 25°C to 3.4 x 10-5 cm2/s at 65°C (melting transition temperature = 16°C). A slight increase in D is observed for 7:3 C20BAS:cholesterol vesicles (1.4 x 10-5 cm2/s at 25°C to 5.0 x 10-5 cm2/s at 65°C) relative to pure C20BAS vesicles. Enhanced diffusion in C20BAS:cholesterol membranes is due to a more rod-like transmembrane C20BAS conformation that more efficiently couples motion at the opposing membrane interfaces via a tilt-until-mechanism. The synthesis and performance of a FRET-based isoprenylcysteine-containing (PL-S-S-MR) molecular beacon that is activated by thiols will also be described. Although applicable to numerous cellular reactions, we are using this molecule to develop a simple coupled fluorescence-based assay that will report the activity of Sadenosylhomocysteine (SAM)-dependent Ictm. SAM is used as the methyl donor by many cellular methyltransferases and the enzymatic reaction results in the transfer of the methyl group to the target molecule and the concomitant production of S-adenosyl homocysteine (SAH). SAH is then rapidly hydrolyzed in cells by the enzyme SAH hydrolase to adenosine and homocysteine (Hcy). In our detection system, the Hcy generated will cleave the disulfide bond between the FRET pair in the beacon producing a fluorescent readout signal.

3:30 PM M2.6
Micro-Patterning and Refunctionalization of Supported Phospholipid Membranes Using Photolithography Approaches. Atul N. Parikh1, Chanel K. Yee1, Annapoorna R.
This paper summarizes several photolithographic approaches developed in our laboratories for patterning and functionalizing supported phospholipid membranes. In particular, we present (1) membrane coupling of phase-separated supported bilayers linked to a model system on a static surface, we ligands from a biomimetic surface.

Fluorescence recovery after photobleaching (FRAP), fluorescence microscopy, different structures of lipid assembly under flip-flop, to a state where all gel phase domains partition to the described. The latest work on stochastic sensing with α-haemolysin top monolayer. During this conversion, we observed a fast, one-way time, and the properties of the resulting lipid membranes and the various fusion conditions were observed. The application of the hybrid membranes has proved to be difficult. In this work, we will report our recent research on formation of biocompatible hybrid lipid membranes on a gold surface and their characterization with imaging surface plasmon resonance (SPR). Different from conventional SPR spectroscopy, SPR microscopy improves on the single spot interrogation of a sample by imaging an area that may consist of a diverse sample matrix. This non-labeling technique can provide vital information about the surface properties of the binding layer on the substrate and allows direct comparison of molecular interactions under identical conditions. Microcontact printing and photolithographic methods were used to generate patterned templates where the state of the hydrophobicity was well controlled. The process of vesicle fusion onto both hydrophilic and hydrophobic surfaces was monitored in real time, and the properties of the resulting lipid membranes and the kinetics of vesicle binding and fusion were investigated. Together with fluorescence microscopy, different structures of lipid assembly under various fusion conditions were observed. The application of the hybrid lipid membranes in sensing of toxin streptolysin O (SLO) and formation of transmembrane pores by the toxin will be discussed.

Sapuri-Butti1, Michael Howland1, Andrea Michelle Smith1, Thomas A. Huser2, Andrew M. Dattebaum3 and Andrew P. Shreve3; 1Applied Science, University of California, Berkeley, CA; 2Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, California; 3Bioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico.

Developing Biocompatible Supported Bilayer Assemblies: In Situ Study of Lipid Membrane Formation on a Patterned Template by Imaging Surface Plasmon Resonance.

Garcia1, Shara M. Dellatore1, Bi-Huang Hu2, Rico C. Gumawain, James A. King1, Phillip B. Messersmith1 and William M. Miller2.

Multiple lines of evidence suggest that mimicking the in vivo niche will be important in controlling stem cell expansion and differentiation. The niche is a complex microenvironment comprised of many different extracellular matrix (ECM) components, cell adhesion molecules (CAM), growth factors and other cells. Given the plurality of soluble and insoluble signals in such a complex system, recapitulating this environment in an ex vivo system will likely require the presence of multiple ligands from a biomimetic surface. Accomplishing this will not only aid in the understanding of how these factors affect the growth and differentiation of stem cells, but may also lead to new therapeutic opportunities for stem cells. We have recently reported the synthesis of linear and cyclic lipid-linked peptide (lipopeptide) moieties and their incorporation into dipalmitoylphosphatidylcholine (DPPC)-based static surfaces with high activity at low lipopeptide loadings. Using RGD-based peptides for the α5β1 integrin as a model system on a static surface, we demonstrated that these lipopeptides support cell binding and spreading at loadings as low as 0.01 mol%. Our results show that cyclic RGD gives increased cell binding when compared to linear RGD at the same mol% for KG-1a and HUVEC cell lines. For the KG-1a cell line, maximal binding was 100% for cyclic RGD and 60% for linear RGD. HUVECs showed a maximal binding of 80% for both cyclic and linear RGD lipopeptides. Although HUVECs reacted near-maximal binding at 0.02 mol% RGD, maximal spreading was not seen until 0.5 mol% RGD, and required a higher dose of linear versus cyclic RGD. A dynamic surface should allow for the rearrangement of lipopeptides within the carrier lipids leading to differences in cell binding and spreading characteristics. We are currently evaluating the effect of static and dynamic surfaces by studying the synergistic and additive effects of multiple lipopeptides on cell binding.

Phospholipids Molecular Films obtained by Langmuir-Blodgett Method Investigated by Fourier Transform Infrared Spectroscopy and Quartz Crystal Microbalance. Herman Sander Mansur and Juliano Oliveira; Metallurgical and Materials Engineering, Federal University of Minas Gerais, Bele Horizonte, MG, Brazil.

Living cell membranes are made of phospholipids assembled in two-dimensional bilayers in such a way that the hydrophobic chains are being shielded from the surrounding water. Phospholipids consist of a water-soluble head, a positively charged polar group, linked to two water-insoluble nonpolar tails, by a negatively charged charged group. Both tails consist of a fatty acid, each 14-24 carbon groups long. Lipid membranes are considered to be multilayered vesicles in living organisms as proteins are, but they are an active mediator of signal transduction and membrane transport. Also, the knowledge derived from the understanding of biomembranes makes it possible to cure diseases such as cancer, AIDS, and genetic disorder. Thin organic films of a thickness of a few nanometers are the source of high expectations as being useful components in many practical and commercial applications such as biosensors, detectors, and electronic components. The possibility to control molecular life is not without limitations, with desired structure and functionality in conjunction with a sophisticated thin film deposition technology enabling the production of biologically, electrically, and optically active components on a nanometer scale. The LB-technique is one of the most promising techniques for preparing such thin films as it enables (i) the precise control of the monolayer thickness, (ii) homogeneous deposition of the monolayer over large areas and (iii) the possibility to make multilayer structures with varying layer composition. The aim of
the present study was to characterize phospholipid deposited through LB technique by ATR-FTIR and QCM sensor. Phospholipid monolayers were deposited from dipalmitoylphosphatidylcholine (DPMC), phospholipid-sidewalls of the long nanotubes and trapping of the PLL molecules in scaffolds for forming guided two-dimensional neural networks. The geometries of the micro scale patterns were so designed to become a powerful tool to control the seeding of cells. Cells micropatterned on the surface were infected with baculoviruses and immobilized with low-setting temperature agarose to inhibit the further infection to neighboring cells. By controlling the dimension of nonfouling micropatterns, we successfully localized each cell in the micropatterned area and used with either a wild-type virus or a GFP-modified virus were differentiated by observing the polyhedral or fluorescence intensity inside the cells using phase contrast optical microscopy and fluorescence microscopy.

M3.2 Secondary Control of Active Biological Transport Systems for Dynamic Nanomaterials Synthesis, Amanda Marie Trent, Andrew K. Boal, Bruce C. Bunker and George D. Bachand; Biomolecular Materials and Interfaces, Sandia National Laboratories, Albuquerque, New Mexico.

In contrast to synthetic materials, biological materials have the ability to assemble, disassemble, and reconfigure based on environmental changes and stimuli. Understanding such energy-consuming processes may enable the integration of dynamic, adaptive assembly mechanisms with nanoscale materials, and permit the development of novel materials capable of responding to external signals. Cellular motor proteins and microtubules play critical roles in materials assembly/disassembly and reconfiguration in biological systems, and represent ideal candidates for nanoscale material research. Recently, kinesin motor proteins have been engineered to carry molecular cargo (such as colloidal gold or magnetic beads) as the protein moves in vitro along microtubule "tracks." Manipulation of this system could potentially be used to dynamically transport and assemble nanoscale materials at synthetic interfaces. Our objective was to introduce a secondary control mechanism for regulating kinesin transport by inserting a "chemical switch" into the protein. In this case, divalent metal ions binding to the switch will inhibit the necessary conformational and mechanical changes for ATP hydrolysis, and effectively stop motility maintaining the protein in a steady state. Chelation of the metal ions should in turn restore motility, enabling cyclic control of kinesin transport. Because metal ions likely inhibit kinesin motility, we first evaluated the sensitivity of both kinesin and microtubules to a number of metal ions. Results suggest that microtubules have varying sensitivity to metal ion species, and may be stabilized through covalent crosslinking. Similarly, Drosophila kinesin motility is differentially affected by metal ions: Cu<sup>2+</sup> > Zn<sup>2+</sup> > Ni<sup>2+</sup>.

M3.3 Guided Neurite Growth on Patterned Carbon Nanotube Substrates. Cengiz Sinan Ozkan and Xuan Zhang; Mechanical Engineering, University of California at Riverside, Riverside, California.

In this paper, we demonstrate the capability to integrate micro and nano fabrication technology to develop substrates that function as scaffolds for forming guided two dimensional neural networks. The substrate fabrication is achieved through a two stage process. The first stage involves developing the micro sized features using standard optical lithography techniques and the second stage involves the synthesis of vertical MWNT arrays using chemical vapor deposition process. The simplicity and reliability of these two techniques in combination affords scalability in developing substrates. The geometries of the micro scale patterns were then designed to address the three important factors in scaffold materials; cell process extension, guidance, and interaction. Neurite extension capability over artificially structured substrates is determined using parallel straight line features. Cell-cell interaction is analyzed using square patterns and finally neurite guidance is characterized over circular features. Surface topography in terms of the length of the nanotubes was also observed to play an important role in process guidance. Neurite processes showed preferential adhesion to the edges of long NT patterns whereas no selectivity was observed in the short NT patterns despite PLL functionalization of both types of substrates. This behavior is attributed to the adsorption of the PLL molecules onto the sidewalls of the long nanotubes and trapping of the PLL molecules in between the nanotubes at the pattern edges during the adsorption process. In the case of short nanotubes during its growth a large percentage of the tubes get pinned on to the substrate. These results in the absence of preferential means for PLL functionalization also show that PLL does not promote capillary action. Also the rigidity of the short NTs do not offer the motile growth cone with a suitable surface for process development. The long NTs in comparison are flexible and undergo conformational and mechanical changes for ATP hydrolysis, and subsequently promote capillary action. Both the neuron and the MWNT scaffold. As the neurite started proliferation of the outgrowth resulted in encompassing the tubes in the vicinity thus causing the nanotubes to cluster together resulting in disruptions in the patterns. We have established that guided neuronal networks can be formed on long vertical MWNT arrays by preferential adhesion to the pattern in comparison to short arrays.

