SYMPOSIUM W
Biological and Bio-Inspired Materials and Devices
April 13 - 16, 2004

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* Invited paper
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 structural properties and their dynamic behaviors. Two key elements in molecular fabrication are chemical complementarity and structural compatibility, both of which confer the weak and non-covalent interactions that bind building block molecules together. Self-assembly provides the opportunity for the spontaneous formation of structures that resemble tissues and cells.

9:00 AM W1.2/01.2
The Creation of Novel Hybrid Materials Through the Coupled Self-Assembly of Chaperonin Proteins and Diblock Copolymers. Linda Katherine Molnar1, Ting Xu2, Jonathan Trent3 and Thomas P Russell4; 1 Center for Nanotechnology, NASA Ames Research Center, California; 2Polymer Science and Engineering, University of Massachusetts Amherst, Massachusetts; 3Astrobiology 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 strategy resulting from the mixing of these two disparate self-assembling systems has been found. The materials utilized were asymmetric diblock copolymer of polystyrene (PS) and polyethylene oxide (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 thicknesses small enough to pass light and the ability to self-assemble. 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 copolymer. AFM images of the resulting thin films with and without chaperonin amorphous chaperonin and upon copolymer assembly, with the formation of the PS-b-PEO copolymer, and enabling the microphase separation of the copolymer. The chaperonins used in these studies, isolated from Sulfolobus shibatae, which lives in geothermal hot springs and grows at temperatures of up to 85 degrees Celsius and pH 2.6, 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-assembly 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 functional designs, and because living systems assemble themselves, 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 can be generated by any of the chemical procedures. 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.

9:15 AM W1.3/01.3
Environmentally Responsive Hydrogels with Tunable Rigidities Constrained Via Peptide Folding and Consequent Self-Assembly. Darrin Pochan1 and Joel Schneider 2; 1Materials Science and Engineering, University of Delaware, Newark, Delaware; 2Chemistry and Biochemistry, University of Delaware, Newark, Delaware.

By using peptide dic in the materials self-assembly design process, one can take advantage of inherent biomolecular attributes, intramolecular structural motifs and secondary structure, in addition to more traditional self-assembling molecular attributes such as amphiphilicity, to define hierarchical material structure and consequently properties. Importantly, intramolecular folding events impart a molecular-level mechanism for environmental reponsefulness 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 thereby, 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 macroscopic 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.

10:00 AM W1.4/01.4

Successful solutions to many problems in science and technology have come from extracting design concepts from biology, 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 can be generated by any of the chemical procedures. 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.
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 controlled pathways of the Drosophila eye pigmentation process. 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
Henggan Pan, Kevin Mailly, 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 alane chain lengths of the primary alkylthiol stabilizing ligands or the surrounding secondary surfactants. Under kinetically controlled silica polymerization conditions, evaporation drives self-assembly of gold NCs through the intermediate NC-assembly, and the final ordered 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 electronic 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 Nanostructures Through Electrosynthesis
Ian McLaughlin1, Chris Costello2, Donghai Wang1, J Eric Hampsley3, Chaojun Li4, C Jeffrey Brinker4 and Yunfeng Lu5; 1Chemical Engineering, Tulane University, New Orleans, Louisiana; 2Chemistry, Tulane University, New Orleans, Louisiana; 3Sandia National Laboratories, Albuquerque, New Mexico.

Conjugated polymer-silica 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-dioctothiophene 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 via the silica mesophase to form a nanocomposite. XRD, TEM, and AFM images revealed a hexagonal, cubic or lamellar mesostructure. PTE incorperation 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 tunable mesoporosity and high crystallinity. 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 Science, 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 driven in three pathways of the DNA base pairing geometry. 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.

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. Lian Huo1 and Masanori Yamada2; EEl, AIST, Tsukuba, Ibaraki, Japan.

Proton conductive membrane is a very important materials for functional electrochemical devices such as fuel cells, battery, electrochemical 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 100°C, 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 a small amount of 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 150°C, 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 Groththus 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 processing concepts. We have been adapting lithographic fabrication approaches for patterning 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. Rivers, 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 examine microporous 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 adsorbed to the channel wall as well as the floor, microtubules almost always move out of the channel, (2) devices where cases of microtubule adhesion dominate, 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 examined a variety of alkane thioles to selectively form monolayers on the gold wall surfaces, and their effectiveness at resisting motor protein absorption and directing MT motion has been compared. Applications of these devices for the manipulation of nanomaterials within MTs and kinesin in conjunction with chemically active surfaces will also be discussed.

2:30 PM *W3.2.4
Bio-inspired Periodic Microlens Arrays with Integrated Pore Structures Created by Multi-beam Interference Lithography. Shu Yang1, Gang Chen2, Chaitanya K. Ural3, Mischa Megenis3, Yong-Jin Han4, Ronen Rapaport5, Edwin L. Thomas2, Chada Bone Formation. Samuel I. Stupp2,3 and Eli D. Sone2; 1Materials Science & Engineering, Northwestern University, Evanston, Illinois; 2Chemistry, Northwestern University, Illinois; 3Feinberg 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 nanocrystals. In one case iron-based magnetic nanocrystals are grown on other self-assembled surfaces, and their effectiveness at resisting motor protein absorption and directing MT motion has been compared. Applications of these devices for the manipulation of nanomaterials within MTs and kinesin in conjunction with chemically active surfaces will also be discussed.

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

3:30 PM *W3.3.1
 Templating of Magnetic and Semiconducting Materials a la Bone Formation. Samuel I. Stupp2,3 and Eli D. Sone2; 1Materials Science & Engineering, Northwestern University, Evanston, Illinois; 2Chemistry, Northwestern University, Illinois; 3Feinberg School of Medicine, Northwestern University, Evanston, Illinois.

