SYMPOSIUM W

Biological and Bio-Inspired Materials and Devices

April 13 - 16, 2004

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* Invited paper

SESSION W1/O1: Joint Session: Self-Assembly
Chairs: Joanna Aizenberg and Jun Liu
Tuesday Morning, April 13, 2004
Room 3005 (Moscone West)

8:30 AM *W1.1/O1.1

The fabrication of novel biomaterials via molecular self-assembly. Shuguang Zhang, Center for Biomedical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Two complementary strategies can be employed in the fabrication of molecular biomaterials. In the "top-down" approach, biomaterials are generated by stripping down a complex entity into its component parts. This contrasts with the "bottom-up" approach, in which materials are assembled molecule by molecule and in some cases even atom by atom to produce novel supramolecular architectures. The latter approach is likely to become an integral part of nanomaterials manufacture and requires a deep understanding of individual molecular building blocks, their structures, assembling properties and dynamic behaviors. Two key elements in molecular fabrication are chemical complementarity and structural compatibility, both of which confer the weak and noncovalent interactions that bind building blocks together during self-assembly. Significant advances have been achieved at the interface of nanomaterials and biology, including the fabrication of nanofiber materials for three-dimensional cell cultures and tissue engineering, the peptide nanotubes for stabilizing membrane proteins and nanocoating molecular and cell organizations. Molecular fabrications of nanobiomateirals have fostered diverse scientific discoveries and technological innovations.

9:00 AM W1.2/O1.2

The Creation of Novel Hybrid Materials Through the Coupled Self- Assembly of Chaperonin Proteins and Diblock Copolymers. Linda Katherine Molnar[1], Ting Xu[2], Jonathan Trent[3] and Thomas P Russell[2]; [1]Center for Nanotechnology, NASA Ames Research Center, Moffett Field, California; [2]Polymer Science and Engineering, University of Massachusetts, Amherst, Massachusetts; [3]Astrobiology Technology, NASA Ames Research Center, Moffett Field, California.

The combination of polymers and proteins to form hierarchically structured multifunctional materials with the processability of polymers while retaining biological function of the protein is being studied. A unique synergy resulting from the mixing of these two disparate self-assembling systems has been found. The materials utilized were an asymmetric diblock copolymer of polystyrene (PS) and polyethyleneoxide (PEO)denoted P(S-b-EO) and a double ring structure-forming protein from a class of heat shock proteins known as chaperonins. Solvent casting has been shown to be a viable and rapid route by which arrays of nanoscopic PEO domains oriented normal to the surface can be produced in a glassy PS matrix in films with thickness several times the period of the copolymer. Here, we show the chaperonin-driven self-assembly of the P(S-b-EO) diblock copolymer thin film cast from an aqueous solution of chaperonin and polymer. AFM images of the resulting thin films with and without chaperonins show that the chaperonins are interacting with the P(S-b-EO) and enabling the microphase separation of the copolymer. The chaperonins used in these studies, isolated from *Sulfolobusshibatae*, which lives in geothermal hot springs and grows at temperatures of up to 85 degrees Celsius and pH 2.0. Structural data and genetic engineering tools have allowed the creation of chaperonin mutants that bind biomolecules or inorganic nanoparticles. The combination of order from the self-assembling properties of diblock copolymers with the genetic adaptation of proteins opens up new possibilities of producing multifunctional materials and the functional components of devices where both organization and specific biological function are required, e.g., sensors, adaptable materials, medical implants, and biocompatible devices.

9:15 AM W1.3/O1.3

Environmentally Responsive Hydrogels with Tunable Rigidity Constructed Via Peptide Folding and Consequent Self-Assembly. Darrin Pochan[1] and Joel Schneider[2]; [1]Materials Science and Engineering, University of Delaware, Newark, Delaware; [2]Chemistry and Biochemistry, University of Delaware, Newark, Delaware.

By using peptidic molecules in the materials self-assembly design process, one can take advantage of inherent biomolecular attributes, intramolecular folding events and secondary structure, in addition to more traditional self-assembling molecular attributes such as amphiphilicty, to define hierarchical material structure and consequent properties. Importantly, intramolecular folding events impart a molecular-level mechanism for environmental responsiveness at the material level (e.g. infinite change in viscosity of a solution to a gel with changes in pH, ionic strength, temperature). The utility in responsive material design with small, 20 amino acid beta-hairpin peptides will be discussed. The self-assembly construction process is predicated on the peptides first intramolecularly folding into the beta-hairpin conformation from a random coil conformation. The resultant gel scaffold network displays unique nano- and microstructure due to the self-assembly process. Importantly, the scaffold assembly is completely reversible with pH or temperature by reversibly folding and unfolding the constituent peptides that, in turn, assembles or disassembles the scaffold, respectively. In addition, the rigidity of the gel scaffold can be tuned via the magnitude of the environmental stimuli, e.g. gels triggered with temperature form a more rigid network when assembled at higher temperatures due to faster folding and self-assembly kinetics. The molecular design and self-assembly principles, including a model to explain the inherent tunability of the final gel networks that underlie the observed morphological and rheological material, will be discussed.

9:30 AM *W1.4/O1.4

Biomimetic Approaches to the Design of Functional, Self-Assembling Systems. George M. Whitesides, Harvard University, Cambridge, Massachusetts.

Successful solutions to many problems in science and technology have come by extracting design or strategy from biology, and applying it in a non-biological context. The use of biomimetic approaches is particularly well suited when designing self-assembling functional systems, because life - from single cells to complex, multicellular organisms - demonstrates an enormous number of successful, functional designs, and because living systems assemble themselves. There are two reasons for studying self-assembly. First, self-assembly is centrally important for life. Biological systems form and are sustained as a result of self-organization. Understanding life, therefore, requires - among other things - understanding self-assembly. Second, self-assembly can generate ordered 3D aggregates of components ranging in size from the molecular to the macroscopic. These structures often cannot be generated by any other procedure. In the past, self-assembly has been best known as a synthetic strategy in the molecular size regime. New examples of its application to nano- and microscale components are now beginning to emerge. As a consequence, self-assembly is becoming increasingly important as a strategy for the formation of useful, nano- and micro-scale structures. This talk discusses the characteristics of self-assembly in living systems and reviews self-assembled functional systems designed according to biological principles.

10:30 AM W1.5/O1.5

Fabrication of Assembled Virus Nanostructures on Templates of Chemoselective Linkers Formed by Scanning Probe Nanolithography. C L Cheung[1], J A Camarero[1], B W Woods[1], J J De Yoreo[1], T Lin[2] and J E Johnson[2]; [1]Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, California; [2]Dept of Molecular Biology, The Scripps Research Institute, La Jolla, California.

The assembly of genetically engineered viruses and proteins on patterned chemical templates has great potential for directing the formation of ordered protein and virus arrays. Moreover, presentation of certain chemical groups on these macromolecules either through genetic modifications or chemical ligation techniques provides a potential route to hierarchical assembly of organic-inorganic nanostrucures. Here we present a general methodology to create nanoscale ordered protein and virus structures by using nano-grafting and dip-pen nanolithography to create patterns of self-assembling molecules that exhibit chemoselective binding to specific sites on engineered proteins and viruses. Using amino-terminated long-chain alkane thiols as the chemical linker, shorter-chain tri-ethylene glycol terminated alkane thiols as a background "protein resist", and the icosohedral cow peas mosaic virus (CPMV) engineered to present cystene groups at specific sites on its surface, we demonstrate the formation of viral arrays. We find that when the chemical templates have dimensions comparable to the size of the virus, they tend to spontaneously form close-packed structures. Using these templates as platforms for investigating the controls on macromolecular aggregation, we examine the kinetics and morphology of array assembly under difference solution conditions by atomic force microscopy. Preliminary results using these templates to direct the growth of virus crystals and comparisons with bulk virus crystallization experiments is also be discussed. This work was performed under the auspices of the U. S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

10:45 AM *W1.6/O1.6

Self-Assembly in Biological Structures. Peter Prevelige, Microbiology, University Alabama Birmingham, Birmingham, Alabama.

In biological systems, dynamic nanometer scale structures self-assemble with sufficient precision that their structures are regular at the level of Angstroms. They do this in a controlled manner, in a noisy environment full of other proteins. To cope with the demands of controlled and precise assembly they have evolved a number of sophisticated control mechanisms. The mechanisms include: well controlled linear assembly pathways, the use of substructure assembly to improve fidelity, controlled conformational switching during assembly, staged assembly, and the use of templates or jigs to assist in form determination. These principles and paradigms are well illustrated in the assembly pathway of the dsDNA bacteriophage. In this talk, a series of vignettes drawn from experimental studies of the assembly of complex biological systems, primarily phage, which serve to illustrate these general principles will be presented

11:15 AM W1.7/O1.7
Surfactant-Assisted self-assembly of water-soluble nanocrystal, ordered arrays, and their integration.
Hongyou Fan, Kai Yang, Kevin Malloy, Sigmon Thomas and Jeff Brinker; Sandia National Laboratories, Albuquerque, New Mexico.

Nanocrystals exhibit size-dependent physics and have many important applications in catalysis, biolabeling, and microelectronics and optics. Current monosized nanocrystals are often organic ligands-protected, therefore, dissolve only in organic solvent. Self-assembly and formation of ordered nanocrystal arrays are limited to only organic solvents. Here we report the synthesis of a new ordered nanocrystal (NC) arrays through self-assembly of water-soluble NC-micelles with soluble silica. The ordered arrays comprise gold nanocrystals arranged within a silica matrix in a face-centered-cubic lattice with cell dimensions that are adjustable through control of the nanocrystal diameter and/or the alkane chain lengths of the primary alkanethiol stabilizing ligands or the surrounding secondary surfactants. Under kinetically controlled silica polymerization conditions, evaporation drives self-assembly of NC-micelles into ordered NC/silica thin film mesophases during spin-coating. The intermediate NC-micelles are water-soluble and of interest for bio-labeling. The robust, 3-D NC mesophase solids are of interest for development of collective optical and electronic phenomena, and, importantly, for the integration of nanocrystal arrays into device architectures. Integration of a MOS capacitor using such an ordered gold NC/silica oxide demonstrated charge storage on the gold nanocrystals and discharge behavior dominated by electron transport within the ordered gold nanocrystal array. Temperature dependent device I-V characteristic and electron tunneling behavior have been observed. Sandia National Laboratory is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under Contract DE-AC04-94AL85000.

11:30 AM W1.8/O1.8
Conjugated Polymer/Silica Nanocomposites with Tunable Mesostructure. Byron McCaughey[1], Chris Costello[2], Donghai Wang[1], J Eric Hampsey[1], Chaojun Li[2], C Jeffrey Brinker[3] and Yunfeng Lu[1]; [1]Chemical Engineering, Tulane University, New Orleans, Louisiana; [2]Chemistry, Tulane University, New Orleans, Louisiana; [3]Sandia National Laboratories, Albuquerque, New Mexico.

Conjugated polymer-ceramic nanocomposites have been extensively researched because they have shown enhanced conductivity, mechanical strength, processability, environmental stability, and other unique properties. Our research focuses on the synthesis of conjugated poly(2,5-thienylene ethynylene) (PTE)/silica nanocomposites with tunable mesostructure. The synthesis approach involves surfactant-induced partitioning, self-assembly and co-organization of 2,5-diiodothiophene monomer and palladium-based catalyst within a poly(silicic acid) matrix. Surfactant choice and self-assembly conditions created hexagonal, lamellar, or cubic silica mesophases. Subsequent polymerization initiated by exposing the monomer/catalyst/silica nanostructures to acetylene gas resulted in the formation of ordered, mesostructured poly(2,5-thienylene ethynylene)/silica nanocomposites as determined by UV-vis, FTIR, XRD, and TEM experiments. PTE formation was verified by a broad UV adsorption between 300 and 600 nm that changed position based on catalyst and monomer concentrations. XRD scans and TEM images demonstrate the formation of hexagonal, cubic or lamellar PTE/silica mesostructure. PTE incorporation within the mesoporous silica was determined by an increase in XRD d-spacing on a series of spin-coated thin films. Also, a robust polymerization mechanism was revealed. Finally, silica removal results in free-standing conjugated polymer particles with mesoporosity and high surface area. This novel approach provides a unique route to synthesize mesostructured conjugated polymers and polymer/inorganic nanocomposites.

11:45 AM W1.9/O1.9
The molecular car and its on-chip infrastructure. Zhigang Suo and Wei Hong; Division of Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts.

A molecule adsorbed on a solid surface has an electric dipole moment. It performs random walks when no external field is applied. However, an electrode can direct the motion of the molecule. For example, one can embed an array of individually addressable electrodes near the surface of a dielectric substrate. Charge the electrodes sequentially, and the molecular dipole moves in a desired way: going forward, reversing, and making a turn. Such a molecule, or its monolayer island, is a molecular car. As an illustration, consider a short-chain molecule with three characteristics: its one end adsorbs to a solid surface, its mid-chain has a group with an electric dipole moment normal to the solid surface, and its other end is a passenger receptor. The molecule has a modular structure. The division of labor offers the flexibility to design separate modules, at the molecular level, to fulfill distinct functions. The car captures a specific passenger molecule in one pool, shuttles it, and then releases it in another pool, all on a single chip. This talk describes the mechanics of the molecular car and its on-chip infrastructure, their design requirements, and our numerical simulation. Thermal fluctuation will be an important consideration. One needs to learn to drive the car in perpetual earthquake.

SESSION W2: Bio-Inspired Devices
Chair: Trevor Douglas
Tuesday Afternoon, April 13, 2004
Room 3003 (Moscone West)

1:30 PM W2.1
Proton conductive membrane synthesized from biological molecular hybrids. Itaru Honma and Masanori Yamada; EEI, AIST, Tsukuba, Ibaraki, Japan.

Proton conductive membrane is a very important materials for functional electrochemical devices such as fuel cells, battery, electrochromic device and sensors. In particular, temperature tolerant as well as anhydrous proton conductor has been attracted much attention for application to advanced polymer electrolyte fuel cells operated above 100C, where the water ion exchange membrane is dried to lose proton conductivity. Here, in this presentation, the anhydrous proton conducting polymer membrane has been synthesized from biological molecular hybrid materials. The chitin phosphate polymer is mixed with imidazole or Uracil(RNA) molecules to form acid/base hybrid materials. The materials show proton conductivity exceeding 1 mS/cm under non-humidified condition up to the 150C, which can be potentially applied to the PEFC membrane operated under high temperature and non humidified condition. The conductive mechanism different from water molecules Vehicle or Grotthuss has been suggested.

1:45 PM *W2.2
Bio-inspired and non-lithographic formation of nanodevices.
Harold Craighead, Cornell University, Ithaca, New York.

Observing biological systems at the nanoscale can motivate new device designs and inspire new material processiing concepts. We have been adapting lithographic fabrication approaches for patterning sensitive biomaterials for integration with electronic and optical devices. This may provide the basis for new sensors and diagnostic device technology. We have used a simple polymer lift-off process, for example, to create high spatial resolution patterns in lipid bilayers, resembling a cell membrane, containing active receptor molecules. Conversely we have been adapting non-lithographic approaches for creating functional devices, using methods that in some ways emulate the way living systems create complex structures. In a simple example of the bio-inspired approach, we have used a scanning electrodeposition process for creating both biological and inorganic nanoscale devices. For example, we have deposited individual conducting nanofibers to act as high sensitivity chemical sensors with high spatial resolution. We have used a similar non-lithographic approach to create nanofluidic systems and nanoelectro-mechanical devices.

2:15 PM W2.3
Engineered Microchannels for Active Nanomaterials Assembly. Andrew K. Boal, Joseph M. Bauer, Susan B. Rivera, Ronald P. Manginell, George D. Bachand and Bruce C. Bunker; Biomolecular Materials and Interfaces, Sandia National Labs, Albuquerque, New Mexico.

Our current research is focused on the development of motor protein integrated microfluidic devices for the active assembly of nanomaterials. These systems, based on surface bound kinesin motor proteins propelling microtubules (MTs) bearing nanoparticle cargo, require the fabrication of a microfluidic channel environment capable

of both directing overall microtubule motion and providing surfaces able to off-load nanoparticle cargo in predetermined locations. To address both of these concerns, we are preparing microfluidic channels with gold walls deposited on oxide surfaces of silicon wafers. Microtubule movement within a channel is largely decided by microtubule-wall collision events, which are in turn highly governed by the chemical nature of the wall surface. Typically, a microtubule-wall collision can lead to one of three scenarios: (1) in environments where kinesin is absorbed to the channel wall as well as the floor, microtubules almost always move out of the channel, (2) devices where casein or kinesin denatures on the channel wall and collisions mostly lead MT redirection within the channel ,and (3) devices where the walls do not absorb kinesin and also redirect the MTs. To optimize the performance of our devices with regards to microtubule guidance and protein resistance, we have used a variety of alkane thiols to selectively form monolayers on the gold wall surfaces, and their effectiveness at resisting motor protein absorption and directing MT motion has been evaluated. Applications of these devices for the manipulation of nanomaterials using MTs and kinesin in conjunction with chemically active surfaces will also be discussed.

2:30 PM *W2.4
Bio-inspired Periodic Microlens Arrays with Integrated Pore Structures Created by Multi-beam Interference Lithography.
Shu Yang[1], Gang Chen[1], Chaitanya K. Ullal[2], Mischa Megens[1,4], Yong-Jin Han[1], Ronen Rapaport[1], Edwin L. Thomas[2], Chada Ruengruglikit[3], Qingrong Huang[3] and Joanna Aizenberg[1]; [1]Bell Laboratories, Lucent Technologies, Murray Hill, New Jersey; [2]Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [3]Food Science, Rutgers, The State University of New Jersey, New Brunswick, New Jersey; [4]Philips Research Laboratories, Eindhoven, Netherlands.

Nature has developed strategies that give biological processes and structures exquisite selectivity and performance. Inspired by the discovery of light-sensitive brittlestars, which have microlens arrays with integrated pores, we have developed a novel, yet simple approach that uses multi-beam interference lithography to create porous hexagonal microlens arrays (period of 1-8 micrometers) that are analogous to the biological structures. The lens size, shape, symmetry and connectivity are controlled by beam wave vectors and their polarizations; while the pore size and porosity are adjusted by laser intensity, exposure time and additive base concentration. The combination of microlenses and pores will allow wide tunability of optical properties when actuating optically active liquids in and out of pores, thus making it superior to traditional microlens arrays.

SESSION W3: Biomimetic Surface Engineering
Chair: Shu Yang
Tuesday Afternoon, April 13, 2004
Room 3003 (Moscone West)

3:30 PM *W3.1
Templating of Magnetic and Semiconducting Materials a la Bone Formation.
Samuel I. Stupp[1,2,3] and Eli D. Sone[2]; [1]Materials Science & Engineering, Northwestern University, Evanston, Illinois; [2]Chemistry, Northwestern University, Evanston, Illinois; [3]Feinberg School of Medicine, Northwestern University, Evanston, Illinois.

Bone is one of the most fascinating materials found in nature, with the outstanding physical properties necessary to support and protect the organs of vertebrates, the capacity to repair itself, and a clever structure that allows it to host cells buried in its hard matrix. This hard matrix forms in a highly dynamic process of biomineralization by templating the growth of mineral crystals on fibrous scaffolds with control of shape, size, and crystallographic orientation. In this lecture we illustrate the use of artificial systems designed to mimic some of these elements of bone growth to template to growth of magnetic and semiconducting crystals. In one case iron-based magnetic nanocrystals are grown on cylindrical nanofibers formed by self assembly of peptide amphiphiles. These nanofibers present on their surfaces amino acids that can coordinate to iron ions, just as acidic bone proteins on collagen substrates capture calcium ions. In another system "quantum confined" cadmium sulfide crystals are grown on other self-assembled and covalently captured nanofibers. These bone-biomimetic approaches to template inorganic crystals could be used to form hybrid one-dimensional arrays of functional crystals on soft substrates that can align, migrate, and deform.

4:00 PM *W3.2
Biocompatible Sulfur Containing Polymers for Surface Passivation and Patterning.
Jane P Bearinger[1], Samuel Terrettaz[3], Roger Michel[2], Christine A Orme[1], Marcus Textor[2] and Jeffrey A Hubbell[3]; [1]MTP/ CMS, LLNL, Livermore, California; [2]ETH, Zurich, Switzerland; [3]EPFL, Lausanne, Switzerland.

Biocompatible polysulfides have been designed to overcome existing stability limitations of monofunctional alkanethioloates and find utility as coatings in the formation of biodiagnostic and bioanalytical devices. Specifically, triblock copolymers of poly(propylene sulfide) and poly(ethylene glycol) (PEG-bl-PPS-bl-PEG) were synthesized to stably chemisorb to gold via the PPS domain and present a hydrophilic passivating surface via the PEG domain. The copolymers were tested for stability and the ability to resist adsorption of proteins and attachment of dermal cells. X-ray photoelectron spectroscopy (XPS) and surface plasmon resonance (SPR) results indicate that PEG-bl-PPS-bl-PEG applied to gold renders surfaces more resistant to oxidation than alkanethiols, as well as resistant to both protein and cells. Fibroblast cell activity was also reduced on PEG-bl-PPS-bl-PEG treated gold, as compared to untreated surfaces. The advent of novel characterization and patterning methods allows for manipulation of these polymers and bio-molecules. We have employed ultraviolet (UV) energy, EC-OWLS (electrochemical optical waveguide lightmode spectroscopy), microcapillary electrochemistry, microchannel flow, and photocatalysis to control sulfide adsorption via oxidation to pattern surfaces. When PEG-bl-PPS-bl-PEG is oxidized, it loses its strong chemisorbed bond to gold and is easily removed from a gold surface. Initial homogenous oxidation studies were performed on PEG-bl-PPS-bl-PEG coated gold surfaces. The PPS polymer backbone, characterized with XPS, changed from a sulfide to a sulfone state upon exposure to UV energy generated in the presence of oxygen. Homogeneous oxidation was also achieved with EC-OWLS on PEG-bl-PPS-bl-PEG coated ITO (indium tin oxide). Further studies were performed to locally oxidize the material. To this end, we first employed a micro-capillary cell setup to perform solution-based electrochemical oxidation of a PEG-bl-PPS-bl-PEG coated gold substrate. Polarization tests were conducted using the micro-capillary electrochemical cell setup in both aqueous and organic environments and scanning XPS confirmed chemical oxidation. Local oxidation has also been achieved with microchannel fluidic patterning with PDMS (polydimethylsiloxane) stamps on gold. Our present patterning focus explores the efficacy of TiO2 as a photocatalyst for oxidation of alkanethiols and the PEG-bl-PPS-bl-PEG sulfur chemistry. We are working with TiO2 nanoparticles and TiO2 bound to Poly(dimethyl siloxane) (PDMS) irradiated with UV. To date, patterning has been imaged with low voltage scanning electron microscopy (SEM) and Atomic Force Microscopy (AFM).

4:30 PM W3.3
Exogenous Pulmonary Surfactants Films.
Coralie Alonso and Joseph A. Zasadzinski; Chemical Engineering, University of California, Santa Barbara, California.

Several commercial exogenous surfactants such as Survanta, Infasurf and Curosurf are all successfully used for lung surfactant replacement therapy to treat RDS (Respiratory Distress Syndrome) although their compositions are quite different. Making use of Langmuir films, a plausible in vitro model for the lung environment we identify common points or differences between commercial surfactants native pig surfactant film. The surface pressure versus trough area isotherms show that all the films meet the requirements regarded as first criteria to define a good surfactant: high collapse pressure and efficient respreading. However, further characterization highlights striking discrepancies. Surface morphologies, as seen by Brewster angle and fluorescence microscopy, are very different and their evolution upon compression and expansion as well. Phase transitions and reorganizations within the film can be seen for Survanta while nothing so drastic appear for other samples. These observations correlate with the surface shear viscosity measurements which show that Survanta films are much more rigid than Curosurf, Infasurf or native pig ones. These results can be explained in terms of molecular interactions and correlate to differences in compositions. Because of a higher DPPC and fatty acid content, the solid fraction / liquid fraction balance in Survanta films is higher than for other ones which leads to more textured and rigid films. The morphology at high surface pressure also relate to the surface viscosity and thus to the composition.

4:45 PM W3.4
The Role of Protein Organization on Synthetic Surfaces on the Behavior of Attached Cells.
Djordje Nikolic and Jeffrey D Carbeck; Chemical Engineering, Princeton University, Princeton, New Jersey.

