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

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Symposium Support
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* Invited paper
8:30 AM *M1.1
Design Rules for Biological Adhesives. Deborah Lockland, 1Chemical and Biomolecular Engineering, University of Illinois, Urbana, Illinois; 2Chemistry, 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 and rearrangement. Our investigations are revealing fundamental design rules for these complex molecular machines.

9:00 AM M1.2
Development and Characterization of a Novel Interface with Nanoscale Features. Jake D. Ballard1, 2, Ludovico M. Dell’Acqua-Bellavitis1, 3, Rena Bizios1, 4, and Richard W. Siegel1, 4; 1Materials Science & Engineering, Rensselaer Polytechnic Institute, Troy, New York; 2Rensselaer Polytechnic Institute, Troy, New York; 3Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, New York; 4Rensselaer Nanotechnology Center, Rensselaer Polytechnic Institute, Troy, New York.

Nanophase materials are emerging as prime candidates for the next-generation of biomaterials because, depending on their nanoscale features, such materials have been shown to elicit selective, desired responses from the cells. Differences in the distribution of cells, including attachment, proliferation, and differentiation, on nanoscale surface features (compared to larger scale features) have been attributed to the type and conformation of proteins (such as fibronectin 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 at understanding how the size and shape of decorate nanoparticles affect the adhesion of different mammalian cells. We present the results that demonstrate the fabrication of stimulus-responsive nanoscale arrays of a genetically engineered polypeptide by SPR that enable the controlled release of a cell-adhesive ligand in response to solution pH changes. In a second example, we describe the fabrication of micro-patterned protein-decorated nanoparticle arrays that transiently functionalize cell-surface proteins, enabling the attachment and recognition of specific cell-surface ligands by receptors. The results show that nanoscale patterns of biotin are then incubated with streptavidin, and the streptavidin patterns provide a versatile and flexible nanoscale architecture of streptavidin and the high affinity of the streptavidin-surface interaction. These patterns are useful to novel cell culture, that is, site-selective cell culture.

11:00 AM M1.6
Protein Adhesion to Bare and Polymer-coated Nanostructures. Maureen Dyer1, Kristy M. Ainslie1, Gaurav Sharma2, Craig Grimes3, 4, and Michael V. Pishko1, 2, 4; 1Chemical
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 the subcutaneous tissue or the intravascular will 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 cellular growth near the tissue-material surface. Further, the microarchitecture 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 analytic 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 without nanowires and was reduced even when the magnetostrictive nanowires were placed in a magnetic field. To enhance resistance to protein adhesion, the possibility of coating the nanowires with hyperbranched polymers, such as poly(ethylene glycol) was investigated. Attempting to improve upon these modifications, silicon wafers were used as model surfaces for developing the hyperbranching procedure. Poly(allylamine) and PEG were grafted in layers, ranging from one PAAm layer to a total of six alternating PAAm and PEG layers. Protein adhesion to the silicon wafers at each stage of polymer layers increased when the 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 formation of a protein coat on the coated implant, thereby increasing its efficacy and lifetime.

Self-assembled monolayers (SAMs) and surface patterning via microcontact printing (μCP) received a lot of attention in the last few years. Applications like two-dimensional patterns of biomolecules, proteins, cells, nanoparticles etc. Microcontact printing has potential to pattern the surface with sub-100 nm features. Molecular printboards were recently introduced by Reinholdt et al. (Angew. Chem. Int. Ed. 2004, 43, 369). This term refers to a chemisorbed layer of host molecules that can act as a template onto which guest molecules can be immobilized. These molecular printboards can serve to “write” or “position” and remove molecules using non-covalent chemistry. We develop a new method for repeatedly creating and “printing” the pattern on a substrate. Here, we present an approach using a covalent reversible reaction – imine formation on the gold and silicon oxide surface. Our platform and chemistry can be used to create a desired pattern that can be removed or rewritten. This platform can be recycled and used for immobilization or reaction with other molecules. The reaction of 11-aminoo-1-decanethiol (11-AUT) with propenal, pentanal and decanal produces well-organized imine monolayers, which can be hydrolyzed to 11-AUT monolayers. The imines undergo after hydrolysis return to their beginning composition. Printing of amines on aldehyde-terminated substrates via microcontact printing and removing of the pattern from the surface was also studied. Laser scanning confocal microscopy was also used to visualize reversible covalent patterning. Lucifer yellow ethylenediamine (N-(2-aminoethyl)-4-amino-3,6-disulfo-1,8-naphthalimide, dipotassium salt) as a fluorescent amine—containing marker was employed to detect the pattern on the glass surface. The dye was coupled to an aldehyde–terminated SAM on glass by imine formation from the solution and by μCP. This experiment also demonstrates the feasibility of recycling the patterned substrate. A “printing the pattern” was possible to develop it in the same place with high resolution. This method allows obtaining features more than three times on the same substrate with no loss of resolution. Exploring the reversible covalent chemistry of imines, we present examples of reversible attachment of peptide monolayers and also patterning the substrates through μCP and DPN. We show that “microcontact chemistry” – chemistry in the contact region between a stamp and a substrate – is a versatile tool to create (bio)chemical surface patterns and arrays.
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 functionality, compact size, and embedded membrane environment, 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 cytoplasm into sub-cellular compartments. 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 inserted a number of proteins, including mecano-sensitive channels, bacterial toxins, pore proteins, and, 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 membranes. We have inserted 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 regarding 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 enables a large number of devices, and the same RNA polymerase could 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 Curtia W. Frank; Chemical Engineering, Stanford University, Stanford, California.

Polymer-tethered lipid bilayers have become widely studied as model biomembranes for both fundamental biological research and in the development of applications such as biosensors. In these biomimetic systems, the water-swollen polymer layer separates the membrane from the substrate. This prevents non-physiological interactions between the incorporated biological components and the substrate. This polymer layer 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 cushions. 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 lipopolymer/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(octadecyl)amine, and the hydrophilic backbone monomer is D-glucose-2-propionate. Impacts of the lipidopolymer/lipid composition and polymer conformation are systematically compared using atomic force microscopy to monitor the monolayer and bilayer homogeneity and roughness. Finally, we will demonstrate more microscopically 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. Ankit 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-streptavidine linkage that has the potential to overcome the major limitations of other model membrane environments: protein-substrate interactions in planar supported bilayers; and destabilizing, layer-defects 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 surface, providing the ability to monitor the step-by-step formation of the assembly in addition to 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 streptavidine monolayer.

2:30 PM M2.4
Model Membrane Assemblies on Self-Assembled Fullerenol Surfaces. Gabriel A. Montane, 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 into biomembrane assemblies have important implications for many science, biophysics, and biotechnology. We report on such architectures that employ fullerene and phospholipids. Self-assembled monolayers (SAMs) of C90 and partially hydrolyzed polycyethylsililated-C60 (PCS-C60) were created using silane chemistry. The hydrophobic and hydrophilic nature of the C50 and C30-C60, respectively, was used to drive the formation of model membranes assemblies consisting of either phospholipids monolayers or bilayers. Also, patterned surfaces of C60 and PCS-C60 were generated 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 biomolecular assemblies appropriate for complex applications.