M3.4 Enhanced Biocompatibility of Titanium Implants by Means of Hydrothermal Electrochemical Treatment. Juan Pablo M. Fuenzalida<sup>1,4</sup>, V. M. Fuenzalida<sup>1,4</sup>, C. Pucheco<sup>2</sup>, A. Sandes<sup>1</sup> and M. Redigol<sup>1,2</sup>; 1Departamento de Física, Universidad de Chile, Santiago, Area Metropolitana, Chile; 2I.P.A.D, Universidad de Valparaiso (UBV) & IPAD, Universidad de Valparaíso (UBV). Santiago, Chile; 3Department of Physics and Astronomy, Vanderbilt University, Nashville, Tennessee; 4Centro para la Investigacion Interdisciplinaria Avanzada en Ciencia de los Materiales, Santiago, Area Metropolitana, Chile.

The goal of this work is to enhance the biocompatibility of titanium
and titanium alloy surfaces intended to be used as implants into the body. The hydrothermal-electrochemical method was used to generate a biomimetic coating on titania surfaces by introducing a small amount of magnesium in the coating. The hydrothermal-electrochemical method has some technological advantages such as low cost, low energy consumption and the capability to coat surfaces with a complex shape, even within internal cavities. Biocompatibility of the coatings was evaluated using two in-vitro assays: a) simulated body fluid (SBF) and b) fibroblast culture. X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), and energy dispersive X-ray analyses were performed on SBF-treated samples. After 28 days in SBF a calcium phosphate layer was detected only in these regions previously coated with CaTiO3. This result suggests that the calcium titanate coating plays an essential role in the precipitation of a calcium phosphate layer from the solution on the biomedical surfaces. The fibroblast culture was performed under standard conditions. Viability, proliferation and cell adhesion were evaluated for 1, 7 and 14 days. The control surfaces were polymeric substrates, with a known high viability. The proliferation and adhesion were evaluated by optical microscopy and SEM. The Elisa-test with MTT was used to quantify the cellular viability. Cells had better adhesion to CaTiO3-coated samples in comparison with no coated samples. The proliferation on CaTiO3-coated samples was in the same range, evidencing a good performance of the CaTiO3 coating. The good performance exhibited by this hydrothermal calcium titanate coating, under two different in-vitro assays, suggests that it is useful to enhance biocompatibility of titanium-based implants. Moreover, the method can be scaled up to industrial production.

M3.5 Magnesium-substituted Hydroxypatite/acylated chitosan Nano-composite as Hydrophobic Drug-loaded Matrix for Blood-contacting Applications. Tso Ying Lin, Yi Ling Lin, San Yuan Chen and Shiang Chuan Chen; Material Science, National Chiao Tung University, ROC, Hsinchu, Taiwan.

A novel hydrophobic drug delivery system based on amphiphilic magnesium-substituted hydroxypatite/acylated chitosan nano-composite with hemocompatibility was developed to investigate drug release behavior, acute cytotoxicity and anticoagulant activity as functions of Mg-HAp and processing parameters. In this work, hydrophobic acyl-groups were used to substitute positive charged amino groups of chitosan to delay thrombogenesis and to encapsulate hydrophobic drug such as paclitaxel (an efficient in-stent anti-restenosis agent) with high drug-loading capacity. In addition, Mg-substituted hydroxypatite (Mg-HAp) nano-crystals instead of Ca-HAp will be used to further reduce thrombogenesis due to the partial hydrophilic surface which was contributed to the hydroxyl groups on nano-crystals. Preliminary results show that the hemocompatibility of acylated chitosan can be much improved compared to the incorporation of Mg-HAp nano-crystals. Furthermore, negative charged surface via the grafting of carboxyl group could further improve the hemocompatibility of this nano-composite. The nanoparticles in the drug delivery system could act as a diffusion barrier, and prolong the release period of hydrophobic bioactive agent. Furthermore, in contrast to common approaches by surface modification, the blood compatibility during polymer matrix degradation can be maintained via the partial hydrophilic surface provided by Mg-HAp incorporation. In addition, degradable rate and release profile could be easily tailored by controlling the amount of Mg-substituted hydroxypatite.

M3.6 Fabrication and Characterization of Patterned Surfaces for Single Protein Arrays by Scanning Probe Techniques. Jongyong Kim1,2, James D. Batten1, Jeffrey G. Forbes2 and Kuan Wang1; 1Chemical Science and Technology Laboratory, National Institute of Science and Technology, Georgia, USA; 2Laboratory of Muscular Biology/National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Health, Bethesda, Maryland.

High-density arrays of single protein molecules will not only enhance the understanding of the biological behavior of individual molecules, but also facilitate development of biosensors that can detect and identify multiple targets with high sensitivity and selectivity. For micron scale patterns, many approaches, including photolithography, microcontact printing, and microfluidic channel networks, are in use and have clearly demonstrated their potential utility. For nanometer scale patterns, new techniques must be developed. Recent advances in scanning probe techniques is a promising approach to fabricate chemically and spatially well-defined patterns at the nanometric scale. We describe the preparation and characterization of nanoscopic patterns on self-assembled monolayers (SAMs) by atomic force microscopy (AFM) based scanning probe nanolithography (SPN). An AFM was then used down stream in the process to characterize proteins adsorbed on surfaces on tissue culture and adhesion forces on the molecular level. An ultra-flat gold surface was produced by evaporating gold onto a SAM. A SAM was then formed with hexadecylamine-glycidyl terminated thiols (EG6-OH) to inhibit nonspecific outside the target area. Nanopatterning was done with a cantilever of the AFM to break the S-Au bonds in the presence of N-hydroxysuccinimide (NHS)-terminated thiols that in turn replaced the dislodged EG6-OH groups. These patterned groups are then reactive groups that can be further functionalized with specific antibody probes for selective and specific immobilization of immunoglobulins to develop biosensors for detection and identification of multiple targets with high sensitivity and selectivity with the aim of developing novel immunoassays. Additionally, the incorporated contractile proteins are being used to develop novel motility assays and nanomechanical assays of contractile proteins of single protein filaments and single molecules.

M3.7 Development of Long, Stiff DNA Tubes as Nanopatterned Substrates for Protein Binding. Ashish Kumar1, Axel Ekani-Nkodo2, Paul W. K. Rothemund2, Eric Winfree2 and Deborah Frygenson1; 1Institute of Standards and Technology, Gaithersburg, Maryland; 2Laboratory of Muscle Biology/National Institute of Arthritis and Biotechnology, Nagoya University, Nagoya, Japan.

We describe progress towards developing DNA Nanotubes into a tool for nano-patterning and assaying protein binding. DNA nanotubes are uniquely accessible equilibrium polymers made of motifs known as double-crossovers (DX units). They are typically 10 nm in diameter, up to 50 microns in length and correspondingly stiff (persistence length >5 microns). We have predicted and thereby manipulated the tube-structure to selectively decorate the tubes along the interior or the exterior surface. This ability allows us to use DNA tubes as protein-luding substrates with unusually high density of binding-sites (>500 within a micron), arrayed along the exterior of a tube in a regular lattice of 14.5 nm x 4 nm. We describe results showing the use of DNA Nanotubes as substrates for proteins such as lipase, restriction enzymes and regulatory proteins.

M3.8 Novel Hepatic Cell Culture on Ultra-Water Repellent Film. Yangtze Wu1, Nagaihiro Sait02, Yasushi Inoue1, Akira It03, Hirokuni Honda3 and Osamu Takei3; 1EcoTopia Science Institute, Nagoya University, Nagoya, Japan; 2Department of Molecular Design and Engineering, Nagoya University, Nagoya, Japan; 3Department of Biotechnology, Nagoya University, Nagoya, Japan.

Hepatic or embryonic stem cells culture was a key process in tissue engineering. They were cultivated in spheroidal culture medium. This is not at all unusual since embryonic stem cell in a living body, for instance, grows in spherical space, that is, a fertilized egg. Such cell cultures were difficult since the spherical growth field must be provided to the cells. Thus, the adhesion of cells on a culture plate must be inhibited in order to get a sphere. They have been cultivated in a conical test-tube culture or a weightless culture. However, such methods are not simple and efficient processes. In order to improve the processes, we attempted to develop the simple and efficient cell culture method for hepatic or embryonic stem cell using ultra-water-repellent (UWR) films as a culture field. The UWR film was prepared on a petri dish by microwave plasma-enhanced chemical vapor deposition using trimethylmethoxysilane as a raw material. The water contact angle was more than 150 degrees. Culture medium containing hepatic cells derived from a mouse (ca. 0.25 mL) was carefully placed on the dish. The shape of culture medium was spherical shape as such as a water drop on UWR films. This contact angle was also more than 150 degrees. The dish was vibrated during the cell culture in one experiment, and was not vibrated in another experiment. In both experiments, we successfully cultivated aggregated cells after 1 day. The aggregated cells united into one within the dish was not vibrated, contrary, they do not united into one but divided into many aggregated cells.

M3.9 DNA-Based Nanotechnology: New Nanoscale-Organized Highly Luminescent CdSe Nanorod-DNA Complexes. Vladimir V. Kislov1, Mikhail Artemyev2 and Gennady Khmoutov2; 1Institute of Radioengineering & Electronics, Russian Academy of Sciences, Moscow, Russian Federation; 2Institute for Physico-Chemical Problems, Belarusian State University, Minsk, Belarus; 3Faculty of Physics, Moscow State University, Moscow, Russian Federation.

We present some new results within the concept of using DNA molecules as building blocks and nanotemplates for controllable fabrication of various bioinorganic nanostructures due to their unique physical-chemical properties and recognition capabilities and the

**M3.10** Effects of Cholesterol on Galactosylceramide Domain Size, Shape and Membrane Binding Properties: A Combined Atomic Force Microscopy and Fluorescence Microscopy Study, Craig D. Blanchedette1, W. Chen Lin1, Timorhy V. Ratt02, M. Michael Fresh 2 and Marjorie L. Longo'; 'Biophysics Graduate Group, UC Davis, Davis, California; 2Chemistry, Lawrence Livermore National Laboratories, Livermore, California.

We are interested in studying the effects of cholesterol on Galactosylceramide (GaCer) domain morphology and binding properties between GaCer and two lectins: gp120, an HIV envelope glycoprotein and Trichosanthes kirilowii (TKA). GalCer has been shown to exist on the extracellular leaflet of the cell membrane in filamentary, netlike, or spheroidal nanostructures.

**M3.12** Formation of DNA/Au Structure on Hydrogen-Terminated Silicon through Direct Metal Drawing Approach and its Observation with an Atomic Force Microscope by Takahiro Ishizaki1, Saito Nagahiro1,3 and Osamu Takai2,3; 1Department of Molecular Design and Engineering, Nagoya University, Nagoya, Japan; 2Department of Materials, Physics and Energy Engineering, Nagoya University, Nagoya, Japan; 3Ectopia Science Institute, Nagoya University, Nagoya, Japan.