Control over the organization of proteins on surfaces on microscopic length scales is important in the development of biosensors and protein micro-arrays, as well as in the organization and control of growth of cells on surfaces. Protein patterning is finding widespread use in studying the functional effects of cell adhesion to substrates, usually using patterns similar to the size of a cell (10 - 50 μm). When cells adhere to surfaces, they form discrete adhesive sites called focal adhesions: protein-rich complexes that range in size from less than 100 nm to approximately 1 to 2 μm. To determine how the distribution of

focal adhesions regulates cell behavior requires patterning on length scales much smaller than the diameter of a single cell. We are developing several strategies to organizing proteins that direct cell adhesion on these lengths scales. In one approach, we have developed a new method for the tailoring of proteins on surfaces, based on patterning of 2 μm colloids functionalized with proteins, which provides control over protein organization on multiple length scales. We used surfaces functionalized in this way to form patterned arrays of cells and to study the role of adhesive contacts on the behavior of anchorage-dependent cells. To separate effects of protein organization from topology, we have developed a new method that combines evaporative silanization with photolithography to produce regularly spaced 2 μm islands of the cell adhesion protein fibronectin on silicon wafers. Several templates with different spacing between islands have been made (1, 2, 4 and 8 μm). The separations between islands were chosen to bracket the average distance between adhesive sites naturally formed by cells on homogeneous coatings of fibronectin. Thus, we can determine the optimal distribution of adhesive sites for activating cell functions. Preliminary results suggest that cell size may be inversely proportional to the distance between islands.

SESSION W4: Poster Session I: Biological and Bio-Inspired Materials and Devices
Chairs: William J. Landis, Christine Orme and Rizhi Wang
Tuesday Evening, April 13, 2004
8:00 PM
Salons 8-9 (Marriott)

W4.1
Biological Functionalization of Carbon Nanotubes.
Ranjani Sirdeshmukh, Kousik Sivakumar and Balaji Panchapakesan; Department of Electrical and Computer Engineering, University of Delaware, Newark, Delaware.

Carbon nanotubes are known for the exceptional electrical and mechanical properties. The size dependant properties of nanomaterials have made them very attractive to develop highly sensitive sensors and detection systems. This is especially true in biological sciences, where the efficiency of a detection system would reflect on the size of the detector and the sample required for detection. At approximately 1.5 to 10 nm wide, and approximately 1.5 to 2 μm long, the use of carbon nanotubes as sensors in biological systems would greatly increase the speed and accuracy of detection and diagnostics, for a highly reduced sample size. If binding proteins to the surface of nanotubes could vary the surface states on them, then it would result in varied electrical and optical properties, which would be very sensitive to the reactions occurring on the surface. It has been seen that proteins have a higher affinity to adhere to metallic surfaces. When metallic nanoparticles such as silver and platinum are electrochemically-deposited on the surface of nanotubes, silver and platinum nanowires are formed. Due to the small sizes of these nanowires (diameters ranging from 10 to 100 nm, and lengths of approximately 1.5 μm), the surface effects due to proteins and the actions of their cell surface receptors could be just as prominent. In this paper, we show the deposition of a fluorescent dye, fluorescein isothiocyanate (FITC) conjugated to an antibody with a concentration of 10 μL of 1 μg/mL antibody in 500 μL of Phosphate buffered saline on the surface of the silver and platinum nanowires and carbon nanotubes through fluorescence and reflection images taken using a multiphoton confocal microscope. The results from the images suggests the strong interaction or binding of the antibodies to the surfaces of the nanowires and nanotubes which lends promise for this technique for applications as drug delivery and detection vehicles in the area of biomedical nanotechnology. We are currently studying the fluorescence intensity as a function of the concentration of proteins on the surface of the nanotubes and nanowires and this will be presented at the conference. Detection, through the occurrence of fluorescence, and diagnostics using this technique could bring the sample size down to single-cell measurements, and could hold immense potential in the area of biomedical nanotechnology.

W4.2
Photochemitry of self-assembled protein cages.
Zachary B Varpness[1,3], Jesse Mosolf[1,3], Dan Ensign[1,3], Michelle Flennikin[1,3], Debbie Willits[2,3], Mark Young[2,3] and Trevor Douglas[1,3]; [1]Chemistry and Biochemistry, Montana State University, Bozeman, Montana; [2]Department of Plant Sciences, Montana State University, Bozeman, Montana; [3]Center for BioInspired Nanomaterials (CBIN), Montana State University, Bozeman, Montana.

We have utilized the self-assembled protein cages from ferritin, ferritin-like proteins, and viral capsids, as size constrained reaction environments for nanomaterials synthesis. The nanoparticles are formed using either a photochemical reduction or via oxidative hydrolysis of precursor ions. The resulting transition metal oxide and metallic nanomaterials are monodispersed and have dimensions commensurate with the internal diameter of the protein cages. We have characterized the physical properties of these materials, and in particular the photocatalytic activity of both the unmineralized and mineralized protein cages. The chemical plasticity of these protein cages towards chemical and genetic manipulations makes these highly versatile materials for imparting function by design.

W4.3
Microtubule Templated Synthesis of Inorganic Nanomaterials.
Andrew K. Boal, Thomas J. Headley, Ralph G. Tissot and Bruce C. Bunker; Biomolecular Materials and Interfaces, Sandia National Labs, Albuquerque, New Mexico.

Protein microtubules (MTs) of polymerized tubulin have been used as templates for the biominetic synthesis of metal oxide and metal sulfide nanomaterials. MTs off a unique biomolecular scaffold as they have a controllable, high aspect ratio (25 nm in diameter and 1-1000 _m long) and can be dynamically grown on a selected region on a substrate. As a first example of MT-based template synthesis of nanomaterials, we have prepared iron oxide coated MTs. Exposure of MTs to anaerobic aqueous solutions of Fe2 buffered to neutral pH followed by aerial oxidation lead to the formation of iron oxide coated MTs. The nature of the iron oxide layer depended greatly on the amount of Fe2 added to MT solutions: low amounts of Fe2 yielded 10-30 nm thick layers of amorphous iron oxides. Higher amounts of Fe2 yielded MTs coated with crystalline layers of FeOOH up to 250 nm thick. On the micron scale, these coated MTs were observed to form large, amorphous bundles. Since iron oxide nucleation was observed to occur selectively on the MT surface, it is likely that the highly negatively charged MT surface is responsible to the formation of the observed structures. Progress has also been made towards the synthesis of both metal sulfide (ZnS, CdS) coated MTs, formed by co-precipitation reactions carried out in the presence of MTs, and the synthesis of zinc oxide coated MTs, formed by the hydrolysis of Zn2 in the presence of MTs.

W4.4
Deposition of Patterened Calcium Carbonate Film using a Binary Surfactant System for Drug Detoxification Applications.
Javier Gutierrez, Laurie Gower, Debra Lush, Vishal Patel and Brian Carey; Materials Science and Engineering, Univeristy of Florida, Gainesville, Florida.

In the Unites States alone, over 300,000 patients enter the emergency room each year due to prescription drug overdose complications. In fact, the leading method of suicide is via overdose of amitriptyline, a popular anti-depressant. The goal of this research is to create a particulate system for drug detoxification that will be easily administered to the overdosant. The specific particulate system of interest consists of a core-shell particle coated with a porous calcium carbonate layer. This particulate system will work by acting as a "micro-sponge" that will absorb the overdosed drug. Then, through degradation, the particle will release the drug back into the body at non-toxic rates. To facilitate research on the calcium carbonate layer, the research will be conducted on flat films before proceeding to work on spherical particles. This flat surface consists of a monolayer of a binary surfactant system upon which the mineral film is deposited. One film-forming surfactant, either stearic acid or arachidic acid, and one "pore" surfactant, cholesterol or diolein, will be used. The porosity of the calcium carbonate film will be achieved through phase segregation of the binary surfactant system.

W4.5
Semiconductor Nanocrystals Arrayed on Cellulose and Cellodextrins.
Jun Feng[1], Yong-Hyun Kim[2], Shengbai Zhang[2], Shiyou Ding[1], Melvin P. Tucker[1], Garry Rumbles[2] and Michael E. Himmel[1]; [1]National Bioenergy Center, National Renewable Energy Lab, Golden, Colorado; [2]Basic Science Center, National Renewable Energy Lab, Golden, Colorado.

We are investigating potentially useful interactions between cellulose and semiconductor particles and nanocrystals. Cellulose has a unique microcrystalline structure composed of repeating units of cellobiose stabilized by interchain hydrogen bonds. Native cellulose has distinctly hydrophobic (1,0,0) and a hydrophilic faces (1,1,0). Plant cellulose tends to form 10 to 100 micron size amorphous bundles and bacterial cellulose is observed as organized long filaments. In preliminary work, we have discovered that both cellulose nanoparticles and cellulose fibers demonstrate strong attraction for certain kinds of semiconductor nanocrystals, including TOPO-(CdSe)ZnS quantum dots (QDs). An initial indication of this interaction was the observation that cellulose exposure caused dispersion of QDs in water, yielding photoluminescent cellulose. Finding ways to overcome the hydrogen bound network of the cellulose crystal is challenging, however, cellulose microfibrils (small bundles of cellodextrins) may be useful for arraying QDs. We have also examined the interactions

between cyclodextrins (CDs) and (CdSe)ZnS QDs. Cyclodextrins have ring structures with discrete dimensions and the inner lumen of CDs is hydrophobic and flexible, which can accommodate the 8 x CH2 chains of the TOPO molecules used to passivate (CdSe)ZnS QDs during growth. We attribute the CD-aided dispersion of TOPO-QDs in water to overall entropic benefits afforded from hydrophobic shielding of these surface ligands. We also found that CD-QD solutions demonstrate a sizable 15-nm red shift. To develop an understanding for this red shift, we have performed first-principles density functional theory calculations that show that cyclodextrin hydroxyls react with the QD surface and that hydroxyl oxygens form coordinate bonds with Zn, which explains the decrease in band gap observed experimentally. We also present AFM and SEM images of QDs arrayed on bacterial cellulose and cyclodextrins.

W4.6
Direct Patterning of Membrane-Derivatized Colloids with in situ UV-Ozone Photolithography. Cheng-Han Yu[1], Atul N. Parikh[2] and Jay T. Groves[1]; [1]Department of Chemistry and Physical Biosciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, California; [2]Department of Applied Science, University of California, Davis, California.

A novel lithography method has been developed for pattering biological materials, e.g. lipid bilayer membranes. 3D patterns of the fluid membranes supported on silica beads have been achieved by in situ ultraviolet photolithography. Deep UV (185-254nm) generates ozone and reactive radicals, which chemically degrade and remove lipids with high spatial resolution, even in entirely aqueous environment. In this technique, we utilize backside UV illumination through the substrate, which also serves as microfabricated photomasks. Membrane-coated colloid particles are allowed to settle gravitationally onto this substrate, in which arrays of patterns are in registry with surface pits that position the particles. This process is essentially based on contact printing and self-alignment mechanisms. Micron-resolution membrane patterns on the colloid particle surface are readily observed by epifluorescence microscopy. The removal of lipid bilayer was confirmed by the ability to refill with small unilamellar vesicles (SUV) of different lipid composition, or proteins which bind to bare silica surface. The uniqueness of this photopatterning technique is that it occurs entirely in liquid environment and does not require development steps. Geometrically asymmetric membranes on colloid particles are of utility for presentation of biological signals (e.g. cell surface signaling molecules) to live cell cultures. They also create new possibilities to understand the general construction of 2D colloidal materials. In addition, the organization of them can be modified by adding biological polymerizable molecules in the membrane composition. This may give certain contributions to develop new biomechanical system.

W4.7
Bio-inspired Crystal Growth Induced by Novel Organic Compounds. Nicholas Bryan Dinsdale and Brigid R Heywood; School of Chemistry & Physics, Keele University, Keele, Staffordshire, United Kingdom.

It is now well established that, to design materials with useful properties (e.g. precisely controlled particle size and morphology) it is highly beneficial to examine the compounds and processes used by organic systems, and exploit similar principles in the manufacture of synthetic materials. Living organisms have developed extremely efficient materials which exhibit a finely-balanced compromise between desirable properties, such as low density and high mechanical strength. This study continues previous work by investigating the effect of various novel organic compounds (e.g. novel calixarene derivatives) not unlike those potentially found in organic systems, on the crystallisation of compounds including barium sulphate and calcium carbonate. Of interest in this study will be the usual properties of crystals, including morphology, particle size and uniformity. The effect of the concentration and substituent chain length of the organic components will also be investigated. More specifically, one key aspect for discussion will be that of the, relatively poorly understood, crystallographic phenomenon of twinning. Large scale multiple twinning has been observed in several of the experients conducted. Twinning occurs when two crystals are intergrown in a symmetrical manner, brought about, for example, by the sharing of a mirror plane or rotational axis. Twinning is thus explainable, for example, by the sudden reversal of layers in the ionic packing (ABCBA) in the case of a mirror plane. However, the cause of twinning is little understood, although it is clear here that the presence of additives favours this process. This study will attempt to suggest means by which this twinning may occur.

W4.8
Effects of Materials Properties on Cell Culture. Bing Shi[2], Aaron Fairchild[1], Zenith Kleine[2], Tom Kuhn[2] and Hong Liang[1]; [1]Mechanical Engineering, University of Alaska Fairbanks, Fairbanks, Alaska; [2]Chemistry and Biochemistry, University of Alaska Fairbanks, Fairbanks, Alaska.

Biomaterials have been widely used in artificial joints, such as total hip and knee joint replacements. Their design, texture, properties, and biotribological performance are of great interests. In this research, we investigate effects of materials properties, such as wettabilty and texture of selected materials on cell culture. We conduct cell culture experiments on conventional materials such as glass, polyurethane, and polyvinyl alcohol. The surface property-cell adhesion relationships are discussed in this presentation.

W4.9
Reflective Interferometric Detection of Tag-Free Biomolecules. Jinghui Lu[1], Benjamin Miller[2] and Lewis Rothberg[1]; [1]Chemical Engineering, University of Rochester, Rochester, New York; [2]Dermatology, University of Rochester, Rochester, New York.

Sensitive and selective schemes to detect biomolecules are important enabling tools in medicine, environmental monitoring and biological research. The vast majority of sensing instruments are based on fluorescent tagging of molecules in the sample under investigation, a time consuming and expensive process. In addition, most of the fluorescent readout schemes currently available require relatively expensive imaging systems and detectors. Simplification of the chemistry to avoid tagging of analytes and development of portable, inexpensive readout schemes continue to be central challenges. Surface plasmon resonance, ellipsometric, and interferometric methods have all proved to be viable ways to avoid tagging but they still tend to require relatively complex detection systems. We describe here a sensing method based on destructive interference of reflected light that is sensitive, quantitative and has a simple format. The reflective interferometric detection technique is able to discriminate surface thickness change as low as 2 Angstroms on a silicon-based biosensor. A simple and effective hydro-affinity surface design in addition to functionalization chemistry has been implemented to guarantee the accuracy and consistency of the technique. We have demonstrated detection of tag-free DNA oligonucleotides binding to their surface-bound complements at the level of femtomoles, and the sensitivity could be further increased substantially. The technique is suitable for highly parallel detection in a microarray format and can generally be applied to any target analyte for which selective attachment chemistry can be developed. This technique can potentially be adapted to work under water.

W4.10
Glycera Jaws: A Biocomposite of Metals, Melanin and Proteins. Dana Novak[1], Helga Lichtenegger[2], John Harreld[3], Nelle Slack[4], Galen Stucky[3] and Herbert Waite[1]; [1]BioMolecular Science and Engineering, University of California, Santa Barbara, Santa Barbara, California; [2]Materials Science and Testing, Vienna University of Technology, Wien, Austria; [3]Chemistry and Biochemistry, University of California, Santa Barbara, Santa Barbara, California; [4]Materials, University of California, Santa Barbara, Santa Barbara, California.

Glycera is a marine polychaete worm equipped with four syringe-like jaws to inject venom into its prey. The jaws have unusually high resistance to wear, especially considering their low degree of mineralization. Unmineralized zinc and both mineralized and unmineralized forms of copper are present in Glycera jaws. We have determined that Glycera jaws contain melanin as a major component. Melanin composes approximately one third of the jaws by mass. In this work we explore the mechanical and structural ramifications of melanin in the jaws. The interaction of melanin with jaw proteins and metals is also examined. Glycera jaws are about 50% protein by weight, which contain an average of about 40 mol % histidine. Certain proteins may have even higher histidine contents. Given their role in other organisms, histidine-rich proteins are likely to interact with zinc and copper in Glycera jaws. In this work we identify and characterize metal-binding jaw proteins and investigate their contributions to material properties of the jaws.

W4.11
Observation of nano surface structures of various plant leaves with ultra water-repellency. Osamu Takai[1], Yunying Wu[1], Masao Kouno[2], Hiroyuki Sugimura[2] and Yasushi Inoue[3]; [1]Center for Integrated Research Science and Engineering, Nagoya University, Nagoya, Japan; [2]Department of Materials Processing Engineering, Nagoya University, Nagoya, Japan; [3]Research Center for Nuclear Materials Recycle, Nagoya University, Nagoya, Japan.

Studies on some plant leaves revealed that the super-hydrophoic property was independent of the shapes of nano-scale asperities but mainly affected by these nanostructure although the surface of these leaves consists of both nano- and microstructures. The results from the natural world provide a guide for constructing artificial

super-hydrophobic surfaces with nano-scale fine roughness by Field Ion Beam (FIB). The water contact angle of such artificial surface was mainly affected by the asperity height. This would be the results of more air trapped in the pores between higher height asperity if the fine-rough surface was made up of nano- (or submicro-) scale feature. This work is supported by JSPS - RFTF99R13101 and ASTF.

W4.12
Macroscopic Spherical Assemblies from Charged Polypeptides and Small Multivalent Counterions. Brandon John McKenna[1], Henrik Birkedal[1], Michael H. Bartl[1], Timothy J. Deming[2,1] and Galen D. Stucky[1,2]; [1]Chemistry, UC-Santa Barbara, Santa Barbara, California; [2]Materials, UC- Santa Barbara, Santa Barbara, California.

Micrometer-sized spherical vesicles have been found to assemble from homopolymer electrolytes and small, multi-charged counterions in water. In contrast to previous efforts, these vesicles do not use preformed templates, do not require block copolymers, and do not necessarily employ nanoparticles. We have investigated the requirements for vesicle formation with regards to both components of the assembly. We have found self-assembly to occur with 3 different polypeptides and a variety of counterions, all of which require a minimum number of charged groups to promote supramolecular crosslinking. Two of the polypeptides are formed by amino acids with positively charged side chains while the remaining polymer is anionic. Assemblies made with positively-charged amino acids can be stabilized by adding an outer layer of silica. We show how the assembly process is controlled by pH and how, in consequence, the pKa's of the charged organic groups can be used to reliably predict sphere formation. By varying the nature of the small counterions, we have determined the requirements for assemblies. The assemblies have been further investigated using confocal microscopy and fluorescent labeling of the different components. The mode of assembly and the chemical interactions leading to assembly are discussed.

W4.13
2D Colloidal Arrays of Monodisperse Phase Separated Membrane Domains Formed in a Supported Bilayer. Yoshihisa Kaizuka[1,2], Sharon Rozovsky[1,2] and Jay T. Groves[1,2]; [1]Chemistry, University of California, Berkeley, California; [2]Physical Bioscience Division, Lawrence Berkeley Laboratory, Berkeley, California.

In cellular membranes, lateral phase separation is widely believed to be involved in a variety of cellular processes, such as intracellular sorting of membrane proteins or immune cell recognition. Phase separated domains, rich in cholesterol and sphingolipids, sometimes called rafts, are currently of interest in membrane biology. Quantitative study of membrane lateral phase separation and its role in cellular processes requires precisely defined model membrane systems. We have recently introduced a variety of supported membrane structures formed by the rupture of giant unilamellar vesicles onto conventional supported membranes (supported intermembrane junction). These systems exhibit a number of phenomena not generally seen in solid supported membranes. Most notably, phase separated structures can form in an environment that allows for free movement and interactions and facilitates imaging analysis. In general, phase separated domains collide and coalesce without bound. However under certain conditions, a rich variety of stabilized superstructures can form. These include monodisperse ordered arrays of 1μm diameter rafts and labyrinthine stripe patterns. The planar configuration enables interferometric imaging of membrane topography with nanometer precision. Characteristics of superstructures and applications to cell membrane studies will be discussed.

W4.14
Calcium Oxalate Precipitation at Phase Separated Phospholipid Langmuir Monolayers. Daniel R. Talham and Isa O. Benitez; Department of Chemistry, University of Florida, Gainesville, Florida.

Calcium oxalate and calcium phosphate are the principal crystalline materials found in urinary stones. The inorganic crystals are always mixed with an organic matrix composed of carbohydrates, lipids and proteinaceous materials that account for about 2% of the total mass, although a much larger percentage of the total volume. To better understand the process of stone formation, it is important to study the interactions between the organic and crystalline components. We have previously performed a series of studies of calcium oxalate precipitation at an interface provided by phospholipid Langmuir monolayers that serve as models for the phospholipid domains within membranes. We observed that the Langmuir monolayers can effectively catalyze the precipitation of calcium oxalate monohydrate (COM) and that the identity of the monolayer has a strong influence on the rate of crystal formation. The present study investigates the role of phase boundaries on COM precipitation by studying phase separated Langmuir monolayers. Brewster angle microscopy is used to monitor COM growth at monolayers in LC/LE coexistence and LE/gas coexistence regimes. Phase separated mixtures of different lipids are also studied. COM precipitation is enhanced at monolayers containing a single lipid in phase coexistence, where the phase boundary is dynamic. Phase separated mixtures of different lipids show no enhancement in crystal formation due to the presence of a boundary.

W4.15
A 1.7-Kilobase Single-Stranded DNA that can be Folded into a Regular Octahedron. William M. Shih[2,1,3], Joel D. Quispe[4] and Gerald F. Joyce[2,1,3]; [1]Chemistry, The Scripps Research Institute, La Jolla, California; [2]Molecular Biology, The Scripps Research Institute, La Jolla, California; [3]The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California; [4]Cell Biology, The Scripps Research Institute, La Jolla, California.

Rigid DNA-based nanostructures may play a role in the construction of miniaturized devices through their ability to direct the assembly of materials on the subnanometer to micrometer scale. A key property of DNA - its ability to be amplified exponentially by polymerases - facilitates both the large-scale clonal production of individual sequences and the directed evolution of sequence lineages toward optimized behaviors. Previous examples of three-dimensional geometric DNA objects, however, are not amenable to cloning because they contain topologies that prevent copying by polymerases. Here we show the design and synthesis of a 1,669-nucleotide, single-stranded DNA molecule that is readily amplified by polymerases and that, in the presence of five 40mer oligonucleotides, can be folded into a regular octahedron structure by a simple denaturation-renaturation procedure. The resulting octahedron, approximately 22 nanometers on a side, was visualized by cryo-electron microscopy and shown to have the predicted structure. No eleven base-pair sequence appears twice in the octahedron. Thus each part of the octahedron is uniquely addressable by the appropriate sequence-specific DNA binder.

W4.16
Fabrication of Nanoporous Silica Nanotubes by Inorganic and Organic Double Templates. Jian-Feng Chen[1], Runjing Liu[1], Dapeng Cao[1,2], Zhigang Shen[1], Jimmy Yun[2], Jiexin Wang[1] and Weilie Zhou[3]; [1]Research Center of Ministry of Education for High Gravity Engineering and Technology, Beijing University of Chemical Technology, Beijing, China; [2]Nanomaterials Technology Pte Ltd, Ayer Rajah Crescent, Singapore; [3]AMRI/Chemistry, Advanced Materials Research Institute/UNO, New Orleans, Louisiana.

Inorganic and organic double templates were used to fabricate silica nanotubes with nanochannels perpendicular to the shells. Needle-like CaCO3 nanoparticles, synthesized by a high gravity reactive precipitation method, were used as inorganic templates and C16H33N(CH3)3Br (C16-CTAB) was used as an organic surfactant template. Field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) were employed to characterize the nanotube and nanoporous structure. It can be concluded that the length and diameter of the nanotube can be controlled by the needle-like inorganic CaCO3 nanorod templates and the nanochannels in the shells can be tuned by different surfactant micelles. The nanotubes with nanochannels perpendicular to the shells have a potential application in chemical bio-catalyst, bio-separation, and drug delivery.

W4.17
In Situ Protein Directed Silica Biomineralization. Diana D. Glawe[1], Francisco Rodriguez[2], Rajesh R. Naik[2] and Morley O. Stone[2]; [1]Engineering Science Department, Trinity University, San Antonio, Texas; [2]Materials Directorate, Air Force Research Laboratories, Wright-Patterson AFB, Ohio.

