SYMPOSIUM N

Biological and Biomimetic Materials-Properties to Function

April 1 – 5, 2002

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* Invited paper

TUTORIAL

ST N: *IN-SITU* AND *EX-SITU* CHARACTERIZATION TECHNIQUES AND IMAGING OF BIOMATERIALS
Monday, April 1, 2002
1:00 p.m. - 5:00 p.m.
Nob Hill A/B (Marriott)

This tutorial focuses on in situ and ex-situ experimental methods that provide information about molecular structure, 3D architecture, surface morphology and dynamics of biomaterials. The tutorial will be comprised of three lectures covering novel methods for 2D and 3D imaging of biostructures (AFM, TEM, tomography), molecular and cell biology techniques, tissue engineering, as well as basic tutorial on Crystallization Laws in Biomineralization as revealed by the above techniques (Ostwald rule and amorphous phases; Fundamental phenomena and parameters; Modification of growth by large and small molecules). Each lecture will cover the basic experimental approach including methods of sample preparation and important technical issues, and examples of applications to both inorganic and organic systems will be presented.

Instructors:
William J. Landis, Northeastern Ohio Universities College of Medicine
Alexander A. Chernov, Universities Space Research Association
James J. De Yoreo, Lawrence Livermore National Lab

SESSION N1: MATERIALS IN NATURAL BIOLOGICAL TISSUES
Chair: Joanna Aizenberg
Tuesday Morning, April 2, 2002
Metropolitan I (Argent)

8:00 AM *N1.1
PROTEIN-MINERAL INTERACTIONS IN SITU IN NORMAL AND PATHOLOGIC VERTEBRATE BIOMINERALIZATION.
Marc D. McKee, McGill Univ, Fac of Dentistry, and Fac of Medicine, Dept of Anatomy and Cell Biology, Montreal, CANADA.

Extracellular matrices (ECMs) in vertebrates are typically multicomponent systems whose constituent proteins form large, extended macromolecular assemblies, which in bones and teeth, are competent for biomineralization. Such organic matrices are considered to consist primarily of two classes of proteins, one structural and commonly fibrillar (such as collagen), and the other inductive for, or supportive of, tissue-specific events (such as calcification and cell adhesion). In mineralized tissues, the structural and material organization of matrix and mineral generally reflects the history of cell activity at the local level. Thus, 'blast'/secretory cells (e.g. osteoblasts, odontoblasts) are directly responsible in each case for initiating and producing an ECM that has an architecture and molecular composition designed for nucleating and guiding crystal growth processes appropriate to the biomechanical properties required for a particular tissue. Although much is known about hard tissue ECMs, the molecular interactions governing their construction have yet to be fully elucidated. Our work focuses on how intra- and inter-fibrillar (collagen) calcification is mediated by proteins given the spatial and stereochemical constraints of molecular and tissue architecture. Using data derived mainly from ultrastructural (electron microscopic) approaches to examine matrix-mineral relationships, and from analyses of transgenic mice having targeted gene manipulations of mineral-regulating proteins, this presentation will focus on our knowledge of protein regulation of crystal growth in vertebrate mineralized tissues. In particular, data will be presented on protein-mineral relationships in normal bone, cartilage and tooth ECMs, and for pathologic, ectopic (extraskeletal) mineralization such as found in urolithiasis, vascular calcification and tumor calcification. The presentation will detail the activities of two noncollagenous proteins, showing that matrix Gla protein (MGP) inhibits the induction/nucleation of calcification in the otherwise 'soft' ECMs of arteries and cartilage, and that osteopontin (OPN) regulates/inhibits crystal growth in 'hard-tissue' (including pathologic) ECMs by binding directly to crystal surfaces.

8:30 AM N1.2
EXTRACELLULAR MATRIX MOLECULES INVOLVED IN BARNACLE'S SHELL MINERALIZATION.
Maria S. Fernandez, Italo Vergara, Alejandro Oyarzun, Jose I. Arias, Renato Rodriguez, Juan P. Wiff, Victor Fuenzalida, Jose L. Arias, Faculty of Veterinary Sciences, Universidad de Chile and Center for Advanced Interdisciplinary Research in Materials Science, Santiago, CHILE.

Austromegabalanus psittacus is a large sessile balanomorph barnacle from the coast of Chile and Peru. Its hard shell is composed of twelve calcareous side plates, six paries and six radii, joined in the form of a truncated cone opened at the top. Plates rest on a basal disk firmly cemented to the substratum. Although the crystalline microstructure of barnacle's shell has been studied to some extend, their organic composition and the mechanisms governing the biomineralization of such highly ordered nanocomposite have remained obscure. By using X-ray diffraction, infrared spectrometry, SEM and TEM electron microscopy, histochemistry, immuno-histochemistry and -ultrastructure, biochemistry and crystallization assays, we have studied the cell-shell interactions, the crystalline microstructure of the inorganic moiety and the localization of particular macromelecules, and tested their crystallization ability. The mineral of the plates and basal disc is calcite showing a (104) preferential orientation. Plates are not solid but porous. While paries have longitudinal canals (from the base to the apex), radii have transversal canals arranged parallel to the base. These canals are not in the center of the plates but displaced to the outside of the shell delimiting a thinner solid outer lamina and a thicker inner one. The inner lamina consists of parallel calcified layers separated by organic sheets. These sheets showed autofluorescence and consisted of chitin surrounded by proteoglycans and other minor proteins. These organic sheets are also organized as several concentric rings around the canals. The shell matrix obtained after decalcification, which surrounded the crystals, also contains a loosely net of such proteoglycans. Mantle epithelial cells cover the entire surface of the inner side of the inner lamina and extend to the plate canals. While isolated chitin does not promote or alter calcite crystallization, the proteoglycan-rich fraction dramatically modifies crystal morphology and size. As we have demostrated in another model of biomineralization, such as the eggshell, hereby we suggest that these structured polyanionic proteoglycan moieties are also responsible for the regulation of the barnacle shell mineralization.

8:45 AM N1.3
HARD TISSUE IN INVERTEBRATE JAWS: PROTEIN AND METAL INSTEAD OF MINERALIZATION.
Helga C. Lichtenegger, Galen D. Stucky, Univ of California,Santa Barbara, Dept Chemistry, Santa Barbara, CA; Julian C. Thimm, Ratneshwar Lal, Univ of California, Santa Barbara, Neuroscience Res Inst, Santa Barbara, CA; J. Herbert Waite, Univ of California, Santa Barbara, Dept of Molecular, Cellular and Developmental Biology, Santa Barbara, CA.

A very common strategy by nature to harden animal tissue is by heavy mineralization as it is observed in teeth and bones of humans and other vertebrates. Quite in contrast, the mandibles of some invertebrates like polychaete worms consist of mainly protein and a low amount of metal. Nevertheless, these materials are hard and can form sharp cutting edges. Due to the low amount of inorganic components, the material is also very light-weight. A combination of x-ray scattering, atomic force microscope (AFM) and spectroscopic techniques was used to investigate the fine structure of polychaete worm jaws, as well as the role of metal for their mechanical properties. The mandibles of two different worm species, Nereis sp. (clam worm) and Glycera sp. (blood worm), containing up to 5% w/w zinc (1) and 13% w/w copper (2), respectively, were studied. In both systems, the metal concentration is higher in parts subjected to higher mechanical stress like the very tip of the jaw. Small angle x-ray scattering (SAXS), x-ray diffraction (XRD) and AFM revealed a preferred orientation of fiber like structures at nanometer scale along the longitudinal direction of the jaw. Element maps from electron microprobe measurements were used to correlate the local element concentrations with the local mechanical properties obtained from indentation (3).
1. G.W. Bryan and P.E. Gibbs (1980) J. mar. biol. Ass. U.K. 60, 641-654.
2. P.E. Gibbs and G.W. Bryan (1980) J. mar. biol. Ass. U.K. 60, 205-214.
3. A.N. Parbhu, W.G. Bryson and R. Lal (1999) Biochemistry 38, 11755-11761.

9:00 AM *N1.4
MOLECULAR MECHANISM OF BACTERIAL MAGNETITE FORMATION AND ITS APPLICATION.
Tadashi Matsunaga, Dept of Biotechnology, Tokyo Univ of Agriculture and Technology, Tokyo, JAPAN.

Magnetic bacteria are of considerable biochemical interest with regard to how they carry out controlled biomineralization of magnetite (Fe_3O_4). Each biomagnetite (bacterial magnetic particles: BMPs) is enveloped by a membrane and aligned into chains, which impart a magnetic dipole to the cell. The size varies from 50 - 100 nm in diameter, and number over 10 per cell. BMPs have a single magnetic domain and superior dispersion characteristics in aqueous solution. In order to isolate and characterize the genes related to biomagnetite formation, we employed two approaches: one is the analysis of non-magnetic mutants generated by transposon mutagenesis, and the

other is the identification of BMP-specific proteins.
By transposon mutagenesis, 60 non-magnetic mutants of *Magnetospirillum-magneticum* AMB-1 have been obtained. Sequence analyses of the flanking region of transposons led to the isolation of *magA* that encodes an iron transport membrane protein on BMPs. SDS-PAGE profiles revealed five specific proteins in BMP membrane fraction. Genes encoding two of these five proteins were isolated; the *mpsA* gene shows homology with acyl-CoA transferase of *E. coli* and the *mms16* encoding protein that exhibits GTPase activity. We have hypothesized that BMP formation starts from the invagination of the cytoplasmic membrane. Mms16 serves to prime vesicle budding similar to eukaryotic small GTPases. MpsA might be involved in Mms16 anchoring to membranes. MagA on the vesicle membrane accumulates iron into the resulting vesicle. Moreover, the whole genome sequence of *M. magneticum* AMB-1 is ongoing and several genes (clusters) as candidates for the role in biomagnetite sysnthesis have been obtained. Furthermore, we developed a novel technology for the display of functional proteins, such as proteinA and receptors, on BMPs through gene fusion using MagA and Mms16 as an anchor proteins. BMPs on which protein A is displayed have been successfully applied to the immunoassay.

9:30 AM N1.5
WHELK EGG CAPSULE BIOMATERIAL, (BUSYCON SPP.): A STRUCTURAL AND MECHANICAL INVERTEBRATE KERATIN ANALOG. Scott Rapoport, Robert Shadwick, Scripps Institution of Oceanography, University of California at San Diego, San Diego, CA.

Whelks, a variety of marine snails, produce an interesting biological elastomer whose apparent purpose is to protect and isolate developing embryos in the marine environment. Our work on this material details the results of quasi-static testing and simple biochemical analyses in order to determine what the relationship of this materials' constituents is to other common structural proteins such as keratin, collagen and elastin. Results indicate that this self-assembling polymer has intrinsic similarities in both mechanical and structural aspects with alpha-keratin. Since invertebrate extracellular keratins have not been previously described, this material represents not only an opportunity to succinctly expand keratins into the invertebrate domain, but also an opportunity to examine structural differences between these various forms of keratin in order to ascertain how these differences might contribute to the development of similar mechanical properties. In other words, given two structural proteins with structural similarities on the secondary and tertiary hierarchical levels, yet with very different macroscale arrangements, what are the important factors that determine what these materials' mechanical response to strain will be? Furthermore, the formation of this bulk material requires several discrete steps to arrive at the native, mechanically distinct form. Therefore, it should be possible to examine the self-assembly process as it pertains to the generation of mechanical function by stabilizing precipitations of fibers artificially with crosslinking reagents.

9:45 AM N1.6
ELECTRON DIFFRACTION STUDIES ON THE BRYOZOA. Simon R. Hall, Sean A. Davis, Stephen Mann, University of Bristol, School of Chemistry, Bristol, UNITED KINGDOM; Paul D. Taylor, Department of Palaeontology, The Natural History Museum, London, UNITED KINGDOM.

Electron microscopy and electron diffraction were used to investigate mineral crystallites dissociated from the skeletal walls of six species belonging to the Bryozoa, a phylum of predominantly marine colony-forming invertebrate animals. Four cheilostome bryozoans (*Flustra foliacea, Membranipora membranacea, Thalamoporella novaehollandae and Cellarinella foveolata*) and two cyclostomes (*Fasciculipora ramosa and Hornera robusta*) were analysed. In each case, an attempt was made to relate the crystal morphology imaged in situ by scanning electron microscopy with the crystallographic orientation of isolated crystals determined by electron diffraction analysis in the transmission electron microscope. The results showed that the calcitic cheilostome and cyclostome skeletons consisted of closely packed arrays of plate-like Mg-containing calcite crystallites, and that the crystallographic *a*-axis was preferentially aligned perpendicular to the top and bottom surfaces of the flattened particles. The results suggest that calcite biomineralization occurs under similar crystallographic constraints in the five species studied, even though the cheilostomes and cyclostomes are separated by over 300 million years in the fossil record of the Bryozoa. Similar studies for the aragonite crystallite skeletons of *Membranipora membranacea* indicated that the crystallographic *b*-axis was preferentially oriented perpendicular to the basal surfaces of irregular plate-like particles. To our knowledge, this is the first time that electron diffraction has been used to study this phylum.

10:30 AM *N1.7
HIERARCHICAL STRUCTURE AND MECHANICAL FUNCTION OF BIOLOGICAL MATERIALS. Peter Fratzl, Erich Schmid Institute of Materials Science, Austrian Academy of Sciences, and University of Leoben, AUSTRIA.

Biological materials, such as wood, tendon, bone or teeth are optimized for their mechanical function by a complex hierarchical design. Wood, for instance, is composed of parallel hollow tubes, the wood cells. The cell wall is a composite of cellulose fibrils (with a diameter of 2.5nm for spruce wood) in a matrix of lignin and hemicellulose. Within the main cell-wall layer (S2), the cellulose fibrils are spiralling around the lumen of the cells. The corresponding microfibril angle (MFA), measured with respect to the tube axis, can vary in a wide range. Combined x-ray diffraction and tensile testing experiments have shown that mechanical parameters, such as Young's modulus and the fracture strain, change by more than an order of magnitude when the MFA varies from 0 to 50 degrees. It turns out that the MFA is one of the major structural parameters by which the growing tree can adapt the material properties in the trunc and in branches to the actual needs. Tendon is a fibrous tissue made of proteoglycan-coated collagen fibrils, which themselves consist of periodically staggered tripel-helical collagen molecules. Simultaneous tensile testing and synchrotron x-ray diffraction has shown that, at a given load, the total strain of the tendon is larger than the strain in the fibrils, which in turn is larger than the extension of the molecules. These hierarchies of deformation can be explained in a model where the composite character of the tendon structure is taken into account. Finally, a combination of local probes (based on x-ray scattering, scanning electron microscopy, infrared spectroscopy and nanoindentation) was used to elucidate the influence of mineralization on the mechanical properties of collagen-rich tissue, such as bone or dentin. Most notably, not only the quantity of mineral particles, but also their size and orientation have a decisive influence on mechanical properties, such as the stiffness for instance. In summary, more information is needed on all hierarchical levels, as well as on the interplay between those hierachies, in order to reach a full understanding of the remarkable mechanical properties of biological materials.

11:00 AM *N1.8
TISSUE REGENERATION IN THE SHELL OF THE CONCH STROMBUS GIGAS. D.M. Zhang, X. Su, A.H. Heuer, Department of MS&E, Case Western Reserve University, Cleveland, OH.

Tissue regeneration in molluscs involves deposition of shell biomaterials during wound repair or on foreign objects introduced near the shell-forming cells. In the present study, tissue regeneration in the shell of giant Queen conch (Strombus gigas) was studied in these two ways: an abiotic substrate (such as a glass cover slide) was inserted between the mantle tissue of the animal and the inner layer of the shell, and the tissue deposited on the substrate (the "flat pearl") was studied; in the case of wound repair, tissue regeneration was stimulated by drilling a hole through shell and allowing the animal to undertake the repair. In both processes, the regenerated materials were removed after time intervals from 6 hrs to 10 days, and analyzed by X-ray diffraction, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The hard tissue mineralization sequences in the two types of tissue regeneration processes were generally similar. Poorly organized spherulitic aragonite deposited initially. This was followed by a transition region composed of a number of layers of organic matrix and aragonite. Finally, aragonite deposition became well organized and the tissue developed into the crossed-lamellar-like microstructure, very similar to the structure of the natural shell. However, two major differences were found between the two processes. An organic sheet, which was absent on flat pearls, formed during wound repair before mineralization took place. The initially formed organic sheet consisted of cross-linked fibrils, which showed alternate light and dark bands with a periodic length of $\sim$ 33 nm under SEM. Later, aragonite spherulites became embedded in the organic sheet, which indicated that the organic sheet acted as a substrate for the mineral formation. The second difference in the two processes lay in the regeneration rate, which was $\sim$10 times slower during wound repair than on the flat pearl. Aragonite was the only type of mineral formed during tissue regeneration.

11:30 AM N1.9
THE STRUCTURE AND PERFORMANCE OF NUTSHELLS. Ulrike G.K. Wegst, Eduard Arzt, Max-Planck-Institut für Metallforschung, Stuttgart, GERMANY; Peter Cloetens, European Synchrotron Radiation Facility, Grenoble, FRANCE.

The structure and mechanical performance of nutshells are interesting both from a biological and a materials science point of view. In nature, their shape combined with excellent material properties at low density (ρ = 1.2–1.4Mg/m^3; Young's modulus, E = 1.5–5GPa; fracture strength, σ_f = 150–340MPa; Moh's Hardness = 2.5–5.0) result in an efficient trade-off between the protection of the seed against herbivory, by making it difficult to 'get in', while still ensuring that the seed can

'get out' during germination. In industry, ground nutshells are valued as soft-grit abrasives. They cleanse precision parts without scratching and pitting and can be reused because their resistance to rupture, deformation and breakdown is high. Presented here are investigations of the microstructure and mechanical properties of a variety of nutshells by different techniques (X-ray tomography, X-ray diffraction, scanning and transmission electron microscopy and energy dispersive spectrometry). They revealed the presence of intracellular calcium oxalate monohydrate (Whewellite) crystals of 10–20μm diameter. Proposed biological functions of this form of biomineralisation are (i) the storage of calcium which is in excess of cytosolic requirements and limits and (ii) a defence mechanism to herbivory through an improvement of the mechanical properties of the tissue. The first is likely: nutshells provide the plant with an effective mechanism to store excess calcium and to dispose of it at regular intervals. The second raises two questions. Does the presence of Whewellite crystals increase hardness and abrasion-resistance of the polymeric seed shell material to such an extent — particularly with respect to the properties of the enamel of teeth - that it enhances defence to herbivory? And does the resulting combination of material properties contribute to the efficiency of nutshells as industrial soft-grit abrasives? Answers are proposed in this contribution.

11:45 AM N1.10
MECHANICAL BEHAVIOUR OF BIOLOGICAL ATTACHMENT SYSTEMS UNDER DRY AND LIQUID CONDITIONS. S. Enders, E. Arzt, Max-Planck-Institut f. Metallforschung, Stuttgart, GERMANY; S.N. Gorb, Max-Planck-Institut f. Entwicklungsbiologie, Tuebingen; GERMANY.

The material properties of biological attachment systems in insects are investigated. Aim of our experiments is to understand the fundamental connections between structure, local mechanical and tribological properties of these systems. The mechanical properties of the materials are probed by indentation tests with a combined Nanoindenter/AFM. Previously, indentation measurements have been carried out on dry samples to avoid adhesion and evaporation artefacts caused by the surrounding liquid. We introduce a test method utilizing a novel liquid cell to overcome these problems and simultaneously to prevent the highly sensitive biological samples from drying out. The data obtained on samples kept in a buffer solution during the measurements are compared with the data from dry samples, and considerable differences in the mechanical properties are found. We demonstrate the main advantage of this technique by performing long-time relaxation tests without the influence of desiccation on the material. Results of first measurements revealed differences in structure and mechanical properties of the counterparts of the attachment surfaces. To get additional information about their deformation behaviour, the surface morphology was characterised by AFM before and after the test. The results provide first elements of a model for functional principles of the mechanical interaction between the co-operating surfaces.

SESSION N2: IMAGING AND CHARACTERIZATION TECHNIQUES
Chair: Christine A. Orme
Tuesday Afternoon, April 2, 2002
Metropolitan I (Argent)

1:30 PM *N2.1
MULTIMOLECULAR COMPLEXES AT THE LEADING EDGE OF MOTILE CELLS. Niels Volkmann[1], Paul Matsudaira[2], David Derosier[3], Susan Lowey[4], Kathy Trybus[4], John E. Heuser[5], Rong Li[6], Thomas Pollard[7] and Dorit Hanein[1]. [1]The Burnham Institute, La Jolla, CA. [2]Whitehead Institute for Biomedical Research, MIT, Cambridge, MA. [3]The W.M. Keck Institute for Cellular Visualization, Brandeis University, Waltham, MA. [4]Dept. of Mol. Physiology & Biophysics, University of Vermont, Burlington, VT. [5]Dept. of Cell Biol., Washington University, School of Medicine, St. Louis, MO. [6]Department of Cell Biol., Harvard Medical School, Boston, MA. [7]Molecular, Cellular & Developmental Biology, Yale University, New Haven, CT.