2:45 PM M2.5
Development of Novel Materials for an Isoprenylcycteine Methytransferase-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 isoprenylcycteine methytransferase (IcmM) 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 2,3-di-O-(eicosanyl)-sn-glycero-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 73 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-untilt mechanism. The synthesis and performance of a FRET-based fluorescein-mycetocysteine-methyltransferase (P-L-S-MR) molecular beacon that is activated by thios 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 S-adenosyl methionine-dependent IcmM. SAM is used as the methyl donor by many cellular methyltransferases and the enzymatic reaction results in the transfer of the partially hydrolyzed C-terminal 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
Micropatternning and Refunctionalization of Supported Phospholipid Membranes Using Photolithography. Approaches. Atul N. Parikh, Chanel K. Yee, Anna Popova R. R. M. 3009 (Mescone West)
This paper summarizes several photolithographic approaches developed in our laboratories for patterning and functionalizing supported phospholipid membranes. In particular, we present (1) a method for isolating and masking out domains of length &lt; 100 nm to 1 mm using a high-contrast g-sultimo UV radiation; (2) maskless multiphoton lithography; and (3) pseudo-epitaxial assembly of phospholipid monolayer/bilayer composites on patterned hydrophilic substrates. Mechanisms of pattern formation in each case will be discussed. We further present strategies for functionalizing the pattern-features by subsequent selective adsorption of lipids and proteins. Applications of these platforms in the investigation of biosensor studies of membrane dynamics will be illustrated. This work was supported by the NSF Center for Biophotonics Science and Technology and a grant from Basic Energy Sciences, Office of Science, U. S. Department of Energy.

4:00 PM M2.7

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

Quan Cheng, Thomas Wilkop and Zhuangzhi Wang; Chemistry, UC Riverside, Riverside, California.

Membrane-based biosensors have received considerable attention in recent years. A sensing device that employs antibody peptide granulin as a channel switch on a tethered membrane has been described. The latest work on stochastic sensing with &alpha;-haemolysin (aHL) shows another excellent example. To enable convenient recording of the signal with the sensing device, fusion of vesicles on solid substrates to fabricate a supported bilayer membrane as the sensing interface has been attempted. However, formation of transmembrane pores by toxins such as aHL on supported bilayer 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 improved the 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 surface hydrophilicity 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 assemblies under various fusion conditions were observed. The application of the hybrid lipid membranes in sensing of streptolysin O (SLO) and formation of transmembrane pores by the toxin will be discussed.

4:15 PM M2.8

Mimicking Nanometer-Scale Heterogeneity Using Gel-Liquid Coexisting Supported Lipid Bilayers.

Wan-Chen Lin, Craig D. Blanchette, Timothy Ratto and Marjorie L. Longo; 1Biophysics Graduate Group, University of California, Davis, Davis, California; 2Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, California; 3Chemical Engineering and Materials Science, University of California, Davis, Davis, California.

Supported lipid bilayers are currently receiving increasing attention. They not only serve as model membranes in the study of cellular processes but also exhibit unique membrane properties such as the presence of liquid- and solid-like phases. We have designed our supported bilayers by using a gel-liquid fusion (on a mica substrate) that consists of phase-separated dilauroylphosphatidyldcholine (DLPC) and dielayerphosphatidyldcholine (DSPC) in order to mimic nanometer-scale heterogeneous structures in biological membranes, or so-called ‘raft’ structures. The size of DSPC domains (range form 30 nm to 40 µm) was controlled by the cooling rate during the supported bilayer formation. We were also able to manage the inter-monomer coupling of phase-separated supported lipid bilayers by controlling the method of vesicle preparation. Using atomic force microscopy (AFM), fluorescence recovery after photobleaching (FRAP), and ligand-receptor binding assays we found that bilayers with partially asymmetric nonbilayer domains exhibit a flip-flop, to a state where all gel phase lipid domains partition to the top monolayer. During this conversion, we observed a fast, one-way DSPC flipping from the interface between coupled DSPC domains and uncoupled DSPC domains and also toward trapped DLPC pools within DSPC domains. Overall, the converting process seemed to be analogous to Ostwald Ripening, which leads to less DSPC-DLPC interface. On the other hand, the interface between coupled DSPC domains and coupled DLPC domains. It also gives us the highest proportion of molecular packing in the coupled DSPC domains. We related these stable and metastable states to hydrophobic mismatch and molecular packing in gel-phase domains. In addition, we observed a depletion of fluorescent probes in the leaflet directly opposite to the gel phase domains, indicating the gel phase domains in single monolayer are able to give an ordering effect on the opposing monolayer. Our results indicate that the hydrophobic mismatch at gel-fluid interface can be one of the important factors affecting the short-wavelength ordering of lipid membranes. In addition, the ability to control lipid domain size and lipid superposition is necessary for further bio-mimetic applications of supported lipid bilayers such as platforms for protein-bilayer interaction studies.

4:30 PM M2.9

Mimicking of the Stem Cell Niche: Ligands Incorporated into a Supported Phospholipid Monolayer.

Wor Jensen, A. Sofia Garcia, Shara M. Delattore, Bi-Huang Hu, Rico C. Gunawan, James A. King, Philip B. Messersmith and William M. Miller; 1Department of Chemical and Biological Engineering, Northwestern University, Evanston, Illinois; 2Biomedical Engineering Department, Northwestern University, Evanston, Illinois.

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 as such a complex system, recapitulating this environment in an ex vivo system will likely require the presentation of multiple ligands from a biomimetic surface. Accomplishing this will not only aid in the understanding of how these factors affect the 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 dipalmitoylphosphatidyldcholine (DPPC)-based solid 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 data shows 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 reached 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.

4:45 PM M2.10


Herman Sander Mansur and Juliano Oliveira; Metallurgical and Materials Engineering, Federal University of Minas Gerais, Belo 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 phosphate group. Both tails consist of a fatty acid, each 14-24 carbon groups long. Bilayers are not only essential parts in lipid membranes 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 bimembranes 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 electronic components. The possibility to synthesize organic molecules, almost without limitations, with designed structure and functionality in conjunction with a sophisticated thin film deposition technique enables the production of tailor-made, through chemical, 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 scale multilayer structures with varying layer composition. The use of
the present study was to characterize phospholipid deposited through LB technique by ATR-FTIR and QCM sensor. Phospholipid dimyristoylphosphatidylethanolamine (DMPE), phospholipid dimyristoylphosphatidylethanolamine (DPPPE) monolayers were fabricated using the Langmuir-Blodgett technique. Briefly, 50 ml of a chloroform solution (HPLC-grade, Sigma) of each phospholipid with a total concentration of 1 mg.ml-1 was spread at the air-water interface of a Teflon-made Langmuir-Blodgett trough (NIMA Tech., UK) containing a subphase of deionized water. The surface pressure was measured by a Wilhelmy plate. Some monolayers were spread using the single-shot method. Fifteen to 30 min were allowed before compression to ensure solvent evaporation.