Numerous examples of nanopatterning and nanostructure are commonly found in nature, most apparent in the marine diatoms and sponges. The diatom cell walls are considered as a paradigm for the controlled production of nanostructure silica. Peptides, such as silcateins, silaffins and poly-L-lysine are capable of precipitating silica from a silane precursor. The biomimetic formation of silica nanostructures using polypeptides allows control of morphology at the structural level desired for advanced applications. Here we demonstrate the deposition of silica precipitating proteins onto conductive surfaces using electrophoresis. The exposure of the protein-coated surface to a silane precursor resulted in the formation of a network of plate-like silica structures. The silica morphologies are distinct from the structures obtained without electrophoretic-mediated deposition of the peptides. The impetus of this peptide mediated silica research stems from the desire to mimic and leverage nature's ability to create repeated silica structures with high fidelity to enable bioengineering of materials and devices for opto-mechanical and biomedical applications, among others.

SESSION W5: Biomolecules-Induced Materials Synthesis
Chair: Janet Moradian-Oldak
Wednesday Morning, April 14, 2004
Room 3003 (Moscone West)

8:30 AM W5.1
Organization of metallic nanoparticles using Tobacco Mosaic Virus templates. Erik Dujardin[1,2], Charlie Peet[1], Gerald Stubbs[3], James N Culver[4] and Stephen Mann[1]; [1]School of Chemistry, University of Bristol, BRISTOL, United Kingdom; [2]NanoScience Group, CNRS - CEMES UPR 8011, TOULOUSE, France; [3]Biological Sciences, Vanderbuilt University, Nashville, Tennessee; [4]Center for Biosystems Research, University of Maryland, College Park, Maryland.

Reflecting the growing interest in biomimetic chemistry as a powerful approach to combine complexity and functionality in new materials [1], the design of versatile nanoparticle-based superstructures has recently evolved into a dynamic discipline where biological concepts and entities are playing a crucial role. In particular, the emergent field of nano-electronics faces challenges, such as the anisotropic organization of spherical nanoparticles, which could be addressed by using biological entities as structuring building blocks. In this respect, we will show how self-assembled cylindrical particles of wild type and recombinant tobacco mosaic virus (TMV) can be used as organic templates for the controlled deposition and organization of Pt, Au, or Ag nanoparticles [2]. Chemical reduction of [PtCl6]2- or [AuCl4]- complexes at acidic pH gave rise to the specific decoration of the external surface of wild-type TMV rods with metallic nanoparticles less than 10 nm in size. In contrast, photochemical reduction of Ag(I) salts at pH 7 resulted in nucleation and constrained growth of discrete Ag nanoparticles aligned within the 4 nm-wide internal channel. The number of encapsulated nanoparticles increased when Ag benzoate rather than Ag nitrate was used due to reduced supersaturation associated with the lower Ag/benzoate redox couple, which enhanced the surface-templating effect of the channel wall carboxylates compared with nucleation in solution. Similar experiments using a mutant TMV with reduced negative charge along the central cavity will be presented that confirmed site-specific deposition involving glutamic and aspartate acid groups. Our results suggest that it should be possible to prepare 1-D arrays for a wide range of inorganic quantum dots by molecular engineering of the internal and external surfaces of self-assembled TMV tubules. Even larger ordered structures could be obtained by subsequently aligning the decorated virus in its nematic liquid crystal phase. [1] E. Dujardin, S. Mann, Adv. Mater., 2002, 14, 775-788. S. A. Davis, E. Dujardin, S. Mann, Cur. Opin. Sol. State Mater. Sci., 2003, in press. [2] E. Dujardin, C. Peet, G. Stubbs, J. N. Culver, S. Mann, NanoLett., 2003, 3, 413-417.

8:45 AM W5.2
Molecular Biomimetics: Genetically Engineered Inorganic-Binding Polypeptides as Molecular Building Blocks. Mehmet Sarikaya[1,2], Candan Tamerler[1], Beth Traxler[3] and Francois Baneyx[2,4]; [1]Materials Science and Engineering, University of Washington, Seattle, Washington; [2]Chemical Engineering, University of Washington, Seattle, Washington; [3]Microbiology, University of Washington, Seattle, Washington; [4]Bioengineering, University of Washington, Seattle, Washington.

Molecular biomimetics is a novel approach where polypeptides are selected through display protocols, further engineered using molecular biology techniques, and used as molecular building blocks in controlled assembly and formation of functional inorganics and hybrid materials and systems in nano- and nanobio-technology. These polypeptides are usually 7-15 amino acids long, and obtained via combinatorial biology using, for example, cell surface and phage display libraries. Once selected, the inorganic-binding polypeptides could be further engineered using genetic engineering techniques (e.g., site directed mutagenesis) to tailor their properties for specific, practical applications. The potential of using engineered polypeptides is enormous due to the premise offered by molecular biology, namely, polypeptides' chemical and physical molecular recognition characteristics of inorganics, their self- and co-assembly in higher order and predictable structures, and the ability to manipulate their molecular composition and structure, and, therefore, functional properties, by genetic engineering protocols (DNA-base technologies). Here we describe procedures of selection of inorganic-binding polypeptides using display technaologies, the rules of binding to inorganics (metals, oxides, and semiconductors) achieved through spectroscopic (e.g., surface plasmon resonance), imaging (atomic force microscopy) and molecular dynamics studies, their conjugation and hybridization with designer proteins and DNA, long-range ordered assembly, and, finally, utility as molecular erectors for practical engineering applications. The research is supported by US-ARO through DURINT Program.

9:00 AM *W5.3
Engineered viral protein cages for biomimetic materials synthesis. Trevor Douglas[1,2], Mark Allen[1,2], Michael Klem[1,2], Deborah Willits[3,2], Yves Idzerda[4,2] and Mark Young[3,2]; [1]Chemistry & Biochemistry, Montana State University, Bozeman, Montana; [2]Center for Bionspired Nanomaterials, Montana State University, Bozeman, Montana; [3]Plant Sciences, Montana State University, Bozeman, Montana; [4]Physics, Montana State University, Bozeman, Montana.

The coat protein of the plant virus Cowpea chlorotic mottle virus (CCMV) forms a self-assembled icosahedral protein cage, 28 nm in diameter, in which nucleic acid is stored. The empty protein cage, devoid of the viral nucleic acid, can be utilized for the synthesis of size constrained inorganic nanomaterials. In addition, the protein cage can be genetically and chemically modified to introduce functionality in a site-specific manner at three topographically distinct interfaces of the viral cage. The inner surface of the viral cage can be modified to alter the electrostatic characteristics of the interior, influencing host-guest relationships between the encapsulated material and the protein cage. The outer surface can be modified for attachment of functional groups for specific interaction with surfaces and other protein cages. And, the interface between subunits can be modified to alter structural transitions changing molecular access to the interior of the protein cage. Through control of protein-mineral interactions at the inner surface of the protein cage we can exert control over the synthesis of inorganic nanomaterials formed within the cage. Control of the functionality at the outer surface has allowed us to pattern the mineralized protein cages on solid surfaces, and manipulation of the subunit interactions has allowed us to control access to the inside of the cage. These virus cages are versatile organic templates for the biomimetic synthesis of a range of composite materials with applications in magnetic storage, MR imaging, drug delivery, and catalysis.

9:30 AM W5.4
Protein-Based Nano-Structured Composite Materials. N. Hadar[1], Y. Wine[1], H. Moscovich[1], F. Frolow[1], Y. Shapira[2], Y. Shacham[2] and Amihay Freeman[1]; [1]Dept of Molecular Microbiology and Biotechnology, Tel Aviv University, Tel Aviv, Israel; [2]Dept of Physical Microelectronics, Tel Aviv University, Tel Aviv, Israel.

The design, fabrication, characterization and application of nano-structured materials based on biological macromolecules has recently gained much attention. The use of biological macromolecules such as DNA and proteins as 'building blocks' for the construction of functional nano-structures via self-assembly offers many advantages including homogeneous 'building blocks' population, self assembly mediated by bio-recognition and a sound basis of structural and biochemical data. The use of proteins as 'building blocks' is particularly attractive due to the large variety of shapes and sizes. Here we describe the use of protein crystals, obtained by methods routinely employed for X-ray diffraction studies, as templates for the preparation of novel composite materials. A unique family of nano-structured materials comprised of three dimensional highly ordered alternating arrays of biological moiety (the protein) and synthetic or metallic moieties embeded within crystal cavities may be thus created. Two kinds of 'filling' will be described: crosslinked synthetic gels obtained via vinyl co-polymerization of pre-equilibrated monomers and metallic silver crystals generated as three dimensional array within the protein crystal voids. The design, fabrication and methods of characterization of these composite materials will be presented. Furthermore, a novel approach to the manipulation of the size and geometry of the voids of such crystals will be also included, allowing for the generation of series of engineered crystals from same 'parent protein'. Our results demonstrates feasibility of this approach, paving the way to the fabrication and use of a new family of protein-based composite materials.

9:45 AM W5.5
Self-assembly of nanomatrials into films and fibers using genetically engineered viruses. Seung-Wuk Lee[1,2] and Angela M Belcher[1,2]; [1]Dept. of Materials Sci. & Eng., MIT, Cambridge, Massachusetts; [2]Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas.

Genetically engineered M13 bacteriphage (viruses) were used to self-assemble various nanomaterials (ZnS, Au, fluorescein, and phycoerythrin) into films and fibers. The filamentous viruses, which were the basic building block of the self-ordering system, were selected to have a specific recognition moiety for desired materials surfaces through phage library display. The M13 viruses coupled with ZnS nanocrystals spontaneously evolved a self-supporting hybrid film

material that were ordered at the nano-scale and micron-scale. Periodic domains continuously propagated over a centimeter length scale, which was verified using various optical and electron microscopy techniques. Anti-streptavidin viruses, which could specifically bind to streptavidin previously conjugated with many nanomaterials, were also used to modulate nanomaterials in the self-assembly virus system. Resulting virus composite films had chiral smectic C structure due to the helical surface of the M13 virus. Additionally, 20 micrometer diameter fibers were fabricated with liquid crystalline virus suspension using wet-spinning process which mimick silk spider's spinning process. After blending with highly soluble polyvinyl pyrolidone, nanoscale diameter fibers were fabricated using the electrospinning process. This approach to aligning nanomaterials in a genetically-engineered M13 virus-based liquid crystal system has several advantages. Monodisperse biopolymers (M13 virus) of specified lengths can be easily prepared by molecular cloning techniques. By genetic selection of a peptide recognition moiety, one can easily modulate and align different types of nanomaterials in 3D ordered structures. We anticipate that our approach using recognition as well as a liquid crystalline self-assembly system of engineered viruses may provide new pathways to organize electronic, optical, and magnetic materials.

SESSION W6: Calcium Phosphates as Biomaterials - Bones and Teeth
Chair: William J. Landis
Wednesday Morning, April 14, 2004
Room 3003 (Moscone West)

10:30 AM *W6.1
Intra- and Extrafibrillar Demineralization of Human Dentin Collagen. Mehdi Balooch, Stefan Habelitz, Guive Balooch, Sally J Marshall and Grayson W Marshall; Preventative and Restorative Dental Sciences, UCSF, San Francisco, California.

Human dentin consists of collagen matrix reinforced with apatite (35% organic and 45% mineral) similar in its nanoscale structure to bone and cementum (apatite). The organic component is responsible for improving the ductility and fracture toughness and mainly consists of collage type-I in the form of fibrils. The distribution of minerals inside (intrafibrillar) and between the fibrils (extrafibrillar) defines the magnitude of energy storage and dissipation during mechanical loading. We have used atomic force microscopy (AFM) and micro-Raman spectroscopy to investigate the kinetics and structural and mechanical property changes during demineralization of human dentin collagen fibrils. For intrafibrillar demineralization studies, single dentin collagen fibrils were isolated. Collagen fibrils were imaged on a glass slide in real-time while immersed in trypsin for 2h and subsequently in 10% citric acid. Structural changes of collagen fibrils, including axial periodicity, diameter, and gap height were determined. We found that gap-overlap depth gradually increased with time (initial rate 0.35 nm/sec), linearly with the square root of time before saturation at 7 nm in approximately 60 minutes, suggesting a diffusion process for dissolution of intrafibrillar mineral. This dissolution was lower than previously reported recession rates of 50nm/s for removal of extrafibrillar mineral in dentin exposed to 10% citric acid. Micro-Raman of partially demineralized dentin showed a phosphate peak (960cm-1), suggesting that intrafibrillar mineral remains after the dentin appears to be demineralized. The peak gradually disappeared during 60 minutes exposure to 10% citric acid, consistent with the AFM conclusion that dissolution of intrafibrillar mineral is orders of magnitude slower than in the extrafibrillar compartment. Single collagen fibril moduli decreased from 2 GPa to 7 MPa during prolonged acid exposure. Recombinant collagen fibrils produced by self-assembly were used as a mineral free control and exhibited modulus of 35 MPa, higher than demineralized dentin collagen. Supported by NIH/NIDCR P01DE09859.

11:00 AM *W6.2
Perturbed amelogenin protein self-assembly alters nanosphere properties resulting in defective enamel formation.
Michael L. Paine[1], Wen Luo[1], Janet Moradian-Oldak[1], Hanson Fong[2], Mehmet Sarikaya[2], Shane N. White[3] and Malcolm L. Snead[1]; [1]Center for Craniofacial Molecular Biology, The University of Southern California, Los Angeles, California; [2]Materials Science and Engineering, University of Washington, Seattle, Washington; [3]School of Dentistry, University of California, Los Angeles, Los Angeles, California.

The outermost covering of vertebrate teeth, enamel, is itself a unique composite bioceramic material that is the hardest tissue in the vertebrate body, containing long-, thin-crystallites of substituted hydroxyapatite (HAP). Enamel functions under immense loads, in a wet, bacterial-laden environment generally without catastrophic failure over a lifetime for the organism. Unlike biogenerated tissue that is mesodermal in origin, such as bone, enamel is elaborated by ectoderm derived cells called ameloblasts. We have focused our investigations on the formation of enamel using cell and molecular approaches and by coupling findings from these techniques to biomechanical investigations at the nanoscale and mesoscale levels. We have employed Koch's postulates at the genetic level to ascertain the role(s) for amelogenin protein by creating "loss of function" transgenic animal models. For amelogenin, the principle protein of forming enamel, we have identified two domains, one domain at the amino-terminus and the other domain at the carboxyl-terminus, that are required for the proper assembly of nanospheres. Nanospheres are believed to control the HAP crystal habit. We have used both yeast two hybrid analysis and plasmon resonance spectroscopy to examine the assembly properties of engineered amelogenin protein. Amelogenin protein was engineered using recombinant DNA techniques to: 1) contain deletions to either of the two self-assembly domains; or 2) contain single amino acid mutations in the amino-terminal self-assembly domain, based upon point mutations observed in humans affected with a hereditary disturbance of enamel formation; all of these alterations reveal significant defects in amelogenin self-assembly into nanospheres. The importance of bio-fabrication of enamel by the protein extracellulat matrix is evident from the disorganization of fields of crystallite which in the wild-type condition are woven together by ameloblasts to form a continuum. This weaving of crystallites imposes unqiue materials properties and provides resistence to fracture, allowing enamel to last a lifetime of use. Supported by grants from the NIH, The National Institute of Dental and Craniofacial Research DE 13404 (MLP) and DE 13045 (MLS).

11:30 AM W6.3
Adsorption of amelogenin nanospheres onto charged surfaces, a model for enamel matrix re-construction.
Janet Moradian-Oldak[1], Csilla Gergely[2], Nicolaos Bouropoulos[3] and Frederic Cuisinier[2]; [1]CCMB, University of Southern California, Los Angeles, California; [2]Odontologie, University of Louis Pasteur, Strasburg, France; [3]Material Science, University of Patras, Patras, Greece.

Amelogenins constitute more than 90% of the secretory stage enamel matrix proteins. The assembly of amelogenin protein into nanospheres has been postulated to be a key factor in the stability of enamel extracellular matrix framework, which provides the scaffolding for the initial enamel apatite crystals to nucleate and grow. We have utilized two different approches to investigate adsortption of amelogenin nanospheres onto surfaces : A) analysis of amelogenin mono or multi-layer formation by sequential adsorption process onto auto-assembled polyelctrolyte films. B) analysis of adsorption of amelogenin onto hydroxyapatite crystals by means of Langmuir Model for protein adsorption. The film building up and amelogenin adsorption experiments were followed by optical wave light spectroscopy (OWLS). The used model surfaces are polyelectrolyte films obtained by auto-assembling of cationic or anionic polymers. Stream potential measurements of a recombinant amelogenin rM179 adsorbed onto silica have indicated that the negatively charged nanospheres were reversebly adsorbed on the surface. A monolayer of amelogenin nanospheres was succesfully formed on a positively charged surface. In addition, by using cationic poly-l-lysine (PLL) we were able to create multilayer films of amelogenin and PLL through sequential adsorption. The formation of amelogenin nanospheres monolayer onto apatite crystals was confirmed by determining the adsorption isotherm of rM179 on synthetic hydroxyapatite crystals by means of Langmuir model for protein adsorption. The adsorption affinity of amelogenin onto apatite and the maximum adsorption sites were 19.7×10^5 l.mol^{-1} and 6.09×10^{-7} mol.m^{-2} respectively with an r^2 value of 0.99. These results indicate that amelogenin nanospheres adsorb onto the surface of apatite crystals as binding units with defined adsorption sites. The sequential adsorption process onto polyelectrolytes is an appropriate model for mimicking the assembly of amelogenin in enamel matrix.

11:45 AM W6.4
Calcium phosphate/collagen bone-like nanocomposite coating by electrochemical co-deposition.
Yuwei Fan, Ke Duan and Rizhi Wang; Department of Metal and Materials Engineering, The University of British Columbia, Vancouver, British Columbia, Canada.

A conception of making better biomaterials for tissue repair is to mimic the micro-environment similar to natural tissue. Bone-like composite of collagen and calcium phosphates (CaP) has been reported to have high potential for bone repair. Here we present a method to prepare calcium phosphate / collagen nanocomposite coating by electrochemical deposition in aqueous solution. The coating was prepared on pre-cleaned silicon wafer or polished titanium plate by controlling the electrolyte pH at 4.9 to 5.5. The electrolyte was prepared with the ion concentration of calcium and phosphate 20mM and 34mM respectively. Soluble collagen was added into the electrolyte. After deposition, the coating was washed by distilled

water and fixed immediately by Kanovsky fixative then gradient dehydrated and critical point dried. The macro- and microstructure of the coating and its demineralized collagen frames were observed. Fluorescent micrograph taken under the excitation of blue light, where the glutaraldehyde fixed collagen has strong green fluorescence emission, showed the collagen-CaP coating has high porosity 3D structure with pores varying from 1 um to 80 um. In the demineralized coating, collagen keeps a similar three-dimension framework. The collagen fibres are as small as 70 nm in dimeter. On partly demineralized sample, about 50 nanometer sized crystals were found located along the fibres. These observations indicate that the fibrillation of collagen and mineralization are formed almost spontaneously. XRD spectrum indicates the coating is a DCPD like mineral at low pH and OCP like mineral at higher pH above 5.3. Cathode electrode reaction causes the increase of pH value locally and the supersaturation of the calcium phosphate. Soluble type I collagen molecules are able to self-assemble into collagen fibrils intrigued by increased pH. At the meantime, the calcium phosphate supersaturated locally near to the cathode will easily nucleate on the self-assembled collagen framework to form nano-CaP/collagen composite coating. In summary, electrochemically prepared coating has the morphology of nano-sized collagen fibrils with CaP nano-crystals. This composite coating is similar to the natural bone in structure, composition and formation process. This study on the co-deposition of collagen and CaP points out a new technique of co-depositing other charged proteins or polysaccharides with CaP by a biomimetic way. This work is funded by NSERC, CIHR. R. Wang is incumbent of the Canada Research Chair and Y.W. Fan is grateful of the Killam postdoctoral fellowship. References: 1. Du C, Cui FZ, Zhang W, Feng QL, Zhu XD, de Groot K, J. Biomater. Mater. Res. 50 (4): 518-527, 2000 2. Roessler S, Born R, Scharnweber D, Worch H, Sewing A, Dard M, J. Mater. Sci.-Mater. in Med. 12 (10-12): 871-877, 2001 3. Shirkhanzadeh, M, J. Mater. Sci, Mater. in Med. 9:67-72, 1998

SESSION W7: Calcium Phosphates as Biomaterials - Bones and Teeth II
Chair: William J. Landis
Wednesday Afternoon, April 14, 2004
Room 3003 (Moscone West)

1:30 PM W7.1
An equilibrium thermodynamic model of the sequestration of calcium phosphate by casein phosphopeptides to form calcium phosphate nanoclusters. Carl Holt and Elaine Little; Hannah Research Institute, Ayr, United Kingdom.

Calcium phosphate nanoclusters are formed when a core of amorphous calcium phosphate is sequestered within a shell of casein or casein phosphopeptides. The nanoclusters can form spontaneously from a supersaturated solution or by dispersion of a precipitate of calcium phosphate, demonstrating that they are thermodynamically stable complexes. The average size and chemical composition of the complexes are largely independent of the solution conditions (pH, temperature, peptide concentration, salt composition and rate of reaction) under which they form. Larger, metastable, colloidal particles can form if there is not enough of the phosphopeptide to sequester all the calcium phosphate, or, transiently, if the salt and peptide solutions are mixed together without sufficient care. A thermodynamic model of the sequestration process is presented which makes use of an invariant ion activity product observed in nanocluster-containing solutions. In any given solution that has thermodynamic stability, the extent of the sequestration reaction can be calculated from the empirical formula of the nanoclusters using the criterion that the solution should have the equilibrium value of the invariant ion activity product. Caseins and other members of the paralogous group of secretory calcium binding phosphoproteins to which caseins belong may be able to sequester calcium phosphate in biological fluids such as milk, blood and saliva and in the extracellular matrix of mineralising tissues.

1:45 PM *W7.2
Modification of Brushite Crystal Growth by Citrate and Osteopontin. Ruikang Tang[1], Christine A Orme[2], John R Hoyer[3] and George H Nancollas[1]; [1]Chemistry, University at Buffalo, Buffalo, New York; [2]Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, California; [3]The Children's Hospital, Philadelphia, Pennsylvania.

An important problem in biomineralization is the control that the organic matrix, incorporating macromolecules or proteins, exerts over the formation of inorganic minerals. This may include the most specific kind of interactions related to the compatibility of crystal growth sites and the organic binding functional groups. It is generally agreed that the proteins most active in mediating biologically directed mineral growth are those that contain acidic amino acid residues, specifically carboxylic acid-rich regions, that interact with mineral surfaces such as the calcium phosphates to influence both the rates of formation and crystal morphology. Brushite (dicalcium phosphate dihydrate) is an important calcium phosphate phase that is frequently involved as a precursor to the formation of thermodynamically stable biological apatites. Using citric acid, a carboxylate-rich molecule, as a model system, its effect on brushite crystal growth has been studied using combined constant composition (CC) and in situ atomic force microscopy (AFM). At a relative supersaturation of 0.265, pH=5.70, ionic strength 0.15M and 37°C, the rate of brushite growth, 1.1×10^{-5} mol m^{-2} min^{-1} was reduced to 5.8×10^{-6} mol m^{-2} min^{-1} in the presence of 1.0×10^{-6} M citrate. The reaction was completely suppressed at 2.0×10^{-6} M. The specific inhibited step direction of this growth was the [001] and accordingly, the morphology of grown crystals in the presence of citrate was altered from plate- to rod-like. This can be explained by establishing an energetic and stereochemical basis for the anisotropic effects of citrate on brushite surfaces. A similar inhibition behavior was shown by osteopontin (OPN), a naturally occurring single-chain polypeptide protein, which is a major tissue calcification controlling agent in humans. Here, we show that citrates and OPN control crystal habit and brushite growth kinetics through anisotropic step pinning due to step-specific interactions, with both size and structure dictating their effectiveness. (The work is supported by NIH grant #DE03223)

SESSION W8: Mechanical Properties of Biomaterials
Chair: Rizhi Wang
Wednesday Afternoon, April 14, 2004
Room 3003 (Moscone West)

2:15 PM W8.1
Mechanical Behavior of Human Stratum Corneum. Kenneth S. Wu[1] and Reinhold H. Dauskardt[2]; [1]Mechanical Engineering, Stanford University, Stanford, California; [2]Materials Science and Engineering, Stanford University, Stanford, California.