Bio-compatible polycrystalline materials have been designed to overcome existing strong limitations of non-functionalized surfaces. These materials exhibit utility as coatings in the formation of diagnostic and bioanalytical devices. Specifically, trilob copolymers of poly(propylene sulfide) and poly(ethylene glycol) (PEG-b-PPS-b-PEG) were synthesized to stabilize chemisorp to gold via thiol bonding. Optical and surface plasmon resonance (SPR) results indicate that PEG-b-PPS-b-PEG applied to gold renders surfaces more resistant to alkanethiols, as well as resistant to both protein and cells. Fibroblast cell activity was also reduced on PEG-b-PPS-b-PEG treated 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), microparticle electrochemistry, microchannel flow, and photocatalysis to control surface adsorption via oxidation pattern surfaces. When PEG-b-PPS-b-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-b-PPS-b-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-b-PPS-b-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-b-PPS-b-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. Microchannel oxidation has also been achieved with microchannel fluidic patterning with PDMS (poly(dimethylsiloxane)) stamps on gold. Our present patterning focus explores the efficacy of TiO2 as a photocatalyst for oxidation of alkane thiol and the PEG-b-PPS-b-PEG polymer chemistry. We are working with TiO2 nanoparticles and TiO2 bound Polymethylsiloxane (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 used as exogenous surfactant films. The surface pressure versus trough area isotherms respreading. However, further characterization highlights intriguing 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 striking is seen for Survanta Exogenous Pulmonary Surfactants Films. Coralie Alonso and Joseph A. Zasadzinski; Chemical Engineering, University of California, Santa Barbara, California.

Several commercial exogenous surfactants are used in the place of lung surfactant to replace for 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 used as exogenous surfactant films. The surface pressure versus trough area isotherms show that all the films meet the requirements regarding as first criteria to define a good surfactant: high collapse pressure and efficient respreading. However, further characterization highlights intriguing 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 striking is seen for Survanta. These observables 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 pressure also correlate 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 is microscopically important. It is essential 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 that range in size less than 100 nm to approximately 1 to 2 μm. To determine how the distribution of protein is correlated with cell shape and motion on these substrates, we have examined the behavior of platelets on surfaces. Platelets are a blood cell that aggregate at sites of injury and form a provisional matrix that is the foundation of a blood clot.

4:00 PM *W3.3
Bio-compatible Polybetaine Surfactants Containing Polymers for Surface Passivation and Patterning. Jane P Bearinger1, Samuel Terrettas2, Roger Michel2, Christine A Orme3, Marcus Textron2 and Jeffrey A Hubbell2; 1MTP/ CMS, LLNL, Livermore, California; 2ETH, Zurich, Switzerland; 3EPFL, Lausanne, Switzerland.
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 organize proteins that directly affect 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 sputtering 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 homogenous coatings of fibronectin. Thus, we have optimized the use 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 Orne and Rizhi Tissot and Bruce C. Bunker; Biomolecular Materials and Interfaces, Sandia National Labs, Albuquerque, New Mexico.

Tu esday 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 1.0 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 cells and to study the role of adhesive contacts on the behavior of anchorage-dependent cells.

Thus, we can determine the optimal distribution of adhesive sites for naturally formed by cells on homogeneous coatings of fibronectin. Therefore, we have optimized the use of adhesive sites for activating cell functions. Preliminary results suggest that cell size may be inversely proportional to the distance between islands.

W4.2 Photochemistry of self-assembled protein cages.
Zachary B Varpness1,2, Jesse Mosolf1,3, Dan Ensign1,3, Michelle Flenniken1,3, Debbie Wittles4,5, Mark Young2,3 and Trevor Douglas1,2; 1Chemistry and Biochemistry, Montana State University, Bozeman, Montana; 2Department of Plant Sciences, Montana State University, Bozeman, Montana; 3Center 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 nanometric 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. Boul, 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 biomimetic synthesis of metal oxide and metal sulfide nanomaterials. MTs offer the unique biomineral scaffold as they have a controllable, high aspect ratio (25 nm in diameter and 1-1000 μm 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 an aeroaqueous solutions of FeCl2 buffered to neutral pH followed by aerochemical oxidation lead to the formation of iron oxide coated MTs. The nature of the iron oxide layer depended greatly on the amount of FeCl2 added to MT solutions; low amounts of FeCl2 yielded 10-30 nm thick layers of amorphous iron oxides. Higher amounts of FeCl2 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 preferentially on the MT surface, it is likely that the negatively charged MT surface is responsible for 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 Patterned Calcium Carbonate Film using a Binary Surfactant System for Drug Detoxification Applications.

In the United States alone, over 200,000 patients enter the emergency room each year due to prescription drug overdose complications. In fact, the leading method of suicide is via overdose of amitryptilie, 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 overdose patient.

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 Cellulodextrins.
Jun Peng1, Yong-Hyun Kim2, Shengzhang Zhang3, Shiyou Ding1, Melvin P. Tucker3, Gary Rumbles2 and Michael E. Himmel1; 1National Bioenergy Center, National Renewable Energy Lab, Golden, Colorado; 2Basic 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 monoclinic structure composed of repeating units of cellulose stabilized by interchain hydrogen bonds. Native cellulose is 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 bagasse cellulose is observed as organized long fibrils. 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 hydrodynamic bound network of the cellulose crystal is challenging, however, cellulose microfibrils (small bundles of cellulose) may be useful for arraying QDs. We have also examined the interactions...
between cyclodextrins (CDs) and (CdSe)Zn QDs. Cyclodextrins have
ring structures with discrete dimensions and the inner lumen of CDs in
hydrophobic character, which can accommodate the 8-14 CH2
chains of the TOPO molecules used to passivate (CdSe)Zn QDs
during growth. We attribute the CD-aided dispersion of TOPO-QDs in
water to overall entropic benefits afforded from hydrophobic
shielding of the surfactant alkyl groups. 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 reach the QD surface and 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 Yu1, Atul N.
Parikh1 and Dwight L. Pohl2, 1Department of Chemistry and Physical Biosciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, California, 2Department of Applied Science, University of California, Davis, California.

A novel lithography method has been developed for patterning 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 microfabrication 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 membrane patterns on colloidal particles allow presentation of biological signals (e.g. cell surface signalling molecules) to live cells. 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 polyelectrolytes 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 liable beneficial to include the contribution of biological and 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) on not only those potentially found in organic systems, on the crystallization of compounds containing 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 compounds 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 experiments 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. More 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 Shi2, Aaron Fairchild1, Zenith Kleine1, Tom Kuhn2 and Hong Liang1, 1Mechanical Engineering, University of Alaska Fairbanks, Fairbanks, Alaska; 2Chemistry 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 biomimetic performance at the interface are of great interest. In our research, we investigate effects of materials properties, such as wettability and texture of selected materials on cell culture. We conduct cell culture experiments on conventional materials such as glass, polyurethane, and polyvinyl alcohol. The effects of biomaterials surface properties on cell attachment and adhesion relationships are discussed in this presentation.

W4.9 Reflective Interferometric Detection of Tag-Free Biomolecules. Jinghui Lu1, Benjamin Miller2 and Lewis Rothberg1, 1Chemical Engineering, University of Rochester, Rochester, New York; 2Dermatology, 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 tags among complex mixtures on a silicon surface.