Electron cryomicroscopy is the principal method for solving the structures of complexes and large protein assemblies that remain beyond the reach of NMR spectroscopy or X-ray crystallography. Combined with three-dimensional image reconstruction this technique allows researchers to view macromolecular structures at molecular ($<30\AA$) to near atomic ($<4\AA$) resolution. We use this approach to study multimolecular complexes involved in the assembly and regulation of the actin cytoskeleton at the leading edge of motile cells. In particular we study the role of Arp2/3 complex in actin filament network assembly (Volkmann et al. 2001, Science **293**, 2456-9), the role of myosin in cell migration (Volkmann et al. 2000, Nature Struct. Biol. 7, 1147-55) and the role of actin binding proteins in providing a scaffold for cell protrusions (Volkmann et al. 2001, J. Cell Biol. **153**, 947-56).

2:00 PM *N2.2
IMAGING SUPPORTED BILAYER-CELL INTERFACES USING HYBRID SUBSTRATES. Steven Boxer, Lance Kam, Caroline Ajo, Stanford University, Dept of Chemisty, Stanford, CA.

Supported lipid bilayers can be assembled on appropriately-treated glass surfaces and exhibit lateral fluidity over large distances. We have shown that supported bilayers can be partitioned and corralled by scratching the membrane, by assembly on surfaces that have been patterned with a variety of materials using photo-or electron beam lithography, and by surprising combinations of blotting, stamping and caulking using hard materials and proteins. These tools provide a basic technology for preparing defined arrays of membrane-associated components for cell screening and for studies of membrane-cell interactions. New applications of these tools to separate out laterally mobile fractions of membrane associated proteins will be described. In order to study cell-supported bilayer interfaces with the maximum possible vertical resolution, we have developed a variation on total internal reflection fluorescence microscopy (TIRFM) that uses high refractive index substrates. Since bilayers do not assemble on most high refractive index materials, we take the simple approach of over coating the high index material with a thin (few to tens of nm) layer of SiO_2. Such hybrid supports have been fabricated with lithium niobate (n=2.3), ZnS (n=2.3) and YAG (n=1.8). By varying the angle of incidence, it is possible to vary the penetration depth of the evanescent field near to the substrate, and by collecting images at many different angles of incidence, reconstruct vertical images with contrast on the tens of nm scale. Height standards that are compatible with supported membranes and living cells will be described, along with quantitative images of cell-surface and cell-supported membrane interfaces.

2:30 PM N2.3
USING ULTRAHIGH-RESOLUTION LASER TWEEZERS TO CHARACTERIZE THE MECHANOCHEMICAL PROPERTIES OF SINGLE BIOMOLECULES. David Izhaky, Nancy Forde, Glenna Woodcock and Carlos Bustamante, Depts of Molecular and Cell Biology, Physics and Howard Hughes Medical Insitute, University of California, Berkeley, CA.

Until recently, scientists had no way of measuring the forces that an individual biomolecule experiences during the course of a reaction. Conventional "bulk" measurements are usually only capable of measuring the properties of an ensemble of molecules; properties peculiar to individual molecules are often lost in the averaging process. However, it is now possible to measure the dynamics of a reaction with unprecedented resolution using single-molecule techniques. We have developed an ultrahigh-resolution single-molecule method based on laser-trapping technology in order to study molecular motors. This technique can be used to measure directly, and in real time, the movements, forces and strains that develop during the course of a biochemical reaction. We are also able to exert external force on motor proteins, enabling us to characterize their mechanochemical properties, and also to alter the outcome of the biochemical reaction. Recent results from the study of single molecules of RNA polymerase, the motor protein responsible for transcription (the copying of DNA into RNA) will be presented.

2:45 PM N2.4
DIRECT OBSERVATION OF BIOMIMETIC NUCLEATION BY SURFACE X-RAY SCATTERING. Elaine DiMasi, Brookhaven National Laboratory, Physics Department, Upton, NY; V.M. Patel, S. Murisamy, M. Olszta, L.B. Gower, University of Florida, Department of Materials Science and Engineering, Gainesville, FL.

Two important aspects of biogenic mineral nucleation are the kinetics and the organic template, which together can determine the crystal polytype and morphology. Nucleation from a liquid subphase onto a surfactant monolayer is an important model system, since the template charge and lattice spacing may be tuned through control of the surfactant and the surface pressure. Studies up to now have relied upon optical and electron microscopy — no in-situ probe on atomic length scales has been available. Using synchrotron x-ray scattering, we have made the first in-situ observations of biomimetic calcium carbonate growth, from its inception as calcium ions collected at a monolayer interface, to a macroscopic amorphous mineral film. The mineral precursor forms beneath an arachidic acid monolayer assembled on a supersaturated calcium carbonate subphase containing $\sim$25μg/ml polyacrylic acid inhibitor. X-ray reflectivity and in-plane diffraction were monitored to determine the thickness, density, and crystallinity of the film. At early times, we detect only the monolayer, incorporating a Stern layer of calcium ions. Subsequently, a film grows at a rate of about 20$\sim\AA$/hour to a maximum thickness of 280$\sim\AA$, 18 hours later. The film density, which remains constant throughout this

growth, is 82% that of calcium carbonate hexahydrate. This suggests that the film is comprised of a hydrated, open structure. In-plane diffraction from the monolayer remained unchanged during the 18 hour interval, with no other peaks appearing at calcite or vaterite positions. However, after 20 hours, intensity from in-plane peaks began to shift as crystallization began. In this system, the polymer concentration and molecular weight drastically affect film growth, with no strong evidence for interactions between the in-plane monolayer structure and that of the mineral film. Hence, this system is probably kinetically, rather than template driven. We hope to clarify the role of the template in future work.

3:15 PM N2.5
IN-VIVO NANOSCALE ACOUSTIC EVALUATION OF DROSOPHILA MELANOGASTER DEVELOPMENT.
Antanas Daugela, Hysitron Inc., Michael Kohane, Durance Co., Hiroshi Kutomi and Thomas Wyrobek, Hysitron Inc, Minneapolis, MN.

Quantitative quasi-static nanoindentation and ultrasonic measurements carried out at the nanoscale can provide a very complex characterization of biological tissues helping to bridge the gap between destructive spectrometric analysis and model based diagnostics. Mechanical properties can be derived from experimental loading-unloading curves. An ultrasonic signal transmitted trough the initial contact enables to look at localized pre-contact phenomena where nanometer scale positioning and pico Newton contact force approach are essential. Ultrasonically transmitted signal can be analyzed using advanced signal processing algorithms. A case study was carried in-vivo on etherized Drosophila melanogaster samples for two lifecycle stages, i.e. larvae and pupae. Mechanical and acoustic properties were measured at three locations (posterior, mid and anterior regions) for (1) ether-immobilized larvae and non-etherized pupae and (2) for the ether-immobilized larvae, over time, during larval development. Discrete ranges of reduced elastic modulus values, viscous energy and ultrasonic absorption energy were obtained for larvae and pupae at the completion of metamorphosis. Variations in mechanical/acoustic properties were correlated to the different stages of metamorphosis.

3:30 PM *N2.6
CONTROLS ON MINERALIZATION BY SMALL- AND MACRO-MOLECULAR GROWTH MODULATORS INVESTIGATED BY IN SITU ATOMIC FORCE MICROSCOPY.
Jim De Yoreo, Christine Orme, Ana Villacampa, Maria Bartelt, Dept. of Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, CA; Kevin Davis, Patricia Dove, Dept. of Geological Sciences, Virginia Polytechnic Institute, Blacksburg, VA; John Hoyer, Childrens Hospital of Philadelphia, Philadelphia, PA; George Nancollas, Dept. of Chemistry, SUNY Buffalo, Buffalo, NY.

Through the process of biomineralization, organisms use modulators of crystal growth to produce morphologies and facets not expressed in purely inorganic systems. In other cases, growth modifiers are used to inhibit nucleation and growth of undesirable crystal phases. In order to understand the physical mechanisms by which mineralization is inhibited or modified, we have used in situ atomic force microscopy to investigate the growth of a number of crystal systems involved in biomineralization including calcium carbonate (calcite), dicalcium phosphate dihydrate (brushite), and calcium oxalate monhydrate (COM). In addition to examining growth in pure solutions, we have determined the effects of both inorganic and organic growth modifiers including divalent cations, simple amino acids, poly-amino acids, citrate, and urinary osteopontin, a protein involved in kidney stone formation. Here we review the growth mechanisms for the pure systems and quantify the thermodynamic and kinetic parameters that control surface morphology and step dynamics. We compare these results to those obtained with solutions containing the growth modifiers. Our results show that while, in all cases, growth in pure solutions occurs on anisotropic growth hillocks generated by dislocations, the mechanisms of modification vary between systems and include both step inhibition and additive incorporation as well as modification of surface energies.
This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract No. W-7405-ENG-48.

4:00 PM N2.7
MECHANICAL AND MICROSTRUCTURAL PROPERTIES OF STRATUM CORNEUM.
Kenneth S. Wu, Department of Mechanical Engineering, Reinhold H. Dauskardt, Department of MS&E, Stanford University, Stanford, CA.

The outermost layer of skin, or stratum corneum (SC), provides mechanical protection and a controlled permeable barrier to the external environment. Studies have demonstrated that temperature, hydration, and the application of topical agents influence the mechanical properties of SC. In the case of emerging transdermal drug delivery technologies, the application and removal of adhesive drug delivery devices necessitates an appropriate balance between patch adhesive strength and SC cohesive strength in the direction normal to the skin surface. However, the out-of-plane cohesive strength, or delamination resistance, of SC is not well understood, and almost no quantitative data or reproducible test methods are available. In this presentation, a mechanics approach is described to study the SC intercellular delamination resistance and fracture mechanisms as well as their relationship to SC microstructure. The effects of temperature and hydration on cohesive properties are also explored. Fracture mechanics-based cantilever-beam specimens were used to reproducibly determine energy release rates to quantify the cohesive energy of SC. Fracture energies of fully hydrated SC are shown to decrease with increasing temperature while dehydrated SC exhibits a more complex variation with temperature. Stress-separation tests showed that fracture energies and peak separation stresses decreased with increasing temperature and hydration although the SC elastic modulus varied marginally with temperature or hydration. Results are described in terms of microstructural changes associated with hydrophilic regions and intercellular lipid phase transitions. The application of these techniques to characterize other soft tissues is discussed.

SESSION N3: ORGANIC BIOMATERIALS—PROTEINS AND PEPTIDES
Chair: Peter G. Vekilov
Wednesday Morning, April 3, 2002
Metropolitan I (Argent)

8:00 AM *N3.1
CREATING BIOMIMETIC MATERIALS THAT RESIST ECTOPIC CALCIFICATION.
Cecilia M. Giachelli, Rachit Ohri, University of Washington, Bioengineering Dept, Seattle, WA.

Ectopic calcification refers to calcium salt crystal deposition in soft tissues and biomaterial devises. Ectopic calcification is recognized as a problem associated with biomaterial implantation and failure, especially in cardiac valve bioprostheses, cardiac assist devices, and intraocular lenses. Until recently, the process of ectopic mineralization was considered unregulated and passive. In the past 5 years, however, a number of key observations have changed this perception. In particular, a number of biomolecules have been identified that effectively regulate biomineral deposition. One of these molecules, osteopontin, is normally expressed in ossified tissues and is invariably found at sites of ectopic calcification in vivo. Osteopontin has been found to inhibit apatite deposition in both solution and cell-dependent crystal growth assays. Furthermore, mice lacking osteopontin display increased calcification of subcutaneously implanted bioprosthetic valve tissues compared to wildtype mice. Thus, osteopontin can be classified as a natural inhibitor of calcification. Evidence suggests that osteopontin contributes to inhibition of calcification by physically interacting with nascent apatite crystals and preventing crystal growth, as well as stimulating cell-mediated resorptive mechanisms. We have prepared recombinant osteopontin in bacteria, and found that the anticalcific effect of osteopontin is absolutely dependent on extent of phosphorylation. Furthermore, several fragments of osteopontin that retain anticalcific activity in vitro have been identified. Using soluble recombinant phosphorylated osteopontin, we have been able to inhibit calcification of bioprosthetic valve tissues in osteopontin null mice. Current studies are aimed at developing effective immobilization strategies to assess the efficacy of surface-bound osteopontin in preventing calcification of bioprosthetic valve tissues. These studies open the door for biological and biomimetic approaches to limit, prevent, and potentially reverse ectopic calcification of implanted biomaterials.
Studies supported by grants from NSF (UWEB, EEC9529161), NIH (HL62329-01), and AHA.

8:30 AM N3.2
BIOLOGICALLY-BASED SELF-ASSEMBLING HYDROGELS.
Brandon L. Seal, Alyssa Panitch, Arizona State University, Department of Bioengineering, Tempe, AZ.

One goal of tissue engineering focuses on using molecular manipulation to incorporate bioactivity into three-dimensional scaffolds. Borrowing from biology, we have cloned the self-assembly regions of fibrinogen to form a scaffolding that interacts with fibrin and that can independently assemble upon demand. Peptides consisting of a binding pocket (BP), ligand, and/or a Factor XIIIa substrate were synthesized and conjugated to acrylated dextran (40 kD, 31% degree of substitution). To make 0.6% fibrin and 0.5, 1, or 2% peptide-dextran conjugated gels, peptide-dextran was combined with fibrinogen and polymerized on a rheometer for 15 minutes at 37°C. The resulting hydrogels were subjected to a frequency sweep between 0.1 and 100 rads/sec using an oscillatory stress of 1 Pa. The

incorporation of BP conjugated to dextran significantly decreased the storage moduli of fibrin gels in a dose-dependent manner. This result suggested that the sequence on BP-dextran interacted with the ligand on fibrinogen and disrupted normal fibrin gelation. When added to fibrin gels, ligand peptide conjugated to dextran also inhibited normal fibrin polymerization, which resulted in a significant decrease in storage modulus compared to fibrin controls. In contrast, Factor XIIIa substrate peptide conjugated to dextran significantly increased (160%) the storage modulus of gels relative to fibrin. The addition of unconjugated dextran did not significantly affect the mechanical properties of fibrin. This work shows that peptides directing assembly can be designed using motifs found in natural proteins. After conjugation with hydrophilic polymers, these peptides can retain biological activity. The peptides used in this study not only alter the mechanical properties of fibrin, but also allow a mechanism for creating a self-assembling network. Furthermore, these materials can be designed with physiologically-relevant enzymatic degradation sites and can support the release of bioactive factors such as adhesion molecules, growth factors, and extracellular matrix proteins.

8:45 AM N3.3
INCORPORATION OF TRIFLUOROISOLEUCINE INTO PROTEINS IN VIVO. Pin Wang, David Tirrell, California Institute of Technology, Division of Chemistry and Chemical Engineering, Pasadena, CA.

Amino acid replacement in proteins is normally limited to 20 the natural amino acids in *in vivo* protein engineering. Incorporation of nonnatural amino acids into proteins extend the scope of protein engineering to include a wide variety of artificial functions. Functional groups orthogonal to those of the naturally occurring amino acids, including alkenes, alkynes, aryl halides, electroactive side chains, and hyper-hydrophobic side chains have been incorporated into proteins *in vivo*. In this study, we prepared two trifluoroisoleucines: 2-D,L-amino-3,3,3-trifluoromethyl pentanoic acid (3TFI) and 5,5,5-D,L-trifluoroisoleucine (5TFI). We were able to incorporate 5TFI into a model target protein, mouse dihydrofolate reductase (mDHFR), in an isoleucine auxotrophic *Escherichia coli* host strain grown in 5TFI-supplemented minimal medium depleted of isoleucine. Incorporation is confirmed by tryptic peptide analysis and matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) of the protein product, which indicates that more than 99% of the encoded isoleucine residues are replaced by 5TFI. Examination of the secondary structures of wide-type mDHFR and the variant containing 5TFI by circular dichroism (CD) suggests that 5TFI causes minimal structural perturbation. In the light of fact that incorporation of trifluoroleucine into the hydrophobic core structures of proteins can enhance thermal and chemical stability, we are investigating the effect of 5TFI to those (and other) properties.

9:00 AM *N3.4
CREATING FUNCTIONAL PEPTIDE ARCHITECTURES AT INTERFACES. Raymond Tu, Matthew Tirrell, Materials Research Laboratory, University of California, Santa Barbara, CA.

Synthetically lipidated peptides possess the self-assembly character of amphiphilic molecules and the biological activity of the peptide headgroups. This enables interfaces to be assembled that deliver biofunctionality in a controllable way. We have studied the ability of peptide amphiphiles to promote cell adhesion at both material surfaces and in interaction with liposomes in suspension. Incorporation of peptide amphiphiles in liposomes greatly increase the uptake of synthetic dyes by cells in dicating their potential utility in targetted drug delivery applications.

9:30 AM N3.5
ADVANCED BIOMATERIALS VIA DESIGNED POLYPEPTIDES FOR SELF-ASSEMBLY: DESIGN PRINCIPLES AND MORPHOLOGY CHARACTERIZATION OF AMPHIPHILIC BLOCK COPOLYPEPTIDES AND Ph SENSITIVE FOLDING OLIGOPEPTIDES. Darrin Pochan, Lisa Pakstis, Bulent Ozbas, Vahik Krikorian, MS&E and Delaware Biotechnology Institute, Univ of Delaware, Newark, DE; Joel Schneider, Department of Chemistry and Biochemistry, Univ of Delaware, Newark, DE; Andy Nowak, Mike Wyrsta, Tim Deming, Departments of Chemistry and Materials, Univ California at Santa Barbara, CA.

The design, synthesis, and self-assembly of polypeptides as synthetic materials that possess the ability to aggregate and/or fold into specifically defined, a priori designed, functional nanostructures is being pursued via two avenues. First, synthetic block copolypetides will be discussed that are observed to form membranes, vesicles, and novel hydrogels with structural and biological properties tailorable by the choice of amino acid (and consequent secondary structure) in the respective blocks. Specifically, non-ionic triblock polypeptides have been designed in an attempt to form a predetermined equilibrium vesicle size or membrane elasticity and ionic diblocks have been designed for tissue engineering hydrogels on assembly in aqueous solution. Understanding these a priori design principles is crucial for the engineering of new materials for drug delivery and biomaterial scaffolding. Second, de novo designed oligopeptides that undergo specific folding events triggered by pH, preceding or concurrent with self-assembly, will be discussed. Specifically, the ability of small (20 amino acid residues) molecules to produce relatively very large (~50 micron diameter) spheres and tubes via a hierarchical self-assembly process will be discussed. The assembled oligopeptides have the added design attribute of being responsive to pH, unfolding/unassembling at pH below 6. Both classes of peptide-based self-assembled materials were characterized via laser confocal, cryotransmission electron, and field-emission scanning electron microscopy combined with small angle neutron and x-ray scattering.

10:15 AM N3.6
SOLVENT ENTROPY EFFECTS IN THE FORMATION OF PROTEIN SOLID PHASES. Peter G. Vekilov, Department of Chemical Engineering, University of Houston, Houston, TX.

Water, the native solvent for proteins and the one from which the formation of most solid phases of interest occurs, is often viewed as an inert medium holding the protein molecules. To illustrate the opposing concept that trapping and release of the water molecules, associated with a protein in the solution, is an important component of the thermodynamics of phase transformationwe chose the mutated hemoglobin, HbC. HbC forms crystals inside red blood cells of patients with the Homozygous Hb CC disease; HbC crystallization is a model of processes underlying numerous condensation diseases. We found a strong retrograde solubility dependence on temperature, corresponding to a high positive enthalpy of 155 kJ mol-1, i.e., the specific interactions favor HbC molecules in the solute state. Then, HbC crystallization is only possible because of the huge entropy gain of 610 J mol-1K-1, stemming from the release of up to 10 water molecules per protein intermolecular contacthydrophobic interaction. Atomic force microscopy studies of the crystallization of this protein revealed the crystals grow by the attachment of single molecules to kinks on the surface. The density of the growth sites is in excellent agreement with a model calculation based on the crystallization free energy. With the second model protein, apoferritin, we determined of the temperature dependencies of the second virial coefficient and the protein solubility between 0 and 40°C and found that the enthalpy of crystallization is close to zero. We imaged with the AFM the configuration of the kinks. We show that the kinks are due to thermal fluctuations of the molecules at the crystal-solution interface. The free energy of the intermolecular bond f = 3.0 kBT = 7.3 kJ/mol (PRL 2000, 85, 353). The crystallization free energy, extracted from the protein solubility, is - 42 kJ/mol. Further analyses reveal that the main component in the crystallization driving force is the entropy gain due to the release upon crystallization of two waters bound to the protein molecules in solution (JMB 2000, 303, 667).

10:30 AM *N3.7
INTERACTIONS, AGGREGATION AND PHASE TRANSFORMATIONS IN PROTEIN SOLUTIONS. Dimiter Petsev, CMMR, University of Alabama, Hunstville, Huntsville, AL; Peter Vekilov, Department of Chemical Engineering, University of Houston, Houston, TX.

We have studied the molecular interactions in solutions of apoferritin, holoferritin and lysozyme by static and dynamic light scattering. A surprisingly strong repulsion between the molecules was found at high electrolyte concentrations (about and above 0.2 M) with all studied proteins. At such conditions the electrostatic interactions are suppressed and we argue that the repulsion is due to the water structuring around the protein molecules giving rise to so-called hydration forces. The molecular forces are ion-specific and addition of divalent ions may lead to attraction instead of repulsion. For example ferritin solutions are stable in presence of Na^{+} but crystallize readily if small amount of Cd^{2+} ions are added. While the interactions in ferritin solutions are temperature independent, those in lysozyme change which leads to phase separation and coexistence of two fluid phases with different protein densities. Analysis of crystal solubility and light scattering data suggests that the interaction between ferritin molecules is mostly to due to changes of the solvent entropy. We also showed that the interactions between apoferritin dimers are more attractive, unlike those between monomers. This is a possible reason for the negative impact of such impurities on the protein crystal structure.