The mechanical and fracture behavior of soft tissues is often crucial to their function, with the underlying cellular and extracellular structures being optimized for the required properties. Understanding these properties is important for a range of emerging tissue engineering and transdermal drug delivery technologies. The outermost layer of skin, or stratum corneum (SC), provides mechanical protection and a controlled permeable barrier to the external environment. Limited studies have demonstrated that temperature, hydration, and the application of topical agents influence the mechanical properties of SC although these have been limited primarily to in-plane as opposed to out-of-plane behavior. We present a mechanics approach to study the out-of-plane delamination resistance and mechanical behavior of human SC tissue in the direction normal to the skin. In addition, stress separation tests were performed to probe cohesive strength. The influences of hydration, temperature, and delipidization were explored. Decreases in debond energies from 5-7 J/m2 to 1 J/m2 were measured for hydrated specimens while dehydrated specimens exhibited more constant debond energies of 3 J/m2. Removing structural intercellular lipids with chemical treatment yielded significant increases in debond energies and less pronounced differences between hydrated and dehydrated specimens at each given testing temperature. The results reveal the highly anisotropic nature of SC mechanical properties including counterintuitive increases in SC debond resistance and strength with delipidization. Using novel micro-stress separation techniques, further tests were performed on specimens with dimensions approaching the length scale of the individual SC cells (30 mm). Probing the SC at this scale facilitates more accurate assessment of cellular bond strength by eliminating the constraints of adjacent cells that can alter mechanical properties.

2:30 PM W8.2
Mechanistic Aspects of Fracture of Human Cortical Bone. Jamie J. Kruzic[1,2], Ravi K. Nalla[1,2], John H. Kinney[3] and Robert O. Ritchie[1,2]; [1]Materials Science and Engineering, University of California, Berkeley, Berkeley, California; [2]Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California; [3]Lawrence Livermore National Laboratory, Livermore, California.

Over the past few decades, there has been much interest in the fracture properties of human bone. As understanding such properties in the context of the inherent hierarchical microstructure of bone is of obvious importance, this study addresses the evolution of the in vitro fracture toughness with crack extension (Resistance-curve behavior) in terms of the salient mechanisms involved. Fracture-mechanics based measurements were performed on compact-tension specimens hydrated in Hanks' Balanced Salt Solution using cortical bone from mid-diaphyses of 34-41 year-old human humeri. Post-test observations

of the crack path were made by optical microscopy and three-dimensional x-ray computed tomography. The fracture toughness was found to rise linearly with crack extension with a mean crack-initiation toughness of K_o 2.0 MPa$sqrt$m for crack growth in the proximal-distal direction. The increasing cracking resistance had its origins in several toughening mechanisms, most notably crack bridging by uncracked ligaments. Uncracked-ligament bridging, which was observed by tomography in the wake of the crack, was identified as the dominant toughening mechanism responsible for the observed R-curve behavior through compliance-based experiments. The extent and nature of the bridging zone was examined quantitatively using multi-cutting compliance experiments in order to assess the bridging stress distribution. The results obtained in this study provide an improved understanding of the mechanisms associated with the failure of cortical bone, and as such are of importance from the perspective of developing a realistic framework for fracture risk assessment, and for determining how the increasing propensity for fracture with age can be prevented.

3:15 PM *W8.3
Spider silk or hagfish silk, that is the question: Alternate routes to the production of high performance protein fibers.
John M. Gosline, Doug Fudge, Paul Guerette and Nimrod Levy; Dept. of Zoology, University of British Columbia, Vancouver, British Columbia, Canada.

Spider dragline silks are renowned for their high strength (ca. 1 GPa) and exceptional toughness (energy to break 150-200 MJm^{-3}), and as a consequence there is considerable interest in the production of genetically engineered materials based on the protein sequence designs of these silks. The properties of dragline silks arise primarily from the β-sheet crystals that crosslink and reinforce the protein networks that exist in these materials. In addition, their 25 - 30% extensibility, which accounts for the exceptional toughness of these silks, arises from protein sequence blocks that encode short lengths of amorphous protein chains between the crystals. These amorphous chain segments are also responsible for the supercontraction of dragline silks when they get wet. That is, wet dragline silks swell with water, shrink to about half their initial length, and become rubber-like, because the amorphous chains in the network are plasticized by water. This hydration sensitivity may limit the use of spider dragline proteins in the manufacture of biopolymer-based materials. Our study of Hagfish slime threads suggests an alternate strategy for the production of protein-based materials. These threads are made entirely from intermediate filaments (IFs), which are 10 nm diameter, self-assembling filaments that are constructed from α-helical, coiled-coil protein dimers. When hagfish threads are tested in tension they show an initial, low-modulus (6 MPa) elastic zone, but above about 35% extension deformation becomes plastic because of the irreversible, α-helix —*drarrow*— β-sheet transformation that occurs. These fibers fail at about 200% extension, but extension beyond about 100% results in a dramatic strain-hardening that arises from the aggregation of the β-sheets into β-sheet crystals that crosslink the IF-proteins to form a robust polymer network. The tensile strength of the hydrated thread is about 200 MPa, but when wet threads are drawn to about 150% extension and dried, their properties become virtually indistinguishable from those of dragline silks. That is, strength and toughness are essentially the same as those given above for dragline silk because the protein networks in dragline silk and in stretched and dried hagfish threads are very similar. Variation in the draw-processing regime allows for the production of a range of material properties. In addition, the silk-like fibres formed from hagfish threads are quite resistant to the effects of water, and because their composition provides many opportunities for chemical modification, we have been able to completely eliminate water-induced contractions. Thus, genetically engineered materials based on hagfish thread IF proteins may offer a more effective process for the production of high-performance protein-based materials.

3:45 PM W8.4
Do Natural Silks Make Good Engineering Materials? Natalie A. Morrison[2], Fraser I. Bell[2], Alexandre Beautrait[2], Joanne Ritchie[2], Christopher Smith[2], Iain J. McEwen[2] and Christopher Viney[1,2]; [1]School of Engineering, University of California Merced, Merced, California; [2]Chemistry, School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh, Midlothian, United Kingdom.

Much of the widespread interest in natural silk as a blueprint for new engineering materials has its origin in the high strength, stiffness and toughness exhibited by silk fibres in constant strain rate tests. These tests typically are completed within minutes; they do not duplicate realistic in-service load histories, and they do not adequately probe the long-term behaviour of the sampled material. Mechanical testing regimes that are specifically designed to explore creep of, or stress relaxation in, silk reveal a rather less promising outlook than constant strain rate tests: both effects are significant, and will restrict the possible applications of silk. We cannot envisage natural silk serving as a long-term load-bearing material without modification. Natural silk will not rival steel as a means of suspending the deck of the Golden Gate bridge! This realisation should not be surprising, since nature has designed silk to be used for only a few hours or days (spider webs) or at most a few months (insect cocoons, which are not required to carry constant, large loads throughout their useful life). Silk also suffers from significant moisture sensitivity. The load-bearing properties of silk are decreased, and creep and stress relaxation are greatly increased, by exposure to a moist environment. This, too, is not surprising, since the original functionalities that confer water solubility to the silk protein stored in the silk-producing glands are not discarded when the protein solution is spun into fibre. The chains are re-folded into a form from which they cannot as a whole re-solubilise in pure water, but this does not prevent marked local changes in chain conformation and packing from occurring within hydrophilic domains. Given the above mechanical and environmental limitations, what realistic applications exist for silk-inspired materials? Webs are designed to remain at least partially intact while dissipating a high-energy impact, and cocoons are really composites designed to optimise toughness. Indeed, even "single" silk threads have a composite microstructure, and we regard this feature as a useful target of attempts to make biomimetic silk. A propensity to creep, with additional platicisation imparted by moisture, can in fact be a bonus in a material required to contain an explosion. And, if the objective is to modify silk so that it exhibits reduced creep and increased stiffness and toughness – albeit at the expense of reduced ductility – then we have demonstrated that this can be achieved by exposing silk to microwave radiation.

4:00 PM W8.5
Surface Roughness and Maximum Load Alter the Mechanical Properties of Lamellar Bone Measured by Nanoindentation.
Eve Donnelly[1], Shefford P. Baker[2], Adele L. Boskey[3,4,5] and Marjolein C. H. van der Meulen[1,6]; [1]Mechanical Engineering, Cornell University, Ithaca, New York; [2]Materials Science and Engineering, Cornell University, Ithaca, New York; [3]Mineralized Tissues Research Laboratory, Hospital for Special Surgery, New York, New York; [4]Biochemistry, Weill Medical College of Cornell University, New York, New York; [5]Graduate Program in Physiology, Biophysics, and Systems Biology, Weill Medical College of Cornell University, New York, New York; [6]Laboratory for Biomedical Mechanics and Biomaterials, Hospital for Special Surgery, New York, New York.

Skeletal function depends critically on bone structural integrity. Diseases such as osteoporosis diminish the load bearing capabilities of bone, leading to fracture and related clinical complications, including death. In osteoporotic patients, the first bone changes that are noted occur in cancellous bone. At the microstructural level, cancellous bone is composed of lamellae consisting of highly oriented mineralized collagen fibers interspaced by less-structured interlamellar regions. However, relatively little is known about the material properties of these structures. While nanoindentation is now commonly used to probe the mechanical properties of mineralized tissues, most such studies have used indentations that are deep relative to lamellar dimensions and have not reported sample surface roughnesses. In this study, nanoindentation was used to assess the mechanical properties of lamellar and interlamellar tissue in rabbit cancellous bone. The effects of surface roughness and maximum nanoindentation load on the measured mechanical properties of lamellar bone were examined. The mechanical properties of lamellar and interlamellar bone in two samples of differing surface roughness were determined at multiple maximum nanoindentation loads. At low loads, the indentation modulus of the lamellar bone was approximately 20% greater than that of the interlamellar bone, while at high loads the measured properties of both layers converged to a composite value. As indentation depth increased relative to surface roughness, the variability of the interlamellar properties decreased substantially, while that of the lamellar properties remained constant. Relatively shallow indentations made on smooth surfaces revealed significant differences in the properties of lamellar and interlamellar bone that support microstructural observations of lamellar bone as more mineralized than interlamellar bone.

4:15 PM W8.6
Nanomechanical Characterization of Polyhema Co-Polymers for Bone Tissue Engineering Applications.
Catherine Klapperich[1] and Jie Song[2]; [1]Boston University, Boston, Massachusetts; [2]Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, California.

We have generated a library of polyHEMA-based hydrogel polymers conjugated with anionic amino acid and peptide ligands that induce the formation of calcium phosphates in vitro under various mineralization conditions, resulting in a polymer/ceramic composite material. The microstructure and crystallinity of the nucleated mineral has been analyzed using SEM and energy dispersive x-ray analysis and vary as a function of mineralization conditions. These

materials were designed as bone mimics, and it was necessary to determine whether the mechanical properties of the composite material were comparable to those of bone tissue. We also wanted to test the crystalline mineral phase separately as a secondary method of determining the relative amounts of amorphous and crystalline mineral phase. We performed mechanical tests using a Hysitron Triboindenter nanoindentation apparatus. First we measured the surface modulus and hardness values of the composite materials. We tested both the hydrated and dry materials. Then, we measured the time dependent mechanical properties of the materials using the Hysitron feedback control module. In stress relaxation experiments, the strain on the material is held constant, while the reduction in stress is measured as function of time as the material relaxes and micro and nanostructural rearrangements dissipate energy. In creep experiments, a constant load was maintained on the sample, while the material was allowed to continue displacing underneath the load. Data from these experiments yielded characteristic time constants that will be used to construct viscoelastic material models of the composite mineralized gel system.

4:30 PM W8.7
Nanowiring Enzymes to Carbon Nanotube Probes.
Charles Patrick Collier, Maria Jose Esplandiu, Ian Ross Shapiro and Vern Garrett Bittner; Chemistry, California Institute of Technology, Pasadena, California.

The observation of spectroscopic signals in response to mechanically induced changes in biological macromolecules can be enabled at an unprecedented level of resolution by coupling single-molecule manipulation/sensing using carbon nanotubes with single-molecule fluorescence imaging. Proteins, DNA and other biomolecules can be attached to nanotubes to give highly specific single-molecule probes for the investigation of intermolecular dynamics, the assembly of hybrid biological and nanoscale materials and the development of molecular electronics. Recent advances in nanotube fabrication and Atomic Force Microscope (AFM) imaging with nanotube tips have demonstrated the potential of these tools to achieve high-resolution images of single molecules. In addition, proof-of-principle demonstrations of nanotube functionalization and attachment of single molecules to these probes have been successfully made. Improved techniques for the growth and attachment of single wall carbon nanotubes as robust and well-characterized tools for AFM imaging are being developed. This work serves as a foundation toward development of single-molecule sensors and manipulators on nanotube AFM tips for a hybrid atomic force microscope that also has single-molecule fluorescence imaging capability. An individual single wall carbon nanotube attached to an AFM tip can function as a structural scaffold for nanoscale device fabrication on a scanning probe. Such a probe can have a novel role, to trigger specific biochemical reactions or conformational changes in a biological system with nanometer precision. The consequences of these perturbations can be read out in real time by single-molecule fluorescence and/or AFM sensing. For example, electrical wiring of single redox enzymes to carbon nanotube scanning probes will allow for observation and electrochemical control of single enzymatic reactions, by monitoring fluorescence from a redox-active cofactor or the formation of fluorescent products. Enzymes nanowired to carbon nanotube tips may enable extremely sensitive probing of biological stimulus-response with high spatial resolution, including product-induced signal transduction.

4:45 PM W8.8
Regular, low density cellular structures- rapid prototyping, numerical simulation, mechanical testing.
Jurgen Stampfl[1], Arthit Pisaipan[1], Martin M. Seyr[3], Mathias H. Luxner[3], Heinz E. Pettermann[3], Alexander Woess[2] and Peter Fratzl[2]; [1]Inst. of Materials Science and Testing, Vienna University of Technology, Vienna, Austria; [2]Max Planck Institute for Colloids and Interfaces, Potsdam, Germany; [3]Inst. of Lightweight Materials and Structures, Vienna University of Technology, Vienna, Austria.

Cellular solids form the basis of many biological and engineering structures. Most models use the apparent density and the mechanical properties of the bulk material as the main parameter for the prediction of the mechanical properties of such structures. In this work the influence of the architecture of periodic cellular solids with identical apparent density is investigated numerically and experimentally. Using computer aided design, structures with 8x8x8 base cells are designed and fabricated. The physical prototypes which are tested experimentally are made from thermosetting polymers by employing Rapid Prototyping (RP) techniques. Various RP techniques are compared regarding their suitability for the fabrication of cellular materials. The used technique must be able to fabricate complex structures with many overhangs without the use of support material. Furthermore, the utilized technique must be able to shape these structures with sufficient accuracy in order to minimze the variation of the geometrical and mechanical properties within one batch of structures with identical architecture. After analyzing the elastic and visco-elastic porperties of the bulk material, the fabricated structures are loaded under uniaxial compression and evaluated regarding the stiffness, maximum strength and energy absorption ability. By building rotated structures, the directional sensitivity of these parameters is investigated. For numerical simulation of the cellular structures linear Finite Element analysis is employed. Three-dimensional models are set up using higher order beam elements. In a first step, the structure is treated as an infinite medium and homogenization via a periodic 'micro-field approach' is used. The entire elastic tensor for different relative densities is evaluated, from which the direction dependency of the Young's moduli is derived. In a second step, simulations of finite structures are performed for direct comparison with experiments. Samples consisting of several basic cells are modeled considering free edges which leads to a better correspondence to the experimental setup. Finite structures of different numbers of cells are modeled to study the influence of the sample size. The experimental and numerical results correspond very well and form a consistent picture of the problem. The multi-disciplinary approach leads to a comprehensive view of effects which govern the mechanical behaviour of the investigated cellular structures.

SESSION W9/O5: Joint Session: Tissue Engineering I
Chair: Darrin Pochan
Thursday Morning, April 15, 2004
Room 3005 (Moscone West)

8:30 AM *W9.1/O5.1
Microdefining Cellular Habitats for Cardiovascular Tissue Engineering.
Tejal Ashwin Desai, Biomedical Engineering, Boston University, Boston, Massachusetts.

Cells in viable tissues respond to mechanical stimuli under both physiological and pathophysiological conditions by changing their metabolism, mass, internal structure, and resorption or production of proteins and extracellular structures, thereby altering their interactions with adjacent cells. In order to begin to understand these complex interactions, cells must be exposed to an appropriate in vivo-like environment. Thus, an important challenge in tissue engineering is to control the 3-D organization of cells in their microenvironments. A key determination in the engineering of these tissues is how, and to what extent, this environment can be controlled in vitro to recreate in vivo-like architecture. Currently, the most common approach to developing a tissue-engineered construct for the restoration, repair, replacement, or regeneration of functional tissues is to allow cells to randomly distribute in an extracellular matrix or polymer scaffold to create a 3-D cell culture environment. However, controlling the cellular microenvironment is essential for the creation of functional tissue engineered constructs. Nonetheless, little work has been carried out in controlling the spatial arrangement of multiple cell populations in 3-D culture. We have utilized micro and nanopatterning and microfluidic delivery techniques to create more physiologic-like tissue engineered constructs. Microtextured substrates have been used to create more physiologic cardiac cell cultures. Microfluidic channels with microtopography, created in polydimethylsiloxane (PMDS) elastomers, will be micropatterned with vascular cell populations (Human Umbilical Vascular Endothelial Cells (HUVEC) and Smooth Muscle Cells (SMC)). The extent to which microarchitecture can influence cellular behavior will be described. Such information will have important implications for implantable tissue engineering constructs and the reduction of immunogenicity in cell -seeded synthetic grafts. This presentation will describe novel tissue engineering approaches for microdefining cell populations, furthering our knowledge of the effects of spatial organization and mechanical stimulation on cell behavior and tissue formation. The proposed technologies and techniques may also offer a more flexible method for the design of tissue engineering constructs. By culturing cells under conditions that are closer to those found in vivo, the relationship between cell function and microenvironment can be more easily studied. In vitro methods for growing cells in tissue-like environments not only has direct application in organ regeneration, but may also be applied to cell-based biosensors, biochips, and high throughput cell-based pharmaceutical screening.

9:00 AM W9.2/O5.2
Fabrication and Evaluation of Uniformly Sized Nanoporous Alumina for Human Osteoblast Cell Culture.
Erin Leary Swan, Ketul Popat and Tejal A Desai; Biomedical Engineering, Boston University, Boston, Massachusetts.

Bone tissue engineering requires the ability to regulate cell behavior through precise control over substrate topography and surface chemistry. Aluminum oxide, or alumina, has been extensively employed as a substrate for bone cell seeding in dental and orthopedic implant applications. However, the current techniques do not allow

precise surface topography and orientation of the porous material. A new method of producing alumina has been developed to improve osteoblast adhesion and proliferation. A two-step anodization process has been optimized for fabrication of hexagonally arrayed nanoporous alumina membranes. The method allows for the creation of uniformly sized pores in the range of 30 to 80 nm diameter determined by anodization voltage. The membranes display uniform pore density and pore size, which is suggested by scanning electron microscopy (SEM). From this process, a pure, uniform alumina membrane with through holes and specific control of nanostructure was produced. In order to test the compatibility of this porous alumina with osteoblasts, adhesion, proliferation, morphology, and matrix production were tracked for various pore sizes and compared to amorphous aluminum oxide. Growth and adhesion results were evaluated by cell counting and microscopic imaging, while matrix production was quantified by enzymatic assays. Also, alumina surfaces were modified by cell adhesion peptides, and osteoblast growth was compared to the unmodified membranes. Nanoporous alumina can be produced with highly defined pores of constant size and density and provides a stable platform for osteoblast culture that is easily tailored to optimize growth and function. The alumina membranes show promise for employment as a substrate for dental or orthopaedic implants.

9:15 AM W9.3/O5.3

Use of Soft Lithography for Multi-layer MicroMolding (MMM) of 3-D PCL Scaffolds for Tissue Engineering.

Yang Sun, Nicholas Ferrell and Derek Hansford; Biomedical Engineering Center, The Ohio State University, Columbus, Ohio.

It is desirable that 3-D scaffolds for tissue engineering have precisely controlled geometries due to their improvement of cellular adhesion and functionality. Surface features smaller (1-10μm) than typical cell dimensions have been shown to have significant effects on cell behavior and cell-surface interactions. In this paper, a soft lithography technique was used to fabricate polydimethylsiloxane (PDMS) stamps of repetitive groove and grid patterns with feature sizes of 5μm width, 5μm depth, and 45μm wide spaces. Several methods were compared for the fabrication of 3-D multi-layer polycaprolactone (PCL) scaffolds with the precise patterns. First, spin coating and oxygen plasma were combined to build 3-D scaffolds with PDMS stamps of the groove pattern. The resultant scaffolds had good alignment and connection between layers; however the upper layer collapsed due to the poor mechanical rigidity. Second, the micromolding in capillaries (MIMIC) technique was used to deliver the polymer into the small grooves by capillarity; however the resultant lines were discontinuous and not able to form layers. Finally, a new multi-layer micromolding (MMM) method was developed and successfully applied in a grid pattern to fabricate 3-D scaffolds. Proper heating and stamping parameters were identified that allowed the successful demonstration of the process on the thermoplastic PCL polymer. Scanning electron microscopy (SEM) characterization showed that the multi-layered scaffolds had high porosity and precisely controlled 3-D structures. Initial cell seeding experiments showed that the micropatterned scaffolds enhanced cellular attachment and proliferation, and encouraged cellular growth into the scaffold structure.

9:30 AM W9.4/O5.4

Design and Fabrication of a Constant Shear Microfluidic Network for Tissue Engineering.

Jeffrey T Borenstein[1], Mohammad Kaazempur-Mofrad[2], Brian K. Orrick[1], Eli J. Weinberg[1,2] and Joseph P. Vacanti[3,4]; [1]Biomedical Engineering Center, Draper Laboratory, Cambridge, Massachusetts; [2]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [3]Department of Pediatric Surgery, Massachusetts General Hospital, Boston, Massachusetts; [4]Department of Surgery, Harvard Medical School, Boston, Massachusetts.

Recent progress in microfabrication of biodegradable materials has resulted in the development of a three-dimensional construct suitable for use as a scaffold for engineering blood vessel networks. These networks are designed to replicate the critical fluid dynamic properties of physiological systems such as the microcirculation within a vital organ. Ultimately, these 3D microvascular constructs will serve as a framework for population with organ-specific cells for applications in organ assist and organ replacement. This approach for tissue engineering utilizes highly engineered designs and microfabrication technology to assemble cells in three-dimensional constructs that have physiological values for properties such as mechanical strength, oxygen, nutrient and waste transport, and fluidic parameters such as flow volume and pressure. Three-dimensional networks with appropriate values for blood flow velocity, pressure drop and hematocrit distribution have been designed and fabricated using replica molding techniques, and populated with endothelial cells for long-term microfluidic cell culture. One critical aspect of the fluid dynamics of these systems is the shear stress exerted by blood flow on the endothelial cells lining the walls of the vessel; a key parameter that is known to initiate a cascade of mechanotransduction phenomena whereby mechanical shear forces yield biological responses that govern the cellular function. In this work, we report the design and construction of three-dimensional microfluidic constructs for tissue engineering that have uniform wall shear stress throughout the network. This type of control over the shear stress offers several advantages over earlier approaches, including more uniform seeding, more rapid achievement of confluent coatings, and better control over endothelial cell behavior for in vitro and in vivo studies.

10:15 AM *W9.5/O5.5

NanoLiterBioReactor: Monitoring Long-Term Mammalian Cell Physiology at Nanofabricated Scale.

Ales Prokop[1,2], Zdenka Prokop[1], David Keith Schaffer[3], Eugene Kozlov[2], John P Wikswo[4], David E Cliffel[5] and Franz J Baudenbacher[6]; [1]NanoDelivery, Inc., Nashville, Tennessee; [2]Chemical engineering, Vanderbilt University, Nashville, Tennessee; [3]Mechanical Engineering, Vanderbilt University, Nashville, Tennessee; [4]Physics and Biomedical Engineering, Vanderbilt University, Nashville, Tennessee; [5]Chemistry, Vanderbilt University, Nashville, Tennessee; [6]Biomedical Engineering, Vanderbilt University, Nashville, Tennessee.