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 samples binding to their surface-bound complement 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 aptamers for which selective attachment chemistry can be developed. This technique can potentially be adapted to work under water.

W4.10 Glyceria Jaws: A Biocomposite of Metals, Melanin and Proteins. Dana Novak,1 Helga Lichtenegger2, John Harrell2, Nelle Slack1, Galen Stucky3 and Herbert Waite4; 1BioMolecular Science and Engineering, University of California, Santa Barbara, Santa Barbara, California; 2Materials Science and Testing, Vienna University Technology, Wien, Austria; 3Chemistry and Biochemistry, University of California, Santa Barbara, Santa Barbara, California; 4Materials, University of California, Santa Barbara, Santa Barbara, California.

Glyceria 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 under load-bearing conditions and are resistant to metalization. Unmineralized zinc and both mineralized and unmineralized forms of copper are present in Glyceria jaws. We have determined that Glyceria 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. Glyceria 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 Glyceria 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 Takai1, Yunying Wu1, Masao Kouno2, Hiroyuki Suginuara2 and Yasushi Inoue2; 1Center for Integrated Research Science and Engineering, Nagoya University, Nagoya, Japan; 2Department of Materials Processing Engineering, Nagoya University, Nagoya, Japan; 3Research Center for Nuclear Materials Recycle, Nagoya University, Nagoya, Japan.

Studies on some plant leaves revealed that the super-hydrophobic 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...
Micrometer-sized spherical vesicles have been found to assemble from homopolymer electrodyes and multicharged 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 amphiphilic monomers. 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 protein 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 how the requirements for sphere formation can be 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.

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. Phaseseparated 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 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 boundary conditions, a rich variety of stabilized superstructures can form. These include monodisperse ordered arrays of 15 nm diameter rafts and labyrinthine stripe scale feature. This work is supported by JSPS - RFTF99R13101 and ASTF.

Macroscopic Spherical Assemblies from Charged Polypeptides and Small Multivalent Counterions. Brandon John McKenna1, Henrik Birkedal1, Michael H. Bartl1, Timothy J. Deming2,1 and Galen D. Stucky1,2,1.

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8:30 AM W5.1
Organization of metallic nanoparticles using Tobacco Mosaic Virus templates, Erik Dujardin1,2, Charlie Peet1, Gerald Stubbs3, James N Culver1 and Stephen Mann1; 1School of Chemistry, University of Bristol, BRISTOL, United Kingdom; 2NanoScience Group, CNRS - CEMES UPR 8011, TOULOUSE, France; 3Biological Sciences, Vanderbilt University, Nashville, Tennessee; 2Center 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 emerging field of nanoelectronics 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 genetically engineered nanoparticles 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]-or [AuCl4]-complexes at acidic pH rise to the 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 on the inner surface of wild-type TMV. The number of encapsulated nanoparticles increased when Ag benzoate rather than Ag nitrate was used due to reduced supersaturation associated with the lower Ag/Ag+ redox couple, which enhanced the surface-tension 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. Solid State Mater. Sci., 2003, in press. [2] E. Dujardin, C. Peet, G. Stubbs, J. N. Culver, S. Mann, Nanlett., 2003, 3, 413-417.

8:45 AM W5.2
Molecular Biomimetics: Genetically Engineered Inorganic-Binding Polypeptides as Molecular Building Blocks, Mehmet Sarikaya1,2, Candan Tamerler1, Beth Traxler3 and Francois Baneyx1,4; 1Materials Science and Engineering, University of Washington, Seattle, Washington; 2Chemical Engineering, University of Washington, Seattle; 3Biomedical Engineering, University of Washington, Seattle, Washington; 4Bioengineering, 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 bio-technology. These engineered polypeptides are usually 7-10 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 applications. The potential of using engineered polypeptides is enormous due to the promise 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 techniques). Here we describe procedures of selection of inorganic-binding polypeptides using display technologies, 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 Douglas1,2, Mark Allen1,2, Michael Kleiman1,2, Deborah Williams1,2, Yves Idzerda1,2 and Mark Young1,2; 1Chemistry & Biochemistry, Montana State University, Bozeman, Montana; 2Center for Bionspired Nanomaterials, Montana State University, Bozeman, Montana; 3Plant Sciences, Montana State University, Bozeman, Montana; 4Montana State University, Bozeman, Montana.

The coat protein of the plant virus Cowpea chlorotic mottle virus (CCMV) forms a self-assembled icosahedral protein cage, 20 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 functional elements 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 the self-assembly of nanoparticles into films and fibers using genetically engineered viruses. Seung-Wuk Lee1,2 and Angela M Belcher1,2; 1Dept. of Materials Sci. & Eng., MIT, Cambridge, Massachusetts; 2Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas.

Genetically engineered M13 bacteriophage (viruses) were used to self-assemble various nanomaterials (ZnS, Au, fluorocine, and phycocerythrin) 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 site for depositing materials 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 composite films had chiral meso-metric 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 mimicked spider’s spinning process. For the spinning process, a virus solution containing highly soluble polyvinyl pyrrolidone, 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 envision 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)


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 collagen type-I in the form of fibrils. The distribution of minerals inside (intrafibrillar) and between the fibrils (extrabrinillar) defines the magnitude of mineralization and deformation 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 intrabrinillar 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 intrabrinillar mineral. This dissolution was lower than previously reported recession rates of 59nm/s for removal of extrabrinillar mineral in dentin exposed to 10% citric acid. Micro-Raman of partially demineralized dentin showed a phosphorous peak at 1080 cm^-1, suggesting that intrabrinillar 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 intrabrinillar mineral is orders of magnitude slower than in the extrabrinillar 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 moduli on demineralized dentin collagen supported by NIH/NIDCR P01DE09859.

11:00 AM *W6.2 Perturbed amelogenin protein self-assembly alters nanoparticle properties resulting in defective enamel formation. Michael L. Paine1, Wen Luo1, Janet Moradian-Oldak1, Hanson Fong2, Mehmet Sarikaya2, Shane N. White3 and Malcolm L. Sneed3; 1Center for Craniofacial Molecular Biology, The University of Southern California, Los Angeles, California; 2Materials Science and Engineering, University of Washington, Seattle, Washington; 3School of Dentistry, University of California, Los Angeles, Los Angeles, California.