11:00 AM N3.8
SELF-ASSEMBLY OF A MODULAR POLYPEPTIDE MODELED SILK-MIMETIC PROTEIN. Chrystelle S. Cazalis, Dept of Chemistry, Emory University, Atlanta, GA; Robert P. Apkarian, Integrated Microscopy and Microanalytical Facility, Emory University, Atlanta, GA; Vincent P. Conticello, Dept of Chemistry, Emory University, Atlanta, GA.

Spider dragline silk fiber displays a unique and technologically significant combination of high tensile and compressive strength. The structural origin of these properties arises from the alternating sequence of crystalline alanine-rich domains and amorphous glycine-rich domains, which undergo microscopic phase separation in the silk fiber. We have designed and synthesized a novel polypeptide A, which emulates the modular structure of crystalline and amorphous elastomeric domains in dragline silk proteins. The sequence of Polypeptide A comprises an alternating arrangement of a self-complementary, amphiphilic silk-mimetic oligopeptide (Ala-Glu-Ala-Glu-Ala-Lys-Ala-Lys) and environmentally-response elastin-mimetic segment (Val-Pro-Gly-Val-Gly). A synthetic gene encoding eleven repeats of the alternating sequence was expressed in Escherichia coli strain BL21(DE3) as a C-terminal fusion to a decahistidine leader sequence to afford a polypeptide with a molar mass of approximately 75kDa.. The regularly alternating pattern of elastin-mimetic and silk-mimetic blocks inside the protein as well as their specific sequence allowed the copolymer to spontaneously self-assemble upon concentration or heating above the phase transition of the elastin-mimetic block. The conformational rearrangement that occurs during the self-assembly process was studied using a combination of FTIR, CD, and solid-state NMR spectroscopy as well as electron microscopy. It involves the conformational rearrangement of the alanine-rich domain from an α-helix to a β-sheet concomitant with formation of a transparent self-supporting membrane. Scanning electron microscopy of specimens that had been processed above the phase transition of the elastin-mimetic segments indicated a morphology that consisted of a matrix of beaded fibrils approximately 10-20 nm in diameter.

11:15 AM N3.9
BIOMIMETIC MINERALIZATION OF SILK FIBROIN FOR COMPOSITE MATERIALS. Smita Jadhav, Sandra Burkett, Amherst College, Department of Chemistry, Amherst, MA.

Silks are spun fibrous protein materials produced by biological systems. Silk has served an important role in textiles and other applications, where it is valued for its strength and elasticity. Recently, the utility of silk for biotechnology and biomedical materials applications has begun to be explored. Biomimetic mineralization of Bombyx mori silkworm silk fibroin to form composite materials is one approach to tailoring the mechanical properties of silk for these applications. Our study of silk-silica composites is inspired by studies of the protein silicatein, which is found in silica spicules of the sponge Tethya aurantia, and can catalyze the hydrolysis and condensation of the silica precursor tetraethoxysilane (TEOS) at room temperature and neutral pH. The synthesis and characterization of silk-silica composites will be described.

11:30 AM N3.10
RECOMBINANT SPIDER SILK FIBERS. Charlene M. Mello, Steven Arcidiacono, Jason Soares, Alfred Allen, David Ziegler and Elizabeth Welsh, US Army Soldier Biological Chemical Command, Natick Soldier Center, Natick, MA; Tom Laue and Susan Chase, Dept Biochemistry and Molecular Biology, University of New Hampshire, Durham, NH.

Spiders produce a family of silk proteins, which represent a tailored set of structural proteins designed over thousands of years of evolution. However, the dragline silk, has captured the interest of many investigators due to its remarkable mechanical properties balancing stiffness, strength and extensibility. Unfortunately, spiders are not capable of producing sufficient quantities for materials applications. Therefore, recombinant spider silks have been produced and a method has been developed for spinning fibers from these recombinant silk proteins. Purified recombinant silk solutions reveal an increase in -sheet content with time and dynamic light scattering suggests molecular self-association is time and temperature dependent in both dilute and concentrated protein solutions. Recombinant silk fibers are 10 to 60 um in diameter and water insoluble. Molecular orientation within the fiber is evidence by birefringence.

SESSION N4: INTERFACE ENGINEERING, PATTERNING AND BIOCOMPATIBILITY
Chair: Angela M. Belcher
Wednesday Afternoon, April 3, 2002
Metropolitan I (Argent)

1:30 PM *N4.1
MANIPULATING CENTRAL NERVOUS SYSTEM CELLS WITH TOPOGRAPHICALLY PATTERNED SURFACES. Andrea M.P. Turner, Harold G. Craighead, School of Applied & Engineering Physics, Cornell Univ, Ithaca, NY; Natalie Dowell, William Shain, NYS Dept. of Health, Wadsworth Ctr, Albany, NY; Ginger Withers, Gary Banker, CROET, Oregon Health Sciences Univ, Portland, OR.

The tools developed by the computer industry and applied to the field of nanobiotechnology have enabled physicists, chemists, biologists, and engineers to fabricate the devices necessary to probe biological systems down to molecular size scales. A major aspect of this merging of technologies is the construction of chemical and topographical patterns on substrates for the control of cell attachment and growth. Surface patterning methods offer the ability to organize cells on surfaces, and to promote and/or inhibit cell attachment to specific locations. We have used conventional semiconductor fabrication techniques to pattern silicon surfaces with micrometer-sized surface features, namely pillars. Standard nanoimprint lithography (hot embossing) techniques were used to fabricate similar patterns in cyclo-olefin polymers for use in time-lapse imaging. We have used these surfaces to study how central nervous system (CNS) cells, in particular rat hippocampal neurons and cortical astrocytes, attach and grow when confronted with topographical cues of various geometries. We have observed that the geometric constraints to which a neuron is exposed have a significant impact on various aspects of neuronal process development, including the rate of neurite (dendritic and axonal) outgrowth, neurite morphology, dendritic branching, and specific protein production, transport and organization. Fluorescence, scanning electron, and phase-contrast time-lapse microscopies were used to analyze and quantify the growth of neurons and astrocytes on surfaces with pillars 1 to 5 microns tall, with widths of 500 nm to 10 microns, and inter-pillar gaps of 1.0 to 10 microns. The results from studies indicate the importance of topographical cues in the development of central nervous system cells in vivo as well as their manipulation in vitro. This research has helped to elucidate some fundamental aspects of the interactions between CNS cells and surface structure.

2:00 PM N4.2
ENHANCED CELLULAR FUNCTIONS ON NANOSTRUCTURED POLYMERS. Anil Thapa, Derick M. Miller, Karen M. Haberstroh, and Thomas J. Webster, Department of Biomedical Engineering Purdue University, West Lafayette, IN.

The objective of the present in vitro study was to determine cellular functions on polymers that mimic the nanometer dimensions of extracellular matrix proteins found in soft tissues. Specifically, micron dimensions of individual fibers in conventional poly-lactic/glycolic acid and polyurethane polymer films were reduced into the biologically-inspired nanometer regime by soaking in either basic (NaOH) or acidic (HCl) solutions, respectively, for up to 60 minutes at room temperature. For adhesion experiments, chondrocytes (cartilage-synthesizing cells; Cell Applications, Inc.), vascular smooth muscle cells (Vec Technologies), bladder smooth muscle cells, and osteoblasts (bone-forming cells; ATCC) were separately seeded (at a cell density of 3,500 cell/cm^2) in cell culture media and were allowed to adhere under standard cell culture conditions (that is, a humidified, 95% air / 5% CO2, 37°C environment) for 4 hours. For proliferation experiments, these cells were seeded (at a cell density of 3,500 cell/cm^2) in cell culture media and were allowed to proliferate under standard cell culture conditions for 1, 3, and 5 days. After the prescribed time period, cells were fixed, stained, and counted. Experiments were run in triplicate and were repeated at three different times. For all cells tested in the present study, adhesion was significantly ($p < 0.1$) higher on nanostructured- compared to conventional-structured (i.e., micron) polymer films. In fact, chondrocyte, bladder smooth muscle, and osteoblast adhesion was more than two times greater on nano- compared to conventional fiber dimension poly-lactic/glycolic acid polymer films. The present study, therefore, provides evidence that polymers which simulate the nanometer dimensions of proteins in tissues may enhance cell functions necessary for the success of a number of implant applications.

2:15 PM N4.3
NANOSCALE PATTERNING OF ANTIGEN AND ANTIBODIES ON SILICON AND POLYMER SUBSTRATES FOR IMMUNOLOGICAL ASSAY ANALYSIS. Andrea M.P. Turner[1], R.N. Orth[1], T.G. Clark[2], Y.F. Chang[2], and H.G. Craighead[1]. [1]School of Applied and Engineering Physics. [2] Department of Microbiology and Immunology, Cornell University, Ithaca, NY.

Nanofabrication of vapor-deposited polymer offers a new capability for nanoscale spatial control of antigen and antibodies patterning on polymer and silicon substrates. Antigen and antibodies are patterned using a polymer lift-off technique creating uniform features below 575 nm. Several patterning techniques are explored for adhering antigens and antibodies. Avidin-biotin technology is used to capture biotinylated antigen and antibodies from solution. Antigen conjugated lipids are used to create antigen-containing lipid bilayers that readily capture antibodies and cell membranes receptors. Patterned protein-A is used to capture antibodies at their Fc terminal on the patterned surface. The biomaterial platform layers are added to the substrate

followed by antigen and/or antibody application. The nanoscale antigen patterns serve as models for foreign substances epitopes and antigen presenting cells. Similarly, the nanoscale antibody patterns serve as model antigen receptors on cell surfaces. The polymer lift-off method involves projection lithography and reactive ion etching to pattern the polymer on the micron- and nanoscale. This technique allows patterning of biological materials to resolutions similar to that of photolithographic equipment. Additionally, this technology offers methods for localizes immunological material to study cellular function, receptor aggregation, signaling, diffusion constants, cell-cell interactions, and cellular movement. These results are compared to results obtained from polymer lift-off patterning of supported lipid bilayers containing conjugates that bind avidin, his-tagged proteins, and DNA oligomers. The polymer protects the surface from specific and nonspecific binding until it is removed. After polymer removal, the nonspecific bonding is low, with a fluorescence signal/background ratio over 150. Patterning of biomolecules is characterized using biological cells, AFM, conjugated fluorescence dyes, and confocal microscopy.

2:30 PM N4.4
FORMATION, CHARACTERIZATION, PROTEIN RESISTANCE, AND REACTIVITY OF $Cl_3Si(CH_2)_11(OCH_2CH_2)_3OH$ SELF-ASSEMBLED MONOLAYERS. Jiehyun Seong, Seok-Won Lee, Paul E. Laibinis, Massachusetts Institute of Technology, Dept. of Chemical Engineering, Cambridge, MA.

We report a detailed study of self-assembled monolayers (SAMs) on SiO_2 substrates containing HO-oligo(ethylene glycol) functionality. First, we formed SAMs of acetoxy-oligo(ethylene glycol)-terminated alkyltrichlorosilanes, $Cl_3Si(CH_2)_{11}(OCH_2CH_2)_3OC(=O)CH_3$. The terminal acetate was included as a protecting group for -OH. The acetate was later reduced to -OH to form thin films of covalently grafted $(CH_2)_{11}(OCH_2CH_2)_3OH$ chains on SiO_2. X-ray photoelectron spectroscopy (XPS), Infrared (IR), ellipsometry, and wetting measurements were used to characterize the SAMs exposing both acetate- and hydroxy- terminated oligo(ethylene glycol) groups. Surface characterizations and fluorescence studies verified that the films exhibited notable resistances toward the non-specific adsorption of various proteins including lysozyme, albumin, fibrinogen, and IgG. The protein resistance was superior to films of 'inert' oligo(ethylene glycol) surfaces prepared by direct reaction of SiO_2 with $Cl_3Si(CH_2)_{11}(OCH_2CH_2)_3OCH_3$. The reactivity of hydroxyl group in oligo(ethylene glycol) terminated SAMs was studied by reacting the -OH with trifluoroacetic anhydride and other reagents. Protein A was covalently immobilized to the hydroxy-oligo(ethylene glycol) films using 1,1-carbonyl diimidazole as a crosslinker. The nonspecific adsorption of protein A to the surface was also reduced by the presence of ethylene glycol units and provided a useful surface for immobilizing antibodies to the SiO_2 substrates. Details on the use of these properties and designed antibody surfaces will also be presented in the talk.

2:45 PM N4.5
HELICAL, DISORDERED, AND WHAT THAT MEANS: STRUCTURAL CHARACTERIZATION OF A NEW SERIES OF OLIGO(ETHYLENE OXIDE)-TERMINATED SELF-ASSEMBLED MONOLAYERS. David J. Vanderah[1], Jennifer Arsenault[3], Thomas Parr[4], Hongly La[2], Curtis W. Meuse[1], Vitalii Silin[1], Richard S. Gates[1]. [1]NIST, Gaithersburg, MD. [2]Univ of Maryland, MD. [3]Appalachian State Univ, Boone, NC. [4]Grinnel College.

Self-assembled monolayers (SAMs) of a series of methyl-capped, linear oligo(ethylene oxide)-terminated thiols of the general formula $MCH_2CH_2O(CH_2CH_2O)_xCH_3$, where M = HS or $HSCH_2$ and x = 2 - 5, were prepared on polycrystalline gold and characterized by reflection adsorption infrared spectroscopy (RAIRS) and spectroscopic ellipsometry (SE). The RAIRS and SE data show that the SAM structures varied with the length of the oligo(ethylene oxide) {OEO} segment and the conditions during assembly, particularly the solvent used. For the longer compounds, x = 4 and 5 and M = HS or $HSCH_2$, both ordered and disordered SAMs were prepared. For the ordered SAMs, the OEO segment adopted the 7/2 helical conformation, oriented normal to the substrate, of the folded-crystal chain polymorph of poly(ethylene oxide). The relative ease of the formation of these ordered SAMs will be discussed. For the shorter compounds, x = 2 and 3 and M = HS, only disordered, "amorphous" SAMs formed. Our data suggests that SAMs with OEO segments less than five ethylene oxide units long are unable to adopt the helical conformation. We will discuss how the order of these SAMs influences the ability of these surfaces to resist the adsorption of proteins.

3:15 PM N4.6
SELECTIVITY OF POLYPEPTIDE BINDING TO NANOSCALE SUBSTRATES. Steve R. Lustig, Anand Jagota, CR&D, The DuPont Company, Experimental Station, Wilmington, DE.

We present theory and new computational methodology for designing polyelectrolytes and polypeptides which can selectively recognize nanoscale substrates. The methodology to design appropriate polymer sequences is illustrated by designing polypeptides for the assembly of multicomponent circuits, although the principles also apply to polymers which template structure during materials synthesis as well as add new biological and chemical functionality to surfaces. Molecular modeling and design of polypeptides enables mechanistic optimization of amino acid sequence via the calculation of molecular statistics and thermodynamics of substrate binding. Optimization permits enhancement of both binding energy and binding selectivity from one or more atomistic surfaces. The molecular modeling is based on united atom chains with explicit backbone and side chain structures which are required to account for molecular geometry, intramolecular interactions and molecule-substrate interactions. Molecular geometry and intramolecular interactions are described by a novel Continuous Rotational Isomeric State (CRIS) method. CRIS permits continuous backbone torsion sampling and is seen to be critical in binding optimization problems where chain flexibility is important. Intramolecular and intermolecular interactions are described by van der Waals dispersion and static electrostatic terms with an effective continuum dielectric solvent. A novel Monte Carlo algorithm permits extremely rapid computation and sampling of polypeptide-substrate binding so that a genetic optimization algorithm can be used to modify the peptide sequence in a search for selective recognition. We illustrate the case of selective polypeptide binding to slightly-positive charged surfaces, for example GaAs(100) with other competitive surfaces. We find that selective sequences start with peptides which actually repel most surfaces and continue with sequences which attract the target surface. Thus polypeptide design requires a delicate balancing of both repulsive and attractive interactions with candidate substrates.

3:30 PM *N4.7
THREE-DIMENSIONAL SUB-MICRON FABRICATION WITH BIOACTIVE PROTEINS BY MULTIPHOTON EXCITATION. Paul J. Campagnola, University of Connecticut Health Center, Center for Biomedical Imaging Technology and Department of Physiology, Farmington, CT; Steven L. Goodman, Imago Scientific Instruments Corporation, Madison, WI.

We have recently introduced a 3-D fabrication methodology that enables the directed assembly of biologically compatible polymers and bioactive proteins, and is capable of <250 nm resolution. This methodology utilizes the intrinsic 3-D confinement of multi-photon excitation to locally initiate photo-activation-induced cross-linking and/or polymerization. This method alleviates some of the limitations of the more conventional lithographic and stamping fabrication methods in terms of flexibility in chemistries; maintaining bio-molecular (protein) bioactivity; producing sub-micron features; and fabricating complex 3-D structures. The current instrument utilizes a titanium sapphire femtosecond laser coupled to a modified laser scanning confocal microscope To maintain protein bioactivity, and to facilitate the use of structures in biomedical applications, cross-linking and polymerization reactions are photo-initiated in aqueous solutions using non-toxic dyes such as Rose Bengal. With this methodology, sub-micron structures have been assembled by cross-linking many different proteins, and by locally polymerizing several types of synthetic polymers into complex 3-D structures. Examples include 300 nm enzyme-based structures that exhibit a high level of bioactivity, sub-micron to >100 um structures fabricated from adhesive proteins and micron-scale sustained release (drug delivery) devices assembled from both hydrogels and proteins. Also demonstrated is the capability to direct cellular adhesion and proliferation onto cross-linked 3-d bio-active surfaces. Enhanced response was shown for fibroblasts onto cross-linked fibrinogen, FGF2, and PDFG matrices relative to non-adhesive BSA. New photo-activators have also been designed and synthesized to increase the efficiency of the fabrication process, particularly in regard to relatively insoluble structural proteins. These new activators have shown to be much more effective in cross-linking Type 1 collagen into stable 3-d structures than existing photo-chemistries and may prove to be powerful in tissue engineering applications. We further demonstrate how Second Harmonic Generation can be utilized to image the resulting protein matrices and probe the extent of cross-linking and organization.

4:00 PM N4.8
CELLULAR INTERACTIONS AND BIOCOMPATIBILITY OF SELF-ASSEMBLING DIBLOCK POLYPEPTIDE HYDROGELS. Lisa Pakstis, Bulent Ozbas, Vahik Krikorian, Darrin Pochan, Univ of Delaware, Dept of Materials Science and Engineering, Newark, DE; Cliff Robinson, Univ of Delaware and Delaware Biotechnology Institute, Dept of Chemistry and Biochemistry, Newark, DE; Andrew P. Nowak, Timothy Deming, Univ of California, Dept of Materials and Chemistry, Santa Barbara, CA.

Self-assembling peptide based hydrogels having a unique nano- and microscopic morphology are being studied for potential use as tissue engineering scaffolds. Low molecular weight (~20 kg/mol), amphiphilic, diblock polypeptides of hydrophilic lysine (K) or glutamic acid (E) and hydrophobic leucine (L) or valine (V) form hydrogels in aqueous solution at neutral pH and at very low volume fraction of polymer (vol. fraction polypeptide $\geq$ 0.5 wt%). The morphology of these hydrogels have been characterized using laser confocal microscopy (LCM), small angle neutron scattering (SANS), and cryogenic transmission electron microscopy (cryoTEM) imaging. Studies of the interactions of the hydrogels with *E. coli* reveal that these materials are non-cytotoxic regardless of the nature of the hydrophilic block in the polypeptide. The inherent biocompatibility of these materials has been assessed through the interaction of the gels with mammalian cells. Hence, the novel hierarchical morphology and biological nature of these gels makes them candidates for scaffolds in tissue engineering applications. Current research is directed at the design and incorporation of binding sites within the polypeptide to target specific cellular interactions with the hydrogel.

SESSION N5: COMPOSITE BIOMATERIALS—BONES AND TEETH I
Chair: William J. Landis
Thursday Morning, April 4, 2002
Metropolitan I (Argent)

8:00 AM *N5.1
DEVELOPING BIO-STABLE AND BIODEGRADABLE COMPOSITES FOR TISSUE REPLACEMENT AND TISSUE REGENERATION. Min Wang, School of Mechanical and Production Engineering, Nanyang Technological University, SINGAPORE.