Phospholipid monolayers were transferred onto the QCM crystal (9.0 MHz) and ZnSe crystal for ATR-FTIR measurements using the vertical dipping method at a mean surface pressure of 30 mN-m-1. Uniform molecular LB films of DMPC, DMPE, DPPPE phospholipids were prepared from monolayers generated on the QCM surface. The ratio and QCM frequency changes results. Also, ATR-FTIR spectra results have also indicated the surface molecular organization and packing.

SESSION M3: Poster Session: Developing Nano-Bio Interfaces
Chairs: George Becher and Henry Hess
Tuesday Evening, March 29, 2005
8:00 PM
Salons 8-15 (Marriott)

M3.1 Nonfouling Surface for a Highly Reliable Plaque Purification of Cells. Jinho Hyun1, Yeonho Je2, Yunna Kim3 and Byungcheol Shin4; 1Department of Biosystems and Biomaterials Science and Engineering, Seoul National University, Seoul, South Korea; 2School of Agricultural Biotechnology, Seoul National University, Seoul, South Korea; 3Advanced Materials Division, Korea Research Institute of Chemical Technology, Daejeon, South Korea.

In the presentation we demonstrate a simple method to micropattern surfaces with a short nonfouling oligoethylene glycol side-chains that enables long-term, spatially resolved attachment and growth of cells for a reliable plaque purification. The conventional plaque purification of cells infected with viruses was routinely performed with an aggregating cell mass. However, it was not adequate to expect the same spatial environments for the cells, and frequently resulted in the experimental error, quantitatively in picking up a cell. Instead, we propose a highly resolved cell microarray with nonfouling micropatterns for a controllable and reliable methodology of a plaque purification. Previously, control of cell-substrate contact area, cell attachment and growth, and cell-cell interactions in micro scale have been demonstrated using microfabricated surfaces. Micropatterning of localized chemical or biochemical domains has the potential to become a powerful tool to control the seeding of cells. Cells micropatterned on the surface were infected with baculovirus and immobilized with low-gelling 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 areas. Mixed cells infected either with a wild type virus or a GFP-modified virus were differentiated by observing the polyhedra 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. Ana Maria Trent, Andrew C. Bunker and Gregory O. Barkerd; 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 materials 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 utilized to 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 motor activity. The resulting dynamic change in the restriction zone will affect the gliding motility assay. Our results suggest that microtubules have varying sensitivity to metal ion species, but may be stabilized through covalent crosslinking. Similarly, Drosophila kinesins are differentially affected by Co2+ and Zn2+ concentration. The secondary control mechanism was introduced by using site directed mutagenesis to insert a high affinity Zn2+ binding site into the neck-linker region of the Drosophila kinesin coding sequence, which was confirmed by DNA sequencing. The response of the mutated kinesin, as well as the control of kinesin motility using different metal ions, is currently being evaluated using both the gliding motility and ATP hydrolysis assay. Overall, the use of metal ions to control kinesin motility will provide a simple mechanism for controlling active transport of nanoparticle and material cargo in synthetic systems.

M3.3 Guided Neurite Growth on Patterned Carbon Nanotube Substrates. Cengiz Sinan Ozyuzer 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 neural cells, and 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 pattern were chosen to address the three important features in scaffolds namely 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 side walls of the long nanotubes and trapping of the PLL molecules between the nanotubes at the pattern edges due to capillary action. In the case of short nanotubes during its growth a large percentage of the tubes get pinned to the substrate. These results in the absence of cell adhesions for PLL functionalized scaffolds also show that the short NTs do not offer the mimetic growth cone with a suitable surface for process development. The long NTs in comparison are flexible and undergo deformation to accommodate the proliferating neurite. The substrate offers potential towards developing three dimensional scaffolds suitable for implants due to guided surface coverage as well as cell viability in two dimensions. The major finding in the formation of the neuronal bridges was an understanding relating the interaction between the neuron and the MWNT scaffold. As the neurite started to extend from the soma the growth cone extended to envelope the nanotubes in the areas where they were treated with PLL. The consequence of the outgrowth of the neurite 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 Pahor Wiff,4, Cesar M. Fuenzalida1,4, V. M. Fuenzalida1,4, C. Pacheco2, A. Sanders1 and M. Redigol1; 1Department of Physics, Universidad de Chile, Santiago, Area Metropolitana, Chile; 2IPAD, Universidad de Vale do Paraiba (UNIVAP), Sao Jose dos Campos, Sao Paulo, Brazil; 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 biocompatible coating to cover these materials, which introduced 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 X-ray energy dispersive spectroscopy (EDS) 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 adhesion were evaluated for 1, 7 and 28 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 larger in comparison with no coated samples. In all cases the viability of CaTiO3-coated samples and the viability on the polymeric surface were 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 biomimetic-based implants. Moreover, the method can be scaled up to industrial production.

**M3.5**

**Magnesium-substituted Hydroxyapatite/Acylated Chitosan Nano-composite as Hydrophobic Drug-loaded Matrix for Blood-contacting Applications.**Tse Ying Liu, 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 hydroxyapatite/acylated chitosan nano-composite with high anti-compatibility was developed. Investigate degradable rate, drug release behaviors, acute cytotoxicity and anti-adhesive 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 enhance hydrophobic drug uptake such as paclitaxel (an efficient anti-tumor agent) with high drug-loading capacity. In addition, Mg-substituted hydroxyapatite (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 through the incorporation of Mg-HAp nano-composites. Furthermore, negative charged surface via the grafting of carboxyl groups could further improve the hemocompatibility of this nano-composite. The nanoparticles in the drug delivery system could act as a diffusion barrier through the incorporation of Mg-HAp nano-composites. In addition, degradable rate and release profile could be easily tailored by controlling the amount of Mg-substituted hydroxyapatite.

**M3.6**

**Fabrication and Characterization of Patterned Surfaces for Single Protein Arrays by Scanning Probe Techniques.**Joonyeong Kin1,2, James D. Batteas 1, Jeffery G. Forbes 2 and Kuan Yuan Chen and Shiang Chuan Chen; Material Science, National Chiao Tung University, ROC, Hsinchu, Taiwan.

High-density arrays of single protein molecules as building blocks and nanotemplates for controllable micro- and nano-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 nanocoated 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 the micrometer scale and adhesion forces at the molecular level. An ultra-flat gold surface was produced by evaporating gold onto silica. A SAM was then formed with hexadecylamine-glycidoxy terminated thiol (EG6-OH) to inhibit nonspecific outside the target. Nanocoating was done with a cantilever of the AFM with the S-Au bonds in the presence of N-hydroxysuccinimide (NHS)-terminated thiol that in turn replaced the dislodged EGS-OH groups. These patterned NHS-bond surface have been selective for the target 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 immunosensor arrays. Additionally, these patterned immune 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 Ekau-Nikolov2, Paul W. K. Rothemund2, Eric Winfree1, Deborah Fyngenson1, 1Department of Physics, UCSB, Santa Barbara, California; 2Computer Sciences, Computation and Neural Systems, California Institute of Technology, Pasadena, California.