The outermost covering of vertebrae teeth, enamel, is itself a unique composite bioceramic material that is the hardest tissue in the vertebrate body and a critical barrier against soft tissue access. 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 proteins by creating knockout 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 carboxy-terminus, that are crucial for the proper function of nanocollagen. We believe that the proteins are a key to the formation of enamel. 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 DNA techology to contain deletions to either of the two self-assembly domains; or 2) contain single amino acid mutations in the amino-terminal self-assembly domain. We have enumerated the results of the experiments performed 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 extracellular matrix is evident from the disorganization of fields of crystals which in the wild-type condition are woven together by ameloblasts to form a continuum. This weaving of crystals imposes unique materials properties and provides resistance 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).

11:45 AM W6.4 Calcium phosphate/collagen bone-like nanocomposite coating by electrochemical co-deposition. Yuwei Pan, 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 200mM and 24mM respectively. Soluble collagen was added into the electrolyte. After deposition, the coating was washed by distilled water.
water and fixed immediately by Kanovsky fixative then gradient dehydrated and critical point dried. The macro- and microstructure of the coating were visualized by scanning electron microscopy (SEM). 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 μm to 80 μm. In the demineralized coating, collagen keeps a similar three-dimension framework. The collagen fibres are as small as 70 nm in diameter. 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 intruded by increased pH. Once 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 R, J. Biomater. Sci., Polym. Ed., 13 (4): 518-527, 2002. 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 independent of the solution conditions (ionic strength, 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 mineralizing tissues.

1:45 PM W7.2
Modification of Brushite Crystal Growth by Citrate and Osteopontin. Rui-Kang Tang1, Christine A Orme 2, John R Hoyer 3, Ruikang Tang1, Christine A Orme 2, John R Hoyer 3, Leslie A Hrabar1, 1Materials Science and Engineering, Stanford University, Stanford, California; 2Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California; 3Lawrence Livermore National Laboratory, Livermore, California.

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 macromolecules related to mineral 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 carboxylic acid-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, 1.1x10^-6 mol m^-2 min^-1 was reduced to 8.5x10^-6 mol m^-2 min^-1 in the presence of 1.0x10^-6 M citrate. The reaction was completely suppressed at 2.0x10^-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-like to rod-like. This can be explained by establishing an energetic and stereochromical 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, further tests were performed on specimens with their effectiveness. (The work is supported by NIH grant #DE03273)

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. Wu1 and Reinhold H. Daaschardt1, 1Mechanical Engineering, Stanford University, Stanford, California; 2Materials 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 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 delpidization 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 testing temperature. These results reveal the highly anisotropic nature of SC mechanical properties including counterintuitive increases in SC debond resistance and strength with delpidization. Using novel macro-stress separation techniques, further tests were performed on specimens with dimensions approaching the length scale of the individual SC cells (30 nm). 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. Kruecz1, 2, Ravi K. Nalla1, 2, John H. Kinney1, 2, and Robert D. Ritchie1, 2, 1Materials Science and Engineering, University of California, Berkeley, Berkeley, California; 2Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California; 3Lawrence 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 fractal characteristics of the material. 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 arise linearly with crack extension with a mean crack-initiation toughness of $K_I = 2.0$ 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 interlamellar (IL) amorphous protein, the production of which was observed by tomography in the wake of the crack, identified as the dominant toughening mechanism responsible for the observed $R$-curve behavior through compliance-based experiments. The extent and nature of the IL zone gave rise to the possibility of a new technique 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. Golding, 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$^{-2}$), 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 primary structures of dragline silks arise primarily from the $\beta$-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 the presence of localized $\beta$-sheet-like regions of amyloid 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 fibrils that are 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, $\alpha$-helix - $\beta$-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 $\beta$-sheets into $\beta$-sheet crystals that crosslink the fibrillar network 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, their mechanical properties are similar to 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 protein-based materials that can form a robust polymer network. In addition, the silk-like fibers 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 route for the production of high-performance protein-based materials.

3:45 PM W8.4  
Do Natural Silks Make Good Engineering Materials? Natalie A. Morrison, Fraser I. Bell, Alexandre Beaumait, Joanne Ritchie, Christopher Smith; 1School of Engineering, University of California Merced, Merced, California, 2Chemistry, School of Chemistry 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 is based on 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 are gradually lost during 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 protein fibres in conditions of wet, humid packing occluded within hydrophilic domains. Given the above mechanical and environmental limitations, what realistic applications exist for silk-inspired materials? Webs are designed to minimize weight at the partial cost 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 in a number of alternative strategies for developing a realistic framework for fracture risk assessment, and for determining how the increasing propensity for fracture with age can be prevented.

4:00 PM W8.5  
Surface Roughness and Maximum Load Alter the Mechanical Properties of Lamellar Bone Measured by Nanoindentation.  

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, fracture rates are high, and with increasing age, failure occurs in cancellous bone. At the microstructural level, cancellous bone is composed of lamellae consisting of highly oriented mineralized collagen fibrils interspersed 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 roughness. 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 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  
Nonmechanical Characterization of Polyhema Co-Polymers for Bone Tissue Engineering Applications.  
Catherine Klapperich and Jie Song; 1Boston University, Boston, Massachusetts. 2Molecular 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 whether 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 Hystron Tribometer in a constant load 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 temperature-dependent mechanical properties of the materials using the Hystron feedback module. Upon completion of the experiments, the strain on the material is held constant, while the reduction in stress is measured as a function of time as the material relaxes and micro and nanostructural rearrangements dissipate energy. In creep experiments, an additional load was maintained on the sample. The load was increased until the material's strain rate was measured to control corresponding time constants that will be used to construct viscoelastic material models of the composite material and gel system.

4:30 PM W36.7
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 and spectroscopy on 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 intramolecular dynamics, the assembly of hybrid biological and nanoscopic 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. Advanced, proof-of-principle demonstrations of nanotube functionality and attachment of single molecules to these probes have been successfully made. Improved techniques for the attachment of single wall carbon nanotubes to flexible and well-characterized tools for AFM imaging are being developed. This work serves as a foundation towards 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 nanotubes using scanning probe lithography is an attractive idea for observing an 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 chemical and biological stimulation of biological responses in real-time with spatial resolution, including product-induced signal transduction.