Microminiaturized cell-culture environments, i.e., NanoLiterBioReactors (NBRs) for growing and maintaining populations of few cultured mammalian cells in volumes three orders of magnitude smaller than in standard environments would lead to major advances in a number of areas. The small NBR volume would reduce the time required for diffusive mixing and thermal equilibrium; allow accurate cell counting; minimize volumes of expensive compounds used for testing; and provide many culture chambers on a single instrumented chip. Such devices would enable development of a new class of miniature, automated cell-based arrays for massively parallel monitoring of the environment of multiple cell lines. Closed-loop adjustments of the environment, e.g., pH and ionic concentrations, could be added to maintain homeostasis. For a nonspecific monitoring of metabolic activity of cells, the biosensor elements might include planar pH, dissolved oxygen, glucose, lactate and redox potential sensors, or even an isothermal picocalorimeter to measure heat response. Given such sensors, one could perform short and long-term cultivations of several mammalian cell lines in a perfused system and monitor their response to test substances/toxins, enabling automated, parallel, and multiphasic monitoring of multiple cell lines for drug and toxicology screening. An added bonus is the possibility of studying cell populations with low cell counts, detached from typical tissue densities, or in controlled physical and chemical gradients. We have fabricated prototype NBRs using glass culture substrates, and connected PDMS microfluidic channels that can be molded using soft-lithography. Input/outlet ports enabled cell seeding and the supply/withdrawal of culture medium into/from 10-120 nL chambers via injectors. The NBRs were sterilized by UV exposure. This system allowed in situ optical/fluorescence microscopy to monitor culture progress. CO2/air supplywas provided by the high oxygen permeability of the PDMS material. Tests were conducted using fibroblast, CHO and Hep2G hepatocyte cell lines. Biocompatibility was determined for different substrates and coatings of extracellular matrix components. A fluorescent PicoGreen DNA assay was used to evaluate the viability and proliferation over 1-5 day period as compared to a plain glass substrate. Glass was found suitable for cell culturing within the NBR environment. For all three lines, viabilities >90% were achieved. The effect of cell seeding density on cell viability and survival was studied in plating experiments using standard well-plate dishes coated with different substrates. A minimum density was noted for some cell lines to achieve a commencement of cell growth. An instrumented NanoBioRector represents a dramatic departure from the standard mammalian culture environment and opens a new paradigm of cell biology, so far largely neglected in literature.

10:45 AM W9.6/O5.6

Increased Function of Bladder Smooth Muscle Cells on Nano-Structured, Three-Dimensional Polymer Constructs.

Megan A Pattison, Thomas J Webster and Karen M Haberstroh; Biomedical Engineering, Purdue University, West Lafayette, Indiana.

Many treatments for bladder diseases or disorders, such as bladder cancer and bladder outlet obstruction, require resection of the bladder wall. When this is necessary, biomaterials are needed as bladder wall replacement materials. For these reasons, the objective of the present in vitro research was to construct a three-dimensional synthetic polymer scaffold that has nano-dimensional surface features similar to what cells experience in the bladder. Three-dimensional polyurethane (PU) and poly(lactic-co-glycolic acid) (PLGA) scaffolds were constructed using solvent casting and salt leaching processes. These scaffolds were then manipulated to possess nano-dimensional surface features by soaking in nitric acid and sodium hydroxide respectively at select concentrations for various periods of time. Human bladder smooth muscle cells were seeded into the scaffolds at a density of

25,000 cells per scaffold to perform cytocompatibility studies. Adhesion and proliferation experiments were performed for 4 hours, and 1, 3, and 5 days respectively. In all cases, control cells were placed in an incubator and subjected to normal atmospheric pressure, while experimental cells were placed in a pressure chamber and subjected to a sustained pressure of 10 cm H2O. This pressure was chosen because of its physiological significance, as the bladder experiences between 0 and 10 cm H2O pressure during most of its normal cycle. Additionally, intracellular and extracellular amounts of collagen an elastin were quantified as a measure cellular attraction to the surface. Results of this study provide evidence that porous, nano-dimensional polymer scaffolds can be constructed using these methods. Additionally, cell counts, quantity of elastin (both intracellular and extracellular), and amount of collagen (both intracellular and extracellular) were increased on substrates having smaller surface features for both types of scaffolds. Exposure to pressure did not alter cellular adhesion or proliferation on materials, and cells experiencing sustained pressure contained the same amount of extracellular elastin, intracellular collagen, and extracellular collagen as control cells. Cells experiencing sustained pressure, however, contained less intracellular elastin than control cells. These results indicate that the 3D, nano-dimensional synthetic scaffolds created and studied in this research may be suitable bladder wall replacement materials.

11:00 AM *W9.7/O5.7
Challenges Involving Biologically-Inspired Hydrogel ECMs for Tissue Engineering. Kevin E. Healy, Materials Science & Engineering, Bioengineering, Univ. California at Berkeley, Berkeley, California.

A critical problem limiting the field of tissue engineering is the lack of engineering design rules to guide the synthesis and fabrication of artificial extracellular matrices (ECMs) or scaffolds. To address this issue, we have created artificial ECMs that are environmentally responsive and tunable with respect to mechanical properties (e.g. G*), biological ligands, tissue adhesion, and protease degradation. Our current approach is to create modular hydrogel ECMs where different properties of the matrix can be manipulated independently, thus creating a system where parametric analysis of the effect of hydrogel properties on cell proliferation and differentiation is possible. For example, we have synthesized and characterized the physical properties of semi-interpenetrating polymer networks (sIPNs) consisting of linear polyacrylic acid (pAAc) chains within a thermo-responsive N-isopropylacrylamide-co-acrylic acid network [p(NIPAAm-co-AAc)]. To impart biomimetic character into the hydrogels, the AAc groups on the linear chains have been functionalized with peptides containing the RGD and other sequences. The system allows for easy synthesis of admixtures of peptide sequences while maintaining the mechanical properties of the matrix. Therefore, studies addressing the effect of ligand type and density, in the context of matrices with various mechanical properties, can be easily performed. These peptide-modified p(NIPAAm-co-AAc) hydrogels with protease degradable crosslinks serve as useful tools for studying cell-material interactions within three dimensional structures and have the potential to be used as injectable scaffolds for tissue engineering applications. In addition, the synthetic strategy we have employed allows for easy control of mechanical and chemical properties of the matrix allowing parametric analysis of the effect of these properties on tissue development both in vitro and in vivo.

11:30 AM W9.8/O5.8
A Novel System for Self-Assembly of Muscle-MEMS Devices. Jianzhong (Jeff) Xi, Jacob Schmidt and Carlo Montemagno; Bioengineering, UCLA, Los Angeles, California.

As microcomponents in engineered systems, biological muscles have unique advantages such as large force transduction, utilization of biochemical fuel, and self-assembly from single cells, over other inorganic actuators for biomedical engineering applications. Successful integration of muscles with inorganic fabricated structures and electronics promises the capability of precisely characterizing muscles' mechanical properties and fabricating self-assembled controllable autonomous structures powered by ubiquitous glucose. However, the use of extracted muscle tissue from animals on these devices is impractical and inefficient, as the tissues must be dissected and incorporated into each device by hand with crude interfaces between the biological tissue and inorganic materials. Integration of muscle with fabricated structures would be optimally achieved through self-assembling muscle cells on MEMS. The construction of self-assembled muscle-powered MEMS structures is complicated by the stringent requirement to spatially direct the cell growth, control the tight connection of these differentiated structures with MEMS structures, and enable the cells and the integrated hybrid to be free to move. Conventional and soft photolithography techniques have been extensively employed to pattern the growth of a variety of cell types and investigate their interaction with substrate in the micrometer level. However, these techniques are only suitable for patterning static cells on a surface, so a novel system of spatially patterning the contractible cells must be developed to enable the cells and the integrated hybrid devices to be free to move. Here we present a novel system of self-assembling myocytes on MEMS devices. This system has shown its capability of spatially and selectively directed growth and differentiation of myocytes into single muscle bundles in situ, attachment of these functional bundles to MEMS structures, and the controlled partial release of the resultant hybrid devices. A novel force transducer capable of in situ characterization of the mechanical properties of muscle at both tissue and single-cell levels has been fabricated using this system. The mechanical properties of the neonatal ventricular myocytes 1-3-day-old Sprague-Dawley rats (NRVMs), such as substrate-induced stress (2-2.5 kPa) and Young's modulus (40 kPa), have been measured using this force transducer. This force transducer has also allowed us to perform dynamic studies of myocytes. Mechanical and dynamic characterization of healthy muscle cells will contribute to better understanding of cardio tissue physiology and further engineering of functional cardiac tissue constructs. Our force transducer has shown the ability to achieve this goal. Furthermore, using this system, we have also created the first self-assembled muscle-powered microrobots. The studies of the characteristics of these microrobots will be also reported.

11:45 AM W9.9/O5.9
Biomimetic Processing of a Biodegradable, Segmented-Polyurethane for Use in Tissue Engineering Devices. Danielle N Rockwood[1], Jean S Stephens[1], John F Rabolt[1], Kimberly Woodhouse[2] and Joanna Fromstein[2]; [1]Materials Science and Engineering, University of Delaware, Newark, Delaware; [2]Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada.

A segmented-polyurethane has been synthesized using an amino acid-derived diisocyanate and a phenyalanine-based chain extender. Contrary to many polyurethanes used for tissue engineering applications, this polymer is biodegradable and should prove to be biocompatible. In addition, the segmented nature of the polyurethane allows for elastomeric behavior thus providing the mechanical properties required to respond to physiological stresses. Combined with these advantageous physical properties, the chemical architecture of this polymer unites the necessary functional components (e.g., hydrolyzable groups to promote in vivo degradation) to satisfy the many of the desirable requirements for a tissue engineering construct. The goal of many tissue engineering devices is to closely mimic natural systems. In the case of tissue constructs, the extracellular matrix (ECM) contains protein fibers that range in diameter from a few microns to nanometer scale. In order to mimic the ECM architecture, electrospinning has been used to create membranes of nanometer scale polyurethane fibers. The nature of the electrospinning process is such that a range of fiber diameters and surface morphologies can be produced depending on the choice of processing protocols1. In addition, Raman spectroscopy has been used to ensure the conformational integrity of the polymer before and after processing. Our overall goal is to seed cardiomyocyte cells on these electrospun membranes with the hope that these cells will eventually synthesize their own proteins. Over time, the polyurethane construct will be bioresorbed and the cells will create their own ECM. 1 S. Megelski, J. Stephens, D. B. Chase and J. F. Rabolt, Macromolecules 35, 8456 (2002)

SESSION W10: Biological and Biomimetic Inorganic Materials
Chair: Christine Orme
Thursday Afternoon, April 15, 2004
Room 3003 (Moscone West)

1:30 PM *W10.1
Biotechnological Route to Structure-Directed Nanofabrication of Siloxanes, Organometallics and Semiconductors. Daniel E. Morse, Molecular Biology, University of California @ Santa Barbara, Santa Barbara, CA, California.

With a precision of nanostructural control that exceeds present human capabilities, biological systems fabricate 3-d multifunctional high-performance silicon-based materials at low temperatures and near-neutral pH. The fundamental molecular biology of silica production in sponges and diatoms is now being elucidated, and aspects of these processes are being harnessed for industrial and technological processes. Working with the silica needles produced by marine sponges, our laboratory discovered that proteins we named "silicateins" catalyze and structurally direct the polymerization of silica from silicon alkoxides at neutral pH and low temperature. The silicateins are true enzymes, closely related to a well-known family of hydrolases. Site-directed mutagenesis of the cloned recombinant DNAs coding for the silicateins confirmed the mechanism of catalysis, and

has been used to increase the rate of catalysis as well. These studies enabled the synthesis of self-assembling "biomimetics" that incorporate the functionalities identified as essential for catalysis, yielding new structure-directing catalysts of polymerization. The silicateins and their biomimetic counterparts catalyze structure-directing synthesis from a wide range of precursors, yielding inorganic silica, organically substituted silsesquioxanes (silicones) and organometallic silsesquioxanes. We recently discovered that the silicateins also catalyze and structurally direct the hydrolysis and polycondensation of molecular precursors of metallo-oxanes such as titanium dioxide, gallium oxide and zinc oxide. These are the first reported examples of enzyme-catalyzed, nanostructure-directed synthesis of semiconductors. Interaction with the template-like protein surface stabilizes polymorphs of these materials (e.g., anatase titanium dioxide) otherwise not formed at low temperatures. In some cases interaction between the condensing metallo-oxane and the protein results in preferential alignment of the resulting nanocrystallites, suggesting a pseudo-epitaxial relationship between the mineral crystallite and specific functional groups on the templating protein surface. We now are using genetic engineering to identify and harness these structure-directing determinants. Potential uses of this low-temperature, neutral pH route for nanostructure-directed synthesis are under investigation for optical and electronic applications, sensors, energy transducers, cosmetics and pharmaceuticals.

2:00 PM W10.2
The Biochemistry of Silica Nanofabrication in Diatoms.
Nils Kroeger, Nicole Poulsen, Manfred Sumper and Rainer Deutzmann; Biochemie I, University of Regensburg, Regensburg, Germany.

The biological formation of inorganic materials with complex form (biominerals) is a widespread phenomenon in nature, yet the molecular mechanisms underlying biomineral morphogenesis are not well understood. Among the most fascinating examples of biomineral structures are the intricately shaped, silicified cell walls of diatoms, which exhibit species specific nanopatterns (see figure). Morphogenesis of diatom biosilica is an extremely rapid process that is accomplished under mild physiological conditions, thus exceeding the capabilities of present-day materials engineering. Elucidating the molecular mechanisms of silica nanofabrication in diatoms is therefore expected to open new synthetic routes to nanostructured silica materials. In search of the biological molecules that control biosilica morphogenesis, a thorough biochemical analysis of the diatom biosilica-associated organic components was undertaken. This led to the discovery of novel organic molecules termed silaffins and long-chain polyamines (LCPA), which are able to drastically influence silica formation. Silaffins are complex proteins/peptides that are heavily posttranslationally modified. These modifications include phosphoamino acids and lysine residues carrying oligo-N-methyl-propyleneimine chains, which represents a novel type of amino acid modification. LCPA contain even longer chains of N-methyl-propyleneimine units, but these are linked to putrescine residues rather than a polypeptide backbone. Native silaffin-1A (natSil-1A) and LCPA, respectively, not only highly accelerate silica formation from a silicic acid solution in vitro but also control the structure of the forming silica, generating silica nanospheres. Spherical particles of 10-100 nm in diameter have indeed been shown to constitute diatom biosilica, yet natSil-1A and LCPA are unable to create the porous nanopatterns that are typical for diatom biosilica, suggesting that additional components are required. Recently, a novel silaffin molecule termed natSil-2 was discovered that lacks an intrinsic silica formation activity, but is able to modulate the activities of silaffin-1A and LCPA. When natSil-2 and natSil-1A (or LCPA) are combined, porous silica structures are precipitated within minutes following the addition of silicic acid. Remarkably, the precipitate displays pore sizes in the range 100-1000 nm, which is characteristic for diatom biosilica nanopatterns. Biophysical measurements indicate that silica nanopattern formation in this in vitro system is mediated by a self-assembled organic matrix of silaffins.

2:15 PM *W10.3
Biological Aspects of Periodic Mesoporous Organosilicas.
Kai Landskron[1], Benjamin D Hatton[1,2], Doug D Perovic[2] and Geoffrey A Ozin[1]; [1]Department of Chemistry, University of Toronto, Toronto, Ontario, Canada; [2]Department of Materials Science and Engineering, University of Toronto, Toronto, Ontario, Canada.

Organic-inorganic nanocomposites play a significant role in biological systems, e.g. in bones, teeth and shells. Periodic mesoporous organosilicas (PMOs) are synthesized by templating and integrating inorganic and organic components on the nanometer length scale and hence can be seen as biomimetic materials. Herein, we introduce a novel PMO consisting of interconnected [Si(CH2)]3 3-rings of SiO2(CH2)2 tetrahedral building units. This PMO can be made with powder or oriented film morphologies. It can be seen as the first example of a family of compounds as the CH2 groups can be substituted by CHR groups demonstrated in the case of R = Et, Br, I. Potentially also biologically relevant groups like amino acids or peptides could be such groups R that could lead to new bio-devices for sensing or separation.

2:45 PM W10.4
Shape, Size and Morphology Control of Inorganic Crystals with Self-Assembled Monolayers. Yong-Jin Han and Joanna Aizenberg; Bell Labs, Murray Hill, New Jersey.

Self-assembled monolayers (SAMs) provide simple, yet sophisticated surfaces to mimic highly ordered organic surfaces used in the process of biomineralization. A careful selection of organic molecules with an appropriate surface (i.e. HS-(CH2)15-COOH on Ag) allow nucleation and growth of oriented calcite crystals and provide opportunities to study the formation of inorganic crystals assisted by organic molecules. We have elucidated the process of calcite crystal formation on SAMs while studying the correlation between orientations of crystals and their final shapes, sizes and morphologies. We have concluded that the orientation of crystals plays a critical role in controlling their final shape, size and morphology by manipulating the ability of a crystal to interact with additives such as proteins and/or ions during nucleation and growth. In this presentation, we report our experimental results demonstrating how underlying organic molecules control and mold the final shape, size and morphology of biominerals from their initial nucleation during biomineralization.

3:30 PM W10.5
Biomimetic Surfaces Directing Fibronectin Matrix Assembly.
Jeffrey R. Capadona[1], David M Collard[1] and Andres J Garcia[2,3]; [1]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia; [2]Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, Georgia; [3]Petit Institute for Bioengineering and BioScience, Georgia Institute of Technology, Atlanta, Georgia.

Cell adhesion to synthetic materials is vital to numerous biotechnological and biomedical applications, including biomaterials, tissue engineering, and in vitro culture substrates. Cell-material interactions are mediated by specific binding of cellular receptors to adsorbed proteins or engineered motifs on the surface of the material. We have previously shown that the quantity, structure, and function of adsorbed fibronectin (FN) are dynamically influenced by the underlying substrate. In an effort in increase integrin-mediated cell adhesion to synthetic surfaces, numerous groups have incorporated bio-recognition sites onto the material's surface, such as the tri-peptide RGD. Although these strategies do increase cell adhesion to synthetic surfaces, the biological activity of these short peptides is significantly lower than that of the complete protein. A critical characteristic of extracellular matrices is the assembly of supramolecular structures. For example, FN matrix assembly modulates cell cycle progression and differentiation. Using mixed self-assembled monolayers (SAMs) of alkanethiols on gold, we have created a model nonfouling surface that can selectively tether peptides at a controlled density. We have engineered a biomimetic surface that incorporates a short peptide (FN-13) that has been shown to induce FN matrix assembly in several cell lines when added to culture media. SAMs presenting saturated levels of ligand demonstrated a ligand-dependent response in FN matrix assembly for confluent MC3T3-E1 osteoblasts. FN-13-tethered surfaces resulted in the assembly of robust, fibrillar FN matrices. In contrast, osteoblasts were unable to assemble robust matrices on substrates presenting control scrambled sequences. No FN matrix was detected on surfaces functionalized with RGD, FN, or unfunctionalized substrates. Significantly, RGD showed the lowest levels of FN matrix assembly, mirroring background levels. We demonstrate that by tethering the short peptide to a nonfouling and nonadhesive support, we are able to create a biomimetic surface that promotes FN matrix assembly in an osteoblast-like cell line. This biomimetic surface provides a promising strategy to the engineering of biomimetic supports.

3:45 PM W10.6
Semiconductor Surfaces with Nanometer Features Composed of TAT Peptides. Youngnam Cho and Albena Ivanisevic; Purdue University, West Lafayette, Indiana.

Atomic force microscopy (AFM) was used to fabricate well-defined peptide templates onto silicon and porous silicon surfaces via Dip-Pen Nanolithography (DPN). DPN is a powerful technique to write specific organic and/or inorganic molecules onto a surface with an AFM tip. In this work, DPN was used to construct arrays of peptides with nanometer features. Prior to patterning on a surface, a two step modification procedure was carried out. Clean surfaces were silanized to terminate them on amine groups, and subsequently reacted with a heterobifunctional cross-linker. TAT peptides (e.g. CGISYGRKKRRQRRR) which exhibit rapid uptake in cells, were

patterned onto the maleimide-modified surfaces in either contact or tapping mode. Several techniques were used for the characterization of the modified surfaces: X-ray photoelectron spectroscopy (XPS), Fourier Transforms Infrared (FT-IR) spectroscopy, contact angle, and Ellipsometry. Transmission FT-IR was used to monitor each reaction step on the porous silicon surface and provided structural information such as peptide conformation. The complementary analysis of XPS, ellipsomentry, and contact angle confirm the binding of the peptide onto the substrates and allowed to quantify the density of immobilized peptides on a given surface. In preliminary studies, the patterned semiconductor substrates showed promise to serve as test templates to track the interaction of healthy and malignant cells with cell-permeating agents such as TAT peptides.

4:00 PM *W10.7
Biological factors that influence calcium oxalate crystallization in plant cells. Mary Alice Webb, Botany and Plant Pathology, Purdue University, West Lafayette, Indiana.

Many plants accumulate crystals composed of calcium oxalate. These crystals occur in different sizes, shapes, and distributions that characterize particular groups of plants. Differing crystal shapes often reflect specific functions within plants. Calcium oxalate crystals can serve as variety of functions, including storage of calcium, structural reinforcement, and protection against pests. Synthesis of these crystals by plants is a regulated biological process, similar to other types of biomineralization such as bone and tooth formation in animals. However, it is not known how plants control crystal growth to fashion different shapes of crystals from calcium oxalate. We are interested in identifying and characterizing biological factors that influence synthesis of calcium oxalate crystals in plants. We are studying crystal formation in grape plants, which produce unique needle-shaped crystals of calcium oxalate in a highly specialized morphology, termed a raphide. In grape we have shown that raphides develop in specialized cells within compartments formed by biological membranes. Each of these cells, as it develops, constructs a highly organized bundle consisting of several hundred raphides. Raphide-forming cells are distributed throughout the plant, and the same crystal morphology and bundle organization are duplicated over and over again in each specialized cell. Our research has focused on identifying cellular factors influencing the fabrication of raphides and organization of raphide bundles within cells. We are interested in identifying genes and proteins, or gene products, that are involved in this process. We have used two different approaches to identify candidate proteins that may mediate raphide development. In one approach we isolated raphides from grape leaves and generated antibodies that recognized raphide-associated proteins (RAPs). We then used the antibodies to clone grape genes putatively encoding these RAPs. Using this approach we have identified and characterized a protein potentially involved in regulating crystal formation, as well as a molecular motor that may participate with other cellular structures in moving and organizing raphides within developing cells. Studies of these proteins and their putative functions are in progress. We are currently developing a second approach specifically targeting discovery of cellular proteins that may promote or inhibit crystal growth. With this approach we can assay a complex mixture of proteins and detect specific proteins within the mixture that may be active in modifying crystal growth. We expect that the same approach can be applied to identify functionally active proteins in a wide range of other mineralizing tissues.

4:30 PM W10.8
Modulation of Calcium Oxalate Crystallization by Biological Molecules. Roger Qiu[1], Chris Orme[1], John Hoyer[2], George Nancollas[3], Salvador Zepeda[4,1], Anita Cody[5] and James De Yoreo[1]; [1]Chemistry and Materials Science, LLNL, University of California, Livermore, California; [2]The Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, Pennsylvania; [3]Chemistry Department, University at Buffalo, Amherst, New York; [4]Department of Chemical Engineering and Materials Science, University of California, Davis, California; [5]Department of Geological and Atmospheric Sciences, Iowa State University, Ames, Iowa.

Biological control of calcium oxalate crystallization is a crucial aspect of urolithiasis since the majority of human kidney stones are primarily composed of calcium oxalate monohydrate (COM) crystals. The mechanisms responsible for modification of COM by biological molecules normally present in urine have not been previously defined at the molecular level. We report here, the first molecular-scale views of COM modulation by the aspartic acid-rich protein, osteopontin (OPN) and two synthetic 27 amino acid peptides, each containing 21 aspartic acids and either serine or glycine spacers. Our atomic force microscopy studies showed that crystal habit and growth kinetics are controlled through anisotropic step pinning on crystal faces. However, the protein, OPN, modulated growth by its effects on steps only on the (010) face, while the peptides controlled growth on steps of both the (-101) and (010) face. The peptide with serine spacers showed much stronger growth modification than the peptide with glycine spacers. These findings were supported by our kinetic studies using the constant composition method that showed comparable relative potencies. In this presentation, the implication of these findings to insights concerning biological control of stone disease will be discussed.

4:45 PM W10.9
Probing Crystal Growth and Adhesion of Calcium Oxalate Crystal Surfaces: Toward an Understanding of Kidney Stone Formation. Michael David Ward[1], Jeffrey A. Wesson[2], XiaoXia Sheng[1] and Taesung Jung[1]; [1]Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, Minnesota; [2]Nephrology Division, Department of Veteran Affairs Medical Center and the Medical College of Wisconsin, Milwaukee, WI.