We describe progress towards developing DNA Nanotubes into a tool for nano-patterning and assaying protein binding. DNA nanotubes are uniquely accessible templates 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-binding substrates with well-defined 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 ligase, restriction enzymes and regulatory proteins.

**M3.8**

**Novel Hepatic Cell Culture on Ultra-Water Repellent Film.**Yuming Wu, Nagahiro Saito2, Yasushi Inoue1, Akira Ito3, Hiroyuki Honda4 and Osamu Takai5; 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 spherical culture medium. This 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 propagate and establish. They have been cultivated in a conical test-tube culture or a weightless state. 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 culture on an 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 such as a water drop on UWR films. This contact angle was 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 without the dish was not vibrated. In 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 Khomutov2; 1Institute of Radioengineering & Electronics, Russian Academy of Sciences, kislov@mail.ru, 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
synthetic availability of desired nucleotide sequences and length. Development of new effective nanofabrication methods with reasonable control over position and size of the features is crucial for practical advancement in nanoscience and nanotechnology. Within activities of INT of Russia (INTC - Interdisciplinary Nanotechnology Consortium) we have developed the complete technological cycle of design, manufacture and application of new model nanomaterials (MNC) for nanoelectronics and some other areas [1, 2]. We have also synthesized novel DNA complexes with positively charged, highly luminescent CdSe nanorods that can self-assemble into fluorescent micro-patterns. DNA-CdSe-nanorods filaments possess strong linearly polarized photoluminescence due to the unidirectional orientation of nanorods along the filament axis [1]. V.V. Kolesov, I.V. Tarasov, S.P. Gubin, G.K. Khomutov, E.S. Soldatov, I.A. Maximov, L.Samanon, "Electronics of Molecular Nanochip", International Journal of Nanoelectronic, 3, No. 1, (2004): [2]. M. Artemeyev, D. Kisel, S. Abramstko, M. Antipina, R.V. G. Khomutov, V.Kiselnii, A. Rachynslyeja, J. Am. Chem. Soc., 2004, 126, 10594-10597.

M3.10 Effects of Cholesterol on Galactosylceramide Domain Size, Shape and Membrane Binding Properties: A Combined Atomic Force Microscopy and Fluorescence Microscopy Study

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 kirilowi (TKA). GalCer has been shown to exist on the extracellular leaflet of the cell membrane in fluorescent probes and fluorescence microscopy for viewing. We have demonstrated the amplification of micro-patterned lipid bilayers with silica microspheres. Patterned lipid bilayers made via stamping or UV lithography typically require fluorescent probes and fluorescence microscopy for viewing. This label-free process allows a patterned bilayer to be observed through regular optical microscopy and even by eye. This is made possible due to preferential adhesion of silica beads onto bilayer regions but not onto the regions of glass in the absence of patterned lipid. Introduction of lipid to the silica beads on a fluorescently labeled bilayer demonstrated lipid mobility through fluorescence migration onto the previously labeled silica beads.

M3.12 Formation of DNA/Au Structure on Hydrogen-Terminated Silicon through Direct Metal Deposition and its Observation with an Atomic Force Microscope


M3.13 Lipid Bilayer Membrane on Gold & TiO2 Solid Supports: From Liposomes to Supported, Planar Bilayers

M3.14 Spatially Restricted Raft-Like Chemical Heterogeneities within Model Phospholipid Membranes
We have developed a method to direct the reconstitution of raft-like lipid microdomains in controlled densities and distributions at 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 nm squares), was prepared on an oxidized silicon surface by stamping pre-ordered bilayers from a polydimethylsiloxane stamp. The patterned bilayers were doped with 1 mol % Texas Red labeled DHPE to enable epifluorescence visualization of the patterned areas and mobility characterization using fluorescence recovery upon photobleaching. The entire bilayer pattern was subsequently exposed to small unilamellar vesicles composed of raft forming mixtures of cholesterol, sphingomyelin, Gml, 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 vesicles intercalated selectively within the voids leading to well-defined patterns composed of three coexisting phases: primary fluid lipid bilayer corralling a two phase mixture comprised of cholesterol-sphingomyelin rich raft-like microdomains surrounded by the non-raft lipid phase. The stability of the patterned heterogeneity reflect a kinetically trapped mixing in a thermodynamic metastable state and depended strongly on the temperature, cholesterol concentration, saturation of phosphotidyl choline and sizes of the patterns. When cholesterol concentration was 30% and higher, the intercalated bilayer remained indefinitely stable at room temperature. At 20 mol % and below, secondary intercalants gradually diffused within the primary POPC bilayer ultimately effacing 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**

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


**M3.16**

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

Christina Elizabeth Innan and Janes Evan Hutchison; Department of Engineering, Myongji University, Yongin, Gyeonggi-Do, South Korea.

We have explored the use of microtubules (MTs) and kinesin motor and Y. S. Kim; 1Electrical Engineering, Myongji University, Yongin, Gyeonggi-Do, South Korea; 2Physics, Myongji University, Yongin, Gyeonggi-Do, South Korea.

In this paper, we characterize consecutive layers of cystamine-glutaraldehyde-streptavidin-biotinylated DNA nanodevice structure on three-dimensional atomic force microscopy (AFM), aiming to apply 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 controlling the sequential interactions between the layers with AFM, 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.
proteins as bioactive components of nano-biohybrid assemblies. In Nature, microtubules are a type of cytoskeletal filament, involved in intricate physiological processes ranging from 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 to transport cargo in natural systems makes microtubules and kinesin 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 synthetized biostructures provide an adaptable and sophisticated platform for integration with synthetic materials ranging from fluorescent molecules to nanocrystals. In some cases, the microtubules act directly as scaffolds for these synthetic components, whereas in others, kinesin motors may actively participate in the integration of synthetic materials with microtubule scaffolding. This biointeractive approach to materials synthesis interfaces biological molecular machinery with hard materials synthesis to create powerful tools for complex biobuild 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.


1 Biogeniengineering, University of Washington, Seattle, Washington; 2Molecular Surface Technology, Washington, Seattle, Washington; 3IBioengineering, University of Washington, Seattle, Washington. DNA nanotubes reach tens of microns in length, enabling transport a wide variety of organic and inorganic targets. 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 synthetized biostructures provide an adaptable and sophisticated platform for integration with synthetic materials ranging from fluorescent molecules to nanocrystals. In some cases, the microtubules act directly as scaffolds for these synthetic components, whereas in others, kinesin motors may actively participate in the integration of synthetic materials with microtubule scaffolding. This biointeractive approach to materials synthesis interfaces biological molecular machinery with hard materials synthesis to create powerful tools for complex biobuild 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.

9:30 AM M4.4 DNA Based Nanobiotechnology. Hao Yang1, Thomas H. LaBean2, Sungha Park3, Ying Peng4, Hanying Li5 and John H. Reif2

1Department of Chemistry & Biochemistry, Arizona State University; Tempe, Arizona; 2Department of Computer Science, Duke University, Durham, North Carolina.