Bone is the substantial unit of human skeletal system, which supports the body and its movement. At the ultra-structure level, the bone matrix is a composite material consisting of bone mineral particles approximately 50nmx25nmx3nm in size, which are mainly substituted, calcium-deficient hydroxyapatite, and collagen, which is a natural polymer. At the macroscopic level, there are two major forms of bone: cortical bone and cancellous bone. Cortical bone is a dense material and hence load-bearing, while cancellous bone is porous, being composed of short struts of bony material. Bone serves as the template for developing new materials for bone repair. As bone itself is a nanoscale composite, a polymer matrix composite containing a particulate bioactive component appears a natural choice for substituting cortical bone. Over the last two decades, a variety of bioactive polymer matrix composites have been developed for tissue substitution and tissue regeneration. The bioactive phase in these composites is normally one of the calcium phosphates, especially synthetic hydroxyapatite (HA) which closely resembles bone apatite and exhibits high bioactivity. If enhanced bioactivity is required, bioceramics having higher bioactivity such as Bioglass and A-W glass-ceramic can be used as the bioactive phase in the composites. For tissue replacement, bio-stable polymers such as polyethylene (PE) and polysulfone (PSU) are used as the matrix polymer. For tissue regeneration, natural, biodegradable polymers such as polyhydroxybutyrate (PHB) and chitin are used as matrices. Furthermore, mechanical as well as biological performance of a particular composite can be controlled by varying the amount of the bioactive phase in the composite, thus meeting specific clinical requirements. The manufacture of bioactive polymer matrix composites generally falls into two categories: the thermo-mechanical production route, and the physico-chemical production route. For bone analogue composite materials, major influencing factors such as shape, size and size distribution of bioactive particles, mechanical properties and volume percentage of the bioactive phase, properties of the matrix polymer, distribution of bioactive particles in the matrix and the particle-matrix interfacial state should be controlled in order to obtain materials of desirable properties. The evaluation of bioactive composites includes the use of various advanced analytical techniques. The composites that we have produced are currently at different developmental stages and it is our goal to finally get them into clinical use.

8:30 AM N5.2
MOISTURE-ASSISTED STRESS CORROSION CRACKING IN CALCIUM PHOSPHATE CEMENT. Victoria C. Jew, Reinhold H. Dauskardt, Stanford University, Dept of MS&E, Stanford, CA.

Cementitiously forming calcium phosphate apatites have shown significant potential as bone mineral substitutes for orthopedic and craniofacial applications due to their injectable, biocompatible and osteoconductive properties. However, application of these materials is limited by their poor tensile properties. Although basic mechanical properties have been well characterized, only limited information has been reported for the fracture behavior of these materials. We have previously examined cyclic fatigue crack growth behavior in calcium phosphate cements, but mechanisms of crack growth are not well understood. To further elucidate these mechanisms, this study investigates the stress corrosion cracking behavior of a hydroxyapatite bone cement. The effects of temperature and of environment, such as physiological saline, water, and air, on the stress corrosion cracking phenomenon are examined over five orders of magnitude of crack growth velocity. Both moisture and an increase in temperature are shown to significantly accelerate subcritical crack growth. Models for the kinetics of moisture-assisted crack extension have been developed. Implications for the integrity and reliability of such synthetic bone mineral substitutes, particularly in load bearing applications, are considered.

8:45 AM N5.3
BIOMINERALIZED MICROPATTERNED SURFACES FOR BONE TISSUE ENGINEERING. Jian Tan and W. Mark Saltzman, School of Chemical Engineering, Cornell University, Ithaca, NY.

Biomaterials have a long and successful history in dental and bone restoration, however, methods do not yet exist to produce well-controlled biomineralized substrates with precisely defined chemical and physical features that promote cell integration and function. In this study, we created a group of parallel grooves/ridges on silicon surfaces. The dimension of the ridges, 4×4×800 μm, was kept constant whereas the spacing was varied from 4 to 16 μm. The surfaces were oxidized and coated with poly-(aspartic acid) using a silane coupling agent as linker to facilitate mineralization under physiological conditions, i.e. in solutions with similar compositions to those of body fluid. The minerals, mainly calcium phosphates, were characterized using scanning electron microscopy and electron microprobe analysis. The thickness of the mineral layer increased with reaction time, about 0.75 μm after 4 days. Mineralized micro-patterned silicon chips were also placed in cell culture medium to test the stability of the mineral layers. SEM studies showed no significant changes in the morphology and thickness of minerals after 2 days in medium. The viability of fibroblasts and osteoblast-like cells on mineralized surface were comparable to that on cell culture plates. There was an enhanced phenotype expression (alkaline phosphatase activity, ALP) of osteoblasts on mineralized surfaces. In addition, the spacing of the grooves/ridges also affected the morphology and function of cells on patterned surfaces. These results suggest that we have produced highly biocompatible materials with potential applications in bone tissue engineering.

9:00 AM *N5.4
BIOLOGICAL INTERFACES AND THEIR BIOMIMETIC APPLICATIONS TO ORTHOPEDIC BIO-COATINGS. Rizhi Wang, Univ of British Columbia, Dept of Metals and Materials Engineering, Vancouver, CANADA.

Synthetic bio-coatings such as hydroxyapatite (HAp) and diamond-like carbon (DLC) coatings can dramatically improve the biological and mechanical performance of metallic orthopedic implants. Their main problem is early interfacial failure. Solutions to increase bio-coating/substrate integrity could be found in nature. It has been known that some biological hard tissues are often composed of multi layers with distinct but strong interfaces. Typical examples are interfaces among enamel, dentin and cementum layers in teeth, and the interfaces in seashells. How could these biological interfaces work effectively to fulfill the heavy mechanical functions? The answers could inspire new ideas to the design and processing of synthetic interfaces. In this presentation, the structure, deformation and fracture mechanisms of teeth and nacre will be described. The role of biological interfaces and their similarity to implant/bone interfaces will be discussed. The recent research on the HAp/Ti and DLC/substrate interfaces will be presented in terms of coating debonding, residual stresses, interfacial irregularities, and cell-coating interactions. The applications of focused ion beam microscope (FIB) in the study of bio-interfaces are demonstrated.

9:30 AM N5.5
MINERALIZATION AT PLASMA-SPRAYED HYDROXY-APATITE-COATED SURFACES BY HUMAN AND MOUSE OSTEOBLAST-LIKE CELLS IN VITRO. Hao Wang, Linn W. Hobbs, Department of MS&E, MIT, Cambridge, MA; Bernd Kinner, Myron Spector, Orthopaedic Research Laboratory, Brigham & Women's Hospital, Harvard Medical School, Boston, MA.

Osteoblast-like cells from mice or rats have been used to study bone formation in vitro on different implant material surfaces. Few previous experiments have utilized human osteoblast-like cells, which represent the in-vivo biology responsible for any integration of prosthetic joint implants. In the present study, human cancellous (metaphyseal) bone fragments were harvested from patients undergoing total knee arthroplasty to serve as a source of human osteoblast-like cells (HOC) that were subsequently cultured. Similarly, calvaria from neonatal

mice were excised, stripped of soft tissue, and subjected to a series of digestions to obtain mouse osteoblast-like cells (MOC). Both HOC and MOC were seeded at a density of 400 cells/square mm onto plasma-sprayed hydroxyapatite (PSHA)-coated discs of Ti-6Al-4V alloy and also onto tissue-culture polystyrene as a control. When cells reached confluence after 2 weeks, the samples were fixed, dehydrated, and examined by light microscopy, environmental scanning electron microscopy (ESEM), and X-ray energy-dispersive analysis (XEDS). HOC were observed to form a dense cell layer on the coated alloy; this cell layer contained isolated particulate clusters confirmed by XEDS to be a calcium phosphate and resembling mineralized bioapatite deposits extensively observed for earlier in-vitro implants in canine models . By contrast, MOC remained discrete in their coverage and occupied only portions of the substrate surface. Clusters of flat acicular calcium phosphate crystallites, 20-50 micrometers in length and resembling brushite morphology, were observed to emanate from the coated substrate. While the abundant mineral formation for MOC suggests that these cells are more active in the mineralization process, the morphological differences from HOC mineralization are of some concern for the use of mice calvaria in-vitro models in modeling human bone formation.

9:45 AM N5.6
BIODEGRADABLE POLYURETHANE SCAFFOLDS FOR BONE SUBSTITUTES. Katarzyna Gorna, Sylwester Gogolewski, Polymer Research, AO/ASIF Research Institute, Davos, SWITZERLAND.

Bone loss often results from trauma, infection, tumour resection and birth defects. If bone defects are of critical size, cancellous bone graft harvested e.g. from the iliac crest is often used to enhance healing. Harvesting of bone graft is traumatic and invokes high morbidity of the donor site. Monocortical defects without repositioned cortex, bicortical and tricortical defects do not heal completely during a lifetime. Possible solutions to this problem might be offered by the use of cancellous bone graft substitutes with adequate biological qualities or by tissue engineered implants. Ideally, the bone substitute should be porous to allow for bone ingrowth, biodegradable to permit the replacement of the implanted matrix with new bone and radiolucent so that the healing process can be followed radiographically. To diminish micromotions at the implant-bone interface, it might be preferable to use bone substitutes having a certain degree of elasticity, instead of stiff ceramics. Candidates for elastic bone substitutes are biodegradable polyurethanes with controllable elasticity. These can additionally be precalcified in vitro and/or loaded with calcium phosphate salts. This study aims at designing porous 3-D scaffolds from biodegradable polyurethanes to be used as bone substitutes, tissue repair implants and delivery devices for drugs and cells. Polyurethanes have been synthesized using aliphatic diisocyanates, biocompatible polyols, chain extenders and calcium complexing moieties. Fillers were calcium carbonate, hydroxyapatite and tricalcium phosphate. Viscosity measurements, GPC, NMR, DSC, SEM, and mechanical tests have been used to characterize the materials. Degradation and calcification in vitro was carried out in phosphate buffer, pH=7.4, 37°C, or in simulated body fluid (1.5 SBF) 37°C. The scaffolds obtained underwent degradation and calcification in vitro, supported the attachment and proliferation of osteogenic cells, and promoted bone regeneration in critical-size segmental defects in the sheep tibiae.

10:30 AM *N5.7
MODULATION OF CRYSTAL NUCLEATION AND GROWTH MORPHOLOGY BY THE SUPRA-MOLECULAR ARCHITECTURE OF AMELOGENIN-GEL MATRIX. Janet Moradian-Oldak , Nikolaos Bouropoulos, Center for Craniofacial Molecular Biology, University of Southern California, Los Angeles, CA; Mayumi Iijima, Asahi University School of Dentistry, Dental Materials and Technology, Gifu, JAPAN, Hai Bo Wen, DePuy, a Johnson & Johnson Company, Warsaw, IN.

The organic matrix mediated enamel mineralization generates the most unusual morphology of the carbonate-apatite crystals formed in biological hard tissues. The formation of enamel crystals takes pace in an amelogenin-rich matrix whose components are continuously secreted, self-assembled and eventually processed to almost complete degradation, leaving mature enamel composed of more than 95% mineral. It is now accepted that amelogenin 'nanospheres' constitute the primary structural entity of the extracellular protein framework. It has been postulated that amelogenin nanospheres arranged in a supra molecular architecture are involved in controlling the oriented and elongated growth of enamel apatite crystals. Our studies are focused on investigating the nano scale structure and assembly of amelogenin gel matrix and the role this organic framework may play in controlling enamel crystal initiation and growth morphology. Using dynamic light scattering together with atomic force microscopy we demonstrated that the carboxy-terminal processed amelogenins formed larger nanospheres than those formed by the full-length molecule indicating their fusion after the removal of the hydrophilic c-terminal. The effects of amelogenins were examined using in vitro experimental systems: a) on the growth morphology of octacalcium phosphate crystals (OCP), and, b) on apatite nucleating potential of the acidic enamelins. a) It was found that amelogenins had remarkable effects on the morphology of growing OCP crystals regardless of the presence of the hydrophilic C-terminal. These morphological changes appear to be consistent with the growth morphology of enamel apatite crystals during maturation. In 10% amelogenin gel, OCP crystals which are usually ribbon-like shaped became more elongated with rod-like shape morphology. b) In 10% gelatin gel, enamelins enhanced the induction of apatite nucleation in the presence of 1.5% amelogenin matrix in a dose dependent manner, suggesting that amelogenin-enamelin interactions play key roles in controlling the nucleation of enamel apatite crystals.

11:00 AM *N5.8
NOVEL BIOMIMETIC MATERIALS USING PEPTIDE NANOFIBERS AND NANOCRYSTALS. Samuel I. Stupp, Jeffrey D. Hartgerink, Elia Beniash, Northwestern University, Evanston, IL.

Synthetic materials inspired by extracellular matrices such as bone may not only offer new materials for medicine but also provide strategies to organize nanostructures over larger length scales for nanotechnology. The organization and binding of nanocrystals bound by organics as it occurs in bone could be of interest for mechanical, electronic, or photonic reasons in materials technology. Biological strategies have been proposed to bind nanocrystals but it is also possible to simply design systems chemically incorporating both synthetic and biological structural units. We discuss here a strategy in which peptide amphiphiles are designed to form nanofibers by self assembly with diameters under 10 nanometers and lengths reaching into the range of microns. The nanofibers can be reversibly assembled and disassembled by pH changes and most importantly they can be reversibly locked into polymers with molecular weights in the range of millions. Three dimensional networks of these nanofibers have been found to mediate the formation of mineral nanocrystals in spatially controlled fashion. In one example nanocrystals of apatite were found to form with crystallographic registry relative to the long axes of the nanofibers. The strategy could be expanded to fabricate microscopic systems with properties of interest in electronics and photonics.

11:30 AM N5.9
FUNCTIONAL BONE-LIKE MATERIALS: A BIOMIMETIC APPROACH. Jie Song, Carolyn R. Bertozzi, Department of Chemistry, Materials Sciences Division, Lawrence Berkeley National Laboratory and Howard Hughes Medical Institute, University of California, Berkeley, CA.

Bone consists of microcrystalline hydroxyapatite and collagen, an elastic protein matrix that is decorated with mineral-nucleating phosphoproteins. Our rational design of artificial bone-like material uses natural bone as a guide. Hydrogel polymers that possess anionic groups suitably positioned for nucleating biominerals, and therefore mimic the natural function of the collagen-phosphoprotein matrix in bone, were designed to direct template-driven biomimetic mineralization of hydroxyapatite. A homogeneous precipitation technique was applied to mineralize crosslinked hydrogels and yielded unique microstructural patterns of hydroxyapatites that grew and tightly adhered onto the nucleating ligands of the tailor-made hydrogels. Alternatively, in situ polymerization of the organic scaffold in the presence of hydroxyapatite yielded highly porous mineralized composites, with interconnected pore sizes ranging from 10s to 100s of microns. Chemical and structural information of the composite materials are obtained at both micro- and nano-levels by SEM-EDS, HRTEM and XRD. Furthermore, chemical handles that encourage specific cell-material interactions were also installed onto the 3-dimensional composite materials. Together, these functional organic-inorganic composites provide necessary porosity, hydrophilic and mineralized environment, as well as biochemical handles to promote cell adhesion and proliferation, therefore serve as promising candidates as interactive biomaterials for bone implants and repair.

11:45 AM N5.10
COMBINED EFFECTS OF CROSSLINK DENSITY AND CONFORMITY ON THE TRIBOLOGICAL PERFORMANCE OF MEDICAL-GRADE ULTRA-HIGH MOLECULAR WEIGHT POLYETHYLENE. A. Chawan[1], A. Chakravartula[1], J. Zhou[1], M. Ries[2], L. Pruitt[1,3], K. Komvopoulos[1]. [1]Department of Mechanical Engineering, University of California, Berkeley, CA. [2]Department of Orthopaedics, University of California, San Francisco, CA. [3]Department of Bioengineering, University of California, Berkeley, CA.

It is strongly believed that the wear of ultra-high molecular weight polyethylene (UHMWPE) is the limiting factor that determines the life of total joint replacements. Recently, it has been proposed that highly crosslinked UHMWPE will reduce the wear encountered in vivo, thereby extending the life of total joint replacements. Highly crosslinked resins have shown little or no wear in total hip prostheses

simulator studies. To date, however, there is little clinical data to support this finding. Furthermore, uncertainty remains in the orthopedic community as to the optimal degree of crosslinking for different joint conformities. Despite numerous simulator studies performed on highly crosslinked UHMWPE, the effects of low and intermediate crosslink densities on the wear behavior has received less attention. Moreover, systematic studies on the tribological behavior of UHMWPE with coupled variations of crosslink density and conformity are sparse. The aim of this investigation is to characterize the tribological behavior of UHMWPE with varying degree of crosslink density for orthopedic applications involving high variations in conformity. To serve this purpose, pin-on-disk wear tests are used to determine the effect of conformity and countersurface roughness on the wear mechanisms and wear rates. Previous studies performed with non-crosslinked materials have shown that wear rate is influenced by the conformity of the two articulating surfaces of total joint replacements, where the wear rate increased with conformity. Slight increases in surface roughness of the material articulating against the UHMWPE have been found to drastically increase the wear rate. Such information is lacking for the new crosslinked UHMWPE resins intended for applications in hip, knee, and shoulder arthroplasty. The results of this study shed light into the combined effects of conformity, surface roughness, and crosslink density on the wear behavior of UHMWPE. This information is vital for the successful design of durable total joint replacements.

SESSION N6: COMPOSITE BIOMATERIALS—BONES AND TEETH II
Chair: Janet Moradian-Oldak
Thursday Afternoon, April 4, 2002
Metropolitan I (Argent)

1:30 PM *N6.1
SURFACE MODIFIED RESORBABLE BIOACTIVE GLASS FOR USE IN TISSUE ENGINEERING. Paul Ducheyne, Ahmed El-Ghannam, University of Pennsylvania, Center for Bioactive Materials and Tissue Engineering, Philadelphia, PA.

In 1969 bioactive glass was introduced as a new biomaterial that has the ability to strongly bond to bone. The mechanism of action of bioactive glass was extensively studied for the last 3 decades. However, the application of bioactive glass is still limited by its poor mechanical strength. In orthopedic and maxillofacial surgery, bioactive glass granules have been applied as any traditional graft material. Its application as a coating on a metallic implant has been challenged by the instability of the glass-metal interface. In other word, the exploit of the bioactivity properties of this material was limited by its mechanical and physicochemical characteristics. Recently, bioactive glass has been successfully taken into an unprecedented application that optimally exploits the bioactivity property of this material. In 1992 a tissue-engineering scaffold has been developed using bioactive glass. The goal is to use the in vitro synthesized bone as a graft instead of using a synthetic material. The surface of a porous bioactive glass template was engineered to mimic the mineral phase of bone. The effect of culture conditions and surface modification on serum protein adsorption and bone cell function is reported. The template physicochemical characteristics related to osteoblast adhesion and bone tissue formation in vitro are discussed.

2:00 PM N6.2
AMELOGENIN INDUCED BIOMINERALIZATION OF A BIOACTIVE FLUOROAPATITE GLASS-CERAMIC. Stefan Habelitz, Mehdi Balooch, Sally J. Marshall, Grayson W. Marshall, University of California, Department of Preventive and Restorative Dental Sciences, San Francisco, CA; Wu Li, Pamela K. DenBesten, University of California, Department of Growth and Development, San Francisco, CA.

Amelogenin (amg) is the major protein component of the developing enamel matrix, playing a key role in the control of nucleation, morphology and texture of apatite crystals during mineralization. We studied nucleation and crystallization of apatite on bioactive glass-ceramic surfaces in the presence of amg to better understand its function and potential use for syntheses of materials using a biological process. Extrusion oriented glass-ceramic rods containing 30vol% rod-like fluoroapatite crystals were cut and polished perpendicular to the c-axes of crystals. Atomic force microscopy revealed fluoroapatite crystals (l =1μm) at a level of approximately 5nm below the surrounding silica matrix. Full-length human amg cDNA was cloned into pRSET A vector and over-expressed in BL21DE3 plysS E. Coli, purified, and dissolved in 0.1% TFA. Glass-ceramic substrates were immersed at 37°C into 400μL of buffered solutions: A. supersaturated calcium-phosphate solution (CPS); B. 0.8mg/ml amg in 0.1% TFA; C. CPS and 0.3mg/ml amg. When immersed in CPS (A) for 24h, apatite grew selectively on substrate fluoroapatite to about 10nm above the level of the silica matrix, as observed by AFM. As a result of the bioactivity of the glass-ceramic, rod-like crystals (150x500nm) precipitated on the surface after 48h. Specimens immersed into amg-solution (B) were covered with a dense layer of nanospheres (d≈15nm), attributed to immobilized amg. Specimens immersed for 24h in solution (C) containing CPS and amg, exhibited growth of needle-like crystals in epitaxial relationship with underlying fluoroapatite to a height of about 100nm at pH 5.7. With increasing pH of solution (C), amg tended to precipitate. At pH 8, large amounts of proteins were attached to the fluoroapatite. Proteins formed a string-like mesh of spheres (h≈250nm). Micro-Raman and EDX spectroscopy of the formed layers showed the presence of proteins and apatite suggesting the formation of a biocomposite.
Support: NIH/NIDCR P01-DE09859, PO1-DE11526, UCSF Academic-Senate.

2:15 PM N6.3
METAL-BONE BIOHYBRIDS. Erik D. Spoerke[1], Naomi Davis[1], David C. Dunand[1], and Samuel I. Stupp[1,2,3], Northwestern Univ, Dept of Materials Science and Engineering[1], Dept of Chemistry[2], and Medical School[3], Evanston, IL.