Kidney stones are biomineralized crystal aggregates, most commonly containing calcium oxalate monohydrate (COM) crystals as the primary constituent. Notably, in vitro studies have suggested that anionic molecules or macromolecules with substantial anionic functionality (e.g., carboxylate) play an important role in crystal aggregation and crystal attachment to renal epithelial cells. In an effort to elucidate these in vivo processes and the role of crystal surface structure and macromolecules in the regulation of kidney stone disease, in situ atomic force microscopy (AFM) has been used to obtain real-time in situ images of crystal growth on various different faces of calcium oxalate crystals. This approach allows direct visualization of crystal surface morphology, which can be interpreted in terms of the bulk crystal structure based on the unambiguous assignment of the growth features (e.g., steps, terraces) that is achievable with AFM. For example, dynamic AFM imaging reveals dramatically different morphologies for the (100), (010), and (12-1), and real time acquisition provides characterization of the growth modes and rates of growth along different crystallographic directions. These measurements also allow for in situ determination of the influence of solute concentration and molecular or macromolecular additives on those rates. We also have used AFM to measure directly the adhesion forces between tip-immobilized molecules and the calcium oxalate surfaces in aqueous media. These measurements reveal the role of different functional groups on adhesion and support an important role for the carboxylate group in processes responsible for kidney stone formation. Furthermore, the adhesion forces depend upon the crystal face (e.g., COM (010) vs. (100)). These force measurements can be performed in the presence of soluble macromolecules, including native urinary macromolecules (e.g., osteopontin), producing profiles of adhesion suppression/enhancement and suggesting a feasible methodology for identifying the most important crystal surface-macromolecule combinations related to stone formation. Building on recent reports from our group [1,2], this presentation will illustrate that the combination of dynamic imaging of crystal growth on various calcium oxalate crystal faces and direct force measurements on those faces can lead to insights that are expected to produce a comprehensive structural model for the interaction of biomolecules with calcium oxalate. (1) Direct Visualization of Calcium Oxalate Monohydrate (COM) Crystallization and Dissolution with Atomic Force Microscopy (AFM) and the Role of Polymeric Additives, Shouwu Guo, Michael D. Ward, and Jeffrey A. Wesson, Langmuir 2002, 18, 4284. (2) Adhesion between Molecules and Calcium Oxalate Crystals: Critical Interactions in Kidney Stone Formation, X. Sheng, M. D. Ward, and J. A. Wesson, J. Am. Chem. Soc., 2003, 125, 2854.

SESSION W11: Poster Session II - Biosensors and Tissue Engineering
Chairs: William J. Landis, Christine Orme and Rizhi Wang
Thursday Evening, April 15, 2004
8:00 PM
Salons 8-9 (Marriott)

W11.1
Hierarchical order in PEG-peptide Block Copolymers Containing Biomimetic Moieties. Ian W Hamley[1], Valeria Castelletto[1], Oleksandr O. Mykhaylyk[1], Harm-Anton Klok[2] and Annette Roesler[3]; [1]Chemistry, University of Leeds, Leeds, United Kingdom; [2]Institut des Materiaux, EPFL, Lausanne, Switzerland; [3]Max Planck Institute for Polymer Reseach, Mainz, Germany.

Nature has evolved ways to create exquisite nanostructures using biopolymers in a range of structural and functional materials. Mankind now wishes to exploit such optimized architectures in rationally designed nanomaterials and peptides are attracting increasing attention as building blocks for design of self-assembled structures. The ultimate goal is the engineering of nanostructure via combinations of these biologically-derived or biologically-inspired

materials with appropriate synthetic polymer components. This talk will be concerned with nanostructured block copolymers in which the recognition properties and responsiveness of peptide sequences are modulated by incorporation of poly(ethylene glycol) (PEG) blocks in diblock and triblock architectures. Characterization of nanostructures in solutions and in the solid state has been performed using small-angle scattering and electron microscopy. In solution, we find PEG-coated fibrils, which aggregate into tangles resembling those formed by β-amyloid peptides. In the solid state, we study the influence of PEG crystallization on microphase-separated structures containing β-sheet peptide domains. By combining peptide and synthetic polymer blocks, unprecedented control over hierarchical order is demonstrated.

W11.2
In-situ Formation of Biphasic Calcium Phosphate Bioceramics from Goniopora Exoskeleton. Murugan Ramalingam[1], S. Ramakrishna[1] and T.S. Sampath Kumar[2]; [1]NUS Nanoscience & Nanotechnology Initiative and Division of Bioengineering, National University of Singapore, Singapore, Singapore; [2]Dept. Metallurgy & Materials Engineering, Indian Institute of Technology, Chennai, India.

Biomaterials derived from natural resources have recently been recognized as human health care substitutes. Calcium phosphate based bioceramics, in particular hydroxyapatite (HA) and beta-tricalcium phosphate (beta-TCP) are widely practiced clinically as bone substitutes due to their chemical composition similarity with human calcified tissues. Recently, bi-phasic calcium phosphate (BCP) bioceramics have been attracted for many biomedical applications owing to their controlled bioresorption, balancing of more stable phase of HA and more soluble phase of beta-TCP upon implantation. The BCP can be prepared from various calcium and phosphorous precursors in desired shapes depending upon clinical requirements. In this study, we report the in-situ formation of BCP bioceramics from goniopora corals, under hydrothermal condition with different HA/beta-TCP ratio and resorption rate. The prepared BCP was characterized by XRD, FTIR, and TGA and evaluated for its phase purity, chemical functionality and thermal stability. The solubility of BCP was conducted in Hanks medium at pH 7.4 under in-vitro physiological conditions and indicating that the solubility of BCP lays in between the resorption levels of HA and beta-TCP. This study explores the ways to utilize our natural marine resources into value added clinical materials in particular BCP, which can be used in osseous defective sites.

W11.3
Modulation of Reversible Color Switch of Polydiacetylene Supramolecules for Sensing Matrix. Dong June Ahn[1], Tai Young Kim[1], Sang Hoon Lee[1], Doo Ho Yang[1] and Jong-Man Kim[2]; [1]Department of Chemical & Biological Engineering, Korea University, Seoul, South Korea; [2]Department of Chemical Engineering, Hangyang University, Seoul, South Korea.

Polydiacetylene-based supramolecules are interesting biomimetic materials in view of application to chemical and biological sensors. These supramolecules are unique in changing color from initial blue to red upon specific binding events, caused by shortening of delocalization length of π-electrons along diacetylenic backbones. Various binding events including viruses, toxins, glucose, and ionic interactions have been reported detectable. However, most of the polydiacetylene-based chemosensors reported to date function via irreversible fashion. Accordingly, once the blue-phase shifts to the red-phase upon a given external stimulus, the backward "red-to-blue" transition does not occur even though the stimulus is removed afterward from the system. Recently, we reported the first example of both thermally stimulated and pH-stimulated reversible polydiacetylene supramolecules made of a novel single-chain diacetylene derivative capable of enhancing the strength of hydrogen-bonding of the resulting assemblies (J. Am. Chem. Soc. **2003**, 125, 8976-8977). This discovery on the role of enhanced hydrogen-bonding in color change should be useful for designing reversible colorimetric sensors. In this presentation, we report a novel technique to modulate the colorimetric reversibility of the polydiacetylene supramolecules. Weakening the hydrogen-bonding strength by ion binding to the supramolecular surfaces makes the assemblies work irreversibly. By contrast, successive enhancement of the hydrogen-bonding by ion desorption completely recovers their colorimetric reversibility. This modulation technique enables one to capture snap shots of the reversibly-working polydiacetylene supramolecular matrix potentially used for continuous monitoring.

W11.4
Fabrication of Super Water-Repellent Surfaces by Nanosphere Lithography. Peilin Chen, Chun-Wen Kuo and Jau-Ye Shiu; Inst. Appl. Sci. & Res. Eng., Academia Sinica, Taipei, Taiwan.

Inspired by the water-repellent behavior of the micro- and nano-structured plant surfaces, superhydrophobic materials, with a water contact larger than 150 degree, have received a lot of research attentions recently. It has been suggested that contamination, oxidation and current conduction can be inhibited on such superhydrophobic surfaces, and the flow resistance in the microfluidic channels can also be reduced using super water-repellent materials. However, to fully utilize the water-repellent properties of the nanostructured surfaces, it is necessary to investigate the relationship between the nanostructure and the water repellent behavior on surfaces, and to fabricate the nanostructured surfaces with desired surface hydrophobicity. We have developed a simple approach for fabricating tunable superhydrophobic surfaces using a combination of nanosphere lithography and plasma etching. It has been found that the water contact angle on these surfaces can be systematically tuned from 132 to 170 degree by trimming the diameters of polystyrene nanospheres using oxygen plasma. The water contact angles measured on these surfaces can be modeled by the Cassie's formulation without any adjustable parameter.

W11.5
Next generation sensing -Stainless sensing via single cell based sensors. Shalini Prasad[1] and Mihri Ozkan[1,2]; [1]Electrical Engineering, University of California Riverside, Riverside, California; [2]Chemical and Environmental engineering, University of California Riverside, Riverside, California.

There is a need to develop small, highly sensitive, accurate, portable sensors that have the detect- to- warn capability with the ability to distinguish accurately between a broad spectrum of chemical agents. Current methods rely on chemical properties or molecular recognition to identify a particular agent. This technology is limited in its capability to detect large number of possible agents both known and unknown, characterize the functionality of the known agents and predict human performance decrements. There is still a major gap in performing functional assays in the laboratory and implementing this concept in the field. This can be overcome by using cell based sensors. We have designed and implemented a novel cell based sensor that determines single cell sensitivity and response time to specific chemical agents by measuring the modifications of the extracellular electrical activity of the excitable cell membrane of mammalian cells. We have isolated and positioned single mammalian cells over individual microelectrodes that function both as sensing as well as measurement electrodes. We have formed single cell arrays over the microelectrode sensing platforms using gradient AC electric fields and measured the modifications to the extracellular electrical activity due to the action of a broad range of chemical analytes. A comprehensive analysis of the electrical activity modifications due to varying chemical analyte concentrations and the associated response time was performed. An integration of all the analyses yielded a unique identification tag associated with each specific chemical agent. This is termed as the Signature Pattern Vector (SPV). Using secondary staining experiments the physiological pathways associated with the generated SPV was confirmed and hence the veracity of the SPV has been established. This forms the basis of developing a stainless sensing technique that is non invasive and accurate based on the electrical activity modifications.

W11.6
Functional self-assembling bolaamphiphilic polydiacetylenes as colorimetric sensor scaffolds. Jie Song[1], Justin S. Cisar[2] and Carolyn R. Bertozzi[1,2,3]; [1]Materials Sciences Division, Lawrence Berkeley National Lab, Berkeley, California; [2]Department of Chemistry, University of California, Berkeley, California; [3]Howard Hughes Medical Institute, University of California, Berkeley, California.

Conjugated polymers capable of responding to external stimuli by changes in optical, electrical or electrochemical properties can be used for the construction of direct sensing devices. Polydiacetylene-based systems are attractive for sensing applications due to their colorimetric response to changes in the local environment. Here we present the design, preparation and characterization of self-assembling functional bolaamphiphilic polydiacetylenes (BPDAs) inspired by Nature's strategy for membrane stabilization. We show that by placing polar headgroups on both ends of the diacetylene lipids in a transmembranic fashion, and altering the chemical nature of the polar surface residues, the conjugated polymers can be engineered to display a range of thermal- and pH-induced colorimetric responses. We observed dramatic nanoscopic morphological transformations accompanying charge-induced chromatic transitions, suggesting that both side chain disordering and main chain rearrangement play important roles in altering the effective conjugation lengths of the poly(ene-yne). These results establish the foundation for further development of BPDA-based colorimetric sensors.

W11.7
Biomimetic growth of bone-like apatite on nano-fibrous scaffolds. Guobao Wei and Peter X. Ma; Biomedical Engineering, University of Michigan, Ann Arbor, Michigan.

One of the challenges for bone tissue engineering scaffolds is the ability to integrate with the host tissues. To achieve this, we incorporated nano-sized hydroxyapatite (nHAP), or/and coated a layer of bone-like apatite on the pore surfaces throughout the scaffolds in contact with physiological environment. Nano-fibrous polymer scaffolds and their nHAP composite scaffolds with interconnected macro-pores were prepared by the combination of a phase separation technique and a porogen sphere leaching process. The in vitro calcification of the scaffolds was investigated by incubating pre-fabricated macro-porous scaffolds in a simulated body fluid (SBF). Bone-like apatite crystal growth became detectable on and in between nano-fibrous network of the polymer scaffolds after 6 days of incubation. The deposited apatite particles reached a few hundred nanometers in size and had nano-structured surface features. A twenty two-day incubation in SBF led to a uniform apatite layer formation, covering all inner pore wall surfaces, and a mass increase of about 50%. Interestingly, it seemed that the maximum particle size did not increase after the initial incubation period, but the number of particles increased with incubation time. The scaffolds maintained the interconnected macro-porous structure, which is important for cell migration and mass transport. Pre-incorporation of nHAP particles into polymer scaffolds (even at a low content of 10w/w%) induced significantly greater amounts of apatite formation as compared to pure polymer scaffolds. In addition, polymer/nHAP composite scaffolds eliminated the 6-day lag time for apatite deposition, which was observed on pure polymer scaffolds. These results indicated that nHAP incorporation promoted in vitro calcification, and therefore, may also have the ability to improve mineralized new bone tissue formation in vivo. The nHAP, incorporated in a polymer scaffold, also enhanced protein adsorption capacity. The demonstrated bioactivity of nHAP, together with well-controlled macro and nano structures, makes the novel nano-composite scaffolds promising candidates for bone tissue engineering.

W11.8
Block Copolymer-Based Biomembranes Functionalized with Energy Transduction Proteins. Dean Ho, Benjamin Chu and Carlo D. Montemagno; UCLA, Los Angeles, California.

Block copolymer-based membrane technology represents a versatile class of nanoscale materials in which biomolecules, such as membrane proteins, can be reconstituted. Among its many advantages over conventional lipid-based membrane systems, block copolymers can mimic natural cell biomembrane environments in a single chain, which enables large-area membrane fabrication using methods like Langmuir-Blodgett deposition, or spontaneous protein-functionalized nanovesicle formation. Furthermore, the single chain composition of the copolymers can be easily tailored to yield a wide variety of properties based upon the functionality desired. For example, variations to the block lengths, chemical composition, and hydrophilic/hydrophobic properties can be incorporated into the synthesis scheme to yield a plethora of application-specific materials. The membrane protein, Bacteriorhodopsin, found in Halobacterium Halobium, serves as a light-actuated proton pump that develops proton gradients towards the demonstration of coupled functionality with other membrane proteins to effect ATP production, or production of electricity. Using quantum dot-labeled, engineered protein constructs, we have demonstrated large-scale insertion of membrane proteins into block copolymer Langmuir-Blodgett films. Following the adsorption of the hybrid protein/polymer membrane to the conductive fluoropolymer Nafion, and towards the fabrication of a multicomponent system capable of facilitating coupled protein functionality, we have demonstrated measurable pH changes based upon light-actuated proton pumping. Light-actuated activity across the protein-functionalized membrane when fully enclosed in a TMOS sol-gel matrix has also been observed using impedance spectroscopy. Initial data has suggested a significant pH change of up to 1.50 in a volume of 100 microliters and surface area of 0.317 square centimeters, a level that is capable of powering a number of proton-gradient dependent proteins towards the buildup of a robust, hybrid protein/polymer device. Recent atomic force microscopy studies of the protein-embedded polymer film have revealed the formation of protein aggregate-based pattern generation with very uniform torus-shaped rings. Subsequent room temperature incubation of samples for various amounts of time has generated evidence of molecular rearrangement within these films in an effort to autonomously seek out low-energy conformations. Current work is focused towards characterizing the effects that various pattern formations can have on the efficiency of protein functionality as well as film stability in an effort to develop a robust polymer membrane that can facilitate coupled membrane protein functionality for the fabrication of a wide variety of nanoscale devices.

W11.9
Relationship between dip-coating fabrication parameters and the elastical properties of latex grafts and stents.
Marcelo Mulato, Wellington F. P. Neves-Junior, Thais Cavalheri dos Santos and Mariselma Ferreira; DFM-FFCLRP, Universidade de Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil.

Latex, either natural or synthetic, is a material that has been extensively used in many industrial applications (tires, gloves, shoes, etc). Besides its wide technological use, new applications of this old material are frequently suggested. On the other hand, there is a need for biomaterial candidates for applications such as band-aids, prosthesis, tissue engineering and sensors. Preferentially, these new materials must be easy to handle, process, mold and use. In addition to that, the success of any new candidate is mainly dictated by the capability to avoid any rejection of the human body. In our work we are mainly interested in the use of natural Latex from the Hevea brasiliensis for medical applications besides gloves. As already demonstrated in the recent past, these material complies with all the above requirements. As starting point, this presentation focuses on the influence of the fabrication parameters on the final elastical properties of the obtained grafts. The grafts were produced by the dip-coating technique. We used a mixture of latex extracted from several clones. The mixture was centrifuged and stored in ammoniac solution, with the addition of 4% sulfur and 2% polivinil-metil ether solution. The elastic properties of the prosthesis were investigated using mainly the tension-deformation experiments. Tests were made using an expansion/contraction rate of 10 and 20 mm/min, and a distended dimension of 1,5cm. The influence of important fabrication parameters were studied such as: dip-coating velocity, final prosthesis thickness which was varied as a function of the number of baths, initial latex viscosity, thermal treatment(temperature and time) for the polymerization, etc. Single and cycled tension-deformation experiments were performed with closed and open samples considering longitudinal and transversal directions. A model is proposed for the anisotropy of the elastic behavior, which involves the sulfur bonds between polymeric chains. The influence of the fabrication process is also investigated by the comparison between the elastical properties of membranes obtained by dip-coating and spin-coating techniques.

W11.10
Degradation Behavior of Novel poly(α-hydroxy acid)-Derived Polyesters. Jay Christopher Sy[2], Xiao-Jun Xu[4,1], Molly M Stevens[3] and V. Prasad Shastri[1,4]; [1]Materials Science and Engineering, University of Pennsylvania, Philadelphia, Pennsylvania; [2]Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania; [3]Materials, Imperial College London, London, United Kingdom; [4]Joseph Stokes Research Institute, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania.

Due to their bioresorbable characteristics biodegradable polyesters are attractive materials for sutures, fracture fixation devices, and drug delivery systems. Recently, we synthesized a new library of degradable polyesters by modifying poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) backbones with hydrophobic diacids in order to tune the hydrophilic-lipophilic balance (HLB) in the polymer. This modification was aimed at creating polymers that exhibit surface eroding characteristics. Surface eroding polymers afford zero-order drug release and linear diminution of mechanical properties, the latter of which is desirable in load bearing applications. Surface erosion behavior in these polyesters was verified by mass loss of pressed pellets as a function of time. The progression of the degradation front within the polymer pellets was analyzed using Scanning Electron Microscopy. The erosion behavior in these polymers was further confirmed by following the release of a water-soluble dye from microspheres. Atomic Force Microscopy tapping mode imaging was used to study the changes in the surface characteristics of thin films as a function of degradation. Three parameters were modified in engineering this new set of polymers: initiating core carbon-chain length; lactide/glycolide chain length; and the diacid linker used to connect lactide/glycolide segments. In general, in polymers with lactide chain lengths >20 repeat units and molecular weights >25K, the C-length of the initiating core had little effect on degradation kinetics. Surprisingly, for molecular weights 25K or lower, polymers with longer initiating cores degraded slower than polymers with shorter initiating cores. This could be explained on the basis of an increased flexibility of the polymer backbone in the former. This is supported by the observation that polymers with longer initiating cores also exhibited lower glass transition (Tg) temperatures. We observed that polymers with shorter lactide repeat units degraded faster than ones with longer lactide blocks even though the former had twice the molecular weight. This is consistent with a surface erosion mechanism as increasing the lactide chain length should increase hydrophobicity and hence diminish water uptake. In this system, it appears that the surface erosion behavior is driven by a combination of hydrophobicity of the diacid linker and long-range order in the

polymer backbone. An observation that is counterintuitive is that increasing the diacid linker length, which should increase hydrophobicity and decrease water penetration, results in a faster degradation. This suggests that in these novel polyesters degradation is strongly influenced by entropic considerations, which are driven by chain flexibility. Understanding the effects of changing the different components of our novel degradable polyester library will allow us to custom design surface eroding polymers for specific applications.

W11.11

Surface modified diamond field effect transistors for enzyme immobilized biosensors. Kwangsoup Song[1,2], Hirofumi Kanazawa[1,2], Yusuke Nakamura[1,2], Syouta Kawamura[1,2], Munenori Degawa[1,2], Hitoshi Umezawa[1,2] and Hiroshi Kawarada[1,2]; [1]Electronics, Information and Communication Engineering, WASEDA University, Tokyo, Japan; [2]CREST, Japan Science and Technology Corporation, Tokyo, Japan.

Diamond meets the requirements of robust biosensing devices because of its wide potential window, chemical-physical stability and biocompatibility. Although research on chemical and biological modifications on diamond surfaces has begun, the sensing region and transducing region necessary for biosensing have not been realized. Here, we introduce enzyme biosensors using electrolyte-solution-gate field-effect transistors (SGFETs) made of polycrystalline diamonds as tranducers. Diamond SGFETs are respected to have high sensitivity, because the channel surface of SGFETs is directly exposed to electrolyte solution not using membrane or passivation layer on channel surface. To form the sensing sites on diamond SGFETs, glucose oxidase (GOD) was immobilized on the channel surface. Glucose-sensitive diamond SGFETs operate based on the biocatalyzed decomposition of glucose by GOD. From this biocatalyzed decomposition, the pH changes induced by a spontaneous hydrolysis of D-glucono-δ-lactone to gluconic acid decreasing pH near GOD immobilized channel surface can be registered with a SGFETs. The threshold voltage of SGFETs immobilizing GOD on channel surface shifted by approximately 30 mV/decade and drain current changed by approximately 40 μA. Since the polycrystalline diamond wafers are easily obtained, diamond SGFETs may be suitable biosensors and transducers in microelectronics for integrated biosensing and signal processing.

W11.12

Directed Osteoblast Adhesion at Metal Particle Boundaries: Promises for Nanophase Metals. Thomas Jay Webster, Jeremiah U Ejiofor and Brian Ward; Biomedical Engineering, Purdue University, Lafayette, Indiana.

Increased functions of osteoblasts (bone-forming cells) have been demonstrated on nanophase compared to conventional ceramics (specifically, alumina, titania, and hydroxyapatite), polymers (such as poly-lactic-glycolic acid and polyurethane), carbon nanofibers, and composites thereof. Nanophase materials are materials that simulate dimensions of constitutent components of bone since they possess particle or grain sizes less than 100 nm. However, to date, interactions of osteoblasts on nanophase compared to conventional metals remain to be elucidated. For this reason, the objective of the present in vitro study was to design, fabricate, and evaluate osteoblast adhesion on nanophase compared to conventional metals (specifically, Ti, Ti6Al4V, and CoCrMo). Results of this study provided the first evidence of increased osteoblast adhesion on nanophase compared to conventional metals. Moeover, directed osteoblast adhesion was observed preferentially at metal particle boundaries. It is speculated that since more particle boundaries were created through the use of nanophase compared to conventional metals, increased osteoblast adhesion resulted. Results of increased subsequent functions (such as proliferation, deposition of calcium-contaning mineral, etc.) on nanophase metals will also be presented. Thus, this in vitro study suggests that nanophase metals have intriguing properties to enhance the efficacy of orthopedic implants.

W11.13

Collagen-inspired nano-fibrous poly(L-lactic acid) scaffolds for bone tissue engineering created from reverse solid freeform fabrication. Victor J. Chen[1] and Peter X. Ma[1,2,3]; [1]Biomedical Engineering, University of Michigan, Ann Arbor, Michigan; [2]Biologic and Materials Sciences, University of Michigan, Ann Arbor, Michigan; [3]Macromolecular Science and Engineering Center, University of Michigan, Ann Arbor, Michigan.