In recent years, a number of research groups have begun developing nanofabrication methods based on DNA self-assembly. DNA is an extraordinarily versatile material for designing nano-architectural motifs, due in large part to its programmable G-C and A-T base pairing rules. These encoded structural motifs are programmed by a set of rules developed for DNA biotechnology: DNA can be manipulated using commercially available enzymes for site-selective DNA cleavage (restriction), ligation, labeling, transcription, replication, kination, and methylation. DNA nanotechnology is further empowered by well-established methods for purification and structural characterization and by solid-phase synthesis, so that any designer DNA strand can be constructed. Here we present our recent experimental and computational efforts to utilize novel self-assembly as well as for templates in the fabrication of functional nano-patterned materials. We have prototyped a new nanostructured DNA motif known as a cross structure[1]. This nanostructure has a 4-fold symmetry which promotes its self-assembly into tetragonal 2D lattices. Each unit cell can be considered as an individual pixel; if unique DNA labels can be assigned to each cross structure, they can be used to construct 2D arrays with individually addressable binding sites. We have also demonstrated a DNA barcode lattice[2] composed of DNA tiles assembled on a long scaffold strand; the system transcribes information encoded on the scaffold strand into a specific and reprogrammable barcode pattern which is visible by atomic force microscopy. We have achieved gold nanoparticle linear arrays templated on DNA arrays comprised of triple crossover (TX) molecules[3]. We have designed and demonstrated a 2-state DNA lattice[4] which display structural first order transitions switched by DNA nanomotors. We have also developed an autonomous DNA motor executing unidirectional motion along a linear DNA track[5].


10:30 AM M4.5 DNA Nanotubes: Living Polymers for Nanotechnology and Molecular Biology. Ashish Kumar1 and Deborah Kuchinik Pygenson1, 2

1Physics, UC Santa Barbara, Santa Barbara, California; 2Biomolecular Science and Engineering, UC Santa Barbara, Santa Barbara, California.

Short sequences of DNA can be programmed to self-assemble into extended structures based on Watson-Crick pairing rules. The most generic programming scheme makes use of "tiles" - building blocks of three or more DNA strands that hybridize into a core of cross-linked double helices with single-stranded sticky ends. In recent years, beautifully and cleverly tiling systems have been programmed, but control over yield, size and defect densities awaits a better understanding of the assembly kinetics. We use tiles known as double-crossovers (DX units) to construct tubular polymers of DNA that are about 10 nm in diameter and corresponding stiffness (persistence length ~5 µm). These nanotubes reach tens of microns in length, enabling study by fluorescence microscopy and, by inference, bulk fluorometry. We have thus begun to characterize tile-based DNA self-assembly in solution. DNA nanotubes have exponential length distribution that withstand dilution but decay via scission upon heating. Many key characteristics can be explained, and some controlled, via DNA structure and sequence. DNA nanotubes are thus uniquely accessible equilibrium polymers that enable new approaches to optimizing DNA-based programming and understanding the "biologically programmed" self-assembly of protein polymers.

11:00 AM M4.6 Directed Metalization of Enzymes with Preserved Catalytic Activity. Amihay Freeman1, Hila Dagan2, Yael Dro2 and Yossi Shacham-Diamand2

1Molecular Microbiology and Biotechnology, Tel Aviv University, Tel Aviv, Israel; 2Department of Physics of Electronic, Tel Aviv University, Tel Aviv, Israel.

Assembly of a limited number of protein molecules into two or three dimensional arrays, providing functionality such as bioscatalysis or biocomputational, is a major component in the fabrication of nano-structured bioships. The performance of such bioships 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 polymeric gels or binding of chemically modified metallic particles. To the best of our knowledge, wiring by means of directed complementary metal coating of the surface of the enzyme, assumed to be optimal for this purpose, was not demonstrated. We have recently successfully developed new technologies for directed protein metallization by controlled electrodeless 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 rate controlled deposition under mild conditions, suitable for working with proteins without denaturation [1]. Subsequently, we developed novel strategies to direct such metallizations to the surface of a soluble protein molecule without impairing its biological activity. Directed metalization experimentally demonstrated enzyme nanostuctures of high 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. Electrocheim Acta, 48, 2978-2988.

11:15 AM M4.7 Synthesis and Properties of Discrete Nanostructures of Quantum Dots/Au with DNA. Aliena Fu1, Christine M.
Colloidal semiconductor nanoparticles (Quantum Dots, QD) are emerging as exciting candidates for fluorescent labeling experiments. Compared to organic fluorophores, they have broad excitation spectra, unusually tunable emission 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. Since small molecules are often soluble and can be added to the buffer, proteins are far more likely than DNA to denature pathways for drug discovery, pharmaceutical screening, and biospecific reagents used for a protein experiment are generally available in very limited amounts. Here we present a method to simultaneously label multiple functional groups on nanohydrogels. We use focused electron beamls to radiation crosslink components and cell proliferation assays, we show that these nanoscale rather than on the microscale. One class of nanoparticles, the molecular motors, are essential for organizing multi-functional hydrogels are capable of inducing appropriate cellular responses in cultures of one or more cell types. In doing so, we illustrate the rate 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 artificial molecular design and matching as needed, will be a useful strategy for tissue engineering.
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 biomolecule, "uncaged" by UV light, and utilized or utilized by many enzymes to catalyse chemical changes in the buffer solution or power micro/nanoscale transport. We will present the characteristics of caged ATP, the design considerations for the integration of caged ATP into miniaturized devices, in particular biomolecules driven by the motor protein kinesin, key factors in controlling the ATP flow from a buffer solution to activate the nanodevice, and methods and designs to stabilize the system against variations of environmental conditions. The feasibility of employing sunlight for the activation of the system will be also discussed.

2:15 PM M5.5
Hybrid Nanodevices based on Biomolecular Motors: A Lateral Integration Approach
Christian Brunner 1,2, Karl-Heinz Ernst 4,2, Henry Hess 2,3 and Vladimir Pravda 2,3
Materials, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland; 2Bioengineering, University of Washington, Seattle, Washington; 3Center of Nanotechnology, University of Washington, Seattle, Washington; 4Molecular Surface Technology, Swiss Federal Laboratories for Materials Testing and Research (EMPA), Duesseldorf, 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 that pH, ionic strength and temperature of the environment must be well-defined. In vitro gliding assays provide a very easy method to assay protein functionality and stability. In addition, biomolecular motors are an interesting class of proteins for the use in hybrid devices. Motor proteins already have been investigated for nanoscale transportation [1] and lab-on-a-chip systems [2]. A simplified molecular shuttle system, consisting of fluorescently labelled taxol-stabilized microtubules driven by kinesin motor proteins, allowed us to easily assay functionality and lifetime of microtubules and kinesin. The functional decay was tested by utilizing optical microscopy to detect motility and disaggregation of microtubules. In the study, we investigated the biocompatibility of different polymers used in microfabrication with the molecular shuttle system. Flowcells were assembled with glass bottom surfaces and covers fabricated of polyurethane (PU), polymethyl-methacrylate (PMMA), polydimethylsiloxane (PDMS) and an ethyl-vinyl alcohol copolymer (EVOH). We found that the lifetime of the system was mainly limited by the stability of the microtubules rather than of kinesin. Also, we showed that without illumination only PU had a substantial negative impact on microtubule stability, while PMMA, PDMS and EVOH setups showed stabilities comparable to glass. However, under the influence of light, microtubules degraded rapidly in PDMS and PMMA setups, even in the presence of oxygen scavenging adenosine, meaning that the high permeability of PDMS and PMMA increased the instability of microtubule filaments in the flowcell. The active transport characteristics of molecular shuttles have the potential to be utilized for cargo transportation and intracellular transport. However, for subsequent applications we need to know the distance cargo can be moved. Lifetime studies of appropriate linkage systems will therefore be the focus of our research in the near future. [1] Hess, H., Bachand, G. D. and Vogel, V. Turning Nanodevices with Biomolecular Motors. Nature Mater., 10, 2110-2116, 2004. [2] Clemmens, J., Hess, H., Doct, R. K., Matzke, C. M., Bachand, G. D. and Vogel, V. Motor-protein roundabout: Microtubules moving on kinesin-coated tracks through engineered networks. Lab Chip, 4, 83-86, 2004. [3] Brunner, C., Ernst, K.-H., Hess, H. and Vogel, V. Lifetime of biomolecules in polymer-based hybrid nanodevices. Nanotechnology, 15(10), Ss40-Ss48, 2004.