Organic monolayer covalently attached to silicon through Si-C bonds is promising material since they have a potential of effective control of electron transfer interface of silicon/organic layers and/or 2-layer/organic layers. The construction of hybrid organic-molecule/silicon devices offers a potential of the future molecular devices. In particular, DNA molecules, which differ from other molecules, offer effective advantages for constructing molecular devices including that it is easy to self-assemble form a uniform network structure on a large scale and they have a complementation and self-replicating function. To realize functional molecular devices, it is vital to immobilize DNA molecule on inorganic material. In order to achieve this, it is necessary to control of the DNA network pattern at a molecular level, however, the details have not been revealed. The formation and stability of the DNA depends on many factors such as underlying material, immobilization procedures, adsorption time, and concentration of DNA used. An accurate interpretation of all these factors is essential for developing functional molecular devices. In this study, we attempt the hybrid DNA/Au structure on hydrogen-terminated silicon through direct metal drawing approach using an atomic force microscope (AFM) and to investigate the effect of some factors on the stability of DNA. To immobilize DNA, gold patterns were firstly immobilized on hydrogen-terminated silicon surface through direct metal drawing approach with an AFM. An organic-molecule, that is, DNA molecule, was then deposited selectively on only the gold patterned. To confirm hybrid structure, the surface was treated by an AFM and fluorescence-labeled spheres. In addition, the surface potential images of DNA/Au structures were also measured by Kelvin probe force microscopy (KPFM).

**M3.13** Lipid Bilayer Membrane on Gold & TiO2 Solid Supports: From Liposomes to Supported, Planar Bilayers, Nanoporous Gold and Curtis W. Frank1; 1Materials Science and Engineering, Stanford University, Stanford, California; 2Chemical Engineering, Stanford University, Stanford, California.

Supported lipid bilayers formed by the fusion of small unilamellar vesicles onto silicon oxide or organic film modified surfaces serve as model membranes in both scientific research and practical applications. They prove invaluable to researchers in the study of the characteristics and behavior of membrane-bounded processes, protein-lipid interactions and biological signal transduction. They enable the biofunctionalization of inorganic solids, such as semiconductors, gold-covered surfaces, and optoelectronic and lab-on-a-chip devices. Applications of supported membranes on solid surfaces potentially include biosensors, the acceleration and improvement of medical implant acceptance, programmed drug delivery, and the production of catalytic interfaces. Scientists prefer the electrical properties of gold and the beneficial biocompatibility of titanium-oxide to support lipid bilayers, yet have been unsuccessful in creating planar lipid bilayers. In this study, we present a novel method to destabilize intact vesicles, transforming them into a planar bilayer structure on substrate materials, such as gold and titanium oxide. The Quartz Crystal Microbalance-Dissipation (QCM-D) frequency and the dissipation value both indicate for method of complete synthetic biomembranes in the construction of two-dimensional complex fluids. The new method eliminates previous restriction to preferred substrates and allows researchers to take advantage of the beneficial electrical and the superb biocompatibility of titanium-oxide surfaces. This shifts the focus to a material-based solution and away from surface-dependent constraints.

**M3.14** Spatially-Restricted Raft-Like Chemical Heterogeneities within Model Phospholipid Membranes. Aappln: Anna Porin-R. Sapuri-Bulti1, Jay T. Groves2 and Atul N. Parikh1; 1Applied Science, University of California, Davis, California; 2Chemistry, University of California, Berkeley, California.
We have developed a method to direct the reconstitution of raft-like lipid microdomains at controlled densities and distributions in specific areas of a pre-formed fluid phospholipid bilayer. A contiguous primary phase, a single, fluid POPC bilayer, displaying a pre-defined array of water-filled empty voids (e.g., 20 um squares), was prepared on an oxidized silicon wafer by brushing a polyelectrolyte multilayer of polyallylamine and poly(methacrylic acid) or poly(ethylene glycol). The primary bilayer was sequentially exposed to unilamellar vesicles of cholesterol, sphingomyelin, and a phospholipid. Lipid compositions of the secondary vesicle phase contained a constant 28 mol % sphingomyelin and varying ratios of cholesterol and POPC. These secondary intercalants gradually diffused within the primary POPC bilayer ultimately affecting the pattern. In all cases, the recognition of raft-bound GM1 by cholera toxin led to long-term stabilization of initial raft patterns. Further, the engineered rafts were shown to retain key properties of cellular rafts including detergent resistance and dissolution induced by selective cholesterol extraction using methyl-beta-cyclodextrin. We envisage the construct to provide a useful model membrane platform for concentrating raft-like functionalities in pre-determined surface patterns.

**M3.15**

**Electrical Characteristics of DNA and DNA-Protein Complexes using Scanning Probe Microscopy.**


Phys. Myongji University, Yongin, South Korea; Physics, Myongji University, Yongin, South Korea.

Scanning probe microscopy (SPM) with a conducting tip is performed on a voltage stressed planar DNA and DNA-protein complexes. We have observed their electrical properties by measuring the difference of DNA molecules on a silicon wafer with varying applied sample bias. After introducing voltage stress to a local area of DNA molecule, apparent height difference of DNA molecules due to the charge injection or removal was measured. Similar experiments were done with different bias polarities and sequential stress steps. We also observed the electrical and physical properties of the DNA-protein complexes through local probing around the binding sites of protein using SPM.

**M3.16**

**Peptide Containing Monolayers for Controlling Non-Specific Protein Binding and Cell Adhesion on Surfaces.**

Christina Elizabeth Ingram and James Evans Hutchinson.

Department of Chemistry and Materials Science Institute, University of Oregon, Eugene, Oregon.

Fabrication of biocompatible surfaces is an area of intense focus in materials science. To maximize biocompatibility, surfaces must be created that prevent non-specific protein binding, while also presenting cell binding functionality normally observed within the extracellular matrix. Ethylene glycol- and amine-terminated alkaneethiol self-assembled monolayers on gold have shown particular promise in preventing non-specific protein binding to surfaces, although additional work must be done to improve the long-term stability of these assemblies. Previous work in our lab has demonstrated that the presence of buried peptide functionalities in alkaneethiol monolayers significantly improves monolayer stability. Using scanning probe microscopy to pattern cellular adhesion surfaces, we have investigated how hydrogen bonding interactions between buried amine groups influence the long-term prevention of non-specific protein binding. We have also investigated the incorporation of amine containing RGD-terminated nanocrystalline dianode particles into ethylene glycol- and amine-terminated dodecynes. Phase separation in these systems should produce surfaces that closely resemble cell binding sites that occur naturally in the extracellular matrix.

**M3.17**

**Surface Analysis of Cystamine-Glutaraldehyde-Steptavidin-Biotinylated DNA Assembly Structure on the Gold Substrata by Atomic Force Microscopy.**


Electrical Engineering, Myongji University, Yongin, Gyeonggi-Do, South Korea; Physics, Myongji University, Yongin, Gyeonggi-Do, South Korea.

In this paper, we characterized consecutive layers of cystamine-glutaraldehyde-steptavidin-biotinylated DNA assembly structure on the gold substrate by atomic force microscopy (AFM), aiming to apply to the biosensors and bioelectronic systems. For the formation of the molecular structures, gold wire was patterned on the silicon substrate first, and the cystamine terminated with thiol was covalently immobilized on the gold surface. Aldehyde group at both extremes of the glutaraldehyde injected subsequently would be bonded with amine group in the cystamine. Finally streptavidin bonded with aldehyde group forms the assembled structure with biotinylated DNA. By characterizing these secondary interactions between the layers with SPM, we can suggest the optimal condition for the detection system of bio-molecules, such as flow rate and density of samples. This is confirmed by the results measured using the cantilever-based biosensors and will be also presented.

**M3.18**

**Diamond Nano Particles Employed as Bio-Probes for Cellular Imaging.**

José De Jesús, Roberto Acosta, Fabrice Piazza, Eduardo Rosas-Molinar and Gerardo-morel.

Department of Physical Sciences, University of Puerto Rico, San Juan, PR, Puerto Rico; University of Puerto Rico, San Juan, PR, Puerto Rico.

The development of novel nano-particles for imaging living cells in vivo without harming or disrupting their intracellular molecular dynamics is an important area of nanotechnology that interests Material science, Physics, Chemistry, and Biology. Dynamic imaging of cells in vivo is required to track structural changes over time and to obtain direct information about native structures. The lack of sufficiently sensitive molecular probes and detection schemes for imaging individual molecules in vivo is a significant barrier to obtaining real-time information on dynamic cellular processes. A variety of probes are currently used for in vivo studies, but their relatively large size (average size 27 kDa in monomeric form) and chemical and photophysical properties limit their use and resolution within the 3-D context of the living cell. Additional difficulties with probe targeting, cell delivery and detection instrumentation contribute to a detection sensitivity level that is estimated to be too low by a factor of 100-1000. Nanocrystalline ultra-dispersed diamond particles are good candidates for biological probes because they are photo-stable, biocompatible and their luminescence can be tailored for a range of excitation and emission wavelengths. We report on the purification, dispersion and transport of nanocrystalline diamond particles. Luminescence and transmission electron microscopy analyses were used to confirm the transport of N-particles inside cells.

**SESSION M4: Supramolecular Assemblies and Biomimicries**

Chair: Henry Hess

Wednesday Morning, March 30, 2005

Room 3009 (Moscone West)

**8:30 AM - M4.1**

**From DNA to Transistors.**

Kinneret Koren, Biochemistry, Stanford University, Stanford, California.

Bottom-up assembly in which the circuit connectivity and functionality is encoded in the molecular building blocks provides a promising route towards integrated molecular electronics. We propose to harness DNA and related proteins with their remarkable molecular recognition to construct a scaffold for molecular electronic circuits. Sequence specific molecular lithography, which relies on the biological process of homologous recombination carried out by the bacterial RecA protein, was developed as a framework for using the information encoded in the scaffold DNA sequence to direct assembly of an electronic circuit. We have demonstrated that this leads all the way from DNA molecules to functional transistors. Carbon nanotubes were incorporated as the active electronic components in these DNA-templated transistors. The realization of complex DNA-based circuits remains an outstanding challenge which will require the development of new concepts and techniques.

**9:00 AM - M4.2**

**Directed Assembly of Multicomponent Biomaterial Systems.**

Erik David Spoerke, George D. Bachand, Bruce C. Bunker, James A. Voigt and Jun Liu.

Chemical Synthesis and Nanomaterials, Sandia National Laboratories, Albuquerque, New Mexico; Biomaterials and Interfaces, Sandia National Laboratories, Albuquerque, New Mexico.

We have explored the use of microtubules (MTs) and kinesin motor
proteins as bioactive components of nano-biohybrid assemblies. In Nature, microtubules are a type of cytoskeletal filament, involved in intracellular processes ranging from the division of the color-changing behavior of some fish. These processes require that microtubules and kinesin motor proteins act as dynamic and adaptive players helping to direct the transport, assembly, and organization of biological nanostructures. This ability and properties in natural systems make microtubules and kinesins motors attractive as components for integration into synthetic nano-biomaterial systems. Our efforts have focused on understanding and controlling the interactions between these bioactive components and technologically valuable materials. The creation of artificial microtubule organizing centers allows us to control the organizational structure of microtubules. These synthetically-organized bio-structures provide an adaptable and sophisticated scaffold for interaction with synthetic materials ranging from fluorescent molecules to nanocrystals. In some cases, the microtubules act directly as scaffolds for these synthetic components, while in more advanced schemes, kinesin motors actively participate with synthetic materials with microtubule scaffolding. This bio-active approach to material synthesis interfaces biological molecular machinery with hard materials syntheses to create powerful tools for complex biobruid materials development. Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Nuclear Security Administration under Contract DE-AC04-94AL85000.