Tissue engineering aims to resolve the problems of tissue and organ donor shortages. One common technique uses a biodegradable scaffold that acts as an artificial extracellular matrix (ECM) to support tissue regeneration. Cells are incorporated into the porous scaffold, and the cell-scaffold composite is cultivated to promote the formation of tissue throughout entire matrix. Recently, research has focused on engineering the internal structure of scaffolds from the micrometer to the nanometer level. Also, it has been suggested that the nano-fibrous architecture and the high surface area/volume ratio provided by the fibrous proteins in the ECM could improve cell adhesion, which consequently affects cell migration, proliferation, and differentiation. Thus, it is important that the scaffold imitates the cells' natural ECM environment until the host cells can repopulate and resynthesize a new matrix. Here, we use a reverse solid freeform (SFF) fabrication method to create highly-controlled macroporous structures in nano-fibrous poly (L-lactic acid) (PLLA) scaffolds. By using a computer-aided design (CAD) program to create a negative template for the scaffold, the three-dimensional (3-D) mold is created on a 3-D printer using a wax. After the template is printed, a solution of PLLA in tetrahydrofuran (THF) is cast into the mold, and is subsequently transferred to a freezer to rapidly induce thermal phase separation which gives the nano-fibrous morphology. After phase separation, the THF is exchanged with water, and the wax is leached out with organic solvents. Water is then exchanged with the solvents, and the sample is frozen and lyophilized to yield a 3-D nano-fibrous scaffold with a uniform fiber mesh throughout the entire matrix. Fiber diameters in these scaffolds are around 150 nm, similar to type I collagen, and the densities of the fiber meshes can be altered by changing the polymer concentration. Macroscopically, the 3-D printer allows feature sizes down to 250 microns, and allows the user to create features with various geometries. In these scaffolds, interconnected macroporous structures (>100 microns) can be created to allow for a uniform cell distribution during the seeding process, and guide cell migration and overall tissue formation in three dimensions. Also, the ability to create the macroporous regions of the scaffold a priori allows for the fabrication of scaffolds with anisotropic pore structures which potentially could be useful in tissues such as bone, where different tissue densities (e.g. trabecular and cortical bone) are common. Preliminary results also indicate that MC3T3-E1 osteoblasts attach and proliferate on these scaffolds. By having the ability to control the macroporous architecture, interconnectivity, orientation, and external shape of the scaffold, this SFF fabrication/phase separation technique has great potential to design and create ideal scaffolds for bone tissue engineering.

W11.14

Kinetics of controlled apatite growth aided by dentin matrix protein 1. Gen He[1], Dave Schultz[2], David Cookson[2], Jianjun Hao[1] and Anne George[1]; [1]Oral Biology, Univ. Illinois at Chicago, Chicago, Illinois; [2]ChemMatCARS, Argonne National Laboratory, Argonne, Illinois.

Bone and dentin biomineralization requires controlled mineral deposition and multi-scale self-assembly into hierarchically ordered biocomposites with unique mechanical properties. Body fluid is supersaturated with respect to calcium phosphate, however, mineral deposition is only restricted to mineralized tissues and only initiated in the gap region of the type I collagen fibrils during bone and dentin formation. Clinical investigations and transgenic animal models emphasize the critical roles of bone/dentin specific acidic matrix proteins in mineral nucleation, growth, and organization. Here we present comprehensive studies on the regulatory roles of a bone/dentin specific protein, namely dentin matrix protein 1 (DMP1) in biomineralization. The kinetics of formation and growth of colloidal calcium phosphate particles in the pseudophysiological buffer (165 mM NaCl, 10 mM HEPES, 2.5 mM CaCl2, 1 mM KH2PO4, pH 7.4) at room temperature was studied by time-resolved synchrotron small-angle X-ray scattering (SAXS), atomic force microscopy, and high resolution transmission electron microscopy (HRTEM). It was found that polydispersed spheroidal calcium phosphate nanoclusters emerged from the solution underwent simultaneous aggregation and deposited as apatite crystals. However, the growth and aggregation of the calcium phosphate particles were inhibited in the presence of DMP1 and the particle size was restricted below 30 nm. With time, these particles were stabilized in the solution, indicated by a significantly stronger scattering intensity compared to that with the controls, which are buffers without additive or with the same concentration of serum albumin. AFM analysis demonstrated that DMP1 self-assembled calcium phosphate clusters into nanorods with uniform size at 20*100 nm. On the other hand, when immobilized on a glass surface, DMP1 actively entraps calcium phosphate clusters from solution. The nucleated amorphous calcium phosphate precipitates ripen and nanocrystals form. Subsequently, these expand and coalesce into microscale crystals elongated in the c-axis direction. Characterization of the functional domains in DMP1 demonstrated that intermolecular assembly of acidic clusters into a beta-sheet template was essential for the observed mineral nucleation. In conclusion, the present study proposes that acidic proteins such as DMP1 plays a dynamic role in calcified tissue formation by facilitating mineral precipitation from physiological buffer and transforming into apatite of definite size and shape by spatially and temporally regulated nucleation, orientation, and organization.

W11.15
Calcium phosphate crystal phase formation on surfaces of collagen films. Marcelo Silva[1,2], Julio Goes[2], Sonia Figueiro[3] and Antonio Sombra[2]; [1]Departamento de Quimica Organica e Inorganica, UFC, Fortaleza, CE, Brazil; [2]Depertamento de Fisica, UFC, Fortaleza, CE, Brazil; [3]Departamento de Bioquimica, UFC, Fortaleza, CE, Brazil.

Considerable efforts have been made in the last years to improve the biocompatibility of materials and devices used for biomedical applications. The goal is to tailor the surface properties in such a way that a favorable interaction of the surface modified material and biological systems is achieved. Examples are the reduction of the non-specific binding of blood proteins to surfaces which are important for the blood compatibility of materials, and the control of the adhesion of living cells to solid inorganic substrates (e.g. surfaces of field effect transistors) through surface attached polymer monolayers.In the present study, we report the growth of calcium phosphate crystal phases on collagen film. The method consists of two steps. first, the films had been soaked in a Ca(OH)2 or CaCl2 aqueous solution solution and second, the film is soaked in a Na2HPO4 aqueous solution (pH 10). The physical and chemical characteristics of the composites were tested. IR spectroscopy, X-rays and SEM analysis showed the calcium phosphate crystal phase formation on film surface, which was attributed to the catalytic effect of collagen molecules carboxyl groups for crystal nucleation, and acceleration of nucleation from released Ca2+ ions. This result provides a guiding principle for obtaining apatite - organic polymer fiber composites. This composite is expected to have an analogous structure to that of natural bone.

W11.16
Abstract Withdrawn

W11.17
Interaction Between Titanium Implant Surface and Hydrogen Peroxide in Biologically Relevant Environments. Julie J. Muyco[2,1], Joanna M. McKittrick[2], John A. Frangos[3] and Christine A. Orme[1]; [1]Chemistry and Materials Science, LLNL, Livermore, California; [2]Materials Science and Engineering Program, University of California, San Diego, La Jolla, California; [3]La Jolla Bioengineering Institute, La Jolla, California.

Titanium is a widely used material for load bearing and dental implants. Over the decades of use, it has been seen that titanium implants are able to be accepted by the body and in fact become integrated into surrounding bone tissue, or osseointegrate. Titanium implants are inherently covered with a layer of titanium oxide, titania, that is a stable passivating layer *invivo*. Hydrogen peroxide is a highly reactive chemical that can be found in the body under inflammatory conditions. Interaction of titanium with hydrogen peroxide leads to formation of a titanium-peroxy gel. Studies have shown that titanium implants pre-treated with hydrogen peroxide have favorable response *invitro* and *invivo* in terms of the ability to form bone mineral. Correlation between the titania thickness and phase, surface morphology, and surface state after exposure to hydrogen peroxide and simulated body fluid will be presented. Methods of characterizing the materials interface include atomic force microscopy (AFM), x-ray diffraction (XRD), and Raman spectroscopy. This work was performed under the auspices of the U.S. Department of Energy by University of California, Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.

W11.18
Fracture of Calcium Phosphate Cement with Second Phase Organic Additions. Victoria C. Jew and Reinhold H. Dauskardt; Materials Science and Engineering, Stanford University, Stanford, California.

Calcium phosphate apatites formed as cements have shown significant potential as bone mineral substitutes for orthopedic and craniofacial applications. The combination of their injectability, biocompatibility, osteoconductivity, and similarity to the inorganic component of bone provides key advantages over other bone mineral substitute materials. However, the application of these cements is limited by poor tensile strength properties. In addition, we recently reported that calcium phosphate cements are susceptible to accelerated cracking under both fatigue and moisture-assisted stress corrosion conditions. In the present work, we report the effect of introducing an organic second phase on the fracture behavior of hydroxyapatite cement. The study focuses on serum albumin, a prevalent blood protein that can be mixed with cement prior to surgical application. The addition of albumin can also be supplemented with biologically active growth-stimulating proteins such as those in the transforming growth factor β (TGF-β) family. Results showing the effect of albumin additions on strengthening as well as on cracking rates in simulated physiological environments are reported. The microstructural changes that occur with the addition of albumin are also considered. The observed trends are used to develop mechanistic models that describe the kinetics of cracking in calcium phosphate cements both with and without organic second phases. Implications on load-bearing applications for calcium phosphate cements are discussed.

W11.19
Using Electrostatic Forces to Shape Materials for Biomedical Applications. Cheryl Lynn Casper[1,4], Jean S. Stephens[1,4], Nori Yamaguchi[1,4], William Yang[2], Cindy Farach-Carson[2,4], Kristi L. Kiick[1,4], D. Bruce Chase[3,1] and John F. Rabolt[1,4]; [1]Materials Science and Engineering, University of Delaware, Newark, Delaware; [2]Biological Sciences, University of Delaware, Newark, Delaware; [3]Dupont Central Research and Development, Experimental Station, Wilmington, Delaware; [4]Delaware Biotechnology Institute, Newark, Delaware.

Electrospinning is a technique that applies an electric field to a polymer solution in order to produce nanometer to micron diameter fibers. The small fiber diameter, high surface area, and interconnected fibrous network make electrospun fibers a desirable choice for a wide range of biomedical applications. When electrostatic forces are used to shape polymers in solution, a number of variables influence the integrity of the polymer chain and its conformation as it is subjected to medium-large ($\geq$ 500 V$\div$cm) electric fields. Concerns exist that architectural changes may occur in these electrospun biopolymers as a result of the electrospinning process. The importance of this issue is compounded when the materials being subjected to the electrical field are biomolecular in nature. Recently we have investigated using poly (ethylene oxide) (PEO) and collagen type I to produce electrospun fibers in order to assess their suitability for tissue engineering and drug delivery applications. To facilitate cell attachment and growth factor binding, heparin was incorporated into the electrospun PEO fibers at a concentration of 4 micrograms per milligram of electrospun fibers. Energy Dispersive Electron Spectroscopy (EDX) and Ultraviolet Spectroscopy were used to confirm the presence of heparin in the electrospun fiber membranes. Structural analysis using Raman and FTIR Spectroscopy was also performed. Finally, the ability of recombinant perlecan domain I, a scalable product derived from a native heparan sulfate containing matrix proteoglycan, onto the surface of collagen I-based scaffolds has been used to increase the bioactivity and growth factor binding ability of the electrospun fibers.

W11.20
Influence of Short-Chain Alcohols on the Mechanical Properties and Area/Molecule of Fluid-Phase Lipid Bilayers. Hung Van Ly and Margie Longo; Chemical Engineering and Material Science, UC Davis, Davis, California.

The mechanics of lipid membranes play an important role in the stability, permeability, and deformability of natural cells and drug-delivery liposomes. We show through micropipette aspiration of giant unilamellar liposomes that short-chain alcohols (methanol, ethanol, propanol, and butanol) can decrease the elastic moduli, toughness, and thickness of the fluid-phase bilayer. We propose the observed changes are caused by decreases in the interfacial tension of the bilayer. We verify this hypothesis by first determining the interfacial tension of the bilayer from elasticity measurements and showing how it decreases with increasing alcohol concentration. Secondly, from the decrease in the interfacial tension, we can predict the increase in the area per lipid molecule, and its value compares favorably to the area per molecule measurement obtained by flow-pipette micropipette aspiration technique. Furthermore, the alcohol-induced interfacial tension reduction will be related to surface adsorption at the bilayer-water interface. We propose that surface adsorption of small molecules, and subsequent drastic modification in mechanical and barrier properties of membranes, impacts a number of areas including alcoholic fermentation, drug-delivery, cell preservation, and cell viability.

W11.21
Abstract Withdrawn

W11.22
Biodegradable Porous Polyurethane Scaffolds for Tissue Repair and Regeneration. Katarzyna Gorna and Sylwester Gogolewski; Polymer Research, AO Research Institute, Davos, Switzerland.

Loss of tissues and internal organs is usually caused by trauma and/or degenerative changes. While small defects may heal spontaneously, critical-size defects require augmentation to heal. In clinical practice autogenous tissues are used to promote healing. Limited availability of tissues calls for development functional structural scaffolds which might potentially be used instead of autogenous tissues and organs. Optimally, the scaffolds should have an interconnected porous structure allowing for the flux of nutrients and the ingrowth of cells, extracellular matrix and blood vessels, and be bioresorbable or

biodegradable to allow the implanted matrix to be replaced by new tissue. The surface of the pore walls should support the attachment and proliferation of the cells involved in the reparative process of the given tissue. Biodegradable polymers are candidate materials for scaffold and can be transformed into scaffolds with various geometrical forms such as membranes or sponges for example. This report presents data on the preparation of porous scaffolds from new biodegradable polyurethanes and/or their composites with nanosize hydroxyapatite or tricalcium phosphate crystals. The scaffolds support the attachment and growth of articular chondrocytes, osteoblasts, human mesenchymal stem cells and myoblasts. Possible applications of these scaffolds are for the treatment of large bone defects, defects of articular cartilage and nerves and cardiovascular tissues, to mention but a few. Structural scaffolds from biodegradable polyurethanes promote healing of critical-size defects of long bones, and mono-, bi- and tricortical defects in the ilium. Elastomeric properties of the polyurethane scaffolds allow the application of load and shear to cell-scaffold constructs, which enhance the attachment and proliferation of chondrocytes. The pore structure and degradation rate of polyurethane scaffolds can be controlled by changing the material composition and synthesis conditions. Increasing the content of the hydrophilic segment in the polymer chain facilitates mineralization of scaffolds designed for cancellous bone graft substitutes.

***W11.23**
Biomimetic Apatite/Amelogenin Coating on Titanium and its Effects on Osteogenic Gene Expression. Chang Du[1], G.B. Schneider[2], R. Zacharias[2], C. Abbott[1], D. Seabold[2], C. Stanford[2] and Janet Moradian-Oldak[1]; [1]University of Southern California, Los Angeles, California; [2]University of Iowa, LA, Iowa.

The promotion of osteogenic cell differentiation by bioactive molecules is a central issue in bone tissue engineering. Biomimetic coating provides a promising vehicle for delivering such bioactive molecules. The potential of amelogenin protein to facilitate osteogenesis has been documented. We have used a recombinant mouse amelogenin rM179 to fabricate biomimetic apatite/amelogenin coating on titanium surfaces. Our studies were aimed to evaluate the incorporation of the protein in the coatings and their effect on osteogenic gene expression. Apatite coating on ultrasonically cleaned titanium was induced in a two-step process following a slight modification of the technique reported by Wen & Moradian-Oldak 2003 (JBMR 64A.483-490). The uniformity of protein distribution was evaluated by fluorescent labeling. The modification of mineral morphology by the protein was investigated with scanning electron microscopy. Gene expressions of Cbfa-1, osteocalcin, collagen type I, BSPII and alkaline phosphatase of Human Palatal Embryonic Mesenchymal cells cultured on the coatings were quantitatively analyzed by Real Time RT-PCR. Surface topography of titanium coating resulting from morphological changes of the apatite crystals was affected by the presence of amelogenin in a dose dependent manner. Real time RT-PCR showed significant increase in Cbfa1 ($p<0.005$), collagen type I ($p<0.0001$) and osteocalcin ($p<0.0001$) in cells grown on the samples (apatite coatings with amelogenin) when compared to blank (apatite coating without amelogenin). We conclude that amelogenin incorporated into apatite coating on titanium induces osteogenic gene expression in the palatal mesenchymal cells and the novel biomimetic coating has a great potential for dental and biomedical applications. Supported by NIH-NIDCR grants: DE-12350 & DE-13414 (JMO), P60DE13076 (GBS, CS) and the ITI Foundation for Oral Implantology (GBS,CS).

SESSION W12: Engineering Cell Structure and Adhesion
Chair: Thomas Webster
Friday Morning, April 16, 2004
Room 2005 (Moscone West)

8:30 AM W12.1
Primary osteoblasts adhesion onto RGD functionalized and crosslinked polyelectrolyte multilayers films. Catherine Picart[1], Rene Elkaim[2], Pierre Schaaf[3], Benoit Frisch[4] and Jean-claude Voegel[1]; [1]INSERM U595, Strasbourg, France; [2]Parogene, Strasbourg, France; [3]Institut Charles Sadron, CNRS, Strasbourg, France; [4]Laboratoire de Chimie Bioorganique, CNRS, Strasbourg, France.

The adhesion of primary osteoblastic cells on top of biocompatible polyelectrolyte multilayers (PEM) films was investigated for native films and for films whose properties have been changed either with a chemical stimuli (film functionalization), with a mechanical stimuli (film stiffening), or with both stimuli combined. For the functionalization, a 15 amino acids peptide containing a RGD (Arg-Gly-Asp) sequence was grafted to poly(L-glutamic) acid and deposited on top of poly(L-lysine)/poly(L-glutamic) (PLL/PGA), PLL/Poly(alginic), and PLL/Poly(galacturonic) films. The buildup of the film and the adsorption of the PGA-RGD could be followed by Optical Waveguide Lightmode Spectroscopy and by Atomic Force Microscopy. The mechanical stimuli was achieved by crosslinking the films with a water soluble carbodiimide in combination with N-hydroxysulfosuccinimide. Fourier Transform Infrared Spectroscopy evidenced the conversion of amine and carboxylic groups into amide groups. The Alkaline Phosphatase activity test was used to assess the primamry osteoblasts adhesion and proliferation on top of the different films over a period of ten days in culture. Whereas the native films are poorly adherent, the RGD-functionalized ones are extremely attractive to cells with a increase adhesion and proliferation of a factor eight. Additionally, the non-functionalized films could be successfully crosslinked thereby becoming much more attractive to cells than the native ones. More interestingly, combining the RGD effect and crosslinking effect lead to a very good cell adhesion and proliferation. We found that the cells did not react similarly on the different types of films investigated : they were either most sensitive to the chemical stimuli, mechanical stimuli, or both stimuli combined.

8:45 AM W12.2
Stable, Nanoscale Glycosphingolipid Films for Use in Sensing Applications. Rory Stine[1], Cara-Lynne Schengrund[2] and Michael Vincent Pishko[1]; [1]Chemical Engineering, Pennsylvania State University, University Park, Pennsylvania; [2]Biochemistry and Molecular Biology, Hershey Medical Center, Penn State Univ., Hershey, Pennsylvania.

We have developed a means of producing thin, oriented lipid mono-layers which are stable under repeated washing and which may be useful in biosensing or surface-coating applications. Glycosphingolipids (GSLs) such as GM1 were used as a representative lipid for this process. Initially, a mixed self-assembled monolayer of octanethiol and hexadecanethiol was constructed on a clean gold surface. This highly hydrophobic surface was then brought into contact with a thin GSL layer that had been deposited at the air/liquid interface of a solution by evaporating a mixture of GSL in hexane on top of a layer of water, leading to a GSL layer on the water with the fatty acid portion of the molecule facing upwards. The GSL layer, now deposited on the gold surface, was then heated to cause intermingling of the fatty acid and alkanethiol chains, and cooled to form a highly stable film which withstood repeated rinsing and solution exposure. Presence and stability of the film was confirmed via ellipsometry, FTIR, and QCM, with an average overall film thickness of 3.5 nm. The orientation of the lipid film, with the polar head group at the air/substrate interface, may be used to coat a substrate surface in a manner that could impart a specific functionality to the interface. This could be ideal for sensing applications if a fatty acid with a specific binding ligand as its head group were used. The GM1 ligand may be used as such, with the saccharide head group showing a high degree of affinity for cholera toxin.

9:00 AM W12.3
An electronic retinal interface for single cell stimulation. Neville Z Mehenti[1], Greg S. Tsien[2], Harvey A. Fishman[3] and Stacey F. Bent[1]; [1]Chemical Engineering, Stanford University, Stanford, California; [2]Electrical Engineering, Stanford University, Stanford, California; [3]Ophthalmology, Stanford University, Stanford, California.

While clinical results of visual prostheses to date have been encouraging, there remain many challenges before useful vision can be achieved. Current retinal prostheses primarily use microelectrode arrays to locally depolarize groups of neurons in a field-effect manner. An amplified signal from an array of photodiodes is commonly used to power the microelectrodes, which due to their large size are spaced at a low density relative to the cells layered in the central retina. Since current implants are located up to 100 microns or more from the cells they will be stimulating, both resolution and power requirements for the devices are adversely affected. A major goal of a retinal prosthesis that would restore useful vision is to connect microelectrodes to individual retinal neurons, thus providing a high-resolution interface with minimal power requirements. To address some of these challenges, we have used cell micropatterning technologies, such as microcontact printing, to direct the growth of retinal neurites to individual microelectrodes to achieve single cell stimulation. Rat retinal ganglion cell (RGC) neurites were isolated and purified through retinal dissection and immunopanning techniques. A laminin micropattern was aligned on a microelectrode array, and RGCs seeded on the array extended neurites along the pattern to contact individual electrodes. Threshold current and charge densities for cell stimulation were measured, and found to be an order of magnitude lower than those found for equidistant cell bodies that were not patterned toward a microelectrode. Since it is unclear what the governing electrical requirements for extracellular stimulation are, detailed studies were pursued to investigate these parameters and their effects. Electrode arrays with different geometries were microfabricated, and spatial and electrical requirements for cell stimulation through micropatterned neurites were characterized using fluorescence imaging techniques. Both physical and chemical approaches to cell patterning were

developed to direct individual neurites to microelectrodes. RGC growth and patterning was also evaluated on different substrates for the microfabrication of an electrode array using soft materials. The development of such an interface may provide the specificity and resolution that are necessary to treat not only retinal degeneration but a variety of neurological disorders as well.

9:15 AM W12.4
Dynamic Microcompartmentation of Biomolecules within Synthetic Cells. Christine D. Keating, Michael Scott Long, Marcus Helfrich and Clinton Jones; Chemistry, Penn State University, University Park, Pennsylvania.

We have prepared synthetic cells in which a lipid bilayer membrane encapsulates a primitive functional mimic of the cytoplasm. This synthetic cytoplasm is comprised of two chemically dissimilar polymers, such as polyethylene glycol and dextran, at a few weight percent in water. Under appropriate conditions of temperature and concentration, these polymer solutions exist as aqueous two phase systems, in which each phase is enriched in one of the polymers. In our case, liposomes are prepared above the phase transition temperature for the polymer solution and then cooled to initiate phase separation within the liposomes. Biomolecules such as proteins and nucleic acids were localized to subvolumes within these synthetic cells by partitioning into one of the encapsulated phases. These structures are exciting in that they enable for the first time the interior volume of synthetic cells to be structured, and biomolecules to be partitioned reversibly among the encapsulated microphases. Ultimately, we hope to control biomolecule association and activity through manipulation of local concentrations within these synthetic cells.

9:30 AM *W12.5
Cell Surface Engineering for Device Applications.
Carolyn Ruth Bertozzi, Chemistry and Molecular and Cell Biology, UC Berkeley, Berkeley, California.

In the design of microscale devices, there is considerable interest in the integration of biological components capable of performing complex functions. Proteins, for example, could serve as molecular motors or scaffolds; their natural ability to assemble into complex structures and transform chemical potential into mechanical force could be exploited in a device context. Living cells are capable of complex transformations such as multi-enzyme metabolic conversions that are useful for the biogeneration of catalysis or bioremediation of toxins. They can transduce signals in response to detection of soluble analytes, and therefore have use in the design of biosensing devices. All of these potential applications of biological components require their integration into a synthetic device environment. To achieve this, methods for controlling the interface between the biological molecule or cell and the surrounding material are paramount. This has motivated us to develop new methods for coating material surfaces with synthetic substrates that are compatible with biological components, and conversely, for decorating cells with new chemical structures that permit their attachment to material surfaces. New technologies for interfacing cells and materials will be presented in this talk.

10:30 AM W12.6
Transport Properties and Surface Microstructure of Lipid-Monolayer Coated Microbubbles. Mark Andrew Borden[1], Gang Pu[1] and Marjorie L. Longo[1,2]; [1]Chemical Engineering & Materials Science, University of California, Davis, California; [2]Biophysics, University of California, Davis, California.