4:45 PM W36.8
Regular, low density cellular structures—rapid prototyping, numerical simulation, mechanical testing, Jurgen Stampfl, Arthit Pisapan, Martin Mart, Mathias H. Luckner, Heinz E. Pettermann, Alexander Woss and Peter Fratzl; 1Inst. of Materials Science and Testing, Vienna University of Technology, Vienna, Austria; 2Max Planck Institute for Colloids and Interfaces, Potsdam, Germany; 3Inst. 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 material as the mean 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 electroforming, compression molding and wood-centered tools for AFM imaging. The 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 whole elastic tensor is derived from different relative densities is evaluated, from which the direction dependency of the Young's module is derived. In the 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 size effect. The experimental 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.

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 inappropriate 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 nanofabrication and microfluidic delivery techniques to create more physiologic-like tissue engineered constructs. Microfibrillar channels with microtopography, created in polymethylmethacrylate (PMDS) elastomers, will be micropatterned with various cell populations (Human Embryonic Lung Fibroblasts (HEL), Human Umbilical Vein 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 stimuli on cell function 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, chemical, and function. 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 process allows for the creation of uniformly sized pores in the range of 30 to 80 nm diameter determined by anodization voltages. 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 uniform pores and specific control of nanostructure was produced. To test the influence of this porous alumina on osteoblast adhesion, proliferation, and 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 attachment peptides, and osteoblast growth was compared to the unmodified surfaces. The modified membranes can be produced with highly defined pores of constant size and density and provide 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 W9.3/05.3
Use of Soft Lithography for Multi-layer MicroMolding (MMM) of 3-D PCL Scaffolds for Tissue Engineering.
Yung 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 to their specific needs of cell growth and function. Surface features smaller than 1-10 μm 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 poly(caprolactone) (PCL) scaffolds with pre-patterned patterns. Multiple layers of these scaffolds 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 stability. Second, micromolding in capillaries (MICM) 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 microcasting (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 micropatterned scaffolds enhanced cell attachment and proliferation, and encouraged cellular growth into the scaffold structure.

9:30 AM W9.4/05.4
Design and Fabrication of a Constant Shear Microfluidic Network for Tissue Engineering. Joesph J. Weinberg, Ryan K. Orrick, Eli F. Weinberg, and Joseph P. Vacanti, Biomedical Engineering Center, Draper Laboratory, Cambridge, Massachusetts; Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; Department of Pediatrics, Massachusetts General Hospital, Boston, Massachusetts; 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 parameter values of physiological systems such as the microcirculation within multiscale microfluidic 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 cell culturing within the NBR (Nano-liter Bio-reactor) chambers via injectors. The NBRs were sterilized by UV exposure. Biocompatibility was determined for different substrates and coatings of extracellular matrix materials. A fluorescent PicoGreen DNA assay was used to evaluate the viability and proliferation over 1-5 day period in comparison to a plan 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 NanoBioReactor 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 W9.6/05.6
Increased Function of Bladder Smooth Muscle Cells on Nano-Structured, Three-Dimensional Polymer Constructs.
Megan A. Patterson, 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 harnesses the properties of 3D structures closer 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 nanodimensional 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 and because we have previously experienced pressures 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 of cellular attraction to the surface. Exposure to pressure did not alter cellular adhesion or proliferation on materials, and cells experiencing sustained pressure preserved the same amount of extracellular collagen, and extracellular elastin as control cells. Cells experiencing sustained pressure, however, contained less intracellular elastin than control cells. These results indicate that 3D, nanodimensional scaffold systems created and studied in this research may be suitable bladder wall replacement materials.

11:00 AM *W9.7/05.7 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 protein 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 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. This system allows for easy synthesis of adlayers 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 a next-generation 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 W9.8/05.8 A Novel System for Self-Assembly of Muscle-MEMS Devices, Jinzhong (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 fuels in 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 further fabricating self-assembled complex 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 to 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-MEMS devices could be 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. Our soft photolithography process was 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 contractile cells must be developed to enable the cells and the integrated hybrid device 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 directing 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 and functions of the neonatal ventricular myocytes 1-3-day-old Sprague-Dawley rats (NIRVs), 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 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 microbots. The studies of the characteristics of these microbots will be also reported.

11:45 AM W9.9/05.9 Biomimetic Processing of a Biodegradable, Segmented-Polyurethane for Use in Tissue Engineering Devices, Danielle N Rockwood1, Jean S Stephens1, John F Rabolt2, Kimberly Woodhouse2 and Joanna Fromstein2; 1Materials Science and Engineering, University of Delaware, Newark, Delaware; 2Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada.

A segmented-polyurethane has been synthesized using an amino acid-derived disiocyanate and a phenylamine-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 advantages, 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 use as tissue engineering constructs. 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 protocols. 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 scaffolds will be able to support cellular attachment and growth. This will enable us to synthetically generate their own proteins. Over time, the polyurethane construct will be biodegraded and the cells will create their own ECM. I. S. Meglasi, J. Stephens, D. B. Chase and J. F. Rabolt, Macromolecules 38, 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, Hong-Ming Chu, Materials Science & Engineering, 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 form the basis 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 marine inorganic polymers. 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 structural attributes identified as essential for catalysis, yielding new structure-directing catalysts of polymerization. The silicafins and their biomimetic counterparts catalyze structure-directing synthesis from a wide range of precursors, yielding inorganic silica- or silicatein-substituted silicateins (silaffins) and organometallic silicatosiloxanes. We recently discovered that the silicafins also catalyze and structurally direct the hydrolysis and condensation of molecular precursors of metallo-oxanes such as titanium dioxide, silicon dioxide 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 polymers of these materials (e.g., nanotitanium dioxide) and these nanostructures form at the template surface. In some cases interaction between the condensing metallo-oxane and the template-directed biological materials yield new structure-directing catalysts of polymerization. We have elucidated the process of calcite crystal formation on SAMs while studying how the molecular interactions are mediated by specific binding of cellular receptors to adsorbed proteins or engineered motifs on the surface of the material. We have determined that the orientation and shape of calcite 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 activity 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 biomimetics from their initial nucleation during biomineralization.

**4:30 PM W10.6**

*Semiconductor Surfaces with Nanomaterial Features Composed of TAT Peptides.* Yellowman Cools and Aliena Ivanovs; 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 the surface, a two-step modification procedure was carried out. Clean surfaces were silanized to terminate them on amine groups, and subsequently reacted with a heterofunctional cross-linker. TAT peptides (e.g., CGYSGRKRRQRR) which exhibit rapid uptake in cells, were
structures in moving and organizing raphides within developing cells.

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 a 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 that encode, or genes 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.