2:30 PM M5.4
In-vitro Applications of Carbon Nanotubes as a Protein Transporter for Internalization in Mammalian Cells
Min Hee Lee, Hyeong Hee, HsiuTsung Chang, Hsiang Ju Kim, and Yong Hoon Kim
Chemistry, Stanford University, Stanford, California.

Covalent and non-covalent functionalization schemes are employed to impart stability to single walled carbon nanotubes in aqueous environments. The water-soluble SWNT suspensions are stable under physiological pH and are used for in-vitro studies. In particular, the SWNT are further modified with various cargos such as fluorescently tagged proteins and the interactions of the SWNT-cargo conjugate with mammalian cells are observed [1]. Detection of fluorescence inside the cells by confocal microscopy and flow cytometry indicates that the SWNT act as a transporter for the internalization of the internalized proteins are key issues that are currently being studied. The ability of the nanotubes for transporting and delivery single and multiple molecules can potentially be integrated in biological systems for drug delivery and biosensing applications. Reference: [1] Lee, Min Hee; Chang, Hsiu Tsung; Lee, Hyeong Hee; Kim, Hsiang Ju; Kim, Yong Hoon. "Single-Walled Carbon Nanotube Transporter for Internalization of Proteins in Mammalian Cells." Artif. Cells Blood Substit. Immobil. Biotechnol. 32, no. 3 (2004): 251-259.

2:45 PM M5.6
Controlled Object Delivery in Aqueous Medium through Large Pores
Frederic Pincet 1, Sophie Cribier 2 and Nicolas Rodriguez 2; 1Laboratoire 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 it is to open a pore in the capsule membrane in order to release its content. This transient 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 reproducibly induce controlled 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 types of pores will be presented and the mechanical way by which the pore is stabilized will be discussed.

3:15 PM M5.6
Creating Functionality At The Nanoscale: Bio-Templated Quantum-dot Structures Interrogated Using Dynamical Spectroscopy
Jennifer Hollingsworth, Marc Achermann, Sohee Jeong and Victor Kilner; Chemistry, Los Alamos National Lab, Los Alamos, New Mexico.

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 prompted impetus for investigations of new bottom-up approaches. Often, these latter approaches are simply "bio-inspired," loosely based on biological structural motifs, e.g., layered self-assembly of amphiphilic molecules. Alternatively, the approach involves "bio-templated" assembly, whereby inorganic nanoparticles, for example, are assembled and sometimes modified using a biological scaffold. This approach to achieving structural control at the nanoscale allows the inorganic components to aggregate, in complex, patterns or shapes. Here, we report the assembly and characterization of semiconducting nanocrystals by "bio-templated" method using mesoporous silica (MT) fibers 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 (NQDs), NQDs and dyes, and NQDs 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 biomolecular semiconductor solutions of nanoscale objects, as well as nano-object positioning. Specifically, in the case of NQD-NQD ET, we resolve up to three donor-acceptor (D-A) distances indicative of at least three unique NQD-NQD spatial arrangements. The ability to resolve multiple D-A distances is unique to time-resolved analytical methods. Further, dynamical methods provide significantly enhanced sensitivity, critical for the exceptionally low concentrations often characteristic of these systems, compared to non-dynamical methods. In the NQD-dye system, we distinguish two dynamical regimes, a fast 90 ps and a slow 8 us component, indicative of two D-A geometries. This system allowed us to study NQD assembly for which the NQDs were not assembled with sufficiently high densities to reveal NQD-NQD ET. Lastly, we studied distance-dependent gold quenching of NQD-MT assemblies situated atop gold-coated surfaces, where the NQD-gold distances were controlled using various sized thiol spacers. In summary, our results demonstrate that ET measurements comprise a versatile tool for characterizing bio-templated assemblies and that ET is an efficient mode of interparticle electronic communication in
The overall goal of this project is the development of a biomimetic, photosynthetic energy transduction system which can convert light energy into a transmembrane potential gradient. This potential gradient will be used to pump protons across a planar supported lipid bilayer (PSLB) to establish a proton motive force. A pH sensitive, electrically conducting polymer support beneath the PSLB provides the mechanism for transmembrane proton pumping. Protons are thus driven via a redox reaction with the quinone molecules 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 interfered 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. Moto Knez, Woo Lee, Pin Myllymäki, Miroslav Malševec, Matti Putkonen, Kornelius Nielsch, Lauri S. Niinisto and Ulrich M. Gösele; 1 Exp. II, Max-Planck-Institut für Polymerforschung, Mainz, Germany; 2 Laboratory of Inorganic and Analytical Chemistry, Helsinki University of Technology, Helsinki, Finland; 3 Max-Planck Research Unit for Enzymology of Protein Folding, Halle, Germany.

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 monocrystalline arrangement of pore channels on a 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 phosphonate 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-step reaction 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 Burini1, Sabato D’Auria2, Mose Rossi2 and Andrea M. Rosai1; 1 Nanomaterials Innovation Center, Istituto Italiano di Nanoscienze, Cnr, P.le A. Galilei 40, Torino, Italy; 2 Institute of Protein Biochemistry (CNR), Napoli, Italy.

Porous silicon (PS) is a nanomaterial which represents a convenient choice for fabrication of devices, due to the large internal surface area, the biocompatibility, and the compatibility with microelectronics technology. A method to define biomolecular nanopatterns on PS, based on Electron Beam Lithography (EBL), is presented here. We demonstrate that it is possible to locally define patterns of organic molecules on 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-patterns. This innovative method 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 ultrastable sugar-binding protein isolated from the thermophilic organisms Pyrococcus horikoshii. These biomolecules have been selected taking into account their high impact in the development of advanced nanosensors for important analyses such as the continuous monitoring of three levels of glucose in diabetic patients and the monitoring of the glutamine levels in patients with cancer pathologies.