Human skeletal repair remains one of the preeminent challenges in tissue engineering. Designing a system for the effective repair or replacement of a material as complex as bone requires the creative integration of materials science, chemistry, and biology. We are working to create a system that integrates the advantages of a metallic structure with a chemically active bioceramic and natural cellular growth to create a promising new implant concept. We have developed a technique to deposit nanostructured organoapatite (OA) on titanium (Ti) implant surfaces. This coating has revealed an osteoconductive character, inducing preferential colonization of preosteoblastic cells on porous Ti mesh. We have also worked to create a series of novel Ti foam structures with the idea of controlling pore size, shape, and orientation as well as density in order to create mechanically, structurally, and biologically appropriate scaffolds for use in skeletal repair. Preliminary experiments in a rotating bioreactor have shown that preosteoblastic cells seeded on OA-coated Ti foam colonize and eventually produce collagenous extracellular matrix throughout the porous microstructure of the foam substrates. Future work involving mineralization of this matrix may lead to promising new in vitro, metal-bone biocomposite implant systems.

2:30 PM *N6.4
EFFECTS OF IONIC FLOW AND AMELOGENINS ON THE LENGTHWISE GROWTH OF OCTACALCIUM PHOSPHATE CRYSTALS IN A MODEL SYSTEM OF TOOTH ENAMEL FORMATION. Mayumi Iijima, Yutaka Moriwaki, Asahi Univ, Dept of Dental Materials and Technology, Gifu, JAPAN; Hai Bo Wen, DePuy, A Johnson & Johnson Company, IN; Thoru Takagi, Tokyo Medical and Dental Univ, Dept Oral Biology, Tokyo, JAPAN; Alan G. Fincham, Janet Moradian-Oldak, Univ Southern California, Center for Craniofacial Molecular Biology, CA.

Tooth enamel crystals are formed under regulated Ca^{2+} and $PO_4{}^{3-}$ ion supply from the layer of ameloblasts into the enamel matrix where amelogenins are major protein component. Enamel crystals have prism-like morphology, which is quite different from that of bone and dentin crystals. The present study aims to evaluate the effects of 1) the ionic flow and 2) amelogenins on the morphology of octacalcium phosphate (OCP) crystals, which is a potent precursor of enamel apatite. To this end, growth of OCP was studied in a model system of tooth enamel formation, using 1) various concentrations of Ca and PO_4 solutions as ionic sources and 2) extracted bovine amelogenins and recombinant murine amelogenins (rM179, rM166). Some parallel reactions were carried out using albumin, gelatin, polyacrylamide gel and agarose gel for a comparison. The length of OCP crystal increased, while the width decreased with an increase in the amount of Ca^{2+} and/or $PO_4{}^{3-}$ ions flow. The length to width (L/W) ratio increased from 3 and 95, while the width to thickness (W/T) ratio decreased from 32 to 8 with an increase in concentration, resulting in longer and narrower ribbon-like crystals at higher concentrations. The effect of amelogenins was unique, regardless type of amelogenins : Crystal size decreased and rod-like and prism-like OCP crystals with small W/T ratio were formed in 10% amelogenins. On the other hand, characteristic ribbon-like OCP crystals grew without protein and with gelatin, albumin, polyacrylamide and agarose. Specific interaction of amelogenins with OCP crystal was ascribed to their hydrophobic nature. The present experimental data supported the view that the amount of ionic flux and amelogenin proteins played key roles in controlling biomineralization processes such as modulation of crystal morphology.

3:15 PM N6.5
FREEFORM FABRICATION OF TWO-STEP BIODEGRADABLE POROUS BONE PROSTHESES. Pierre Bourque, Paul Calvert, John Szivek, University of Arizona, AZ; Suzanne Maxian, Temple

University, Philadelphia, PA; Catherine Green and Ranji Vaidyanathan, Advanced Ceramics Research Corp, Tucson, AZ.

Freeform fabrication is attractive for the production of prosthetic materials because it allows arbitrary shapes to be formed directly from computer representations such as tomography data. It also allows internal porosity on a scale of 100 microns or more to be built into the structure as pages for bone in growth or vascularization or both. The samples prepared here comprise an assembly of 100-200 micron struts made from polybutyleneterephthalate using extrusion freeform fabrication of molten polymer. This material is slowly biodegradable and is expected to provide mechanical strength during the initial bone ingrowth. After this, the new bone will become load-bearing and the implant material will be slowly resorbed. In order to promote bone ingrowth into the pores, these were filled with a fluid mixture of polycaprolactone and tricalcium phosphate. Initial cell culture studies showed very slow penetration into the pores. Attempts to increase this by raising the phosphate level caused either intractably high viscosities of the impregnating mix or the need to use diluting solvents with accompanying concerns for toxicity. We therefore developed a technique for coating these porous structures with a water-based latex of polycaprolactone, phosphate and surfactant which is anneal after application. Cell culture studies with rat calvarial cells and with human osteoblasts show that these coatings are biocompatible. We believe that cells become attached deep into the pores. We also will discuss the extension of this coating method to lattices of glycollide-lactide copolymers and results from rat implantation studies.

3:30 PM N6.6
POROUS POLYLACTIDE/BIOACTIVE GLASS COMPOSITES FOR BONE TISSUE ENGINEERING AND SOFT-TO-HARD TISSUE INTEGRATION. Kai Zhang, University of Minnesota, Department of Chemical Engr and Materials Sci; Yunbing Wang, Marc A. Hillmyer, University of Minnesota, Department of Chemistry; Lorraine F. Francis, University of Minnesota, Department of Chemical Engineering and Materials Science, Minneapolis, MN.

Porous polylactide/bioactive glass composites are candidate materials for bone tissue engineering and artificial interfaces between soft and hard tissues, such as the cartilage/bone interface. In this work, porous polylactide/bioactive glass composites were prepared by phase separation of a polymer solution containing 0-29 Vo% bioactive glass particles ($\sim$1.5μm). The composite microstructures are porous with large pores (>100μm) in network of smaller (<10μm) interconnected pores and glass particles distributed homogeneously. The composites have dense top surface layers that can be removed after fabrication. The microstructure of the composites was not affected much by glass content. The bone bonding ability of the composites was studied in vitro by soaking composites in simulated body fluid (SBF). More apatite formed on the composites than on the porous polylactide alone after two weeks of soaking. This hydroxycarbonate apatite formation demonstrates the composites potential for bone tissue engineering and integration with bone. Mechanical tests showed that an increase in glass content increases the elastic modulus of the composites, but decreases their tensile strength. The mechanical properties can be enhanced through glass surface modification by 3-aminopropyltrimethylsilane. After modification, results showed the composites microstructure remained porous with more glass particles incorporated in the polymer matrix. The presence of silane on the glass surface did not affect the in vitro apatite formation ability of the composites.

3:45 PM *N6.7
ANTI-INFLAMMATORY PROPERTIES OF CERAMIC MATERIALS: A NEW APPROACH TO BIOMATERIALS DESIGN. John A. Frangos, Richard Suzuki, Department of Bioengineering, Univ of California, San Diego, CA; Julie Muyco, Joanna McKittrick, Materials Science and Engineering Program, University of California, San Diego, CA; Lars Bjursten, University of Lund, SWEDEN.

The prolonged inflammatory response to an implant is one of the primary causes for the failure to integrate into tissue. Recent evidence demonstrates that the inflammatory response is mediated by the reactive oxygen species generated by macrophages, leukocytes and surrounding connective tissue. We recently demonstrated that peroxynitrite, a potent inflammatory mediator produced in vivo by the reaction of nitric oxide and superoxide, is rapidly decomposed by titanium dioxide. We will present in vitro and in vivo results supporting the hypothesis that the excellent of biocompatibility of titanium and zirconium implants is due to the anti-oxidant properties of the surface oxides.

SESSION N7: POSTER SESSION
BIOMATERIALS
Thursday Evening, April 4, 2002
8:00 PM
Metropolitan Ballroom (Argent)

N7.1
HOOKS IN NATURE AND TECHNOLOGY: THEIR PERFORMANCE AND FAILURE. Ulrike G.K. Wegst, Eduard Arzt, Max-Planck-Institut für Metallforschung, Stuttgart, GERMANY; Elena V. Gorb, Stanislav N. Gorb, Max-Planck-Institut für Entwicklungsbiologie, Tübingen, GERMANY.

Nature's hooks have, directly and indirectly, played an important role in our everyday life for centuries. The plethora of hooked tips on the flower-head of the Fuller's Teasel *Dipsacus sativus*, for example, is still used industrially for raising a regular nap upon cloth. Another example is the hook-and-loop fastener mechanism of burrs which inspired Georges de Mestral to patent what is now commonly known as Velcro. The design of hooks in nature is diverse, even for one type of application, and they are believed to be optimised for the use of the respective organism. This raises a number of questions. How efficient are hooks both natural and man-made? How do they perform, how do they fail, and how do natural and man-made hook-and-loop fasteners compare? In order to answer these questions, the mechanical performance and failure mechanisms of natural and man-made hooks are analysed and described, and two types of failure maps are presented. One correlates geometry with material properties, failure modes and load at failure. Another illustrates - in conjunction with property charts for natural and man-made materials - how man-made materials compare with those of natural hooks, and how the most appropriate material can be selected for a given geometry and a desired failure mode.

N7.2
MAGNETIC MATERIAL ARRANGEMENT IN APIS MELLIFERA ABDOMENS. Darci M.S. Esquivel, Eliane Wajnberg, Geraldo Cernicchiaro, Bruno E. Garcia Centro Brasileiro de Pesquisas Físicas, CME, Rio de Janeiro, RJ, BRAZIL; Daniel Acosta, Avalos Pontifícia Universidade Catolica, Dept. Física, Rio de Janeiro, RJ, BRAZIL.

Honeybees are the most studied insects in the magnetic orientation research field. Different experiments aiming the localization of sensory magnetic particles in honeybees were reviewed (Vcha 1997). Based on the ferromagnetic hypothesis for magnetoreception a few remanent magnetization measurements were performed with honeybees showing the presence of magnetite nanoparticles in their abdomens (Wajnberg 2001 and ref there in). Isolated magnetite nanoparticles of about 3 x102 nm3 and 103 nm3 volumes, depending on the hydration degree of the sample and aggregates of these particles were suggested from Electron Paramagnetic Resonance (EPR) study. A low temperature transition (52 K - 91 K) was observed (El-Jaick 2001). This report is focused on hysteresis curves of Apis mellifera abdomens organized parallel and perpendicular to the applied magnetic field of a SQUID magnetometer (Quantum Design) from 5K to 310K. The differences in the hysteresis curves for the two abdomen orientations suggest that particles are preferentially oriented relative to the bee axis body. The saturation (Js) and remanence (Jr) magnetizations, coercive field (Hc) and initial susceptibility (Xini) are obtained. The smooth changes on these curves in the 120-150K range can be related to the Verwey transition. The anomalous increase of Jr, Xini and Js and decrease of Hc in the 20-30K range could be due to superparamagnetic particles with a low blocking temperature. The Jr/Js derivative curves present a $\sim$50K blocking temperature. Moreover other magnetic iron oxides than magnetite can be contributing to these values. These results point to the complexity of this system that requires complementary experiments and theoretical developments since the available models only consider non-interacting particles, temperature independent Magnetic anisotropy constant, etc.
El-Jaick, L. J. et al. Eur. Biophys. J. 29 (2001) 579 Vcha, M. Biol. Bratislava 52 (1997) 629 Wajnberg E. et al. JMMM 226-230 (2001) 2040.

N7.3
MAGNETIC MATERIAL IN TERMITES BY MAGNETIC RESONANCE. O.C. Alves, Dept de Fésico-Química, Universidade Federal Fluminense, Niterui, Rio de Janeiro, BRAZIL; E. Wajnberg, D.M.S. Esquivel Centro Brasileiro de Pesquisas Físicas, Rio de Janeiro, BRAZIL.

Biomineralized magnetic materials related to magnetoreception, which is a mechanism to detect the geomagnetic field, have been largely studied. Magnetic material was observed in the migratory ant species Pachycondyla marginata (P.m.) in two different structures, clusters and isolated particles, which were associated to different broad magnetic resonance lines. The termite specie Neocapritermes opacus (N.o.) is an interesting model for magnetoreception studies because it

is the only prey of this ant species. Magnetic resonance spectra of one oriented N.o. worker, with the body axis parallel or perpendicular to magnetic field, do not present any evidence of the cluster structure. Previous magnetization measurements using a SQUID magnetometer showed that the magnetic particles present in both insects are within the expected pseudo-single-domain or multi-domain regions, but the material quantity in termite is about one order of magnitude higher than those of the ant. Magnetic resonance spectra confirm the relative higher concentration of magnetic material in the termite than in the migratory ant. The resonance linewidths of the isolated particles of P.m. and the N.o. parallel or perpendicular to the magnetic field presents similar temperature dependence, characteristic of magnetic nanoparticles about 13 nm in diameter. On the other hand, the temperature dependence of the resonance field for N.o. perpendicular to the magnetic field presents a sudden drop at about 100 K that is not observed for N.o. parallel to the magnetic field. This behavior can be attributed to the Verwey transition, which depends on the thickness of the magnetic layer and suggests that in N.o. termites the magnetic particles could be arranged as a thin film perpendicular to their body.

N7.4
DISPLAY OF ESTROGEN RECEPTOR ON BIOMAGNETITE AND DETECTION OF ESTROGEN-LIKE CHEMICALS.
Tomoko Yoshino, Takahashi Masayoshi, Yoshiko Okamura, Takeyama Haruko, Tadashi Matsunaga, Tokyo Univ of Agriculture and Technology, Dept of Biotechnology, Tokyo, JAPAN.

In recent years a variety of expression systems for display of heterologous proteins on the surfaces of bacteria, bacteriophages, viruses and yeasts have been extensively developed. Different types of surface proteins have been used as anchors to display enzymes, single-chain antibodies and random peptides.
Magnetospirillum magneticum AMB-1 synthesizes intracellular nano-sized biomagnetites (bacterial magnetic particles: BMPs), which aligned in chains and enveloped by membranes. We have displayed functional proteins on the surface of BMP using MagA as an anchor protein, which is an iron translocating protein. By protein analysis of biomagnetite membranes, membrane specific protein Mms16 was isolated and characterized. In this study we attempted to display estrogen receptor on BMP using this protein as an anchor molecule for estrogen-like chemical detection.
An expression plasmid containing the *mms16* and DNA sequence for the estrogen receptor hormone binding domain (ERHBD) was transconjugated into AMB-1. ERHBD displayed BMP complexes were magnetically purified from ruptured recombinant AMB-1. Expression of ERHBD on BMPs was confirmed by a binding assay using alkaline phosphatase-conjugated 17β-estradiol. Luminescence intensity obtained from ERHBD-BMPs was 50 times higher (101.9 kcount/s) than wild-type BMPs, indicating successful ERHBD display and 17β-estradiol binding. Furthermore, a competitive binding assay for alkaline phosphatase-conjugated 17β-estradiol to ERHBD was performed using bisphenol A as a competitor. Luminescence intensity obtained from the binding of 17β-estradiol to ERHBD decreased with the addition of bisphenol A. Bisphenol A inhibited the binding of 17β-estradiol to ERHBD at 10^3-fold higher concentrations of alkaline phosphatase-conjugated 17β-estradiol. Therefore, we have successfully demonstrated that estrogen-like chemicals can be detected and evaluated using ERHBD-BMP.

N7.5
MICROSTRUCTURE AND THERMAL EXPANSION PROPERTIES OF OSTRICH EGGSHELL. Alejandro Heredia, L.F. Lozano, C.A. Martinez-Matias, M.A. Peña, A.G. Rodriguez-Hernandez, R. Velazquez, M.V. Garcia-Garduño, V. Basiuk-Evdokimenko*, L. Bucio and E. Orozco, IF-UNAM, MEXICO. *ICN-UNAM, MEXICO.

The science of biomimetics has the potential to enrich many areas of technology and to design new materials but this design should be based on the understanding of biomolecular mechanisms that make possible the preferential arrangement, preferential growth, porosity, particle size, association with biopolymers, etc. All these properties make the biological materials renowed models to mimic them due to their strength and thoughness. This is the case of ostrich eggshells. Formation of biominerals should be mimicked to synthesize composites based on crystal orientation and combinations of biomacromolecules related with common biological crystals, as in eggshells, bones and teeth, that is to say obtain ideas from the structures of living organisms to generate new hybrid materials. The aim of all kind of composite studies is the fabrication of organic-inorganic materials with controlled structures based on biologic ions that may lead to the fabrication of new materials with high performance and high function as well as environment benignity. The structural arrangement of calcite crystals reached in ostrich eggshell is discussed in terms of the analysis of the eggshell microstructure and thermal expansion. The crystal orientation of crystallites is not homogeneous along the eggshell thickness: the microstructural characterization by scanning and transmission electron microscopy (STEM), powder X-ray diffraction (XRD) and thermal expansion experiments (TEE) show that two main characteristic arrangement of calcite exist as well as high stability to thermal expansion in a wide range of values (from room temperature to near 450 Celsius degrees). In the outer surface the c-axis of calcite microcrystals appears closely aligned perpendicular to the outer eggshell surface; while in the inner surface, the hexagonal axis of the crystallites are directed along the eggshell surface.
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N7.6
THERMAL PROPERTIES OF MINERALIZED AND NON-MINERALIZED TYPE I COLLAGEN IN BONE. L.F. Lozano, M.A. Peoa Rico, A. Heredia, A.L. Gomez-Cortez, R. Velazquez, E. Orozco, L. Bucio, UNAM, Instituto de Fisica, DF, MEXICO; J. Ocotlan-Flores, UNAM, Centro de Instrumentos, DF, MEXICO.

The research about the structural stability of bone, as a composite material, compromises a complete understanding of the interaction between the mineral and organic phases. The thermal stability of human bone and type I collagen extracted from human bone by different methods was studied in order to understand the interactions between the mineral and organic phases when is affected by a degradation/combustion process. The experimental techniques employed were calorimetry, spectroscopy and gas chromatography techniques. The extracted type I collagens result to have a bigger thermal stability with a Tmax at 500 and 530 Celsius degrees compared with the collagen present in bone with Tmax at 350 Celsius degrees. The enthalpy value for the complete degradation/combustion process were similar for all the samples, being 8.4 +/- 0.11 kJ/g for bone collagen, while for extracted collagens were 8.9 +/- 0.07 and 7.9 +/- 1.01 kJ/g. These findings demonstrate that the stability loss of type I collagen is due to its interactions with the mineral phase, namely carbonate hydroxyapatite. This cause a change in the molecular properties of the collagen during mineralization, specifically in its cross-links and other chemical interactions, which have a global effect over the fibers elasticity, but gaining tensile strength in bone as a whole tissue. We are applying this characterization to analyze the diagenetic process of bones with archaeological interest in order to identify how the enviromental factors affects the molecular structure of type I collagen. In bone samples that proceed from an specific region with the same enviromental conditions, the enthalpy value per unit mass found to diminish exponentially with respect to the bone antiquity.
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N7.7
COMPARATIVE ANALYSIS OF HEALTHY AND TUMORAL HUMAN BONE TISSUES BY SPECTROMETRIC AND CALORIMETRIC TECHNIQUES. A. Acosta-Romero, Instituto de Fisica, UNAM, MEXICO DF; I. Belio-Reyes, Escuela de Odontologia, Univ. Autonoma de Sinaloa, MEXICO; L.F. Lozano, M.A. Peóa-Rico, A. Heredia, J.L. Ruvalcaba, L. Bucio, Instituto de Fisica, UNAM, Mexico DF, MEXICO.

Hyaline collagen can be present in bone tissue in such manner that no fibroblast cells are able to be seen. Hystologicaly, the hyaline collagen is difficult to distinguish from osteoid. The osteoid in the mineralization process (the accumulating hydroxyapatite carbonate crystals) is made by the osteblasts, and the look is much a like hyaline collagen. There are illness where could be useful to distinguish microscopicaly between hyaline collagen and osteoid. From the scope of the mineralization process, in this work we present the physical and chemical results of the experimental analysis employing differential scanning calorimetry, low vaccum scanning electron microscopy, FT Infrared spectroscopy and chemical analysis by Rutherford Backscattering Spectrometry (RBS) and Proton induceed X-ray emission (PIXE) in order to extract the physical and chemical features that characterize the hyaline colagen and the osteoid.

N7.8
LOW TEMPERATURE FORMATION OF HYDROXYAPATITE FROM EGG SHELL BY WET CHEMICAL SYNTHESIS.
M. Sivakumar, Xingguo Cheng, M. Olszta and L. Gower*; Department of Material Science and Engineering, University of Florida, Gainesville,FL 32611-6400, USA.

Porous hydroxyapatite [Ca10(PO4)6(OH)2, HA] is a potential implant material as a bone substitute owing to its excellent osteoconductive properties. The present methods of preparation of HA range from conventional wet synthesis, solid-state reaction, hydrothermal process and calcinations of animal skeletal bone. In this study, hydroxyapatite was synthesized from egg-shells by a wet chemical method at low

temperature. Analytical grade of diammonium hydrogen phosphate and calcium chloride, in addition to the soluble fraction of organic matrix extracted from egg-shells, were used as the starting materials. The analytical techniques of XRD, FT-IR, TGA and DTA were employed to characterize the precipitation products that resulted from precipitation of hydroxyapatite from the egg-shell. It is evident from the XRD and FT-IR data that the crystal structure and characteristic groups (phosphate and hydroxyl) present are the hydroxyapatite phase of calcium phosphate. The elemental analysis of synthesized hydroxyapatite and in-vitro stability of the samples in phosphate buffer solution of pH 7.2 at 37°C are in progress.