3:45 PM W10.0
Probing Crystal Growth and Adhesion of Calcium Oxalate Crystal Surfaces: Toward an Understanding of Kidney Stone Formation, Michael David Ward1, Jeffrey A. Wesson2, Xiaoxin Sheng1 and Taesung Jung1, 1Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, Minnesota; 2Nephrology Division, Department of Veteran Affairs Medical Center and the Medical College of Wisconsin, Milwaukee, WI.

Kidney stones are biomimetic mineral aggregates, most commonly containing calcium oxalate monohydrate (COM) crystals as the primary constituent. Notably, in vivo 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 (121), and real-time acquisition provides characterization of the growth modes and rates of growth along different crystallographic directions. These measurements also allow investigation of adhesion processes and the role 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 carbohydrate group on the (010) face of COM. Furthermore, the adhesion forces depend upon the crystal face (e.g., COM (010) vs. (100)), and our AFM 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 surfaces, 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.
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-sheets by X-ray scattering. By combining peptide and synthetic polymer blocks, unprecedented control over hierarchical order is demonstrated.

W11.2  
In-situ Formation of Biphasic Calcium Phosphate Bioerodibles from Goniopora Exoskeleton, Murugan Ramalingam1, S. Ramakrishna2 and T.S. Sampath Kumar3; 1NUS Nanoscience & Nanotechnology Initiative, School of Biological Sciences, National University of Singapore, Singapore, Singapore; 2Dept. 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 bioerodibles, in particular hydroxyapatite (HA) and beta-tricaium 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) bioerodibles have been attracted for many biomedical applications owing to their controlled bioreosorption, 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 bioerodibles from goniopora exoskeleton and precursors containing hydrogen-bonding 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 media at pH 7.4 under in-vitro physiological conditions and indicating that the solubility of BCP lay 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 Ahn1, Tai Young Kim1, Sang Hoon Lee1, Doo Ho Yang2 and Jong-Man Kim3; 1Department of Chemical & Biological Engineering, Korea University, Seoul, South Korea; 2Department 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 can change their 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 to date. However, most polydiacetylene-based chemosensors reported 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 delocalization capable of enhancing the strength of hydrogen-bonding of the resulting assemblies (J. Am. Chem. Soc. 2003, 125, 8576-8577). This discovery on the role of enhanced hydrogen-bonding in color change should open new pathway 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  

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. 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 superhydrophobic properties. We have designed and implemented a novel cell based sensor that determines single cell sensitivity and response time by using 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 sensors as well as measurement electrodes. We have formed single cell array sensors using 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 analytic 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 were confirmed and hence the verity of the SPV has been established. This forms the basis of developing a medical diagnostic 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. Je Song1, Justin S. Cisar2 and Carolyn R. Bertozzi3; 1Materials Sciences Division, Lawrence Berkeley National Lab, Berkeley, California; 2Chemical and Environmental engineering, University of California Riverside, Riverside, California; 3Howard 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 placing polar headgroups on both ends of the diacetylene lipids in a transmembrane fashion, and altering the chemical nature of the polar residue, 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 colorimetric response of the poly(ene-yne). These results establish the foundation for further development of BPDAs-based colorimetric sensors.
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 composite scaffolds made of nanofibrous macroporous scaffolds 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 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 10 w/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. Moreover, nHAP incorporation 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.

Block copolymer-based membrane technology represents a versatile method for fabricating protein-functionalized nanoscale devices. Focused towards characterizing the effects that various pattern formations can have on the efficiency of protein functionality as well as design of fabrications of a wide variety of nanoscale devices.

Due to their biocoercible characteristics biodegradable polymers are attractive materials for sutures, fracture fixation devices, and drug delivery systems. Recently, a number of degradable polymers by modifying poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) backbones with hydrophobic diacids in order to tune the hydrophilic-hydrophobic balance (HLB) in the polymers. This modification was accomplished in block copolymers of PLA with diacid-containing chain ends. Due to their bioresorbable characteristics biodegradable polymers are extensively used in many industrial applications (tires, gloves, shoes, etc). However, new applications with integrated nano-sized macropores were frequently suggested. On the other hand, there is a need for biomaterial candidates for applications such as band-aids, prostheses, 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 natural Latex 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 anisotropy of the elastic behavior, which involves the sulfur bonds between polypeptide chains. The influence of the fabrication process is also investigated by the comparison between the clastoproperties of membranes obtained by dip-coating and spin-coating techniques.
polymer backbone. An observation that is counterintuitive is that increasing the diacid linker length, which should increase hydrophilicity and 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 the polyester side chains and their positions on the degradation rate is crucial for us to custom design surface eroding polymers for specific applications.

W11.11 Surface modified diamond field effect transistors for enzyme immobilized biosensors, Kwangsong Song1, Hirofumi Kanazawa1,2, Yusuke Nakamura1,2, Syota Kawamura1,2, Munemori Degawa1,2, Hitoshi Umezawa1,2 and Hiroshi Kawarada1,2

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 transducers. 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 SGFETs operate based on the bio-converted decomposition of glucose by GOD. From this bioconverted decomposition, the pH changes induced by a spontaneous hydrolysis of D-glucono-o-lactone to gluconic acid decreasing pH near GOD immobilized channel surface can be registered with SGFETs. The immobilized biosensors.

W11.12 Directed Osteoblast Adhesion at Metal Particle Boundaries: Promises for Nanophase Metals, Thomas Jay Webster, Jeremiah Eujofor 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 constituent 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 study was to design, fabricate, and evaluate osteoblast adhesion on nanophase compared to conventional metals (specifically, Ta, Ti, Ti6Al4V, and CoCrMo). Results of this study provided the first evidence of increased osteoblast adhesion on nanophase compared to conventional metals. Moreover, 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-containing 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, Yuji J. Chen1 and Peter X. Ma1,2,3

Biomedical Engineering, University of Michigan, Ann Arbor, Michigan; 2Biologic and Materials Sciences, University of Michigan, Ann Arbor, Michigan; 3Macromolecular 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 a temporary extracellular matrix (ECM) to support cell growth and tissue regeneration. Cells are incorporated into the porous scaffold, and the cell-scaffold composite is used 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 scaffold proteins in the ECM, 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 ECM. Here, we use the reverse solid freeform (SSF) 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 using a 3-D printer using 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 leaves the nano-fibrous scaffold. After washing the scaffold, the THF is exchanged with water, and the wax is leached out with organic solvents. Water is then exchanged with the solvents, and the scaffold 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 printed scaffold 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 is a priori allows for the fabrication of scaffolds with anisotropic pore structures which potentially could be useful in tissue engineering, 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, internal structure, and external shape of the scaffold, this SSF fabrication/phase separation technique has great potential to design and create ideal scaffolds for bone tissue engineering.