Motor vehicle accidents are a 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 10⁻²s⁻¹ are responsible for activating pathophysiological cascades which result in delayed neuronal dysfunction and cell death. The exact time-course of these atraumatic injuries and the opportunity exists for therapeutic intervention to repair the damaged tissue. To date, no electro-system allows for a sensitive measurement 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 extraco or 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 electrical recording during the traumatic event and post-injury. This technology is enabled by our recent discovery that thin gold films patterned on silicon membranes remain electrically conductive after stretch cycles > 20%. Our stretchable MEA is fabricated on a soft, biocompatible 250 μm thick elastomeric silicone membrane and consists of a 2x2 25 μm 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 ~ 30%, strain rate ~ 10⁻¹s⁻¹) and preliminary data on growing cell cultures on the array.

SESSION M6: Biological and Bio-inspired Templates for Materials Synthesis and Organization
Chair: George Bachand
Thursday Morning, March 31, 2005
Room 3009 (Moscone West)

8:30 AM M6.1

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 such approach is to use biomolecules as scaffolds because of the specificity they provide. Such scaffolds not only 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 using CPMVnano 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 arrays 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 fixed between the size of the molecule and the limits of lithography. However, this approach lacks control over the fluorophore to fluorophore distance thus leading to significant quenching by resonance energy transfer. CPMV provides a platform on which the 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 Deryng1, Warren C. W. Chan2 and Sangeeta N. Bhatia1; 1Biomedical Engineering, 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 dots (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 have 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 the organelles (DAPI, Mitotracker, etc) are able to permeate cell membranes, the size and surface properties of QDs prevent passive diffusion across the lipid bilayer. Micronjection of QDs into the cell's cytoplasm, however, does achieve the desired staining as demonstrated in our model system. 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 cell organelles over days of culture without loss of signal. Furthermore QDs serve as an ideal model for these investigations, questions about the potential cytotoxicity of these cadmium-containing nanocrystals have surfaced. Our findings suggest that photobleaching of the surface can lead to release of cytotoxic levels of free cadmium salts. These effects can be minimized by appropriate surface coatings and reductions in dose. Coated QDs were shown to be useful for long-term labeling of live cells without inhibition by short-wavelength laser excitation. While organic dyes tend to show luminescence with temperature changes, QDs have proven to be insensitive to changes in temperature which makes them ideal for applications such as tracking cell migration in 3D environments.

9:15 AM M6.3
Fabrication of Core-Shell Drug Nanoparticles for Therapeutic Delivery. Aliyar S. Zahi1, Cheryl Rumbarger2 and Michael V. Pishko2,3; 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 for 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 biopolymers, 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. 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, and composed of two molecular 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 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 nanoparticles. Preliminary biocompatibility studies to study phagocytosis of the PEG modified drug nanoparticles 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.

9:30 AM M6.4
Small Water-Soluble InAs/ZnSe QDs for Biological Imaging. Jochen P. Zimmer, Sang-wuk Kim and Moongi G. Bumbac; Institute of Biotechnology, Massachusetts Institute of Technology, Cambridge, Massachusetts.

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 multicolor coded 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 available. One disadvantage of many QDs for biological imaging, however, is their toxic potential. Heavy metal QDs like CdSe, CdTe, and PbS elicit particular concern. Here we report the
9:45 AM M6.5
The Design of Potent Polypeptide Inhibitors of Anthrax Toxin.

Hiroshi Nishikawa, Kazuo Magara, Ravi Kanae

We have been able to specifically template the formation of ordered arrays of magnetic nanoparticles, conducting nanoparticles, and conducting photonic materials. The use of nanometric coatings on these oxygenated objects on aromatic SAM templates can be realized by micro-contact printing of nanomaterials-conjugated self-assembled proteins, patterned assembly of nanocrystals and QDs has also been achieved.

10:30 AM M6.6

Andrew McMillan, Janine Howard, Chad Paavola, Jonathan Trent, Hiroshi Kaga, Suzanne Chan, Elizabeth Wilson-Kubalek, Andrew McMillan, Janine Howard, Chad Paavola, Jonathan Trent, Hiroshi Kaga, Suzanne Chan, Elizabeth Wilson-Kubalek

By means of self-assembly, immobilization of biologically active molecules on surfaces and with controlled chemical composition and structure can be achieved. When immobilized on a substrate, such as a glass slide, these structures can then be used in research applications.

11:00 AM M6.9
Nanoengineered Calcium Phosphate Materials for Controlling Cell Activity and Gene Transfer.

Hyojin Kim, Selvarangan Panranagaran, Andrew McMillan

We have shown that our approach to introducing DNA can be used to create designer proteins with application potential such as improved cell cycle control, cell proliferation, and tissue regeneration.

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; Bioscience 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 thin film architectures 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 immobilize proteins in ways similar to 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 been used to promote bio-immobilization. The proteins used for these studies were either natural luminophores 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. Bacs1, Carlee Ashley1, Eric Carnes2, Deanna Lopez2 and C. Jeffrey Brinker2,1. Chemical 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 enzymes such as molecular motors or pumps into 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 gelled phospholipid-reservoir, the cell wall is modified to form a 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 nano-cavities in extended nanostructures. By monitoring in-situ structure development, 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 solutions demonstrates 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 unexpected lipid/silica ratios and the d-spacing dependence of this phase on cell concentration. While CDSA forms a highly biocompatible, multilamellar 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.

SESSION M7: Nanostructured Interfaces - Protein and Cell Adhesion
Chair: George Bachand
Thursday Afternoon, March 31, 2005
Room 3000 (Moroney West)

1:30 PM *M7.1
Jun Liu1, Zhengrong Tian1 and Qingshu Huo1. 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 how the molecules on the electrode respond to the specific binding. Our method achieved at least attomolar level sensitivity (aM) to 18-molar) using conventional cyclic voltammetry, many orders of magnitude lower than those reported by typical electrochemical methods. The general scheme is applicable to the detection of a 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-cyuan Lin, Hung-Chen Liao, Chia-Ching Lin, Shang-Shiu Hsiao, Hsin-Chun Chen; Materials Science and Engineering, National Chiao Tung University, Hsinchu, Taiwan.

Novel nanocomposite 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 dimercaptosuccinic acid (DMSA). The surface characteristic of novel ZnO arrayed nanorods was investigated by field-emission scanning electron microscope (FESEM), energy dispersive spectroscopy (EDS), transmission electron microscope (TEM), X-ray photoelectron spectroscopy (XPS), Raman spectra, Fourier transform infrared spectra (FTIR), atomic force microscope (AFM), and contact angle measurement. Moreover, surface grafting density of carbonyl groups of thiol-modified ZnO nanorods and immobilized proteins were examined by dying with C.I. Basic Blue 17 and Coumarin 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 four-inch). 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 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 sputum lotion. For these reasons, the protein-immobilized ZnO 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 cytocompatibility of novel nanocomposite arrayed nanorods is now in progress in our group.