N7.9
TISSUE ENGINEERING OF TENDON IN ANISOTROPIC COLLAGEN GELS. Sarah Calve, Ellen Arruda, Robert Dennis, Karl Grosh, Univ of Michigan, Ann Arbor, MI.

Anisotropic collagen gels are developed in order to create a 3-D tendon construct it in vitro and to enable studies of tendon growth and formation. Tendons are essentially parallel fibers of collagen, ~70% of the dry weight, interspersed with fibroblasts that are responsible for the development and maintenance of tendon physiology. Tendon fibroblasts in culture attempt to align isotropic collagen gels, constrained only by cross-links, and when grown alone will spontaneously form macroscopic tendon-like structures that are under cell-mediated tension. We are currently reproducing these results with fibroblasts obtained from rat Achilles tendon to generate aligned collagenous structures along with developing an anisotropic collagen gel. Collagen gels as an isotropic solid from fibril condensation and native cross-linking. A stress applied to the solid in a channel die stretches the fibrils in one direction and allows for cross-linking while under strain, thereby 'freezing' the chains in a partially oriented network. Temperature, pH and chemical reagents have been shown to control the density of cross-links in collagen. Mott and Roland have termed anisotropic elastomers developed by a similar cross-linking process double networks. Alignment from collagen double networks will stiffen the collagen gel making it more robust and better able to withstand a mechanical stimulus. This will permit us to characterize the influence of mechanics on the development of tendons, which is a factor in maturation. We will also have a substrate that will allow for the study of the behavior of fibroblasts from tendon and other parts of the body on a pre-aligned substrate. This study will help us see if environmental cues influence the fibroblasts that create the different supporting connective tissues of the body (e.g. tendon, cornea, epidermis, fascia) or if the fibroblasts are physically distinct and retain their it in vivo behavior.

N7.10
AN ORGANIC MATRIX FOR INORGANIC CRYSTAL GROWTH. Lara A. Estroff and Andrew D. Hamilton, Yale University, Department of Chemistry, New Haven, CT.

A carboxylate containing organic hydrogelator[1] has been designed to study the effect of the microenvironment on the growth of calcium carbonate crystals. Recent work[2] has suggested that mineralization in the nacreous layer of bivalve mollusk shells occurs in a hydrogel rather than from a saturated solution. If this is so, the chemical environment of nucleation differs greatly in regards to diffusion, ion activities, water "structure" etc. as compared to the conditions present in crystallization from solution. The design and characterization of an artificial hydrogel for crystal growth allows the systematic investigation of different variables affecting crystallization from medium other than the solution phase. The bis-urea, dicarboxylic acid organic hydrogel has been characterized using cryo-TEM and X-ray diffraction. From these results a possible model of the molecular packing in the gel has been developed. The growth of calcium carbonate crystals in the colloidal gel has also been studied. The appearance of the calcite crystals grown in the organic hydrogel changes dramatically over time. Initially the crystals appear relatively unaffected. Crystals removed from the gel at progressively longer times show severely affected surfaces resulting from dissolution. After even longer times, there appears to be overgrowth occurring in addition to continued dissolution suggesting that the crystal is ripening and rebuilding itself. Crystals grown in the gel etch differently than control calcite crystals indicating that there may be gelator occluded in the calcite crystals. Therefore, the carboxylate functionality designed into the gelator appears to promote strong interactions between the gelator molecules and the growing crystals. This provides a unique microenvironment in which the molecules that form the matrix also actively participate in the crystallization.
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(2) L. Levi-Kalisman, G. Falini, L. Addadi, S. Weiner, *J. Structural Biology*, **2001**, *135*, 8-17.

N7.11
AN ASSAY FOR MINERALIZATION INHIBITION. Sajiv Boggavarapu and Paul Calvert, University of Arizona, AZ.

Many synthetic and biological molecules inhibit or retard the crystallization of biominerals such as calcium phosphate and carbonate. The effect is normally monitored by determining the change in inhibition time for a spontaneous precipitation from a supersaturated solution. We have been studying the formation of composite materials by growing precipitates into a hydrogel. The gel is initially formed with a solution of soluble calcium salt and is then immersed in carbonate or phosphate solution to induce mineralization. We have been using this method to assay mineralization inhibitors. A drop of inhibitor solution is injected into the solid calcium-containing gel in a petri dish. The gel is then covered with the anion solution to induce mineralization. Circles of inhibition are seen whose diameter depends on the efficiency and quantity of inhibitor used. This method has been applied to both carbonate and phosphate mineralization. It lends itself to comparative assays of large numbers of inhibitors. An analysis of the coupled diffusion and precipitation processes will be presented.

N7.12
BIOMIMETIC APATITE DEPOSITION ON SOL-GEL DERIVED SILICA GEL. Kanji Tsuru, Masaaki Kubo, Satoshi Hayakawa, Akiyoshi Osaka, Okayama Univ, Faculty of Engineering, Okayama, JAPAN.

Nucleation and crystal growth mechanism of apatite formation on porous silica gel derived by sol-gel procedure was investigated by the use of several simulated body fluids (SBF's) that had different concentrations of Ca(II), P(V), and OH^- but had the same degree of supersaturation for hydroxyapatite. Induction time of apatite crystallization in SBF's was evaluated by thin film X-ray diffractometry. The effect of each ion on the induction time increased in the order: Ca(II)-rich SBF < P(V) rich SBF ll OH^- rich SBF, while that for the rate of initial crystal growth of apatite increased in the order: P(V) rich SBF < Ca(II) rich SBF < OH^- rich SBF. It suggested that amorphous calcium phosphate (ACP) deposited as the precursor of apatite and ACP transformed into apatite nuclei by the incorporation of OH^-. Moreover, the transformation was accelerated by large amount of ACP and was assisted by the rearrangement of silicate units in the gel surface. Ca(II) was richer in ACP deposited in Ca(II)-rich SBF and OH^--rich SBF than that deposited in P(V)-rich SBF. Ca(II)-rich ACP was favorable for crystal growth.

N7.13
CALCIUM PHOSPHATE MATERIALS FROM AOT REVERSE MICELLES-A MODEL FOR BIOMINERALIZATION. Christabel E. Fowler, Henry C. Margolis, The Forsyth Institute, Boston, MA; Mei Li, Stephen Mann, University of Bristol, Dept of Chemistry, Bristol, UNITED KINGDOM.

Organized assemblies of surfactants have been shown to provide suitable environments for the controlled growth of inorganic materials. Since certain biomolecules (e.g. amelogenin within developing tooth enamel) may function similarly in the process of biomineralization, model systems using surfactants may provide insight into the mechanisms of mineralized tissue formation, as well as provide a route to the synthesis of new materials with controlled dimensional, structural and morphological specificity. In this study, the surfactant bis(2-ethylhexyl)sulfosuccinate sodium salt (AOT) was used to stabilize reverse micellar arrangements, and calcium phosphate materials synthesized within the aqueous regions. AOT was used previously to prepare inorganic materials with unique morphologies, however there have been no investigations using high surfactant concentrations, where extended reverse phases exist. Different molar ratios of AOT, aqueous calcium/phosphate solutions, isooctane, and m-xylene were mixed at room temperature. Products were analyzed using TEM, EDAX, ED, SEM, DLS and polarized light microscopy. An array of morphologies was obtained, dependant on the ratios of the reaction constituents. One sample consisted of bundles of aligned crystals of hydroxyapatite, with an ED pattern exhibiting the "arc" familiar to ED of developing enamel. A variety of other morphologies were obtained, including amorphous nanoparticles, and hollow spheres. The results show that the organized medium can lead to precise control over the inorganic structure obtained. The procedure may be suitable for the preparation of other minerals, providing a route to the formation of new materials with interesting and novel properties. The attainment of aligned HA crystals also promotes the system for use as a suitable model for the formation of tooth enamel.
Supported by NIDCR grant DE-13237.

N7.14
THE EFFECT OF SURFACE COVERAGE ON THE PROPERTIES OF BIOMINERALS. Wendy J. Shaw, Daniel Stevens, Lindsey van Schoiack, Allison A. Campbell; Pacific Northwest National Labs, Richland, WA.

The secondary structure of biomineralization proteins is thought to play an important role in the formation of biominerals, however, there is very little experimental evidence to explain the underlying mechanisms. To begin to elucidate these mechanisms, our investigation focused on the peptide consisting of the N-terminal 15 residues of the salivary protein, statherin (DpSpSEEKFLRRIGRFG), both for its own biomineralization properties as well as its relevance as a model for other tooth and bone proteins. The secondary structure of the peptide as well as the growth kinetics and electrostatic charge have been studied as a function of surface coverage to provide further insight into the formation mechanisms. The peptide/HAP system was studied with several different techniques as a function of surface coverage: constant composition kinetics (CCK) was used to determine crystal growth, the zeta potential was measured to give insight into the surface charge, and solid state NMR (SSNMR) was utilized to determine the secondary structure and dynamics in the central region (L8-G12) of the surface immobilized peptide. Previously, we have found that at a monolayer coverage, the peptide is largely helical, though slightly less helical at the two ends, with approximately 20°-30° distribution in the torsion angles that were measured. Preliminary results at other coverage levels indicate that there is no a change in secondary structure as a function of surface coverage. CCK results have also been obtained at a monolayer coverage. The CCK evidence at a monolayer coverage suggests that charge interactions, rather than crystal face specific sites induce bonding. With the current constraints, many models describe these observations, however, one possibility is that the lack of change in secondary structure at lower coverage levels suggests that the helical solubilized peptide maintains the secondary structure as it binds to the surface, altering the structure slightly to find an energy minimum.

N7.15
PREPARATION AND PHYSICOCHEMICAL PROCESS OF HA NANOSTRUCTURED MATERIAL. Shulin Wen, Yunjing Song, College of Material Science and Engineering, Shandong University, Jinan, CHINA.

The investigations of the preparation and physicochemical process of the HA nanostructured materials with high performance have been performed at present study. The HA preparation started from the ethanol solution of calcium nitrate tetra-hydrate and P_2O_5 as raw materials. And the characterization of the effects of reacting temperatures on the grains sizes, their morphology, and their crystalline degrees (some amorphous HA formed at certain condition) of the reacting products carried out in the meanwhile. The physicochemical processes and the conditions for different reactions stages of the HA preparation have been traced and characterized by the TG-DTA (thermogravimetric and differential thermal analysis), the FTIR (Fourier-transformed infrared spectroscopy) and other methods. The investigation of the chemical reactions for the HA preparation showed that the $PO(OH)_{3-x}(OR)_x$ was synthesized when the P_2O_5 was added to the ethanol solution. and then was transformed to the HA completely at the temperature of 500° for hours. The grain sizes and shapes and other microstructural features of the HA reacting products at different temperatures were observed and characterized by the SEM, the TEM and the XRD. The results show that the mean diameters of these product grains were as fine as 30-40nm at the temperature of 500°C, and as fine as 50-70nm at the temperature of 800°C, and as fine as 100-200nm at the temperature of 1250°C. The XRD pattern of the present HA powders heated at 500°C for 2 hrs coincided very well with the JCPDS standards showing its superior purity and therefore, with high performance for later applications.

N7.16
ENTROPIC BARRIERS IN NANOSCALE ADHESION STUDIED BY VARIABLE TEMPERATURE CHEMICAL FORCE MICROSCOPY. Salvador Zepeda , Yin Yeh, University of California, Davis, Davis, CA; Christine A. Orme, James J. DeYoreo, Aleksandr Noy, Lawrence Livermore National Laboratory, Livermore, CA.

Intermolecular forces drive a variety of phenomena in biological systems, such as cell adhesion, protein folding, and molecular recognition in ligand-receptor pairs. Understanding the dynamics of these interactions is critical for modeling and controlling these processes. The advent of ultra-sensitive force measurement techniques has enabled direct measurement of the bond strength on the relevant length scales. Recent measurements have pointed out the importance of kinetic factors in bond strength and the necessity to explore the whole energy landscape of a chemical bond. Still, little is known about the sensitivity of the of the bond strength to changes in temperature despite the fact that the analysis of thermal response provides a way to determine thermodynamic characteristics of the binding interaction. We used atomic force microscopy to measure the temperature dependence of the interaction forces in a well-defined system presenting a finite number of identical interactions between the force microscope tip and sample surface. The tip of the scanning probe microscope was modified with distinct chemical functionalities to change the chemical nature of the interaction. We modified the microscope to allow rigorous control over the temperature in the microscope fluid cell over a wide range of temperatures. Using this setup we measured interaction forces between different tip-sample functionalities as a function of temperature in a series of solvents and solvent mixtures. Some measurements show an increase in the interaction force with the temperature. We will discuss these results as well as the theoretical framework for interpretation of such measurements. We will focus on the relative importance of thermodynamic and kinetic factors affecting the bond strength in the presence of solvent medium, specifically on the entropic contributions to the interaction strength.

N7.17
SOLUTION-ASSISTED TRIBOLOGICAL MODIFICATION OF A BIOMINERAL USING AN ATOMIC FORCE MICROSCOPE. Tom Dickinson, Razal Hariadi, and Steve Langford, Washington State University, Pullman, WA.

When a surface is subjected to tribological loading, bonds experience time dependent distortions and spatial deformations. In the presence of simultaneous chemical stimulation (e.g., from a solution), this can lead to bond breaking, bond formation, and nuclear rearrangement. We present new studies of combining mechanical and chemical stimuli on a model biomineral: brushite ($CaHPO_{4.2}H_2O$), particularly under conditions of solution supersaturation. Thermodynamically, the system tends towards deposition or crystal growth; we show that nucleation and growth on the surface can be controlled on the nanometer size scale using simultaneous mechanical stimulation with an AFM tip. New details of this process are presented with strong support of suggested models using analysis of small perturbations in the frictional force. Careful analysis of the 'noise' in the cantilever motion during contact scanning shows that on single crystal surfaces we are very sensitive to the presence of sub-critical cluster formation and re-dissolution, we find that the amplitude of the noise increases by factors of 2-4. We take this as indirect evidence for the presence of these precursors to recrystallization. Furthermore, rich noise spectra are observed on crystal surfaces with low symmetry when one changes the scan direction we observe modulated signals at frequencies corresponding to calculated times between asperity-lattice row encounters. Again, under supersaturation, the noise levels rise in comparison with pure solvent. Finally, we present structures and surface modifications that can be induced by these mechanical/ chemical synergisms.

N7.18
SPATIO-TEMPORAL PATTERNS IN FERRITIN CRYSTAL GROWTH. Olga Gliko, Peter G. Vekilov, Univ of Houston, Dept of Chemical Engineering, Houston, TX.

The loss of stability of equidistant step trains leads to bunches of steps spreading along the crystal face, interspersed with bands of lower step density. The step bunches leave trails of higher defects density in the crystal lattice, and in this way lower the perfection and the utility of the grown crystals. To investigate the mechanisms underlying the appearance and the evolution of the step bunches, we developed a novel phase-shifting interferometry setup, in which the growing face of a protein crystal substitutes one of the mirrors. The phase shifting algorithm employs five-image sequences captured with a phase shift of $\pi/2$; digital processing of the sequence allows reconstruction of the surface morphology with a depth resolution < 5 nm over a viewfield as wide as 1 mm. A second data collection routine is based on the time traces of the growth rate and local slope variations recorded with time resolution of 0.2 s. We applied this technique to the study of the unsteady kinetics during the crystallization of the protein ferritin. We found that the source of growth layers is always 2D-nucleation. For small crystal sizes and at supersaturations $\sigma = \ln(C/C_e) < 3$ (C-concentration, C_e-solubility) the distribution of 2D-nuclei is uniform, and surface remains flat as crystals grow. For higher supersaturations and crystals larger than 100 μm, 2D-nuclei eventually localize at the facet edges and corners. Numerical modeling in our laboratory has linked this to higher interfacial supersaturation at the edges. We determined the supersaturation dependencies of normal and tangential growth rates are. The effective kinetic coefficient found for $\sigma < 3.6$ is 6×10^{-4} cm/s is equal to the one obtained by AFM for apoferritin and ferritin; at higher supersaturations the value increases by 1.5×. The local slope does not depend on supersaturation. Surface profiles show strong fluctuation of local slope that imply step bunching. While the local slope increases with increasing distance from the layer sources, the amplitudes of its

fluctuations decrease. The time traces show fluctuations of the growth rates and local slope as strong as 100% of the respective average values. The amplitude of these fluctuations decreases as the crystal size increases. The frequency of local slope fluctuations increases with supersaturation. Since crystal size, the location on the facet, and the mean supersaturation are major parameters characterizing the protein supply field, we conclude that the fluctuations are rooted in the coupling of the interfacial processes of growth to the bulk transport in the solution. A means to control the undesired unsteadiness would be by changing the rate of transport in the solution.

N7.19
TEMPLATE-DIRECTED NUCLEATION OF AMINO ACIDS. Alfred Y. Lee, Allan S. Myerson, Illinois Institute of Technology, Dept. of Chemical Engineering, Chicago, IL; Abraham Ulman, Polytechnic University, Dept of Chemical Engineering, Chemistry & Material Science, Brooklyn, NY.

Control of crystal morphology is very important in pharmaceutical technology and design of drug delivery systems. Here we demonstrate that self-assembled monolayers (SAMs) of rigid thiols on gold can serve as heterogeneous nucleants and modify the morphology of L-alanine and DL-valine crystals. Powder x-ray diffraction and interfacial angle measurements reveal that functionalized SAMs induce the formation of L-alanine and DL-valine crystals in different crystallographic directions and that the nano-templates are unable to resolve DL-valine into its optically pure enantiomers. The observation of crystal nucleation and orientation can be attributed to the strong interfacial interactions in particular, hydrogen bonding, between the surface functionalities of the monolayer film and the individual molecules of the crystallizing phase during nucleation. Molecular modeling studies are also undertaken to examine the molecular recognition process across the interface between the surfactant monolayer and the crystallographic planes. Similar to studies of solvent and additive interactions on crystal habit surfaces, binding energies between SAMs and particular amino acid crystal faces are calculated using Cerius2 and the results are in good agreement with the observed nucleation planes of the amino acids. In addition to L-alanine and DL-valine, the interaction of SAMs and mixed SAMs of rigid thiols on the morphology of alpha-glycine are investigated. These results demonstrate that binding energy calculations can be a valid method to screen self-assembled monolayers as potential templates for nucleation and growth of organic and inorganic crystals.

N7.20
INTERACTIONS OF SILICA PRECURSORS WITH AMINO ACIDS, PEPTIDES AND PROTEINS. Thibaud Coradin, Olivier Durupthy, Aurelie Coupe, Jacques Livage, Chimie de la Matiere Condensee, Universite Paris VI, Paris, FRANCE.

Diatoms provide a very nice example of the strategies used by Nature to build carefully designed silica. The biosilicification process takes place at the interface between silica precursors and biomolecules produced by the cells. If we want to understand how this process takes place, we must first study the nature of the interactions that may arise between proteins and the available silica species. In this context, we have chosen a highly diluted sodium silicate solution, acidified to neutral pH. We have also selected different amino acids and their homopeptides that exhibit different functionnalities. Using the molybdosilicate method, we demonstrate that amino acids interact poorly with silicic acid. In sharp contrast, some homopeptides, p-Lysine and p-Arginine are able to induce silica formation. The condensation rate depends highly on the polymer chain length. These studies suggest that the distribution of amino groups along the peptide chains allows silica oligomers formation that may serve as nuclei for silica polymerization. Parallel studies were performed using colloidal silica that confirm the strong influence of the amino containing polypeptides on silica formation. Finally, extension of these studies for more complex biomolecules will be presented. Both approaches indicate that monomeric (silicic acid) and pre-condensed (particles) silica precursors could be involved in the biosilicification processes.

N7.21
MEMBRANE-MEMBRANE INTERACTION AND ADHESION STUDIES USING MICROPRINTED SUPPORTED LIPID MEMBRANES AND MEMBRANE-COATED MICROBEADS. Annapoorna R. Sapuri, Micheal M. Baksh, Jay T. Groves, University of California, Dept of Chemistry, Berkeley, CA.

Cell-cell and cell substrate interactions play a crucial role in numerous biological processes including the immune response. Studies of cell-cell recognition reveal an important role for lateral rearrangement of receptors and ligands in apposing membranes during recognition. In order to better characterize the physical and chemical properties of collective molecular binding at the junction between fluid lipid membranes, we have developed a system in which membrane-coated microbeads of various compositions interact with microprinted arrays of supported lipid membranes. Brownian motion of the microbeads allows them to sample multiple sites on the microprinted substrate. The relative density of beads over different regions of the patterned substrate can thus reveal information about binding energies and discrimination characteristics. This intrinsically high-throughput technique is easily multipexed and may also be a more direct probe of adhesion energy than other techniques, such as shear-flow analysis.

N7.22
FLEXIBILITY AND FLUIDITY OF LIPID BILAYERS AT A MEMBRANE-MEMBRANE-SOLID JUNCTION. Yoshihisa Kaizuka, Jay T Groves, Univ of California Berkeley, Dept of Chemistry, Berkeley, CA.