Bone and dentin mineralization 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 creation of the nanometer level. Also, it has been suggested that the nano-fibrous scaffolds emerged from the solution underwent simultaneous aggregation and deposition as apatite crystals. However, the growth and aggregation of the calcium phosphate particles were inhibited in the presence of DMPI 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 of controls, which are buffers without DMPI or with a concentration of serum albumin. AFM analysis demonstrated that DMPI self-assembled calcium phosphate clusters into nanorods with uniform size at 20±100 nm. On the other hand, when immobilized on a glass surface, DMPI actively entraps calcium phosphate clusters from solution. The nucleated amorphous calcium phosphate precipitates ripen and nanocrystals form. Subsequently, these expand and coalesce into micrometer crystals elongated in the c-axis direction. Characterization of the functional domains in DMPI 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 DMPI plays a dynamic role in controlling 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 Silva1,2, Julio Goes2, Sonia Figueiredo1 and Antonio Sombrã1,2; 1Departamento de Química Orgânica e Inorgânica, UFC, Fortaleza, CE, Brazil; 2Departamento de Física, 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 on 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 crystallites on collagen film. The methods used comprised two steps. first, the films had been soaked in a Ca(OH)2 or CaCl2 aqueous solution for 30 min, and the second, the films were soaked in a Na2HPO4 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 and calcium phosphate crystal phase formation on surfaces of collagen films.

**W11.16** Abstract Withdrawn

**W11.17** Interaction Between Titanium Implant Surface and Hydrogen Peroxide in Biologically Relevant Environments. Julie J. Muycc1, Joanna M. McKittrick1, John A. Frangos2 and Christine A. Orme2; 1Chemistry and Materials Science, LLNL, Livermore, California; 2Materials Science and Engineering Program, University of California, San Diego, La Jolla, California; 3La Jolla Bioengineering Institute, La Jolla, California.

Titanium is a widely used material for load-bearing and dental implants. Over the decades of its 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 on titanium. 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 in vitro and in vivo in terms of the ability to form bone mineral. Correlation between the titanium 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 Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.

**W11.18** Fracture of Calcium Phosphate Cement with Second Phase Organic Additions. Victoria C. Jaw 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. A major limitation of their injectability, bioabsorbability, 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, recent studies have demonstrated 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 focused 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 Casper1,2, Jean S. Stephenson1,2, Nori Yamaguchi3,4, William Yang2, Cindy Farach-Carson2,4, Krissi L. Kick3,4, D. Bruce Chao1,5 and John F. Rabolt1,6; 1Materials Science and Engineering, University of Delaware, Newark, Delaware; 2Biological Sciences, University of Delaware, Newark, Delaware; 3DuPont Central Research and Development, Experimental Station, Wilmington, Delaware; 4Delaware 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 diameters, high surface area/volume ratios, and interconnected fibrous network make electrospin 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 (≥500 V/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 electrospin PEO fibers at a concentration of 4 micrograms per milligram of electrospin fibers. Energy Dispersive Electron Spectroscopy (EDX) and Ultraviolet Spectroscopy were used to confirm the presence of heparin in the electrospin 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 prolylcarboxylylase, on the surface of collagen I-based scaffolds has been used to increase the bioactivity and growth factor binding ability of the electrospin 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 that 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 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: a) alcoholic fermentation, drug-delivery, cell preservation, and cell viability.

**W11.21** Abstract Withdrawn

**W11.22** Biodegradable Porous Polyurethanes Scaffolds for Tissue Repair and Regeneration. Katarzyna Gornia 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 of 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 cells that will provide the necessary strength required for 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 some preliminary results of functionalized films from new biodegradable polyelectrolytes 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 the treatment of large bone defects, defects of articular cartilage and nerves and cardiovascular tissues, to mention but a few. Structural scaffolds from biodegradable polyelectrolytes promote healing of critical-size defects and osteoblast adhesion and differentiation on top of the different films over a period of ten days in culture. While the native films are poorly adherent, the RGD-functionalized ones are extremely attractive to cells with an increase adhesion and proliferation of a four-fold functionalized film. Additionally, the films were 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 remote control of the cells 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.

8:30 AM W12.1
Primary osteoblasts adhesion onto RGD functionalized and crosslinked polyelectrolyte multilayers films. Catherine Picard,4 Rene Elkaff3, Pierre Schard,4 Benoit Frisch1 and Jean-claude Vogel1.1, INSEM U595, Strasbourg, France; 2Parogene, Strasbourg, France; 3Institut Charles Sadron, CNRS, Strasbourg, France; 4Laboratoire de Chimie Biologique, 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(algic), and PLL/Poly(galacturonie) films. The buildup of the film and the adsorption of the PGA-RGD could be followed by ellipsometry, FTIR, and QCM, with an average overall film thickness of 15 nm. The orientation of the lipid film, with the polar head group at the air/substrate interface, may be used to coat a substrate 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 was 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 W12.2

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, three different methods of coating 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 was then deposited on the gold surface, was then heated to cause intermingling of the fatty acid and alkanethiol chains, and cooled to form a very stable film which withstood repeated rinsing and solution exposure. Presence and stability of the film was confirmed by ellipsometry, FTIR, and QCM, with an average overall film thickness of 15 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 was used. The GM1 ligand may be used as such, with the saccharide head group showing a high degree of affinity for cholera toxin.

9:30 AM W12.3
An electronic retinal interface for single cell stimulation. Neville Z Mehtani1, Greg S. Tsien2, Harvey A. Fishman3 and Stacey F. Bent1.1 Chemical Engineering, Stanford University, Stanford, California; 2Electrical Engineering, Stanford University, Stanford, California; 3Ophthamology, 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 limited 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 prosthetic 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 these challenges, we have developed microelectrode patterns, 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 cultured 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, additional studies were pursued to investigate these parameters and their effects. Electrode arrays with different geometries were microfabricated, and single 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 microenvironment, using an electrode array. 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 and3Dan Jones; Chemistry, Penn State University,
University Park, Pennsylvania.