The rapid and specific detection of dilute analytes requires purification and concentration steps, which could be accelerated by active transport on the molecular scale. To this end we have previously designed the "molecular shuttle", an active nanoscale transport system that utilizes surface-adhered kinesin motor proteins to transport biologically relevant materials. Each shuttle contains streptavidin-tagged cargo, including microbeads, DNA or quantum dots. However, most analytes are not tagged with streptavidin, and these analytes need to be selectively loaded into the shuttle. We have thus begun to characterize tile-based DNA self-assembly in functional DNA nanoscale cargo carriers.


11:00 AM M4.6 Directed Metalization of Enzymes with Preserved Catalytic Activity. Amihay Freeman1, Hila Dagan2, Yael Droz1 and Yossi Shacham-Diamand2; 1Molecular Microbiology and Biotechnology, Tel Aviv University, Tel Aviv, Israel; 2Department of Physical Electronics, Tel Aviv University, Tel Aviv, Israel.

Assembly of a limited number of protein molecules into two or three dimensional arrays, providing functionality such as biocatalysis or biosensing, is a major component in the fabrication of nano-structured biochips. The performance of such biochips strongly depends on the sensitivity of the supporting microelectronic device and highly effective signal transduction, enabling reliable measurements of the signals generated by the conjugated proteins. 'Wiring' of enzymes performing oxidation or reduction of biochemicals to electrodes was mainly demonstrated by using conducting polymer gels or binding of chemically modified metallic particles. To the best of our knowledge, wiring by means of directed complementary metallic coating of the surface of the enzyme, assumed to be optimal for this purpose, was not demonstrated. We have recently successfully developed methodologies for directed protein metalization by controlled electrodes deposition, as first step on the way to sub-nanometric wiring based on pure metal conductivity. Employing silver as first model we introduced new mild reducing agents allowing rapid controlled deposition under mild conditions, suitable for working with proteins without denaturation [1]. Subsequently, we developed novel strategies to direct such metalizations to the surface of a soluble protein molecule without impairing its biological activity. Directed metalization of immobilized enzyme structures provided a great importance and its impact on their enzymatic activity will be described. [1] Y. Shacham-Diamand, A. Ingberg, Y. Sverdlov, V. Bogush, N. Croitoru, H. Moscovich and A. Freeman, 2003. Electroless processes for micro and nanoelectronics. Electrochimica Acta, 48, 2079-2090.
Colloidal semiconductor nanoparticles (Quantum Dots, QD) are emerging as exciting candidates for fluorescent labeling experiments. Compared to organic fluorophores, they have broad excitation spectra, tunable emission wavelengths, photostability, and long fluorophore lifetimes and high photo stability. My work is based on the optical properties of QDs and to build DNA directed assembly of QD and Au nanoparticles. Discrete nanostructures with different numbers of Au around the central QD were synthesized and purified by gel- electrophoresis. Researching on the optical properties of QD with metal nanoparticles in the vicinity will help in understanding the interaction between metal and semiconductor nanoparticles, and help in designing better nanoprobes with enhanced photoluminescence and less photo blinking than QD alone.

11:30 AM M4.8

Nanostructured Bioactive Hydrogels from Self-Assembling Modular Artificial Proteins. Stephen Fischer, Lixin Mi, Brian Chung, Sarah Sundelacruz, Michael Curtis and James L. Harden; Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, Maryland.

We have utilized de novo protein design and recombinant DNA methods to design an array of self-assembling proteins with useful biomaterial properties. These proteins have been engineered to include molecular recognition elements that (1) direct their self-assembly into multi-component nanostructured hydrogels with tailored topologies and (2) interact with the surface receptors of a number of different cell types in order to guide their growth and development. The overall protein design is based on a modular, multi-block architecture that includes independent inter-chain binding domain pairs and flexible biofunctional linker domains. The self-assembling end blocks are amphiphilic helices that serve as smart crosslinking agents of the hydrogel, whereas the central linker domain is a water soluble, disordered sequence that encodes specific binding and signaling functions of extracellular matrix (ECM) constituents. Production of these materials through recombinant DNA methods gives unmatched control over their specific structural and biofunctional attributes. In this talk, we present studies of a group of proteins with associating end domains that are engineered to self-assemble specifically into heterotrimer bundles. This bundling leads to the formation of a regular network structure in the hydrogels, which enables the presentation of specific bioactive signals to target cells in a localized and regulated fashion. Through the use of microscopic and cell proliferation assays, we show that these multi-functional hydrogels are capable of inducing appropriate cellular responses in cultures of one or more cell types. In doing so, we illustrate the utility of flexibility and modularity in a biomaterial as a means to induce desired cellular responses. We believe that such a combinatorial approach to biomaterials for artificial ECM applications, in which the end user can choose from a library of bioactive modularity and matching as needed, will be a useful strategy for tissue engineering.

11:45 AM M4.9

Protein-Functionalized Nanohydrogel Arrays. Peter Krsko1, Vasili Papsiropoulos2, Patricin Soteropoulos3 and Matthew Libera1, 1, 1, Stevens Institute of Technology, Hoboken, New Jersey; 2Center for Applied Genomics, PHRI, Newark, New Jersey.

Protein microarrays rely on a highly structured interface between a biosynthetic surface and a physiological system. Like DNA arrays, they involve the surface immobilization of proteins which are then probed with a solution containing target molecules such as oligonucleotides, antibodies, other proteins, or drug candidates. Protein arrays are providing compelling opportunities to study fundamental aspects of protein function as well as to develop new pathways for drug discovery, pharmacological screening, and immunological studies. However, they bring two significant challenges. First, proteins are far more likely than DNA to denature upon binding to a synthetic substrate and consequently alter their higher-order structure. Second, high-throughput methods such as PCR are not available to protein studies, the biospecific reagents used for a protein experiment are generally available in very limited amounts. Here we present a method to simultaneously address both of these issues using functional nanohydrogels. We use focused electron beams to radiation crosslink amine-functionalized poly(ethylene glycol) [PEG]. Pulsed electric irradiation creates hydrogels on the order of 200 nm diameter which, when swollen, are patterned as arrays on glass or silicon at submicron spacings, and we can pattern ~7500 nanohydrogels in a 100 micron diameter area in ~10 seconds. This is an areal density ~10,000 times greater than a microarray DNA chip, and, using, anti-laminin and anti-fibronectin antibodies we show that these proteins maintain their biochemical fidelity with high fidelity. We can read nanohydrogel arrays using a standard microarray scanner, and we can measure protein binding to individual nanohydrogels using fluorescence microscopy. Our most recent experiments compare the performance of nanohydrogel arrays to that of standard protein microarrays probing them with oligonucleotides which specifically bind nucleic acid-binding proteins (NBPs) isolated by screening a human colorectal adenocarcinoma and fetal lung tissue expression library.

SESSION M5: Supramolecular Assemblies and Bionanodevices II

Chair: Henry Hess
Wednesday Afternoon, March 30, 2005
Room 3009 (Moscone West)

1:30 PM M5.1


Man-made labs-on-a-chip and factories-on-a-chip on the microscale have conceptual similarities to the complex analysis and fabrication systems of living cells. However, the cell allows extremely more complex analysis and fabrication than any man-made device. Furthermore, several functions in the living cell occur on the nanoscale rather than on the microscale. One class of nanomachines, the molecular motors, are essential for organizing material in the cellular analysis and fabrication. In achieving their tasks the motors often act in ordered supramolecular assemblies. We have here used state-of-the-art nanofabrication to reconstruct important aspects of such ordering on a chip. In the work we have focused on myosin II as a generic motor protein that normally propels actin filaments in muscle contraction. By appropriate surface functionalisation and suitable assay solutions (ionic strength, viscosity) high-quality myosin induced actin filament sliding could be selectively localized to the floor of nanostructured channels (100-700 nm wide) and 100-500 nm channels. These channels had been produced by electron-beam lithography and, after adsorption of myosin motors, the channel floor was functionalized with trimethylchlorosilane (TMCS) using chemical vapor deposition. The channel walls were made up of polymer inhibiting poly(methylmethacrylate) (PMMA) that also covered surfaces between channels. In consistence with theoretical arguments (based on flexibility of actin filaments and motor energy content) we always found complete rectification of myosin induced actin movement for TMCS track widths less than 400-500 nm (>90 filaments observed during > 15 minutes). Similar results (>90 filaments studied) were obtained in experiments where nanostructuring was achieved with electron beam or nanocontact lithography but where the channel floor was made up of polymer resist mr-0006. Guidance of actin filaments along TMCS/PMMA channels was highly efficient with complete guidance even along curvatures of radius down to 1 µm. Since we have shown that actin filaments can be guided along tracks of CdSe quantum dots this suggests that cargo transportation in circles of very small radius should be readily realized. Thus whereas the short persistence length of actin filaments requires very narrow myosin binding tracks to ensure rectification it has the advantage to facilitate transportation along strongly curved paths. We have shown that myosin motors may retain their ability to propel actin filaments more than a week after their initial adsorption to a TMCS surface. Furthermore, as mentioned above, high-throughput nanopatterning lithography may be used to produce channels for positioning and rectification of motor function. Thus, commercially viable semi-synthetic nanodevices containing the actin-myosin motor system may be realized in the not too remote future.
include field-deployable "smart dust" devices since these rely on a large number of stand-alone micro/nanodevices fabricated at low cost. Here we explore the potential of caged ATP, which is widely used as a tool in cell biology, for energy storage and controlled activation. Caged ATP can be stored in the buffer solution of a bionanodevice, "uncaged" by UV light, and utilized as fuel by many enzymes to catalyze chemical changes or power nanodevices. We will present the characteristics of caged ATP, the design considerations for the integration of caged ATP into miniaturized devices, in particular bionanodevices driven by the motor protein kinesin, key factors in controlling the release of ATP from the system, and designs to stabilize the system against variation of environmental conditions. The feasibility of employing sunlight for the activation of the system will also be discussed.

2:15 PM M5.5

Hybrid Nanodevices based on Biomolecular Motors: A Lifetime Study. Christian Brunner1,2, Karl-Heinz Ernst4,2, Henry Hess2,3,2,3, and Viola Vogel1,2.1 Materials Technology (ETH), Zurich, Switzerland; 2Bioengineering, University of Washington, Seattle, Washington; 3Center of Nanotechnology, University of Washington, Seattle, Washington; 4Molecular Surface Technologies, Swiss Federal Laboratories for Materials Testing and Research (EMPA), Dübendorf, Switzerland.

The lifetime of biomolecules determines the lifetime of hybrid devices. For the commercialization of hybrid devices the longevity of the biological components is crucial. Thus, it is essential to know the least stable part in the system. Most biomolecules are only biologically active in a very narrow band of chemical and physical conditions, meaning the stability of biomolecules is often a limiting factor. Our studies show that the lifetime of nanodevices based on biomolecular motors will scale with the stability of the motor proteins. To extend the stability of nanodevices, motors are often immobilized at the nanoscale. This can be done by entrapment into capsules, addition into a hydrogel, or immobilization at the nanoscale. In all cases, the stability of the motor proteins is of importance. We will present data on the stability of different cellular motors in a range of conditions.

2:45 PM M5.6

Controlled Object Delivery in Aqueous Medium through Large Pores, Frederic Pincet1, Sophie Cribier2 and Nicolas Rodriguez2.1 Laboratoire de Physique Statistique, Ecole Normale Superieure, Paris, France; 2Laboratoire de Physico-Chimie Moléculaire des Membranes Biologiques, Institut de Biologie Physico-Chimique, Paris, France.