Lipid membranes supported on solid substrates such as silica have proven useful in a wide range of physical and biological studies. Lipid molecules and peripherally tethered membrane proteins exhibit free lateral diffusion in the supported membrane. However, transmembrane proteins generally drag on the underlying solid substrate, becoming imobilized, and topographical fluctuations of the membrane can be strongly damped by the substrate. We have recently introduced a supported membrane junction consisting of a conventional supported bilayer membrane, on top of which a second bilayer membrane is deposited by rupture of a giant vesicle. This second (upper) membrane is stably associated with the lower supported membrane and the solid support, but it exhibits properties different from membranes supported directly on a solid substrate. Although individual lipid molecules diffuse freely in both upper and lower membranes, collective fluid movements, such as the thermal motion of phase separated fluid domains, can only be observed in the upper membrane. Such domains in the same membrane mixture deposited directly on the silica substrate are fixed in place. We observe this by examining membranes consisting of two coexisting fluid phases, an unsaturated phosphatidylcholine (PC)-rich liquid ndisordered phase and a cholesterol and sphingomyelin-rich raft-like phase. Independent fluorescent labels can be used to identify the two phases. Coexisting phases can be imaged in either the lower supported membrane or the upper membrane, allowing for direct comparison. The lower supported membrane provides a lubricating layer that enables a greater degree of fluidity and topographical flexibility in the upper membrane. This has been employed to study interactions between membranes and may also prove to be a useful strategy of interfacing solid materials with flexible and fluid cell membranes.

N7.23
APPLICATIONS OF A NOVEL BIO-MIMETIC COLORIMETRIC MEMBRANE ASSAY FOR STUDYING ANTIMICROBIAL PEPTIDES. Revital Halevy[1], Elad Landau[1], Lizuan Zhang[2], Kolusheva Sofiya[1], Robert E.W. Hancock[2], Raz Jelinek[1] . [1]Department of Chemistry, Ben-Gurion University of the Negev, Beersheva, ISRAEL. [2]Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, CANADA.

We have developed a new colorimetric model membrane for studying peptide-membrane interactions. The assay consists of vesicles composed of polymerized polydiacetylene (PDA), and lipids, such as phospholipids, lipopolysaccharides, and others. The vesicles undergo dramatic visible blue - red transitions induced by interaction with membrane peptides. We show that the colorimetric transitions are directly related to biological association of the peptides with the phospholipid moieties within the vesicles, and a correlation exists between the degree of blue-to-red transition and mode of peptide-membrane binding. We demonstrate the application of the new colorimetric assay for studying various antimicrobial peptides, including cecropin-melittin (CEME) conjugates and indolicidin derivatives.

SESSION N8: POSTER SESSION
TISSUE ENGINEERING
Thursday Evening, April 4, 2002
8:00 PM
Metropolitan Ballroom (Argent)

N8.1
SELF-ASSEMBLY OF HYDROGELS FROM ELASTIN-MIMETIC BLOCK COPOLYMERS. Elizabeth R. Wright, R. Andrew McMillan, Emory Univ, Dept of Chemistry, Atlanta, GA; Alan Cooper, Univ of Glasgow, Chemistry Dept, Glasgow, Scotland, UNITED KINGDOM; Robert P. Apkarian, Emory Univ, Integrated Microscopy and Microanalytical Facility, Dept of Chemistry, Atlanta, GA; Vincent P. Conticello, Emory Univ, Dept of Chemistry, Atlanta, GA.

One approach for the production of thermoreversible hydrogels is the

synthesis of amphiphilic triblock copolymers. Block copolymers of this design are able to self-assemble into well-defined mesoscopic aggregates depending on the solvent in which the system is dissolved. Traditionally triblock copolymers that are able to aggregate into microdomains have been designed as ABA-block copolymers, whereby the solvent is specific for the A-blocks and is incompatible with the B-blocks. The microdomains contain a dense core of B-blocks and a flexible corona of A-blocks, the resultant structures are spherical, rod, thread, or disk-like in nature. In the production of BAB-block copolymers, a different aggregation behavior occurs. The solvent is specific for the A-block only. The resulting structure consists of a solvent-swollen network of spherical particles of the insoluble end blocks that are linked together through the soluble mid-block. Triblock copolymers have traditionally been synthesized with conventional organic components. However, triblock copolymers could be synthesized by the incorporation of two incompatible protein-based polymers. The polypeptides would differ in their hydrophobicity and confer unique physiochemical properties to the resultant materials. One protein-based polymer, based on a sequence of native elastin, that has been utilized in the synthesis of biomaterials is poly (Valine-Proline-Glycine-Valine-Glycine) or poly(VPGVG). This polypeptide has been shown to have an inverse temperature transition that can be adjusted by non-conservative amino acid substitutions in the fourth position. By combining polypeptide blocks with different inverse temperature transition values due to hydrophobicity differences, we expect to produce amphiphilic polypeptides capable of self-assembly into hydrogels. Our research examines the design, synthesis and characterization of elastin-mimetic block copolymers as functional biomaterials. The methods that are used for the characterization include rheology, variable temperature 1D and 2D High Resolution-NMR, cryo-High Resolution Scanning Electron Microscopy and Differential Scanning Calorimetry.

N8.2

LIPID EXCHANGE RATES OF CONVENTIONAL AND POLYMER STABILIZED LIPOSOMES. Awad Ahmed, Nicole Heldt, Gregory Slack, Yuzhuo Li, Department of Chemistry and Center for Advanced Materials Processing,Clarkson University, Potsdam, NY.

Polymer-stabilized liposome systems consisting of polyethylene glycol bound lipids (PEG-lipids) and conventional (nonpolymer stabilized) liposomes were investigated for lipid migration between membranes. In order to monitor the exchange rate of lipids between liposomes, 1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphocholine (PY-PC), a phospholipid, with pyrene attached to the hydrophobic tail, was used to label both liposome systems. Labeled and unlabeled liposome systems were mixed and fluorescence spectroscopy was used to examine the lipid transfer rate. More specifically, the relative excimers to monomer (E/M) peak intensities in the fluorescence spectra before and after mixing were employed to deduce the kinetic information. The E/M provides a means to determine the number of PY-PC molecules that are in close proximity to form collision complexes of ground and singlet state. After mixing labeled and unlabeled liposome systems, the E/M ratio for PY-PC in polymer stabilized liposome systems decreased by 66% over a period of 80 minutes, while the E/M for PY-PC in conventional liposome systems decreased 70% in less than 2 minutes. This suggests that the exchange rate for lipids in polymer stabilized liposome systems is much slower than the lipid exchange rate of conventional liposome systems. In addition, the exchange rate for both conventional and polymer stabilized liposome systems is accelerated at elevated temperatures.

N8.3

DRUG DELIVERY SYSTEMS AS INFECTION-RESISTANT AND BIOMIMETIC IMPLANT MATERIALS. Jórg Michael Schierholz, Centre of Advanced European Studies and Research, Dept Implant Materials, Bonn, GERMANY.

Exposure to invasive medical devices represents one of the most important risk factors for nosocomial infection. Devices predispose to infection by damaging or invading epithelial or mucosal barriers, by supporting growth of microorganisms and thus serving as reservoirs, by impeding host defense mechanism, and, when contaminated, by directly infecting patients, thus accounting for approximately 45% of all nosocomial infections. The adverse effects for intravascular devices include thrombosis, infection as well as restenosis of the artery. Invasive devices such as catheters, drainages and metal pins act as an vector for local infections. Incrustation and infection of urinary catheters, which are mainly independent from the choice of the material, cause a great number of nosocomial urinary tract infections. Bone substitutes like hydroxy-apatite and metallic endoprostheses are particulary disintegrated from bone healing with and without infection. Traumatized tissues and implants represent foreign body substrata and are recognized as ideal surfaces for bacterial colonization by minimal or smaller inoculi of bacteria. Within hours of contamination, bacterial adhesion propagates and resistance develops creating foci of recurrent infections refractory to host defences and antibiotics. The presence of subcutaneous foreign material resulted in a decrease of the minimal infection dose of staphylococci from $> 10^6$ to $<10^3$ cfu. The presence of the foreign body also enhances infectivity, whereas inflammation decreases the effectiveness of host defense mechanisms and alters susceptibility of bacteria to antibiotics. The morbidity risk and complications of these devices and procedures may be considered in emerging disease. Thus, bacterial adhesion to biomaterials and the lack of successful tissue integration are recognized as the major barriers to the expanded use of artificial devices For reducing device-associated adverse reactions such as failure of tissue integration, infection, thrombosis, encrustation and intima hyperplasia, local delivery of pharmaceutical active substances from the devices seems to be prudent. Manipulations of molecular bonding (covalent and non-covalent) structures allowed drug release across a period of weeks. New polymer coating materials are being developed that bind to metallic devices, and to those made of other materials. In comparison to superficial coating systems, sustained delivery of rifampicin and miconazole and other rifampicin combinations from short-term and long-term catheters gave excellent antibacterial in vitro and in vivo results (1-3). The combined use of medical devices with pharmaceutical-biological activities such as antibiotic active catheters may reshape the medical landscape if both clinical efficacy as well as efficiency is well demonstrated. Drug-releasing scaffolds for improved tissue-engineering are the next future step for improved tissue-integration.

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1. J.M. Schierholz, C. Fleck, J. Beuth, G. Pulverer. The antimicrobial efficacy of a new central venous catheter with a long-term broad-spectrum activity, JAC, 46(1), 45-50 (2000).
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3. J.M. Schierholz, J. Beuth, Implant infections - A haven for opportunistic bacteria. J.Hosp.Inf. 49(2), 87-93 (2001).

N8.4

INFLUENCE OF HYDROGEL STRUCTURE ON OSTEOBLAST FUNCTION. Patricia S. Arauz and Rebecca Kuntz Willits, Department of Biomedical Engineering, Saint Louis University, St. Louis, MO.

Uses of hydrogels in biomedical sciences range from drug delivery systems to adhesives because of their swelling capacity, biocompatibility and mechanical strength. The focus of this project is the use hydrogels as adhesives and tissue engineered constructs for orthopedic applications. Specifically, this study examines the effect of gel composition and structure on cell growth and function. Two polymers were chosen for analysis poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA). Formation of PEG hydrogels was accomplished by cross-linking acrylate modified PEG using a photoinitiator; PVA hydrogels were formed via freeze-thaw process (18h freeze-6h thaw, 7 cycles). The hydrogels were cut into discs with a diameter of 2 cm and thickness of approximately 1.5 mm. While swelling equilibrium was found to vary with concentration of the gels, the composition of the gel had little effect. Equilibrium was reached for both 10% PVA and 10% PEG after 4-5 hours, while the 15% PVA required approximately 7 hours. To further examine these changes in swelling, the hydrogels will also be characterized by electron microscopy and viscoelasticity. Interactions between the cells and the gels were determined by seeding with an osteoblast-like cell line ($1.35X10^5$ cells/gel) stained with a fluorescent dye specific for DNA. Preliminary tests determined that over a 2 day period the cells survived with minimal change in cell number. Both composition and concentration appeared to have an effect on the cell number, although more studies are necessary for definitive conclusions. Ultimately, a correlation between gel structure and cell function will aid in the formation of an optimal hydrogel for orthopedic applications.

N8.5

GUIDANCE CHANNEL DEVELOPMENT: CONTROLLED RELEASE OF NGF FROM PLGA SCAFFOLDS. Sarah E. Stabenfeldt and Rebecca Kuntz Willits, Department of Biomedical Engineering, Saint Louis University, St. Louis, MO.

Four categories of stimuli have demonstrated influential effects on neural regeneration: mechanical, electrical, chemical and surface characteristics. Our lab is interested in developing a nerve guidance channel that optimally combines these various stimuli. Previously, the effect of composition and surface characteristics of a synthetic microtube on neurite outgrowth was examined. For this study, four microtube constructs were fabricated: porous and nonporous microtubes of both poly(lactic-co-glycolic acid) 85:15 (PLGA) and poly(L-lactic acid) (PLLA). Data analysis indicated a significant difference between the neurite extensions within porous and nonporous constructs; the connectivity of neurites between cells was increased in a nonporous construct over a porous construct. However, little or no difference was exhibited when comparing the composition

of the tubes. Because recent studies have noted that administration of certain neurotrophic factors, such as nerve growth factor (NGF), increase neurite outgrowth and neuronal regeneration, additional neurite stimuli sources are being incorporated into guidance channels. The focus of the current project is to characterize the release of NGF into aqueous and tissue-like environments. Protein-loaded PLGA scaffolds have been fabricated by standard dissolution-evaporation techniques with the inclusion of poly(ethylene glycol) 400 (PEG 400) as protective element against protein denaturation. Release profiles of the model protein, ovalbumin (OVA), within both aqueous and three-dimensional environments will be examined through protein assays and fluorescence techniques. The release of active NGF from a scaffold into a collagen gel containing PC-12 cells will assess the bioactivity of NGF as these cells respond to small amounts of active NGF with neurite extensions. The results from this study will aid in the development of a conduit that acts simultaneously as a guidance channel for neuronal extensions and a reservoir for the release of chemical stimuli such as nerve growth factor.

N8.6

ENHANCED INTRACELLULAR DELIVERY OF CATIONIC LIPOPLEXES FOR GENE THERAPY WITH PH-SENSITIVE SYNTHETIC POLYMERS. Charles Y. Cheung, Jasmine K. Zia, Patrick S. Stayton, Allan S. Hoffman, University of Washington, Department of Bioengineering, Seattle, WA.

Cytoplasmic delivery of DNA from nonviral gene therapy vectors is essential for therapy efficacy. The endosomal release of internalized vectors, therefore, represents a vital component in gene delivery. Lysosomal degradation of these vectors reduces overall transfection efficiency, requiring that the vectors be released from endosomes prior to their maturation to lysosomes for effective vector delivery. Poly(propylacrylic acid) (PPAA) is a synthetic, pH-sensitive polyanionic polymer that has the potential to enhance cytoplasmic delivery of DNA by mediating the acid-catalyzed disruption of endosomal membranes. PPAA has previously been shown to enhance nonviral gene transfection efficiencies in vitro upon incorporation into DOTAP cationic lipoplexes. DOTAP lipoplexes containing either poly(methylacrylic acid) or poly(ethylacrylic acid), which are less hydrophobic poly(alkylacrylic acids) than PPAA, showed minimal enhancement in transfection efficiencies over DOTAP lipoplex control formulations lacking any polymer, implying that the added hydrophobicity of PPAA is essential for transfection enhancement. In addition to enhancing the gene transfection efficiency, PPAA also significantly improved the serum-stability of DOTAP vectors. Whereas DOTAP control vectors were completely inefficient at transfecting cells in media containing as little as 10% fetal bovine serum, transfection with DOTAP vectors containing PPAA maintained high levels of gene expression in media containing up to 50% serum. DNA condensation studies in the presence of 10% FBS indicated the release of the plasmid DNA from DOTAP control lipoplexes, whereas DNA was retained within DOTAP lipoplexes incorporating PPAA. Further investigations with specific serum proteins suggest that serum lipoproteins play a significant role in DNA instability within control lipoplexes, but that addition of PPAA protects against DNA release from lipoplexes. These results may help explain the serum-stabilizing effects provided by PPAA to DOTAP lipoplexes.

N8.7

INORGANIC-SPECIFIC PEPTIDE TEMPLATES ISOLATED FROM A PHAGE DISPLAY PEPTIDE LIBRARY. Rajesh Naik, Sarah Stringer, Laura Sowards, Sharon Jones, Lawrence Brott, Gunjan Agarwal, & Morley Stone Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB, Dayton, OH.

Many biological organisms contain specialized structures composed of inorganic materials. Cellular processes in vivo facilitate the organized assembly of mineral building blocks into complex structures. The structural hierarchy and complexity across a range of length scales are providing new ideas and concepts for materials science. Proteins that direct biomineralization can be used to control the production of nanostructured materials and facilitate the fabrication of new structures. Here, we demonstrate the isolation of inorganic-specific peptides from a combinatorial phage peptide display library. These peptides act as templates in inorganic material synthesis and provide a means of understanding how some of the biological systems may be carrying out materials chemistry in vivo. The overall goal of this research is to use biological templates in bottoms-up microfabrication. By spatially controlling the deposition of the templating peptides on a substrate, it is our hope that we can then control the mesoscopic characteristics of a material can be pre-determined.

N8.8

ASSEMBLY AND CHARACTERIZATION OF ULTRATHIN FILMS OF REACTION CENTER FOR BIOMOLECULAR RECOGNITION. D. Masci[1], A. Krasilnikova[1], C. Coluzza[2]. [1]BIOAG, CRE Casaccia ENEA, Roma, ITALY. [2]Dept of Physics, University of Rome, La Sapienza, Roma, ITALY.

Protein film assemblies are a subject of numerous modern investigations for applications in biomolecular devices. In particular, ultrathin films of photosynthetic reaction center (RC) from Rhodobacter sphaeroides, being coupled with electrochemical or optical trasductor, is a promising candidate for recognition of specific herbicides. In order to obtain such smart sensors, layer-by-layer assemblies of mono and multilayers of photosynthetic RC films were prepared using the Langmuir-Blodgett technique. Similarly to other proteins, RC are not ordinary amphiphilic molecules, hence, it is necessary to embed them in a membrane-like structure before spreading at the air-water interface. For this purpose we reconstituted RC into egg phophaditilcholine by gel filtration to obtain proteoliposomes. Langmuir monomolecular films from RC proteoliposomes were prepared by drop method after studying of the compression isotherm of pure lipids. Time stability of monolayer film was measured, and in the pressure range from 30 to 25 Nm^{-1} a very good stability was observed. Bilayers and multilayers were successively deposited at 25 Nm^{-1} by the Z-type method. Due to high two-dimensional packing density of the proteins on the air-water interface, their chemical-physical properties might dramatically change from the values known for solutions and membranes. Therefore, the protein properties in the deposited films were analysed by UV-visible spectrophotometry and FT-IR spectroscopy. These results together with transient absorbance measurements at 860 nm confirm the protein to preserve its properties typical to those in solutions, and to not denaturate. Superficial distribution of the deposits was investigated by AFM technique.

N8.9

EFFECT OF RGD TETHER LENGTH ON CELL-ADHESION IN RGD ACTIVATED AMPHIPHILIC COMB COPOLYMERS. William Kuhlman, Anne Mayes, Dept of MS&E; Linda Griffith, Lily Koo, Division of Bioengineering and Environmental Health, Massachusetts Institute of Technology, Cambridge, MA.

Novel Poly(methyl methacrylate)-g-Poly(oxyethylene) (PMMA-g-POEM)comb copolymers have demonstrated considerable promise as materials for tissue engineering because of their ability to resist non-specific protein absorption while promoting selected responses from cells using tethered cell signaling molecules. These amphiphilic graft copolymers consist of a long (Mw=25-250k) hydrophobic MMA backbone with short (n=9) PEO side chains. When films or blends of this comb are exposed to water, the PEO side chains preferentially segregate to the surface rendering it resistant to nonspecific protein absorption. Cell-surface signaling can then be controlled by selectively functionalizing PEO end groups. Comb polymers offer a distinct advantage over other surface modification techniques in that they provide a convenient route to control clustering of ligands, an important factor in promoting strong cell-surface interactions. In this study, we examine the influence of PEO tether length on cell-surface signaling, particularly its effects on cell adhesion and motility. Because integrins on the cell surface have a fixed size and limited number of available physical conformations, optimization of tether length may improve cell-surface signaling by allowing polymer tethered ligands to adapt without strain to the conformation of integrins on the cell surface.

N8.10

CREATING CHARGES ON PORE SURFACES OF MESOSTRUCTURED SILICA TO MIMIC BIOLOGICAL ION CHANNELS. Nanguo Liu, Dhaval A. Doshi,University of New Mexico, Department of Chemical and Nuclear Engineering, Albuquerque, NM; Kui Yu, C. Jeffrey Brinker, Sandia National Laboratories, Albuquerque, NM.

Biological ion channels which are 0.2-2 nm in diameter and 1-2 nm in length show high ion selectivity, rapid switching between open and close states and inhibition by some ions. Because of these unique functions, they have many potential applications in biosensors, molecular switches, molecular devices, nanoreactors etc. Channel proteins carry negative and positive charges that are very important in the electrodiffusion process of ion transportation. Modeling and experiments indicate that small charged pores might have similar behavior to biological ion channels. To mimic the performance of biological ion channels, we synthesize some robust silica mesostructures with different pore sizes and pore surface chemistries. In this work, we create -NH_2 or -COOH groups on the pore surfaces of mesostructured silica that can carry positive or negative charges, respectively. We use different surfactants, such as cetyltrimethylamonium bromide (CTAB), $C_{16}H_{33}(OCH_2CH_2)_nOH$, n$\sim$10 (Brij56), $C_{16}H_{33}(OCH_2CH_2)_nOH$, n$\sim$20 (Brij58), and block copolymer $EO_{20}PO_{70}EO_{20}$ (P123), as structure directing agents to create very ordered 3-D networks constructed of silicon dioxide. The ordered 3-D mesophase can guarantee the existence of

trans-membrane pores. One-pot and post-graft synthesis methods are employed for the creation of charges and for controlling the charge density on the pore surface based on component change. Pore sizes of different mesostructured silica films are characterized by SAW and they are from 1.5 to 7 nm in diameter, in the range of biological ion channels. The trans-membrane current will be characterized by a patch clamp amplifier technique. Other characterization methods are TEM, XRD and FTIR.