Microbubbles stabilized by a lipid/emulsifier monolayer shell are important for a variety of reasons in fundamental and engineering science. The shell composition inspired by naturally occurring microbubbles and recently innovated by methods of rational design, can be engineered to serve an array of functions. Our results obtained from electrochemical, optical and fluorescence microscopy on lipid monolayer-coated microbubbles and model Langmuir monolayers demonstrate the relationship between composition, microstructure and transport properties of the lipid shell and provide insight into microbubble stability. We show that the monolayer shell reduces surface tension and impedes gas transport. The stabilization mechanism is determined by the phase state of the monolayer acyl chain region. Surface tension is maintained at the equilibrium value for expanded phase lipids and completely diminished for condensed phase lipids. The shell resistance to gas permeation is only significant for rigid-monolayer forming lipids and increases monotonically with acyl chain length. Shedding of excess shell material during dissolution occurs in a quasi-continuous manner for soft-monolayer forming lipids. In contrast, shells composed of rigid-monolayer forming lipids exhibit crumpling and shedding in distinct cycles. We propose a qualitative mechanism involving zippering of apposing monolayer portions at a critical point. The emulsifier partitions into the fluid phase and, in the case of a single hydrophobic chain, squeezes out at surface pressures below that required for lipid shedding. The size and shape of the crystalline lipid domains are controlled by lipid hydrophobic chain length and quench rate; a rich shell microstructure is observed.

10:45 AM W12.7
Toward control of synapse formation: Specific binding of neurexin expressing cells on patterned lipid bilayer containing GPI-linked neuroligin. Sophie Pautot[1], Hanson Lee[2], Camin Dean[2], Ehud Isacoff[2] and Jay T Groves[3,1]; [1]MSD, LBNL, berkeley, California; [2]MCB, UC Berkeley, Berkeley, California; [3]Chemistry, UC Berkeley, Berkeley, California.

In recent years membrane constituted proteins in supported pattern bilayers have been used to visualize intercellular communication between cells. Different lipid compositions can be micro-patterned in supported bilayers and long range lateral mobility required for activation of cellular response may be controlled. Here we micro-pattern domain of the supported bilayer with the GPI-linked ectodomain of neuroligin (NLG), a post synaptic membrane protein known to bind to neurexin and induce synapse formation. We show that neurexin expressing cells bind specifically to the supported bilayer that contains NLG , and that by controlling NLG pattern we can direct the cell binding and the synapse formation to specific location on our substrate. This micro-scale patterning of functional membrane-associated proteins is the first step toward cell based technological applications.

11:00 AM W12.8
Membrane Protein-Functionalized Hybrid Materials.
Jacob Schmidt, Bioengineering, UCLA, Los Angeles, California.

In millions of years of evolution, biological systems have developed abilities unmatched by manmade technologies. Membrane proteins are diverse and highly versatile components of these 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 common environment of lipid bilayer membranes, the possibilities of engineering devices functionalized by membrane proteins are very attractive. The functional lifetimes of these proteins may be extended from hours to years through incorporation into polymer membranes, allowing their properties to be fully exploited and resulting in new classes of materials. Our initial work has centered on the creation, study, and characterization of the biomimetic polymer membranes. Several techniques allow the insertion of protein into the membranes. We have compared the protein insertion processes between natural lipid bilayers and our polymer monolayers and begun studies of protein function and diffusion within the membranes. As a benchmark, we have been employing a model system of voltage-gated pore proteins, which have electrically controllable porosities. I will present our recent work in engineering this hybrid protein/polymer system. I will report on the progress of this work, the characterization of the membranes, protein insertion processes, and the yield and functionality of the composite. What we learn in this work is being applied to other proteins, such as the mechanosensitive channel protein MscL and the piezoelectric protein prestin, directed toward compact devices for mechanical sensing and acoustic sensing and transduction. 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 huge number of devices, as the same processes can be used for engineering other membrane proteins. This technology is particularly attractive as the purification and processing techniques will apply to most membrane proteins with some variation. This implies that once the learning curve necessary for successful production, insertion, orientation, 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.

11:15 AM W12.9
Adhesion Kinetics of MC3T3-E1 Pre-Osteoblasts to Osteoconductive Porous Titanium Scaffolds.
Jean-Philippe St-Pierre[1,2], Maxime Gauthier[2], Louis-Philippe Lefebvre[2] and Maryam Tabrizian[1]; [1]Biomedical Engineering, McGill University, Montreal, Quebec, Canada; [2]Industrial Materials Institute - National Research Council Canada, Boucherville, Quebec, Canada.

Porous metallic scaffolds have recently gained recognition as a promising avenue toward the regeneration of damaged bone structures [1]. Interest in these materials resides in their ability to provide guidance for bone growth by presenting a favorable structure for cellular adhesion and proliferation. They are also characterized by mechanical strengths and elastic moduli comparable to that of trabecular bone [1,2], making them potential candidates as implants for craniofacial reconstruction applications. A powder metallurgy process to obtain porous metallic materials has been recently developed [3]. In this process, a metallic powder, a solid polymeric

binder and a foaming agent are dry-mixed and molded. The molded powder is then heated in a three-step thermal treatment that includes foaming through the decomposition of the foaming agent, the elimination of the polymer and sintering. This last step promotes solid-state diffusion and creates metallurgical contacts between the metallic particles, which provide mechanical strength to the foam. This process is quite versatile and flexible and permits the adjustment of the final microstructural parameters of the foams. Cellular viability assays demonstrated the absence of cytotoxicity effects on macrophages in contact with porous titanium produced through the process described above, as well as in the presence of high quantities of the processing residuals. The aim of this study is to assess the potential of these highly porous titanium foams ($\leq$ 81% porous) as an osteoconductive material. This has been achieved through a study of the morphology of MC3T3-E1 subclone 14 pre-osteoblasts (ATCC, USA) adhering to the surface of titanium foams with three different pore sizes ranging from 136 to 434 μm after an incubation of 3 hours at 37°C. The cells are imaged with a scanning electron microscope after having been fixed with 2.5% Glutaraldehyde in 0.1M sodium cacodylate, dehydrated in serial ethanol and amyl acetate baths and critical point dried. This analysis has demonstrated that cells adhere strongly to the titanium matrices because they have a spread out aspect and they produced extensive extracellular matrix networks throughout the three-dimensional scaffolds. These observations are compared to those observed on non-porous titanium discs with varying surface roughness. To complement this morphological study, the adhesion behaviour of these pre-osteoblasts to porous titanium are quantified at different times from 1 to 18 hours after having been seeded and their proliferation and differentiation rates, as well as their capacity to produce a mineralized extracellular matrix impregnating the three-dimensional titanium scaffold, are measured in vitro. REFERENCES: 1. Wen CE et al. J Mater Sci – Mater Med. 13 (2002) 397-401. 2. Lefebvre LP et al. 29th Annual Soc for Biomater Meeting. April 30-May 6, 2003. Reno, USA. 3. Lefebvre LP et al. US Publ No US2003/0044301 A1. Pub Date Mar 6, 2003.

SESSION W13: Tissue Engineering II
Chairs: Ching-Chang Ko and Rizhi Wang
Friday Afternoon, April 16, 2004
Room 2005 (Moscone West)

1:30 PM W13.1
Role Of Time And Distance In Biological Growth Processes.
Paul Calvert, Textile Sciences, University of Massachusetts, Dartmouth, Dartmouth, Massachusetts.

Using inkjet printing methods, we have been able to deposit sub-micron layers of hydrogels containing bound enzymes, which remain active. For instance, layers containing alkaline phosphatase can be mineralized by exposure to organic phosphates and calcium salts. In studying these processes, it has become clear that suitable sequences of enzymes, substrates and inhibitors can be used to form localized or stratified structures within a layer of gel. This raises the possibility that timed release of reagents by cells, into the adjacent extra-cellular matrix, could be important in the formation of tissues. A combination of experiment and modeling provides evidence for how this approach could be applied to biomimetic materials.

1:45 PM W13.2
The early stage of new bone formation on plasma-sprayed and electrochemically-deposited hydroxyapatite coatings.
Hao Wang[1], Alexandra E Porter[2], Myron Spector[3], Zhou Xiang[3] and Linn W Hobbs[1]; [1]MIT, Cambridge, Massachusetts; [2]University of Cambridge, Cambridge, United Kingdom; [3]Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.

Hydroxyapatite (HA) is widely used in coatings for orthopedic implants because it is believed to accelerate early bone formation, following orthopedic surgeries. In this study, Ti-6Al-4V implants with two different HA coatings (plasma-sprayed HA and electrochemically deposited HA) and bare Ti-6Al-4V (as controls) were implanted into trabecular bone in canine model for 6 hours, 7 days, and 14 days, respectively. The bone/implants interfaces were studied by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM results show a higher bone apposition ratio on HA coatings; TEM results also confirm the faster mineralization on HA. The present time periods chosen fit into gaps in our prior assessments, but largely confirm the mineralization sequence inferred previously - which occurs in three distinct, and probably unrelated, stages likely coordinated with different proteins present at different time periods. (The last of these is type I collagen arriving between 6 and 7 days.) The earlier protein precursors are being sought using immunohistochemical TEM methods.

2:00 PM *W13.3
Mimicking the Extracellular Matrix One Step at a Time.
Alyssa Panitch, Harrington Department of Bioengineering, Arizona State University, Tempe, Arizona.

The extracellular matrix is a complex biological scaffold with properties that vary depending on multiple factors including: tissue type, disease state, position within the body and internal and external signals. Several attempts have been made to mimic the structural, mechanical and signaling environment of the matrix. Many of the mimetic strategies have involved incorporation of purified biological molecules, biomimetic peptides and synthetic polymers and ceramics. However, due to the complexity of the native extracellular matrix, many of the efforts to mimic its activities have fallen short. In an effort to begin to understand which material properties are crucial in the design of successful artificial extracellular matrices, artificial proteins, biopolymers and synthetic polymers have been systematically altered to incorporate biological signals encoding degradation, controlled release, crosslinking and cell adhesion. Information garnered from both analytical evaluation and *invitro* testing of these materials will be used to develop next regeneration materials that can serve as biointeractive artificial extracellular matrices for regenerative medicine.

2:30 PM W13.4
Biomolecular separation gels as an inorganic growth medium.
Scott R. J. Oliver, Department of Chemistry, SUNY-Binghamton, Binghamton, New York.

Polyacrylamide gels are commonly used to separate biomolecules, based on their highly ordered porous network formed when the polymer is combined with water. We are using this well-studied system, as well as other swollen polymer matrices such as polydimethylsiloxane (PDMS), as a sacrificial scaffold in which to grow inorganic materials. Our system is biomimetic, as we infiltrate the void space of the polymer with a solution-bound inorganic precursor, followed by inorganic condensation polymerization. Depending on the polymer and conditions, we obtain one of a variety of inorganic microstructures. These shapes include spheres, bowls, nets and coral-like matrices. We also completely fill the original swollen polymer, to create and inorganic complement of the swollen polymer. Our initial attempts to chemically or thermally remove the polymer template to give a free-standing inorganic material will also be discussed. Methods of characterization center on optical microscopy, SEM, TEM and powder X-ray diffraction.

2:45 PM W13.5
Surface Engineering of Nano-fibrous Poly(lactic acid) Scaffolds.
xiaohua liu, Youngjun Won and Peter X Ma; Biologic and Materials Sciences, University of Michigan, Ann Arobr, Michigan.

The architectural design and surface properties of scaffolds are important aspects in tissue engineering. The porous scaffolds provide the environment to accommodate cells and guide their growth, while the nature of surface of the scaffolds can directly influence cells attachment, proliferation, and ultimately new tissue regeneration. In this work, a highly porous poly(lactic acid) (PLLA) scaffold with nano-fibrous architecture of pore walls has been fabricated by mimicking the structure of natural collagen using a novel thermally induced phase separation method developed in our group. A universally effective surface modification method was developed, and a biomacromolecule gelatin was successfully grafted onto the surface of nano-fibrous PLLA scaffolds by physical entrapment alone or physical entrapment followed by chemical crosslinking. The surface composition, morphology, and properties were examined using ATR-FTIR, XPS, SEM and contact angle measurements. The surface of scaffolding by chemical crosslinking after physical entrapment always had higher surface density of gelatin than that only by physical entrapment. The surface coverage of gelatin on PLLA reached as highly as 39.4% using physical entrapment followed by chemical crosslinking. The surface hydrophilicity increased with the amount of gelatin on the surface of the scaffold. MC3TC-E1 osteoprogenitor cells were cultured for 6 weeks in solid walled PLLA scaffolds, nano-fibrous PLLA scaffolds, and surface-modified nano-fibrous PLLA scaffolds, respectively. The osteoblasts proliferated in all three types of scaffolds, but the cell numbers were always significantly higher in the surface-modified nano-fibrous scaffolds than in the other two types of scaffolds, and the cell numbers in nano-fibrous scaffolds was higher than that in the solid walled scaffolds. These results demonstrate that the surface-modified nano-fibrous architecture serves as a superior scaffolding for tissue engineering.

3:15 PM W13.6
Nano-biotechnology: Better Orthopedic Implant Materials Through the Use of Nanophase Ceramic:Polymer Composites.
Tyler A Smith and Thomas Jay Webster; Biomedical Engineering, Purdue University, Lafayette, Indiana.

Nanotechnology embraces a system whose core of materials is in the range of nanometers. Although various definitions are attached to the word "nanomaterial" by different experts, the commonly accepted concept refers nanomaterials as those materials with the basic structural unit specifically in the range of 1-100 nm (nanostructured), crystalline solids with grain sizes 1-100 nm (nanocrystals), extremely fine powders with an average particle size in the range of 1-100 nm (nanopowders), and fibers with a diameter in the range of 1-100 nm (nanofibers). Nanotechnology thus creates materials and products that potentially outperform, at several boundaries, the existing materials in terms of mechanical, electrical, catalytic, and optical properties. However, despite their promise to mimic the surface roughness cells experience in vivo, the use of nanophase materials in biological applications remains largely unexplored. The objective of the present in vitro study was, therefore, to determine whether when added to a polymer composite, nanophase compared to conventional ceramics enhance functions of osteoblasts (or bone-forming cells). Results from this study provided the first evidence that functions (specifically, adhesion, synthesis of alkaline phosphatase, and deposition of calcium-containing mineral) of osteoblasts are enhanced on three-dimensional poly-lactic-co-glycolic acid (PLGA) composites containing nanophase compared to conventional titania (70:30 wt.% PLGA:titania). Since the chemistry, material phase, porosity (%), and pore size of the composites were similar, this study implies that the nano-structured surface features created by adding a nanophase compared to conventional ceramic, was a key parameter that enhanced functions of osteoblasts. In this manner, this study adds another novel property of nanophase ceramics: their ability to enhance osteoblast functions in vitro in three-dimensional composite form. For this reason, nanophase ceramics deserve further attention as orthopedic tissue engineering materials.

3:30 PM W13.7
Enzyme-Inspired Self-Assembling Peptide Amphiphile Nanofibers. Hannah Storrie[1] and Samuel I. Stupp[2,1,3]; [1]Department of Chemistry, Northwestern University, Evanston, Illinois; [2]Department of Materials Science, Northwestern University, Evanston, Illinois; [3]Feinberg School of Medicine, Northwestern University, Chicago, Illinois.

Mimicking the action of enzymes in self-assembling materials is a new frontier in biomaterials design. Enzyme mimics would provide access to chemically active materials capable of providing specific signals and substrates to cells in vitro and in vivo. Peptide amphiphiles provide a robust system for the introduction of epitopes required for enzyme activity. We have chosen to explore mimics of alkaline phosphatase (ALP) for biomineralization applications. The active site of ALP contains two zinc ions, one bound via two histidine residues and an aspartic acid residue and the other bound via two aspartic acid residues and one histidine residue. Additionally, an arginine residue and a serine residue are required to coordinate the ligand in the binding pocket. To mimic this protein structure, we haved designed a novel PA that contains a branched peptide head group with one arm for zinc binding and a second arm for ligand binding coupled to an aliphatic tail by a crosslinkable cysteine tetramer region that allows the self-assembled structure to function across a broad pH range. PA's form gels at low concentrations (<1% w/w) and provide a three-dimensional scaffold for cell growth that is similar to the extra-cellular matrix. We have show ion-dependent self-assembly of branched PA's in the presence of zinc ions and confirmed histidine-zinc binding interactions by NMR, which demonstrates a specific interaction of zinc ions with the PA, and indicates its ability to act as a structural mimic of ALP in vitro.

3:45 PM W13.8
In Vitro And In Vivo Tests Of Hydroxyapatite-Gleatin Nanocomposites For Bone Regeneration: A Preliminary Report. Ching-Chang Ko[1], Ying-Lien Wu[1], R A Narayanan[2] and Wei-Shou Hu[2]; [1]Oral Science, University of Minnesota, Minneapolis, Minnesota; [2]Chemical Engineering and Materials Sciences, University of Minnesota, Minneapolis, Minnesota.

A biomimetic process has been developed to fabricate hydroxyapatite-gelatin (HAP-GEL) nanocomposites for bone regeneration (Chang and Ko et al. 2003). We hypothesize that this newly developed HAP-GEL is osteoconductive and is suitable for tissue engineered scaffolds. This preliminary study is aimed to characterize cell affinity and osseous regeneration of the HAP-GEL. The HAP-GEL was synthesized according to the procedures described in the previous publication. The attachment and proliferation of human fetal osteoblasts on HAP-GEL discs were evaluated using three different gelatin contents (2g, 3g, and 4g). The cells were seeded onto each disc and incubated at 34 degrees Celsius in 5% CO2 air atmosphere. At different time points of cultivation, cells were stained with Fluorescein DiI to determine their viability and morphology. To assess the cell proliferation, cells were detached at Days 1, 4, and 7 by trypsinzation and counted. For in vivo tests, HAP-GEL rods were implanted into the proximal femur of Sprague-Dawley rats (Animal Protocol ID#0212A38202). One month after the implantation, the femurs were harvested and fixed in 10% formalin. The undecalcified HAP-GEL-bone sections were prepared through the EXAKT microgrinding system and stained with Stevenel's Blue and Van Gieson's Picro-Fuchin. New bone formation was assessed by bright red. Four hours after attachment, most cells appeared round in all discs; cell spreading was observed after 24 hours. The highest gelatin content supported a significantly higher cell growth than the others at 7 days. Thus all compositions support satisfactory attachment, spreading and growth. In vivo results showed excellent interfacial bone regeneration. No necrotic tissues were found. In conclusion, the HAP-GEL not only mimics the biochemistry and nanostructures of bone but also supports the attachment, proliferation and differentiation (bone formation) of osteoblasts. The HAP-GEL we developed provides a suitable surface for regeneration. (Supported by NIH/NIDCR 1R21-DE015410-01, N01-DE-22635, 3M/ESPE Dental, and MDRCBB)

4:00 PM W13.9
Synthesis of Calcium Carbonate Nanoparticles for Drug Detoxification. Debra Holly Lush, Vishal Patel, Allison Kurz, Piyush Sheth, Javier Gutierrez and Laurie Gower; Materials Science and Engineering, University of Florida, Gainesville, Florida.

Nanoparticulate systems are being developed for use in pharmaceutical and industrial controlled release applications. In the Unites States alone, over 300,000 patients enter the emergency room each year due to complications from overdose of prescription drugs. In fact, the leading method of suicide is via overdose of amitriptyline, a popular anti-depressant. There currently exists no quick and effective method to detoxify these patients. The goal here is to synthesize "soft" emulsion particles coated with an inorganic shell with tailorable porosity and degradation properties, which when introduced to the blood intravenously, act as drug "sponges" for patients overdosed on these lipophilic drugs. This is done via a biologically inspired mineralization process of surface-induced deposition of calcium carbonate coatings templated onto charged emulsion particles. The experimental technique includes the addition of ammonium carbonate into a combination of oil-in-water microemulsion, calcium chloride, magnesium chloride, and a highly acidic polymer. Nanoparticles with diameters of about 200nm have successfully been synthesized. Current experiments on these particles are directed towards in vitro testing of drug uptake capabilities and templating porosity of the inorganic shell using binary surfactant systems in order to achieve molecular filtration.

4:15 PM W13.10
Minimal functional model of hemostasis in a biomimetic microfluidic system. Rustem Ismagilov, Matthew K Runyon and Bethany L Johnson-Kerner; Chemistry, The University of Chicago, Chicago, Illinois.

This presentation describes the development of a minimal model of hemostasis. Hemostasis is a complex functional system that consists of 80 coupled biochemical reactions that involve biomolecules and cells, both in solution and on surfaces. Hemostasis is responsible for repairing damaged blood vessels and preventing excessive bleeding. It maintains blood in a fluid, clot-free state under normal conditions, but creates a localized solid clot in response to vascular damage. The complexity of hemostasis is associated with a finely tuned self-regulation, essential for its function. This self-regulation is believed to be the basis of two essential features of hemostasis: i) threshold response: there is no response to small regions of internal vascular damage present throughout the circulatory system, but full response to substantial damage of a blood vessel. ii) local response: a clot formed in the region of substantial damage is confined to that region. A simple model of hemostasis was developed. Using this model, only three non-biochemical reactions were required to create a functional biomimetic microfluidic system capable of repairing itself when damaged. Some of the predictions of the model were successfully tested with human blood. This biomimetic system suggests that the function of hemostasis is highly dependent on the geometry of the junctions between vessels, and suggests a hypothesis that hemostasis influenced the evolution of mammalian vascular networks.

4:30 PM W13.11
Biomimetic Encapsulation of Enzymes. Rajesh R Naik[1], Heather R Luckarift[2], Jim C Spain[2] and Morley O Stone[1]; [1]MLPJ, Bldg 651, Air Force Research Laboratory, Dayton, Ohio; [2]Airbase Technologies Division, Air Force Research Laboratory, Tyndall Air Force Base, Florida.

The successful use of enzymes for applications in catalysis and sensors is dependent on the host material used for immobilization of the enzymes. One of the most widely used method for immobilizing

enzymes is sol-gel silica encapsulation. Entrapment of biomolecules by the sol-gel process involves the hydrolysis of the alkoxysilane precursor by water, acid or base catalysis, to form hydroxy derivatives. The biomolecule prepared in a buffered solution (pH 7) is subsequently added to the hydroxy derivatives or sol, and gelation is initiated. The hydrogel is slowly aged at low temperature over a period of several weeks. The sol-gel method is a generic immobilization technique used to entrap biomolecules. Here we describe the entrapment of enzymes in a silica matrix using a biomimetic approach. The entrapment process is a one-pot procedure wherein the silica matrix is biologically synthesized in the presence of the enzyme to be encapsulated.

4:45 PM W13.12
Biohybrid Architectures from Amphiphilic Macromolecules.
Jeroen Cornelissen, Gerald Metselaar, Irene Reynhout, Dennis Vriezema, Alan Rowan and Roeland Nolte; Organic Chemistry, University of Nijmegen, Nijmegen, Netherlands.

Biohybrid Architectures from Amphiphilic Macromolecules In Nature structural information is transferred in a hierarchical fashion from the smallest building blocks, i.e. amino and nucleic acids, to the complex architectures formed by them. These principles can be applied also in the formation of peptide derived polyisocyanides. Polymers of isocyanides have a helical conformation in which side group n is more or less on top of side group (n + 4). The distance between these side chains is very well suited for the formation of beta-sheet-like hydrogen bonds between the peptide groups present in these side chains. Both in the formation and their final structure these polyisocyanopeptides resemble the properties of proteins [1]. The stereochemical infomation present in the monomer is transferred to the stable helical conformation of the polymer and in certain cases, i.e. acid catalyzed polymerization, the polymerization of optically active monomers is highly stereoselective. If a macromolecular initiator, such as polystyrene, is used for the isocyanide polymerization, a amphiphilic rod-coil diblock copolymer is obtained. In a selective solvent for the isocyanide segment, these macromolecules self-organize an form a selection of morphologies. In water the aggregate formation is, among other factors, dependent on the ratio between the two blocks and the interaction between like blocks. This results next to micellar and bilayer type aggregates, in the formation of superhelices [2]. In a slight modification these types of block copolymers give large and stable vesicular aggregates. These are able to encapsulate enzymes that stay active in the inner compartment of the vesicle, while substrate molecules still can diffuse through the polymer membrane. This results in a new type of microreactor [3]. The concept of self-organizing block copolymers is aqueous dispersion could be taken one level further. Systems are recently developed, where the hydrophilic segment of the macromolecules consists of a protein or an enzyme. Three different approaches were applied in connecting the biomacromolecule with the hydrophobic polystyrene segment; i.e. covalent, by cofactor reconstitution and by using the biotin-streptavidine couple. In all three cases different aggregation behavior was observed, resulting in different aggregate morphologies. 1. Cornelissen, J.J.L.M.; Donners, J.J.J.M.; de Gelder, R.; Graswinckel, W.S.; Metselaar, G. M.; Rowan, A.E.; Sommerdijk, N.A.J.M.; Nolte, R.J.M. Science 293 (2001) 676-680. 2. Cornelissen. J.J.L.M.; Fischer, M.; Sommerdijk. N.A.J.M.; Nolte, R.J.M. Science 280 (1998) 1427-1430. 3. Vriezema, D.M. et al. Angew. Chem. Int. Ed. 42 (2003) 772-776.