The delivery of encapsulated drugs and objects in general has been a hot topic for the past decades. One way to achieve this is to open a pore in the capsule membrane in order to release its content. This transitory pore must last long enough for the delivery to significantly take place. For most of the potential applications of such an approach, an accurate timing of the delivery is necessary. Here, it will be shown how it is possible to reprogram the time of pore opening and vesicle delivery in an aqueous buffer. Several videos will show the opening up and the closing of the pore as well as the release of incorporated objects. This potentially allows to trigger immediate drug delivery in physiological systems. The different pore sizes will be presented and the mechanical way by which the pore is stabilized will be discussed.

3:15 PM M5.6


The ability to construct ordered two- and three-dimensional structures on the nanometer scale is essential for the development of next-generation optical, electronic, and magnetic materials and devices. The limitations of top-down approaches in providing routes to nanoscale assembly have provided impetus for investigations of new bottom-up approaches. Often, these latter approaches are simply nobio-inspired loosely based on biological structural motifs, e.g., layered self-assembly of amphiphilic molecules. Alternatively, the approach involves noble-bio-template assembly, whereby inorganic nanoparticles, for example, are assembled and sometimes functionalized using a biomolecular scaffold. This approach to achieving structural control at the nanoscale allows the inorganic components to assume, in aggregate, complex patterns or shapes. Here, we report the assembly and characterization of semiconductor nanocrystals as nanoscale scaffolds. More importantly, we characterize the assembly by observing optical signatures of the assembly process induced by long-range, Förster-type energy transfer (ET) between nanocrystal quantum dots (QDs), QDs and dyes, and QD-dye and metal surfaces. Significantly, the power of this spectroscopic technique to reveal precisely the positions of nanoscale objects is not diminished in the biomolecular system. In contrast, imaging techniques such as high-resolution transmission electron microscopy (HR-TEM) are not amenable to in-situ analyses of biological systems. In contrast, ET methods allow real-time, in situ analysis in biologically relevant buffered aqueous solutions of assembly/disassembly processes as well as controlled object positioning. Specifically, in the case of QND-QND ET, we resolve up to three donor-acceptor (D-A) distances indicative of at least three unique QND-QND spatial arrangements. The ability to resolve multiple D-A distances is unique to time-resolved dynamical methods. Further, dynamical methods provide significantly enhanced sensitivity, critical for the exceptionally low concentration often characteristic of these systems, compared to non-dynamical methods.

In conclusion, our studies demonstrate that ET measurements comprise a versatile tool for characterizing bio-template assemblies and that ET is an efficient mode of interparticle electronic communication! in
Materials as R. r-v 20%, strain rate Armstrong and structure to changes in pH is Nernstian from pH 4 to 9. The pH structure, a monocrystalline arrangement of pore channels on a surfaces with gold and titania is achieved by electrochemical methods covalently. In the case of gold membranes thiol-capped active linker then subsequently reoxidized at the sol-gel/PSLB interface, releasing transduce proton gradient potentiometrically and/or varying the pore diameter from 10 nm to 400 nm, enzyme-catalytic which act as adhesive interfaces for binding proteins and enzymes yields in a one-step reaction are achieved by simply forcing the spectrally; both detection modalities are facilitated by electrically conducting polymer support beneath the PSLB can constructing the device on an electroactive planar waveguide. A convenient choice for the fabrication of biodevices, due to the huge internal surface area, the biocompatibility, and the compatibility with nanowires, ca. 1-2 nm in diameter, are then formed by electropolymerization in the porous sol-gel network to create a conductive, pH sensitive electrode. In order to facilitate the vesicle fusion, a second thin sol-gel layer is then applied. The response of this structure to changes in pH is Nernstian from pH 4 to 9. The pH response is completely blocked by fusion of vesicles to form a PSLB, demonstrating the role of the support polymer a lipid-soluble quinone shuttle into the PSLB provides the mechanism for transmembrane proton pumping. A proton donor such as ascorbic acid, introduced into the aqueous volume above the PSLB, initiates the proton pumping via a redox reaction with the quinone molecule, which are then subsequently reoxidized at the sol-gel/PSLB interface, releasing protons into the pores of the sol-gel. Development of an artificial, membrane-based proton pump interfaced to a planar semiconductor is a crucial first step toward creation of light-driven proton pumping devices based on semiconductor-supported lipid membranes.

Nanoscale Flow-Through Reactors for Biocatalysis based on Gold and Titania Membranes. Mato Knez 1,2 , Sabato D’Auria 1,2 , Mose’ Rossi 2 and Andrea M. Rossi 1,3 , IN anotechnology and Microsystems, lEN Galileo Ferraris, Torino, Italy; 2Institute of Protein Biochemistry (CNR), Napoli, Italy.

Three-Dimensional Protein Nanopatterns on Porous Silicon. Stefano Boring 1 , Sabato D’Auria 1,2 , Mose’ Rossi 2 and Andrea M. Rossi 1,3 , Nanotechnology and Microsystems, lEN Galileo Ferraris, Torino, Italy; 2Institute of Protein Biochemistry (CNR), Napoli, Italy.

Porous alumina membranes are well-established template-systems for the synthesis of a large variety of nanostructured materials. When we introduce imprint lithography in the fabrication process of our porous structure, a new type of arrangement of the channels cm²-scale is obtained and the deviation of the pore diameter is reduced to less than 2%. However, alumina membranes do not provide high compatibility for biomolecules and therefore are not suitable for biomolecular reactions. In order to achieve well-defined and biocompatible membranes, the chemical composition of the alumina template surface needs to be modified. Coating of alumina membrane surfaces with gold and titania is achieved by electrochemical methods and atomic layer deposition (ALD). Subsequently wet-chemical methods can be applied to adsorb monolayers of linker molecules which act as adhesive interfaces for binding proteins and enzymes covalently. In the case of gold membranes thiol-capped active linker molecules and for titania membranes phosphate capped ones are used. A typical model system for the protein reaction is the well-known "avidin-biotin" system and for the enzyme catalysis the reaction of luciferase with luciferin. Due to high surface area of this system, high yields in one reaction step are achieved by simply forcing the reactants to pass the membrane. The high potential of the reactors can even be improved by stacking of several tailor-made membranes in order to achieve multi-step reactions (e.g. for peptide synthesis). By varying the pore diameter from 10 nm to 400 nm, enzyme-catalytic reactions with small molecules as well as with large-sized can easily be performed. We thank the german ministry for education and research (BMBF, project number 03N8701) for the financial support.

Three-Dimensional Protein Nanopatterns on Porous Silicon. Stefano Boring 1, Sabato D’Auria 1, Mose’ Rossi 2 and Andrea M. Rossi 1, Nanotechnology and Microsystems, lEN Galileo Ferraris, Torino, Italy; 2Institute of Protein Biochemistry (CNR), Napoli, Italy.

Porous silicon (PS) is a nanostructured material which represents a convenient choice for the fabrication of micro-devices, due to the huge internal surface area, the biocompatibility, and the compatibility with microelectronics technology. A method to define biomolecule nanopatterns on PS, based on Electron Beam Lithography (EBL), is presented here. We demonstrate that it’s possible to locally expose the material surface in order to bind proteins within submicrometer regions. Furthermore, due to the sponge-like structure of the substrate, one can exploit the internal surface, obtaining three-dimensional bio-potential devices, this innovation will be described in details, and demonstrated in case of three different kind of proteins: the glucose-binding protein isolated from E. coli, the glutamine-binding protein isolated from E. coli and an intractable sugar-binding protein isolated from the thermophilic organism Pyrococcus horikoshii. These biomolecules have been selected taking into the account their high impact in the development of advanced nanosensors for important analyses such as the continuous monitoring of glucose levels of patients and monitoring of the glutamine levels in patients with cancer pathologies.


Motor vehicle accidents are leading cause of traumatic brain injury (TBI) which is due to brain deformations resulting from deceleration of the head against the interior of the car. It is believed that induced deformations greater than 10% strain applied at strain rates greater than 10⁻² per second are responsible for activating pathophysiological cascades which result in delayed neuronal dysfunction and cell death. The extended time-course of these cascades suggests that there is an opportunity exists for therapeutic intervention to rescue the damaged tissue. To date, no electro system allows for a sensitive measure of neuronal health and function prior to, during, and after TBI. Existing micro-electrode arrays (MEAs) fabricated on rigid substrates allow for the simultaneous recording from up to 100 sites invivo, but only before and/or after TBI. With current MEA systems, it is difficult to record from identical neurons pre and post-injury, and the stability of the preparation must be broken to make these contacts preventing long-term studies. We propose a novel technology to develop stretchable micro-electrode arrays, capable of simultaneous mechanical stimulation and recording of neuronal activity in vitro, allowing for continuous recording during injury and post-injury. This technology is enabled by our recent discovery that thin gold films patterned on silicone membranes remain electrically conductive after stretch cycles > 20%. Our stretchable MEA is fabricated on a soft, biocompatible 250μm thick elastomeric silicone membrane and consists of a 2x2 25mm thick gold micro-electrode array directly patterned on the silicone. We report on the stretchable micro-electrode array fabrication process, electro-mechanical behavior under large and rapid strain (strain ~ 20%, strain rate ~ 10⁻³/s) and preliminary data on growing cell cultures on the array.

Functionalization of Group III-Nitride Surfaces for Biosensor Applications. Barbara Baum 1, Georg Steinhoff 1, Oliver Purrucker 2, Motomu Tanaka 3, Martin Stutzmann 3 and Martin Eckhoff 3, 1Walter Schottky Institute, Technical University of Munich, Garching, Germany; 2Institute for Biophysics, Technical University of Munich, Garching, Germany.

Group III-nitrides are promising substrate materials for biophysical applications as they combine high chemical inertness, excellent electronic characteristics and optical transparency. Recent results have shown long term chemical stability under physiological conditions and have proven the biocompatibility of this material system [1]. AlGaN/GaN electrolyte gate field effect transistors (EGFETs), operated as ion-sensitive devices, feature an almost Nernstian pH-response of about 56mV/pH [2]. Furthermore, electrical recording of extracellular action potentials with AlGaN/GaN EGFET as recently been demonstrated successfully at original to noise ratios due to the extremely low noise levels were obtained [3]. Utilization of such devices for the sensitive electronic detection of specific biomolecular interactions is a promising approach for the realization of novel biosensors. To this end a detailed understanding and control of the inorganic/organic interface of AlGaN-based devices is required. We have studied the covalent functionalization of AlGaN-surfaces by deposition self assembled layers of two different kinds of silane molecules. The deposition of octadecyltrimethylsilane (ODTMS) resulted in hydrophobic AlN- and GaN-surfaces, suitable for subsequent deposition of lipid monolayers. For immobilization of single strangled oligonucleotides on AlGaN-surfaces the formation of self assembled monolayers (SAMs) of amidopropyltrichlorosilane (APTES) was studied. Subsequently, amino-labelled 20-mer oligonucleotides were immobilized via Schiff-base formation. The effect of surface pretreatments and the influence of different process parameters on the properties of the resulting functionalization layers

SESSION M6: Biological and Bio-inspired Templates for Materials Synthesis and Organization

Chair: George Bachand

Thursday Morning, March 31, 2005
Room 3000 (Moscone West)


Significant challenges exist in assembling nanoscale devices and be able to address or measure responses at the same level. Even though the scale gap between the size of the molecule and the limits of lithography is shrinking, simpler and inexpensive approaches are being pursued to develop bottom-up self-assembly techniques. One approach is to use biomolecules as scaffolds because of the specificity that they offer. Such scaffolds naturally offer the promise of exquisite control of positioning multiple components on the molecular scale but also can bridge the scale gap by interfacing with lithographically defined structures or undergo further self-assembly into extended structures by themselves. Using genetically engineered 30 nm Cowpea Mosaic Virus (CPMV), we have demonstrated the usefulness of engineered proteins as scaffolds for bottom-up self-assembly of ordered nanostructures. In this presentation two specific examples of CPMV, modified to express cysteine residues on the capsid exterior, will be presented. In the first example, gold nanoparticles were attached to the viral scaffold to produce a pattern with specific interparticle distances. The nanoparticles were then interconnected using thiol-terminated conjugated organic molecules that can act as molecular wires, resulting in a three-dimensional conductive network. The possibility of using the conductive network as a functional electronic device will be discussed. In the second example, we demonstrate the use of the cysteine mutant to enhance the detection sensitivity in array based biosensors. In fluorescence based detection assays, signal intensity is determined by the amount of label that can be localized at the reaction site. Traditionally, this is done by increasing the number of fluorophores attached to the target antibody or DNA. However, this approach lacks control over the fluorophore to fluorophore distance thus leading to significant quenching via resonance energy transfer. CPMV provides a platform on which such fluorophores can be arranged with controlled intermolecular distance without self-quenching, leading to increased assay sensitivity and decreased false negatives. Further these scaffolds can be used in simultaneous multianalyte detection.