We have prepared synthetic cells in which a lipid bilayer membrane encapsulates a liquid volume within a monolayer shell. The fluid within these capsules is isolated from the aqueous phase by the lipid monolayer. In order to control the lifetime of the liquid volume, we have designed and fabricated monolayers that can be pumped from a single hydrophobic chain, squeeze 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
GPL-linked neurologin. Sophie Pautot1, Hanson Lee2, Camin
Dean2, Ethel Isaccoff and Jay T Groves1,3, 1MSD, LBNL, Berkeley, California; 2MB, UC Berkeley, Berkeley, California; 3Chemistry, UC Berkeley, Berkeley, California.

In recent years membrane constituted proteins in supported pattern bilayers have been used to visualize intercellular communication between cells. Different cell conformations can be micro-patterned in supported bilayers and long range lateral mobility required for activation of cellular response may be controlled. Here we present a new method for controlling the distance between cells in a lipid bilayer that contains a pattern of membrane proteins, such as NLG1, and that by controlling NLG pattern we can direct the cell binding and the synapse formation to specific location on our substrate. This novel micro-scale patterning of functional membrane-associated proteins is the first step toward cell based technological applications.

11:00 AM W12.8

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 transduction such as multiple sensor transduction that are useful for the biogenration 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 W12.5

Transport Properties and Surface Microstructure of
Lipid-Monolayer Coated Microbubbles. Mark Andrew Borden1,
Gang Pu1 and Marjorie L. Longo1,2; 1Chemical Engineering &
Materials Science, University of California, Davis, California; 2Biophysics, 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 monolayer Langmuir monolayers demonstrate the relationship between composition, microstructure and transport properties of the lipid shell and provide insights 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 stretch 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, shedding of rigid monolayers exhibit crumpling and shedding in distinct cycles. We propose a qualitative mechanism involving zipperering 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 that are necessary 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.
In a three-step thermal treatment that includes foaming through the decomposition 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 (≥ 81% porous) as an osteoconductive material. This has been achieved through an analysis 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 anhydrous 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 promote mineralized extracellular matrix impregnating the three-dimensional titanium scaffold, are measured in vitro.


SESSION W13: Tissue Engineering II
Chairs: Ching-Chang Ko and Rizhi Wang
Friday Afternoon, April 16, 2004
Room 203 (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 sequence of enzymes, substrates and inhibitors can be used to form localized and structured matrices with a wide range of gel. This raises the possibility that time delayed reagents by cells, into the adjacent extracellular 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 Wang1, Alexandra E Porter2, Myron Spector3, Zhou Xiang3 and Alyssa Panitch4; Biomedical and Materials Sciences, University of Michigan, Ann Arbor, Michigan.

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 generalization inferred previously, which occurs in three distinct, and probably unrelated, stages likely correlated with different proteins present at different time periods. (The last of these is type I collagen arriving between 6 and 7 days.) The earlier precursors are being sought using immunohistochemical TEM methods.

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

The extracellular matrix is a complex biological scaffold with proteins that vary depending on the type of tissue, disease state, position within the body and external signals. Several attempts have been made to mimic the structural, mechanical and signaling environment of the matrix. Many of the mimicking strategies have incorporated proteins, biopolymers, organic 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 failed 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 or cell adhesion. Information garnered from both analytical evaluation and in vitro testing of these materials will be used to develop next generation 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.
Polymeric chelate is 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 polymethylacrylate (PDMS), as a sacrificial scaffold in which to grow inorganic materials. Our system is biomimetic, as it replicates 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 microribbons. These ribbons include spheres, bowls, nets and corallike matrices. We also completely fill the original swollen gel, to create and inorganic complement of the swollen gel. 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.
S. Panitch, Youngjun Won and Peter X Ma; Biologic and Materials Sciences, University of Michigan, Ann Arbor, Michigan.

The architectural design and surface properties of scaffolds are important aspects in tissue engineering. The porous scaffolds provide the environment to accommodate cell growth and minimize the distance between the cell and the hydrogel. While the nature of surface of the scaffolds can directly influence the attachment, proliferation, and ultimately tissue regeneration. In this work, a highly porous poly(lactic acid) (PLLA) scaffold with nano-fibrous architecture of porous PLLA scaffold. The scaffolds were fabricated by mimicking the structure of natural collagen using a novel thermally induced phase separation method developed in our group. A ubiquitously effective surface modification method was developed, and a nanofibrous gelatin scaffold 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 by only physical entrapment. The surface coverage of gelatin on PLLA reached as high as 35.4% by using physical entrapment followed by chemical crosslinking. The surface hydrophilicity increased with the amount of gelatin on the surface of the scaffold. MC3T3-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.
Nanotechnology embraces a system whose core of materials is in the range of nanometers through nanoscale dimensions. In this context, 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 residual sizes 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, was a key parameter that enhanced functions of osteoblasts in 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 Storrie1 and Samuel I. Stupp2,3,4
1Department of Chemistry, Northwestern University, Evanston, Illinois; 2Department of Materials Science, Northwestern University, Evanston, Illinois; 3Feinberg 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 for zinc binding and a second arm for ligand binding coupled to a structural unit specifically in the range of 1-100 nm (nanostructured), specific interaction of zinc ions with the PA, and indicates its ability 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 cell 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 have designed a novel PA that has a branched peptide head group with an 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 gels at low pH concentrations (<1% w/v) and provide a three-dimensional scaffold for cell growth that is similar to the extracellular matrix. We have show ion-dependent self-assembly of branched PAs in the presence of zinc ions and confirmed histidine-zinc interactions by MRI, 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-Gelatin Nanocomposites For Bone Regeneration: A Preliminary Report. Ching-Chang Ko1,2,3,4, Ying-Lien Wu1,2,3,4, R A Narayanan1,2,3,4, Wei-Shou Hu1,2,3,4
1Oral Science, University of Minnesota, Minneapolis, Minnesota; 2Division, Air Force Research Laboratory, Dayton, Ohio; 3Feinberg School of Medicine, Northwestern University, Chicago, Illinois.

A biomimetic process has been developed to fabricate hydroxyapatite-gelatin (HAP-GEL) nanocomposites for bone regeneration. We hypothesized 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.

4:00 PM W13.9
Synthesis of Calcium Carbonate Nanoparticles for Drug Detoxification. Debra L. Nims1, Matthew K Runyon and Bethany L Johnson-Kerner
1Chemistry, 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 restoring damaged blood vessels and preventing 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-linear reaction-diffusion equations 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 threshold response of the junctions between vessels, and suggests a hypothesis that hemostasis influenced the evolution of mammalian vascular networks.

4:30 PM W13.11

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.
index.

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 information 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.M.; de Gelder, R.; Grawinckel, 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) 772-775.

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