2:15 PM M7.3
Biomimetic Surfaces for Chromophore Binding. Albina Ivanisevic2,3, Brandy Perkins2 and Rosangelly Flores2. 1Purdue University, West Lafayette, Indiana; 2Biomedical Engineering, Purdue University, West Lafayette, Indiana; 3Chemistry, Purdue University, West Lafayette, Indiana.

The key reaction in the visual cycle is the photoisomerization of a chromatophore molecule, 11-cis-retinal, to all-trans-retinal. The objective of this study was to construct an artificial, biomimetic surface to accommodate the binding of 11-cis retinal. A five step surface modification procedure was applied to SiOx surfaces to develop a pseudo-physiologically nourishing environment conducive to support the binding of 11-cis-retinal. The surface at the end of this modification scheme was terminated on peptide nucleic acid (PNA) that was covalently attached to a hetero-biunifunctional cross-linker. A number of techniques were used to verify binding of the chromatophore to the artificial surface. Upon the completion of each modification step, contact angle measurements, tapping mode atomic force microscopy (TM-AFM), and X-ray photoelectron spectroscopy (XPS) were carried out for the surface characterization. Throughout the study the collection of analytical techniques confirmed the binding of 11-cis retinal to the biomimetic surface. The successful mimicking of this biological reaction can be applied to the construction of novel biosensors and transducers.

2:30 PM M7.4
Interaction between Recombinant Ferritin Molecules and Solid Substrates Measured with an Atomic Force Microscope: The Effect of the Sequence in the N-terminal Domain. Tomohiro Hayashi1,2, Masahiko Haru1,2; 1Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology, Yokohama, Japan; 2Local Spatio-Temporal Functions Laboratory, Frontier Research System, RIKEN (The Institute of Physical and Chemical Research), Wake, Saitama, Japan.

An atomic force microscopy (AFM) was used to probe the interaction between ferritin molecules and solid surfaces (silicon and highly ordered pyrolytic graphite (HOPG)) in water environment. Ferritin molecules are immobilized onto an AFM tip using PBLH (poly-l-lysyl-l-histidine) with Langmuir-Schaefer method. Our emphasis was focused on two things. One is the force-distance curve on approach, which depicts what kind of force ferritin experiences in approaching to the surface. The other is adhesion energy estimated from the curves on receding. We used several types of recombinant ferrins, which specifically possess strong affinity with their target materials (in this work, titanium surface and carbon nanocones). The
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 phage library. Our results showed that the sequence of N-terminal has a significant effect on both short- (van der Waals, dipole-dipole, etc.) and long- (electrostatic) ferritin-substrate interactions. That is, the sequence in the N-terminal domain affects the 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 DUV 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 TiOx Modified Biomimic Surfaces: Nanostructuring, Surface Characterization, and Osteoblast Cell Growth Studies. Jigberto Advincula1,2, Athena Advincula2 and Jack Lemons3,1 Department 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 deposition (LbL) methods. The polyelectrolytes used were PEI, PSS, PSS, and PAM. The organic-inorganic composite systems were characterized using XPS, SEM, and AFM, and tensile 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 number 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 coated substrate. Higher adhesion of osteoblast cells at 1 and 24 hours and formation of bone nodule 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 materials prepared using sol-gel processes. The particles generated can be in the form of toxic metallic ions, inorganic metal salts, or polymeric debris bound by proteins. The large surface area characteristics of nanometer-scale debris particles may lead to undesirable biological interaction. 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 microwaved 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 diamond terminated with hydrogen promotes markedly better MSC adhesion than oxygen terminated diamond. We hypothesize that oxygen-terminated diamond creates a hydrophobic surface that resists protein adorption (coating) and dramatically reduces MSC in a manner that is not quanitatively measured. While oxygen-terminated diamond promotes MSC adhesion and coating, hydrogen-terminated diamond readily adsorbs a collagen layer that allows for a significant increase in adhesion of MSCs in cell culture. We believe our nanoacne approach, designing materials from simple starting molecules like the carbon atoms from methane, and surface design with biomimetic peptides will produce novel biomaterials with ideal mechanical properties and excellent adhesion of osteoblasts to the substrate.

3:30 PM M7.8 Fabrication and Evaluation of Micro to Nano Hierarchical Structures of Nanoporous Architecture for Bone Biotemplating. Ketel Popat, Vivek Mukhatri 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, refined and extended selection fabrication routes for producing metal-oide films with controlled nano to micro architecture, potentially moving us closer to the goal of osteointegration. We propose using these unique films for bone biotemplating, where control the growth and differentiation of osteoblasts. We hypothesize that controlled nanoarchitecture can promote osteoblast differentiation and matrix production, and enhance short-term osteointegration. Moreover, the ability to create model nanodimensional constructs 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 that control the growth and differentiation of osteoblasts. We hypothesize that controlled nanostructures can promote osteoblast differentiation and matrix production, and enhance short-term osteointegration. Moreover, the ability to create model nanodimensional constructs 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 that control the growth and differentiation of osteoblasts. We hypothesize that controlled nanoarchitecture can promote osteoblast differentiation and matrix production, and enhance short-term osteointegration. Moreover, the ability to create model nanodimensional constructs 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 that control the growth and differentiation of osteoblasts. We hypothesize that controlled nanoarchitecture can promote osteoblast differentiation and matrix production, and enhance short-term osteointegration. Moreover, the ability to create model nanodimensional constructs 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 that control the growth and differentiation of osteoblasts. We hypothesize that controlled nanostructure sandents of various length scales are required by cells to attach and form bone nodules in vitro.
Team there are the in-vivo model and thirdly, assist in PM M7.11. To achieve this it is essential to develop 180 and 3000 min dissolution, PM M7.9 (NIRT) program under Grant No. DMR-0402891. at Tang, Lijun Wang and can enahle a hrnao PM M7.10 IniInic Ruikang Living neural networks (LNN) New the dissolution reactions control the formation of extraordinarily the visualization and 111easure1111ent of cell-cell interaction in the for1111e three require1111ents 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 microbe 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 shown to be less effective in the number of applications for mammalian cells with excitatory cell membranes.

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 cell's 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 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 microbe 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 excitatory cell membranes.

4:15 PM M7.9
Nano Structured Platforms for Cellular Analysis Devices. Shilini Prasad1, Satyajith Ravindran2, Cengiz Ozkan3 and Mihr Ozkan3,1; 1 EE, University of California, Riverside, Riverside, California; 2 CEE, University of California, Riverside, Riverside, California; 3 ME, University of California, Riverside, Riverside, California.

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4:30 PM M7.10
Nanostructured Ceramic Platform for Living Neuronal Network Chips. Dimitri Routsevitch1, Michael Stowell2, Maria Pantages2 and Oleg Polyakov1; 1 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 prostheses 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 detecting 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: microengineering of the anodic alumina to provide tailored neuron/substrate interface; hybrid micromachining of patterns for neural growth guidance; using encapsulated nanoelectrodes 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, hydroxyapatite (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±0.3 x 10^-11 and 3.3±0.5 x 10^-11 mol mm^-2 min^-1 at 180 and 300 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 carious 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 09226).