9:00 AM M6.2 Use of Quantum Dots for Live Cell Applications. Austin Derfus, Warren C. W. Chan, and Sangeeta N. Bhatia; 1Bioengineering, University of California, San Diego, La Jolla, California; 2Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada.

Labeling of live cells with fluorescent probes allows tracking of mixed cell populations both in vivo and in vitro. While the time scale of observation for conventional organic dyes is limited due to photobleaching, semiconductor quantum dot (QDs) have surfaced as a bright, photostable alternative. Furthermore, the emission properties of QDs can be tuned by size and composition, permitting the synthesis of a large set of probes to monitor many cell types simultaneously. While several groups have been successful in labeling cell populations with receptor-mediated endocytosis, we found that complexing the QDs with some transfection reagents (notably cationic liposomes) or using electroporation allows improved labeling of cell populations (as quantified by flow cytometry). With these methods of serial labeling, cell populations can be tracked through multiple doublings and/or days in culture. While these methods deliver QDs to the cytoplasm of live cells, the nanoparticles occur in aggregates rather than as single QDs. Monodisperse particles are desirable for a number of uses, but most notably for delivery into the nucleus and other organelles, where the large size of an aggregate is a barrier to entry. While organic dyes used to label organelles (DAPI, Mitotracker, etc) are able to permeate cell membranes, the size and surface properties of QDs prevent passive diffusion across the lipid bilayer. Microinjection of QDs into the cell’s cytoplasm, however, does achieve the desired staining associated with fluorescence microscopy. In the cytoplasm, we demonstrated the active trafficking of QDs to the mitochondria and the nucleus by conjugation to canonical localization peptides. The photostable nature of the QD labels allows observation of these organelles over signal. Unlike QDs, modification to serve as an ideal model for these investigations, questions about the potential cytotoxicity of these cadmium-containing nanocrystals have surfaced. Our findings suggest that photoxidation of the surface can lead to release of cytotoxic levels of free cadmium, and these effects can be minimized by appropriate surface coatings and reductions in dose. Coated QDs were shown to be useful for long term live cell labeling of hepatic tissue in vitro without deleterious effects on cell growth, migration, or differentiation. In vivo, however, must be critically examined, as our results suggest Cd release is a possibility over time. Rather than signal an end to the future of QD labeling, these findings suggest design parameters to minimize heavy metal toxicity in biological applications.

9:15 AM M6.3 Fabrication of Core-Shell Drug Nanoparticles for Therapeutic Delivery. Aijaz S. Zehri, Cheryl Rumberger, and Michael V. Pishko; 1Chemical Engineering, Penn State University, University Park, Pennsylvania; 2Chemistry, Penn State University, University Park, Pennsylvania; 3Materials Science and Engineering, Penn State University, University Park, Pennsylvania.

A layer-by-layer (LbL) self-assembly technique was used to encapsulate core charged drug particles in a polymeric nanoshell. This approach provides a new strategy in the development of polymeric vehicles in controlling drug release and targeting to diseased tissues and cells specific to a human illness, such as cancer. Encapsulation of the drug within a polymer can help regulate its release at the diseased site. Therefore, a nanoshell composed of two bio-polymers, poly-L-lysine and heparin sulfate, were assembled step wise onto core charged drug nanoparticles. The exterior surface of the nanoshell was functionalized with biocompatible and targeting functional moieties, polyethylene glycol (PEG) and folic acid, respectively. In vitro, the novelty in this LbL assembly technique is that both hydrophobic and hydrophilic drugs may be delivered in a controlled and site-specific manner. Drug nanoparticles of dexamethasone were fabricated using a modified solvent evaporation technique. Optimization studies illustrated that dexamethasone nanoparticles within a size range of 314.0 to 154.7 nm can be synthesized by adjusting the surfactant concentration, drug concentration, and organic to aqueous volume ratio. The surface morphology of the encapsulated drug nanoparticles were viewed by transmission electron microscopy (TEM) and scanning electron microscopy. The TEM images indicated that the nanoshell was approximately 5 nm, composed of two polymer layers. Characterization of the surface chemistry and charge of the nanoshell required the use of x-ray photoelectron spectroscopy (XPS) and zeta potential, respectively. XPS data collected for PEG modified drug nanoparticles confirmed that the PEG surface layer was greater than 80%. The composition of the repeat unit in a PEG molecule. Also, zeta potential results re-confirmed PEGs presence at the surface. The chemisorption of PEG molecules neutralizes the surface of the nanoshell and this was illustrated by the measured neutral zeta potential of the drug particles. Preliminary biocompatibility studies to study phagocytosis of the PEG modified drug particles were performed using a flow cytometric assay. Results from our model drug delivery system, PEG modified fluorescent beads, suggest that the neutral charge of the nanoshell results in a decrease in phagocytosis after 24 hours of incubation. The results to date hold promise in using the LbL technique to control the surface chemistry when fabricating a nanoshell for drug delivery.


Due to their desirable optical properties, semiconductor nanocrystals, or quantum dots (QDs), hold promise as biological imaging agents. Unlike organic dyes, QDs exhibit continuous absorption profiles at wavelengths ranging from the ultraviolet to the band edge, narrow emission profiles, and high photostability. These features facilitate multiplexed analyses difficult with traditional fluorophores. High quantum efficiencies further suit QDs to biological imaging, especially for wavelengths in the infrared (IR), at which relatively few dyes are bright and stable. One disadvantage of many QDs for biological imaging, however, is their toxic nature. Heavy metal containing QDs like CdTe, CdSe, and PbSe elicit particular concern. Here we report the
synthesis of InAs/ZnSe core-shell QDs that have been tuned to emit at near-IR wavelengths well suited to biological imaging, from 750 to 850 nm. These cores contain high solubility and stability required for medical use in humans or already present in the body. The InAs cores are smaller than 2 nm in diameter, a size previously nearly unknown and not well characterized for InAs. The ZnSe shell grown on these oxidized core materials can be varied to control stability in air. Additional ligand exchange with dihydrogen acid permits solubility and stable fluorescence in water.

9:45 AM M6.5
The Design of Potent Polyvalent Inhibitors of Anthrax Toxin. Amit Joshi1, Kunal Gujraty1, Saleem Basha1, Jeremy Morglide2 and Ravi Kane3; 1 Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York; 2 Department of Pathobiology and Laboratory Medicine, University of Toronto, Toronto, Ontario, Canada.

We report the design of potent nanoscale therapeutics based on the concept of polyvalency. Polyvalency, the simultaneous binding of multiple ligands on one biological entity to multiple receptors on another, is a phenomenon that is ubiquitous in nature. We are using a biomimetic approach, inspired by polyvalency, to design potent inhibitors of anthrax toxin. Since the major symptoms and death from anthrax are due primarily to the action of anthrax toxin, the toxin is a prime target for therapeutic intervention. We used plasmon to identify peptides that bind to various protein targets, and synthesized polyvalent inhibitors of different sizes and valency. These polyvalent inhibitors are several orders of magnitude more potent than the corresponding monovalent inhibitors and can neutralize anthrax toxin in vivo. We will describe the design, characterization, and testing of inhibitors that prevent the assembly of anthrax toxin and inhibitors directed towards the cellular receptor for the toxin. The inhibitors developed during this work may enable the successful treatment of anthrax during the later stages of the disease when antibiotic treatment is ineffective.

10:20 AM M6.6
Self-assembling biomolecules that form highly ordered structures have attracted interest as potential alternatives to conventional lithographic processes for patterning materials. Genetic engineering can be used to create designer proteins that are capable of recognizing, binding, and organizing materials with a high degree of specificity. By genetically functionalizing self-assembling proteins, template-driven synthetic processes can be used to direct the formation of arrays of materials with order defined by the nanoscale structure of the template. We have developed a technique for patterning materials on the nanoscale that relies on the genetic manipulation of a self-assembling protein cage called a chaperonin. Chaperonins are composed of multiple protein subunits that naturally associate to form rings 20 nanometers in diameter, which can also be induced to form higher-order structures such as two-dimensional crystals and filaments. By engineering affinity for inorganic materials at defined locations on the chaperonin structure, we have been able to specifically template the formation of ordered arrays of magnetic nanostructures, conducting nanoparticles, semiconductor quantum dots, and inorganic nanowires. The natural affinity of chaperonins for spherical and/or elongated liposomes and lipid tubules enables patterning through self-assembly onto surfaces with complex geometries in addition to flat surfaces. The synthesis and properties of patterned arrays of inorganic materials using engineered proteins will be presented, with an emphasis both on device applications and on practical limitations of using such systems.

11:00 AM M6.7
Nanomaterials through Self-Assembled Monolayer and Protein Hybrid Assembly. Hong Ma, Melvin T. Zin, Mun-Sik Kang, Qing-nlin Xu, Joel S. Horwitz, Mehmet Sarikaya and Alex K.-Y. Jen; 1 Dept of Materials Science & Engineering, University of Washington, Seattle, Washington.

The synthesis and processing techniques are now established for obtaining high quality monodisperse nanocrystals of various semiconducting and metallic materials, fullerenes of varying properties, single- and multi-wall carbon nanotubes, as well as polymeric dendrimers and other nanophase systems. The next key step towards applications in device fabrication is to incorporate nanocomponents into functional and desired nanostructures without mutual aggregation. In this presentation, we will highlight the design and synthesis of new fused-ring molecules, such as the anthracene-based aromatic self-assembling molecules with tunable energetic parameters (pi-pi stacking, hydrogen bonding, dipole-dipole interaction) and geometrical factors to achieve highly-ordered and rigid self-assembled monolayer (SAM) templates. Through genetically engineered proteins for inorganic (GEP), the selective integration of nanocrystals onto a medium like SAM templates can be performed by micro-contact printing of nanomaterials-conjugated self-assembling proteins, patterned assembly of nanocrystals and CNFs has also been achieved.

11:15 AM M6.8
Nanotechnology, NASA Ames Research Center, Moffett Field, California; 2 Department of Materials Science and Engineering, University of Alabama - Birmingham, Birmingham, Alabama; 3 Department of Pathology, Univ. of Alabama - Birmingham, Birmingham, Alabama; 4 Dept of Physics, Univ. of Alabama - Birmingham, Birmingham, Alabama.

The excellent biocompatibility and bioactivity of calcium phosphate nanostructured surfaces and nanoparticles offer a promising pathway for controlling key bioengineering processes such as cell cycle regulation, gene transfer, and patterned cell growth. The ionic dissolution products from these materials are known to affect, for example, the cycle of cells responsible for bone tissue formation. This effect may be genetically mediated as expression of various families of genes has been shown to be upregulated by Ca2+ ions. These include genes for cell-signaling molecules, growth factors, DNA synthesis and repair, and extracellular matrix proteins. If the temporal sequence of the cell cycle can be matched to the kinetics of calcium phosphate dissolution, there is a clear opportunity to exert control over the cell cycle using these nanoengineered biomaterials. The release of ions according to a prescribed time sequence and concentration may enable control of cell proliferation and tissue growth at desired rates and locations. Calcium phosphate surfaces are also known to provide an excellent substrate for strong cell adhesion, differentiation, and proliferation. This biological affinity manifests itself even more forcefully in the common observation of trans-membrane cellular uptake of calcium phosphate nanoparticles carrying DNA fragments. Because of their tunable biodegradability and potential influence on cell cycle, these DNA-carrying nanoparticles may be suitable for gene transfer. Basic investigations of controlled cell response and gene transfer induced by calcium phosphate nanostructures have been limited, however, because of the heterogenous nature of calcium phosphate nanoparticles that are commercially available and commonly employed in these studies. In this work, we have focused on the use of a novel technique known as nanoparticle beam pulsed laser deposition (NBPLD) to create calcium phosphate nanoparticles of well-controlled size, structure and chemical composition that are delivered to flat silicon surfaces or dispersed in balance solutions and subsequently exposed to cells for gene transfer experiments. Nanoparticle sizes can be tuned in the 3-20 nm range with nanoparticle concentrations may be varied between 5×1011 and 1012 cm2 -2 on flat surfaces and between 106 and 1018 cm-3 in solution. We have targeted the control over phase make-up of calcium phosphate nanoparticles composed of nonresorbable hydroxyapatite and other bioresorbable calcium phosphate phases by adjustment of the process parameters. Precise control of the bioreversible phase dissolution in these nanoparticles may be useful in adjusting the timing of cell stimulation and gene transfer that often must be synchronized with other biological processes. In-vitro measurements of gene transfer efficiencies as a function of calcium phosphate nanoparticle size, composition, and chemical environment (silicon surface or balance solution) will be discussed.

11:30 AM M6.9
Methods to Preferentially Integrate Active Proteins within Mesoporous Silica Thin Films. Andrew M. Dattelbaum, Mac G. Brown, Aaron S. Anderson, Min S. Park and Andrew P. Shreve; 1 Materials Science & Engineering, University of Alabama - Birmingham, Alabama; 2 Department of Biochemistry and Molecular Biology Division, Los Alamos National Lab, Los Alamos, New Mexico.

In this presentation we will discuss ways to preferentially integrate active biomolecules, such as proteins, within the nanometer-sized pores of mesoporous silica thin films. The ability to immobilize active biomolecules into bulk mesoporous powders for enzymatic catalysis is known. The inorganic framework has been shown to stabilize immobilized proteins, which are located within the protective confines of water-filled nanopores. There are, however, several advantages to immobilization of biomolecules in this thin film architecture including the ability to better characterize immobilized materials by spectroscopic or electrochemical methods, as well as being more amenable to sensing and imaging applications. Here we show that mesoporous thin films can be used to immobilise proteins in ways similar to the preparation of bulk powders using cytochrome c as an example. We also show that functional groups may be grafted into the nanometer-sized pores of...
mesoporous thin films, and then patterned using deep-uv light to preferentially accumulate proteins within functionalized regions of the film. Both electrostatic and specific covalent interactions have used to promote bio-immobilization. The proteins used for these studies were either natural luminescent proteins or were labeled with luminescent molecules. The effectiveness of preferentially integrating active proteins within mesoporous thin films will be demonstrated using fluorescence imaging microscopy techniques.

11:45 AM M6.10
Developing Complex Structures and Functions through Cell-Directed Self-Assembly. Helen K. Basu1, Carlee Ashley1, Eric Carnes1, Deanna Lopez2 and C. Jeffrey Brinker2,3. 1Chemical and Nuclear Engineering, University of New Mexico, Albuquerque, New Mexico; 2Center for Micro-Engineered Materials, University of New Mexico, Albuquerque, New Mexico; 3Sandia National Laboratories, Albuquerque, New Mexico.

The integration of biological building blocks, from molecular assemblies to whole, living cells, into functional platforms is important to applications across the field of nanotechnology. Hybrid materials for tissue engineering, addressable arrays for bio-sensing, or the harnessing of tissues such as molecular motors or pumps in engineered systems all require the development of device fabrication schemes that incorporate the biological unit while maintaining functional bio/inorganic interfaces. We report a cell-directed self-assembly (CDSA) approach to the integration of biological materials with a nanostructured inorganic host that insures long-term functionality through a porous, buffer-filled environment and 3-dimensional confinement. During immobilization of S. cerevisiae cells in a porous, buffer-filled silicon novel interface through which it both directs the assembly of the inorganic host phase and provides a fluid, membrane-like environment for the localization of proteins and nanocrystals in extended nanoscale arrays, we show the cells attract and maintain a fluid region of aggregated lipids near the cell wall, directing a structural transformation of the system as it transitions through the interface to the inorganic phase. Replacing the cell with several proteins demonstrated that the living cell is necessary for the formation of the lipid interface and subsequent ordering of the inorganic phase, serving as a site for lipid aggregate nucleation and ordering during CDSA. The living cell’s response to osmotic stress is likely an important part of its ability to direct the structure of its inorganic host, including both the presence of a lamellar phase at an unexpected lipid/silica ratio and the d-spacing dependence of this phase on cell concentration. While CDSA forms a highly biocompatible, multimolecular phospholipid reservoir that maintains cell viability within the nanostructured host, we believe it will also become an important tool in allowing cells to efficiently organize proteins and nano-objects for localization at the cell surface or incorporation into the cell.

1:30 PM M7.4
Generalized Electrochemical Methods for Sensitive and Label Free Detection at the Nano-Bio Interfaces. *Jian Liu1, Zhengrong Tiao1 and Gisheng Bie1. 1Chemical Synthesis and Nanomaterials, Sandia National Labs, Albuquerque, New Mexico; 2Department of Chemistry, University of Arkansas, Fayetteville, Arkansas.

Electrochemical methods are widely investigated for interrogating the nano-bio interfaces. However, the molecular binding at the interfaces does not automatically generate a signal that can be recognized by analytical tools. Recently we have developed generalized methods for highly sensitive and label-free analysis based on self-assembled nanomaterials. The detection depends on how the molecules in the electrolyte solution diffuse onto the electrodes, or on the way the molecules on the electrode respond to the specific binding. Our method achieved at least attomolar level sensitivity (aM) (10^-18 mol) using conventional cyclic voltammetry, many orders of magnitude lower than those reported by typical electrochemical methods. Through this approach we can detect a wide range of biological and chemical species. * Sandia National Laboratories is a multi program laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the Department of Energy under Contract DE-AC04-94AL85000.

2:00 PM M7.2
Immobilization of Proteins on Arrayed ZnO Nanorods Grown on Thermoplastic Polyurethane (TPU) Flexible Substrate for Biomedical Applications. Ting-ju Liu, Hung-Chou Liao, Chia-Ching Lin, Shiang-Shing Hsu, Chi-Sheng Hsiao and Sun-Yuan Chen. 1Materials Science and Engineering, National Chiao Tung University, Hsinchu, Taiwan.

Novel composite arrayed nanorods consisting of ZnO nanorods were grown on thermoplastic polyurethane (TPU) flexible substrate via solothermal process in low temperature (348 K) and proteins [human serum albumin (HSA) and collagen] were then immobilized to the surface of ZnO nanorods modified (-SH and -COOH functional groups) by dimercaptopropanoic acid (DMSA). The surface characteristic of novel arrayed ZnO nanorods was investigated by field-emission scanning electron microscope (FESEM), energy dispersive spectroscopy (EDS), transmission electron microscope (TEM), X-ray photoelectron spectrum (XPS), Raman spectra, Fourier transform infrared spectrum (FTIR), atomic force microscope (AFM), and contact angle measurements. Moreover, surface grafting density of carboxyl groups of thiol-modified ZnO nanorods and immobilized proteins were examined by dying with C.I. Basic Blue 17 and Coomassie brilliant blue (CBBG) assay, respectively. The results display that ZnO nanorods show a single crystal wurtzite structure with a diameter of about 60-200 nm and can be grown on polymer surface over a large area (diameter 4-10 mm). This should be a significant breakthrough in optoelectronics, sensors, transducers and biomedical sciences fields. In addition, DMSA modification and proteins immobilization of the ZnO nanorods also have been proved by C-1s, O-1s, S-2p, and N-1s scan spectra of XPS, EDS, dye assay, Raman and FTIR spectra. Furthermore, immobilization of proteins caused the water contact angle to reduce, in other words, the surface hydrophilicity was increased. Better cell surface hydrophilicity lead to better biocompatibility and hemocompatibility. Besides, TPU has been approved for in vivo implantation by FDA and ZnO is stable and can be applied in suntan lotion. For these reasons, the protein-immobilized nanorods on TPU substrate might be applicable to biomedical device such as biosensor or cells-scaffold. Based on this preliminary study, the investigation for immobilization of antibodies instead of proteins and cyto-compatibility of novel nano-composite arrayed nanorods is now in progress in our group.
special sequences in the N-terminal domain of each sub-unit were obtained by the peptide phage display method, which enables us to select a target-specific sequence from a library. Our results showed that the sequence of N-terminus has a significant effect on both short-ranged (van der Waals, dipole-dipole, etc.) and long-ranged (electrostatic) ferritin-substrate interactions. That is, the sequence in the ferritin N-terminus is responsible for the long-range effective charge of ferritin, resulting in the direction of long-range interaction. In addition, the sequence affects the adhesion energy by factors of more than 10. By analyzing the results of AFM force-distance curve and quartz crystal microbalance (QCM) measurements under different conditions (salt concentration and pH), we discuss the mechanism of ferritin-substrate interactions in terms of DLVO force (van der Waals attraction and electrostatic interaction) and hydration force. Moreover, we propose special characteristics of target-specific sequence picked up through the selection process of the peptide phage display method.

2:45 PM M7.5 Sol-gel TiO2 Modified Biomimetic Surfaces: Nanostructuring, Surface Characterization, and Osteoblast Cell Growth Studies. Rigoberto Advincula1, Ma. Athena Advincula2, and Jack Lemon3, 1Department of Chemistry, University of Houston, Houston, Texas; 2Department of Biomedical Engineering, University of Alabama at Birmingham, Birmingham, Alabama.

The design of surfaces that induce controlled, guided and rapid healing is a main goal in immobilizing molecules to surface of implants. To this goal, nanostructured biomimetic and bioactive sol-gel derived titanium oxide composites were prepared using electrostatic layer-by-layer (LbL) method for the preparation of self-assembled monolayers (SAMs), and molecular imprinting techniques on titanium alloys. Previous studies were made on ideal flat surfaces on Au-Coated glass and Si-wafers. Chitosan and poly(acrylic) acid (PAA) were deposited layers by layers with the thickness of 125 nm via LbL method. Polyethylene glycol (PEG) derivatives were chemisorbed on the LbL films. Adhesive peptide Arg-Gly-Asp (RGD) were imprinted on titanium oxide matrices by molecular imprinting techniques. The surface topography, composition and adhesion strength of the different layered composites were investigated by surface plasmon spectroscopy, ellipsometry, SEM, XPS, AFM, and pull test. The biological activity was determined by the initial adhesion of MC3T3 cells after 24h. The stability and selectivity of the RGD-imprinted film was determined by measuring the absorbance of bound integrin by ELISA after rebinding of RGD guest molecules. Higher cell numbers observed on chitosan compared to PEG was dependent on surface composition, wettability and topography. ELISA results indicate selective recognition of the template for RGD guest molecules. The biocompatibility of the oxide was determined by cell adhesion of MC3T3 cells and mineralization in extended cultures and compared with a commonly passivated alloy substrate. Higher adhesion of osteoblast cells at 1 and 24 hours and formation of bone nodules at 14 days were found compared with the passivated substrate. These results were attributed to the rougher, porous surface, hydrophilicity and increased hydroxyl groups content of the material. The results indicate that higher hydrogen termination required by cells to attach and form bone nodules in vitro.

3:30 PM M7.6 Fabrication and Evaluation of Micro to Nano Hierarchical Structures of Nanoporous Architecture for Bone Biotemplating. Ketel Popat, Vivek Mukhathay and Tejal Desai, Biomedical Engineering, Boston University, Boston, Massachusetts.

Surfaces that contain micro- and nanoscale features in a well-controlled and engineered manner have been shown to significantly affect cellular and subcellular function. In this work we have developed and implemented microfabrication routes for producing metal-oxide films with controlled nano to micro architecture, potentially moving us closer to the goal of osteointegration. We propose using these unique films for bone biomimetic and bioactive substrates that control the growth of osteoblasts. We hypothesize that controlled nanoscale architectures can promote osteoblast differentiation and matrix production, and enhance long-term osteointegration. Moreover, the ability to create novel nanotextured surfaces that mimic physiological systems can aid in studying complex tissue interactions in terms of cell communication, response to matrix geometry, and effects of external chemical stimuli. We have used photolithographic techniques to create localized patterns of nanoscale (0-100 nm) features that are surrounded by either a non-adhesive background or by a different porosity. It is probable that osteoblast requires a hierarchical substrate that presents variations at both the micron and mm scale to express fully differentiated behavior. By localizing nanostructured regions, we will restrict spatially the signals that these materials present to the cell. The spatial segregation of signals is required for the normal functioning of many types of cells in-vivo, and we suspect that this principle holds for osteoblasts as well. For example, localized patches of porous alumina can be created using photolithographic and combination and wet and dry etching techniques. By understanding biomimetic substrates for cellular adhesion and differentiation we can more effectively design biomaterials interfaces.

3:45 PM M7.7 Incorporation and Controlled Release of an Anti-Inflammatory Drug Using Electrospun Biodegradable Polymers Coated with Conducting Polymers for Neural Tissue-Microelectrode Interface. Mohammad Reza Abidian1, Matt Meier2, and David C. Martire3, 1Biomedical Engineering, University of Michigan, Ann Arbor, Michigan; 2Materials Science and Engineering, University of Michigan, Ann Arbor, Michigan; 3Macromolecular Science and Engineering, University of Michigan, Ann Arbor, Michigan.

The interface between microfabricated neural microelectrodes and neural tissue plays a significant role in the long term performance of these systems. It is thought that biocompatible polymer coatings can stabilize the interface between microelectrode and living tissue at the site of implantation. The ability of neural electrodes to record high signal over extended periods of time remains a significant problem. The engineering of bioactive electrode coatings has been investigated for its potential to promote in-growth of neural tissue, reduce shear stress, and enhance signal transport from electrodes to ions at the electrode-host interface. We have developed a new approach for preparing anti-inflammatory drug loaded conducting polymer nanofilms. This approach entails electrochemical deposition of poly(3,4-ethylene oxydiene-oxithiophene) (PEDOT) on the electrode sites and around an anti-inflammatory drug (dexamethasone) loaded into electrospun poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PS) films. Incorporation and sustained release of drug from nanotubular PEDOT scaffolds was demonstrated. The electrical properties of the drug loaded polymer functionalized microelectrode were examined with impedence spectroscopy and cyclic voltammetry. The impedance results revealed that the impedance of gold electrode sites significantly decreased from 800 kΩ to 8 kΩ at PEDOT deposition on the electrode sites and around the PPA layers. The surface morphology of the coated electrodes was examined by optical microscopy and scanning electron microscopy. Scanning electron microscopy (SEM) and focused ion beam (FIB) showed the nano tubular structure of conducting polymers on the electrode sites.

4:00 PM M7.8 Human Mesenchymal Stem Cell Adhesion and Spreading on Nanoporous Diamond and Plasma Nitrided Titanium Alloy Surfaces. William C. Clemen, Susan L. Boll, and Shane Aaron Catledge, and Yogesh K. Vohra, 1Physiology & Biophysics, University of Alabama at Birmingham, Birmingham, Alabama; 2Biomedical Engineering, University of Alabama at Birmingham, Birmingham, Alabama; 3Physics, University of Alabama at Birmingham, Birmingham, Alabama.

Adverse effects of joint replacements are generally the result of the wear debris and corrosion products of the materials used in these implants. The wear debris produced can be in the form of toxic metallic ions, inorganic metal salts, or polymeric debris bound by proteins. The large surface area characteristic of nanometer-scale debris particles may lead to undesirable biointeraction. The adverse effects can be reduced by using less toxic materials and by improving the hardness to reduce the amount of debris particles. We report surface modifications of titanium alloy (Ti-6Al-4V) and of nanostructured diamond using microwave plasma chemical vapor deposition (CVD) to produce nitrided titanium and oxygen-terminated diamond surface layers, respectively. Preliminary data indicates that the adhesion of osteoprogenitor cells (human mesenchymal stem cells, MSCs) to nitrided titanium is nearly equivalent to that of Ti-6Al-4V, while hardness was improved over 4-fold. This data indicates that the plasma nitriding technique will reduce the possibility of implant loosening over time. Less cell adhesion is observed on diamond coatings compared to all other surfaces we have tested. However, by replacing only the atoms that terminate the diamond carbon lattice, we are able to increase or decrease cell adhesion without reducing the hardness of the surface. We find that oxygen-terminated diamond promotes MSC adhesion while hydrogen-terminated diamond resists protein adsorption. Hydrogen-terminated diamond promotes adhesion. When exposed to a collagen-containing solution, hydrogen-terminated diamond readily adsorbs a collagen layer that allows for a significant increase in adhesion of MSCs in cell culture. We believe our nanoscale approach, designing materials from simple starting molecules like the carbon atoms from methane, and surface modifications with biomimetic peptides will produce cartilage materials with ideal mechanical properties and excellent.
biocompatibility. Acknowledgements: We acknowledge support from the National Science Foundation (NSF) - Nanoscale Interdisciplinary Research Team (NIRT) program under Grant No. DMR-0402891.

4:15 PM M7.9
Nano Structured Platforms for Cellular Analysis Devices. Shalini Prasad1, Sathyajith Ravindran2, Cengiz Ozkan3 and Milhi Ozkan4, 5: 1 EE, University of California, Riverside, Riverside, California; 2 CEE, University of California, Riverside, Riverside, California; 3 ME, University of California, Riverside, Riverside, California.

Understanding cell interaction and behavior in a controlled and in-vitro environment is essential for developing diagnostic and testing micro-devices. These devices should have the capability to record measurable changes to the cells physiology due to micro/nano scale interactions between the cellular processes and the external micro-environment. To achieve this it is essential to develop substrates that are bio-compatible and non-biodegradable. There are three requirements that need to be satisfied. First the substrates should demonstrate scaffolding properties namely they should promote cell adhesion, proliferation and ensure viability over periods suitable for measurement of recordable events based on the applications (> 24 hours). Secondly, the substrate should allow for cells to undergo morphological changes associated with their growth and development that mimic the in-vivo model and thirdly, assist in the visualization and measurement of cell-cell interaction in the form of signal transduction. We present here a comparison among three substrates that demonstrate nano porosity and surface roughness namely: porous alumina, vertically patterned multiwalled carbon nanotube substrates and metallic nanowires substrates for developing miniature cellular analysis devices. We determine the capability of each genre of substrates for promoting cellular outgrowth and measurement of in-situ variations to the cellular electrical activity associated with cellular morphological changes. These substrates are suitable for devising a number of applications for mammalian cells with excitable cell membranes.

4:30 PM M7.10
Nanostructured Ceramic Platform for Living Neuronal Network Chips. Dmitri Routkevitch1, Michael Stowell2, Maria Pagonis1, and Oleg Polyakov1j Synkera Technologies Inc., Longmont, Colorado; 2 University of Colorado, Boulder, Colorado.

Unlocking the mechanism of neural growth and communication is needed for understanding and treatment of many degenerative diseases, as well as for neural prosthesis and restoration of damaged neural connections. Living neural networks (LNN) can enable a broad array of new tools for neural research. Furthermore, LNNs being capable of detect minute environmental perturbations, are an attractive target for chemical and biological sensing. However, producing reliable LNNs is challenging, and requires control over the neuronal growth and formation of synaptic junctions, high charge density high-resolution neuronal contacts, overall biocompatibility and reproducibility. Substrates for LNNs that would satisfy these requirements are not available. In this presentation we will describe our approach to addressing this opportunity via use of self-organized nanoporous alumina ceramic as a platform for guided growth and interfacing of LNNs. The core of the approach is in synergy of several ideas: nano-engineering of the anodic alumina to provide tailored neuron/substrate interface; hybrid micromachining of patterns for neural growth guidance; using encapsulated nano-electrodes arrays and routing the excitation/response signals to the bottom of the chip to provide soft high resolution electrical contacts to neurons.

4:45 PM M7.11

Since many inorganic biomaterials are apatite-like, hydroxypatite (HAP) is commonly used as a model for biological minerals. Our research has shown that both surface energy and particle size during the dissolution reactions control the formation of extraordinarily stable nano-sized HAP crystallites. The in vitro demineralization kinetics of human tooth enamel surfaces is investigated using nanomolar-sensitive constant composition (CC) methods (pH=4.50, ionic strength=0.15M, temperature=37°C and relative undersaturation with respect to HAP = 0.90). Following an initial rapid removal of surface polishing artifacts, the demineralization rates decrease as the reactions proceed (e.g. 5.4±0.5 x 10^-11 and 3.5±0.5 x 10^-11 mol mm^-2 min^-1 at 180 and 3000 min in dissolution, respectively). This is in accordance with our new model for HAP dissolution, resulting in hollow enamel cores and nano-sized remaining crystallites, resistant to further dissolution. It is significant that the demineralization of human enamel crystallites may be suppressed when the particles approach nanometer size. This study shows that demineralization of enamel in acidic medium follows our previously proposed nano-dissolution model, which can be used to mimic cavity lesion formation. The model also suggests that nano-sized biomaterial crystallites may show a remarkable degree of self-preservation in the fluctuating physiological milieu. Supported by the National Institute of Craniofacial and Dental Research (DE 03252).

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