N8.11
SYNTHESIS OF BIOCOMPATIBLE SURFACES BY DIFFERENT PLASMA POLYMERIZATION TECHNIQUES. Elena Garreta, Salvador Borros, Carles Colominas, Nuria Agullo, Material Science Lab, Department of Physical Chemistry, IQS, Universitat Ramon LLull Barcelona, SPAIN; Joan Esteve, Applied Physics Department, Universitat de Barcelona, Barcelona, SPAIN.

Nowadays, there is an increasing interest in finding new materials that can be used as biomaterials. Such interest is mainly focused to solve the problems related with the non-desired reactions that sometimes are produced in the human body when biomedical implants are employed. Polymers are widely used in this field, both, as polymeric materials themselves and as a polymeric surface on a different substrate. Concerning the later, and in order to improve the biocompatibility of the material, surface modification techniques appear to be quite interesting for this subject. Thus, surface modification techniques become a good choice to improve the biocompatibility of the material. In the present work, three techniques of assisted polymerization have been studied: Plasma Polymerization with a radiofrequency source, Pulsed Plasma Polymerization and Hot Filament Polymerization. Different monomers have been tested, such as, acrylic acid, anhydride maleic and glycidil metacrylate. The kinetics of the thin film growth were studied and the optimal polymerization conditions were established for each monomer and activation source. Polymeric layers have been characterized by IR, AFM and XPS. The conventional plasma polymerization technique has turned out to be the most aggressive, since the polymer obtained with every monomer has an elevated fragmentation grade. However, it is well known that pulsed plasma and hot filament polymerization are soft ionization methods and it is possible to obtain homogeneous polymeric surfaces with no fragmentation, as the results of this work showed. The polymer obtained by hot filament keeps the monomer structure and it is possible to tailor the thin film functionality, changing the monomer used. These functionalized polymeric surfaces could turn into a biomaterial, joining biological macromolecules or living cells. In this way, the material biocompatibility would be increased and also other mechanical properties like adhesion could be improved. Therefore, it could be a promising material for biomedical applications, such as bone implants.

N8.12
SURFACES ENERGETIC ENGINEERING OF PECVD POLYMERIC FILMS FOR BIOMEDICAL APPLICATIONS: BACTERIAL AND CELL ADHESION RESPONSE. Pasqua Rossini, Giacomo Ceccone, Pascal Colpo, and Francois Rossi, EC-JRC, Institute for Health and Consumer Protection, ISPRA (VA), ITALY.

Organic films (Polyethylene-like, Polyacrylic acid-like and Poly-ethyleneglycole-like) with different surface free energy have been deposited by plasma enhanced chemical vapour deposition (PECVD) in order to study their bio-reactivity (proteins adsorption, bacterial adhesion, cell seeding) when in contact with biological fluids (blood, saliva). Plasma and surface diagnostics have been used to optimise and control the deposition processes. For the study of the plasma phase, Mass Spectrometry, Optical Emission Spectroscopy and Fourier Transformed Infrared Spectroscopy (FTIR) have been used, whilst the films have been characterised by using, X-Ray Photoemission Spectroscopy (XPS) and FTIR. The coatings superficial properties (surface energy) have been studied by means of contact angle measurements, while their bioreactivity when in contact with biological fluids so with proteins, cells, bacteria has been investigated by a Quartz Crystal Microbalance by monitoring their absorption kinetics. Moreover, optical and electron microscopy have been used to observe the influence of biological interactions on films' morphology. The relation between film deposition parameters and biological response will be discussed.

N8.13
ANTIMICROBIAL COATINGS OBTAINED IN AN ATMOSPHERIC PRESSURE DIELECTRIC BARRIER GLOW DISCHARGE. Sabine Paulussen, Dirk Vangeneugden, Olivier Goossens, Erik Dekempeneer, VITO (Flemish Institute for Technological Research), Materials Department, BELGIUM.

Since the early 1990s there is a fast growing interest, both from industry and the academic world, in plasma processes at atmospheric pressure. Up to now, applications are mainly situated in the area of cleaning, sterilization and activation of plastic surfaces. The current focus shifts towards the deposition of organic and inorganic functional coatings on various substrates. This paper addresses the development of plasmapolymer coatings that prevent bacteria from adhering to medical devices, implants, textile fibers, etc. The two main parameters affecting bacterial colonization onto surfaces are the surface energy and its smoothness. Atmospheric plasma deposition of a smooth and thin coating can affect both properties, thus allowing the preparation of non-adhesive surfaces. Hexamethyldisiloxane, 2-hydroxyethylmethacrylate and ethyldiazoacetate were used as monomers. The resistance of the polymer coatings to bacterial adhesion is further improved after adding antimicrobial substances like silver salts, quaternary ammonium and phosphor salts and pyridine like compounds to the precursor solution. The antimicrobial compounds are incorporated in the plasmapolymer network through covalent linking. Like the classical low-pressure plasma techniques, the atmospheric pressure plasma process allows treatment of a variety of objects, foils, fibers, etc in a sterile environment. In addition, the atmospheric pressure set-up allows continuous processing, which is especially interesting for the treatment of textile and plastic foil, and gives rise to significantly higher deposition rates.

N8.14
NANOPOROUS ELECTROSPUN POLYMER FIBERS FOR TISSUE ENGINEERING APPLICATIONS. J.S. Stephens, J.F. Rabolt, Univ of Delaware, Dept of MS&E and Delaware Biotechnology Institute, Newark, DE; M.C. Farach-Carson, Univ of Delaware, Dept of Biology, Newark, DE.

Nanoporous fibers have been electrospun from a series of volatile solvents and characterized by field emission scanning electron microscopy (FESEM), atomic force microscopy (AFM), and Raman spectroscopy (1). Pores ranging in size from 20 - 1000 nm (having depths ranging from 50 - 70 nm) have been observed on the surface of both amorphous (e.g., polystyrene (PS), poly(methyl methacrylate)) and semicrystalline (e.g., polycarbonate, synthetic spider silk) polymer fibers. The size and density of the nanopores can be varied simply by changing the electrospinning conditions. The nanoporous nature of electrospun PS fibers is ideal for incorporating heparin binding growth factors to promote cell growth, and these studies are currently under investigation. In addition, the nanoporous PS fibers have been successfully filled and coated with silver nanoparticles and they are currently under evaluation for their anti-viral properties and use as rigid scaffolds.
(1) J.S. Stephens, S. Frisk, S. Megelski, D.B. Chase, J.F. Rabolt, Applied Spectroscopy, 55, Oct 2001.

N8.15
DESIGNING 3D SPHERICAL PORE NETWORK IN NANO FIBROUS TISSUE SCAFFOLDS. Victor J. Chen and Peter X. Ma, University of Michigan, Department of Biologic and Materials Sciences, and Department of Biomedical Engineering, Ann Arbor, MI.

Each tissue or organ type has its specific characteristic architecture depending on its physiological function. This architecture is critical for cell-cell, cell-matrix, cell-nutrient, and cell-signal molecules interactions. Collagen has a nano fibrous architecture (fiber diameter: 50-500 nm) and exists in nearly all types of tissues in the body. We hypothesize that nano fibrous architecture provides excellent environment for cell adhesion, migration, proliferation, and differentiated function for a variety of cell types. To mimic the nano fibrous architecture and to overcome the concerns over immunogenicity and disease transmission associated with natural collagen, synthetic nano fibrous extracellular matrices (scaffolds) have been developed in our lab. To provide porous network for cell seeding and mass transport channels required in a tissue engineering scaffold, we developed a novel technique to generate interconnected spherical pores with adjustable interpore openings and nano fibrous pore walls in this work. Paraffin spheres were prepared with a dispersion method, and thermally assembled into 3D structures. Polymer solutions were cast over the paraffin assembly and phase-separated to form nano fibrous architecture. The paraffin was then leached out to form the 3D spherical pore network. The interpore openings were adjusted by the thermal assembling conditions. With the versatility of these techniques for the polymer types and architectural parameters, nano fibrous scaffolds with tailored structure and properties can be designed for a variety of tissue engineering applications.

N8.16
IN VITRO DEGRADATION OF POLY(PROPYLENE FUMARATE)/POLY(PROPYLENE FUMARATE)-DIACRYLATE NETWORKS. Mark Timmer[1], Catherine G. Ambrose[2], Antonios G. Mikos[1]. [1]Department of Bioengineering, Rice Univeristy, Houston, TX. [2]Department of Orthopaedic Surgery, University of Texas Health Science Center, Houston, TX.

Biodegradable materials show promise in orthopaedic applications

because they can provide a temporary support for bone formation and regeneration, avoiding any long-term stress-shielding effects. One such material being investigated for these applications is poly(propylene fumarate) (PPF), an unsaturated linear polyester, that can be combined with the crosslinking agent poly(propylene fumarate)-diacrylate (PPF-DA) to form degradable polymeric networks. PPF/PPF-DA can be crosslinked by UV photo-polymerization, which facilitates manufacturing by stereolithography techniques. These networks show low water absorption and can provide a range of mechanical properties by varying the PPF/PPF-DA double bond ratio. This study examined the effect of PPF/PPF-DA double bond ratio, medium pH, and the incorporation of a β-tricalcium phosphate (β-TCP) filler on the in vitro degradation of PPF/PPF-DA. Cylindrical specimens were submerged in buffered saline at 37°C and the change in mass and compressive mechanical properties were monitored over a 52-week period. All formulations showed an initial increase in modulus and yield strength over the first 12 weeks, achieving maximums of 1310 ± 100 MPa and 46 ± 4 MPa, respectively in the β-TCP composite. Increasing double bond ratio demonstrated greater degradation with a 17% mass loss over the entire time of the study. Samples in the lower buffer pH 5.0 compared to physiological pH 7.4 did not show any differences in mass loss, but exhibited a faster decrease in the compressive strength over time. The inclusion of the ceramic filler showed minimal mass loss and maintained the mechanical properties at the level following their initial increase. These results show that the degradation of PPF/PPF-DA networks can be controlled by the double bond ratio, accelerated at a lower pH, and prolonged with the incorporation of the β-TCP filler.

N8.17
DESIGN AND *IN VITRO* PRODUCTION OF AN AUTOLOGOUS TRABECULAR BONE SUBSTITUTE. Galateia Kazakia, University of California at Berkeley, Department of Mechanical Engineering, Berkeley, CA; Eric Nauman, Tulane University, Department of Biomedical Engineering, New Orleans, LA; Bernard Halloran, University of California at San Francisco, Veterans Administration Medical Center, Department of Medicine, San Francisco, CA; Tony Keaveny, University of California at Berkeley, Departments of Mechanical Engineering and Bioengineering, Berkeley, CA.

Repair of trabecular bone tissue in cases of trauma, abnormal development and disease is of major clinical importance. Problems posed by autologous and allogeneic bone grafting techniques can be circumvented by inducing the patient's native cells to produce and mineralize autologous bone matrix upon a scaffold *in vitro*. A composite bone substitute is consequently created that is osteoinductive and immunologically inert. Ideally, the scaffold material must possess mechanical integrity, morphology and porosity appropriate to the site of implantation. Sintered trabecular bone harvested from an appropriate location inherently has these physical traits and is composed of hydroxyapatite, proven to be a good substrate for mineralization. The primary goal of this study was to quantify the effect on the apparent level properties produced by bone marrow stromal cell (BMSC) mineral deposition onto a sintered trabecular bone scaffold. Thirty-six trabecular bone cylinders (18 matched pairs) were machined from 18 human vertebral specimens. All cylinders were sintered and one cylinder from each matched pair was seeded with rat BMSC. After 5 weeks in culture, cylinders were evaluated for changes in size, density, volume fraction, and mechanical properties, and were also analyzed histologically. Apparent density and volume fraction were found to increase by a factor of four as a result of BMSC seeding. Statistically significant increases in strength and energy absorption were also found. These results, as well as those of the histological evaluations, provide a basis for the design and *in vitro* production of an autologous trabecular bone substitute with tailored mechanical properties.

N8.18
CALCIUM CARBONATE REINFORCED NATURAL POLYMER COMPOSITES FOR BONE GRAFTS. Samar J. Kalita, Susmita Bose, Howard L. Hosick*, Steve A. Martinez** and Amit Bandyopadhyay, School of Mechanical and Materials Engineering. *School of Molecular Biosciences. **College of Veterinary Medicine, Washington State Univ, Pullman, WA.

Challenges in tissue engineering have always motivated scientists and engineers to develop new biomaterials that can restore the structural features and physiological functions of natural tissues. A novel ceramic-polymer composite was processed with bio-active ceramics dispersed in a natural bio-active polymer for bone graft applications. A commercially available caster bean extract polymer (CBP) was used. It is a natural vegetable polymer extracted from the oily caster beans of the dicotyledonous class. During processing of these composites, in situ random interconnected porosity was generated similar to natural bone. Hg-porosimetry results of these composites show that most of the pores are between 50 to 150 microns. Compression tests were performed on cylindrical samples to determine the mechanical properties. Average compression modulus was calculated as 178 MPa, while the failure strength was 7 MPa. The failure strength is quite comparable to human cancellous bone. Cytotoxicity and cell proliferation studies were conducted with modified human osteoblast cell-Line (OPC-1) to show that these composites are biocompatible. The composites showed good cell attachment with a continuous increase in cell growth up to two weeks. This paper will present physical, mechanical and in vitro test results of these novel composites.

N8.19
IN VITRO ASSESSMENT OF A NOVEL BORATE-BASED BIOACTIVE GLASS. Roger F. Brown, Heather K. Teitelbaum*, Nona L. Adams, and Richard Brow*, Departments of Biological Sciences and *Ceramic Engineering, Univ Missouri-Rolla, Rolla, MO.

This research was undertaken to develop and characterize a bioactive, calcium-containing, borate glass as an alternate to the widely-used 45S5 glass. We report here on a borate glass that does show evidence of bioactivity. X-ray diffraction analysis of samples of this glass incubated for three weeks in simulated body fluid reveal formation of a hydroxyapatite surface layer, an important predictor of bioactivity. The ability of the borate glass to support the attachment, growth, and function of bone cells was tested with the established Saos-2 human osteosarcoma cell line. The osteoblast-like Saos-2 cells were seeded on wafer-shaped samples of the glass and incubated in alpha-MEM medium supplemented with 25 mM HEPES buffer and 10% fetal calf serum. SEM analyses show the cells attach to the borate glass and assume a well-spread morphology essentially identical to that of Saos-2 cells on 45S5. Growth kinetics measurements reveal replication of Saos-2 cells on the borate glass although at a slightly slower rate than those seeded on 45S5 glass. Maintenance of osteoblast function was assessed by spectrophotometric measurement of alkaline phosphatase activity in lysates of cells recovered after culturing for five days on the glass samples. Three replicate experiments revealed alkaline phosphatase activity in Saos-2 cells recovered from the borate glass that is about 75% of that measured for cells grown on 45S5 glass. These initial in vitro results suggest the borate glass tested is bioactive and does support the attachment, growth, and continued function of osteoblastic cells. Follow-up tests are underway with samples of the borate glass implanted in the tibia of rats to assess in vivo performance

N8.20
Abstract Withdrawn.

N8.21
INTERNAL OSTEOSYNTHESIS FIXATION PLATE AND CALLUS BONE: A BIOMECHANICAL APPROACH. Suyambulingam Veerabagu, Kazu Fujihara, Seeram Ramakrishna, Dept of Mechanical Engr, Bioengineering Division, National University of Singapore, Singapore, SINGAPORE; Ganeshwararao Dasari, Dept of Civil Engineering, National University of Singapore, Singapore, SINGAPORE.

In orthopedic surgery, bone plate and screws are often used to treat the diaphyseal (shaft of a long bone) fractures. The objective of the paper is to evaluate bending properties and strain fields in bone and internal osteosynthesis fixation plate system. A combined experimental and finite element model study was carried out to understand the complex material behavior of internal osteosynthesis fixation plate. The enhancement of strain appearance on the plated bone is as crucial issue as bending property of bone plates[Karneszis et al.,1998]. For instance, external loadings (axial, bending and torsion loadings) induced by body movement create strain in a bone-plate system. Strain gives the stimulus to the damaged bone tissue and encourages callus formation. However, the stiffness mismatch between metal plates and bone tissue leads to a situation that the majority of the load is transferred by the plate rather than by the underlying bone. As a result, micro strain to enhance bone healing is restricted. The low elastic modulus of composite plates ensures only a part of load is transferred through bone plate and the remaining load is borne by bone itself, thus creating movement at fracture. The movement induced by external loading gives stimulus to the damaged bone tissue and encourages further callus formation. Therefore it is necessary to calculate magnitudes of strains induced in internal osteosynthesis fixation plate system subjected to loading. The purpose of this study is to compare and investigate the biomechanical behavior of various polymeric biomaterials polytetrafluoroethylene, polyacetal, polymethylmethacrylate, polyethylene terephalate, polyetherether-ketone, silicone rubber, polysulfone kept over cortical callus bone as well as cancellous bone.
References:
[1] A. Karneszis, A.W. Miles, J.L. Cunnigham, Learmonth. Biological internal fixation of long bone fractures: a biomechanical study of a noncontact plate system. Injury 1998;29:689-695.

SESSION N9: BIOMIMETICS, SENSORS AND NANOTECHNOLOGY
Chair: Joanna M. McKittrick
Friday Morning, April 5, 2002
Metropolitan I (Argent)

8:00 AM *N9.1
PEPTIDE SEMICONDUCTOR NANOCRYSTAL INERACTIONS AND ORGANIZATION OF HYBRID MATERIALS.
Angela M. Belcher, Christine Flynn, Sandra Whaley, Chuanbin Mao, Seung-Wuk Lee, Ioana Pavel, The University of Texas at Austin, Dept of Chemistry and Biochemistry, Austin, TX.

Biological systems have a unique ability to control crystal structure, phase, orientation and nanostructural regularity of inorganic materials. We are currently investigating the principles of natural biological molecular recognition in materials and developing new methods to pattern useful non-biological electronic and magnetic materials. Two types of peptide combinatorial approaches will be discussed to identify proteins that select for and specifically bind to materials such as semiconductor wafers, semiconductor and magnetic nanoparticles, carbon nanotubes and conducting polymers. These approaches utilizes the inherent self-organizing, highly selective properties of biologically derived molecules. We have selected peptides that can specifically bind to and discriminate zinc-blende 3,5 semiconductor surfaces. We have also selected peptides that can nucleate 2,6 semiconductor nanoparticles. Peptides still bound to phage or bacteria will be compared with free peptides and surface bound peptides. Ordering of nanocrystal using major and minor phage coat protein engineering as well as long range ordering using different biologically liquid crystalline phases will be discussed.

8:30 AM N9.2
THE FABRICATION OF AN OPTICAL DEVICE BASED ON THERMOPROTEIN RESPONSE. Rajesh R. Naik, Lawrence L. Brott, Amelia K. Carpenter, Sean M. Kirkpatrick, Morley O. Stone, Air Force Research Laboratory, Materials and Manufacturing Directorate, Wright-Patterson Air Force Base, OH.

Biological organisms have the ability to sense and respond to thermal energy. Some organisms have adapted highly unusual structures and macromolecules to respond to thermal energy with exquisite sensitivity. One such molecule is the thermo-sensitive protein TlpA from Salmonella. We and others have demonstrated the incredibly rapid denaturation-renaturation cycle that this protein possesses[1]. A central challenge has been harnessing this behavior in an appropriate signal transduction device. We have chosen to use polymer-based diffraction gratings as the signal transduction device. We will present work that utilizes a controlled phase separation process driven by a holographic two-photon initiated photopolymerization (H-TPIP) process[2]. We hypothesize that the resulting grating contains periodic domains of low crosslink density enriched in TlpA. We speculate a structure is being formed which is similar to the hybrid gratings we have fabricated using the silaffin peptide R5[3]. These thermo-protein domains respond to thermal stimulation by changing the grating spacing - thus producing a signal that is easily monitored.
1. R.R. Naik, S.M. Kirkpatrick, M.O. Stone, Biosensors and Bioelectronics 16: 1051-1057 (2001).
2. S.M. Kirkpatrick et al. Applied Physics A 69: 461-464 (1999).
3. L.L. Brott et al. Nature 413: 291-293 (2001).

8:45 AM N9.3
BIOMIMETIC MATERIALS FOR SELECTIVE RECOGNITION OF BIOLOGICALLY SIGNIFICANT MOLECULES. Mark E. Byrne, Biomaterials and Drug Delivery Laboratories, School of Chemical Engineering; Kinam Park, Department of Industrial and Physical Pharmacy, Department of Biomedical Engineering; Nicholas A. Peppas, Biomaterials and Drug Delivery Laboratories, School of Chemical Engineering, Department of Biomedical Engineering; NSF IGERT Center on Therapeutic and Diagnostic Devices, Purdue University, West Lafayette, IN.

The study of recognitive proteins and protein binding domains reveal molecular architectures with specific chemical moieties that provide a framework for selective recognition of a target analyte in aqueous environment. Since proteins are heteropolymers of amino acids, proper matching and positioning of chemical residues can lead to artificial macromolecular structures capable of specific recognition. By analyzing binding proteins, we have been successful in designing biomimetic polymer networks that specifically bind biologically significant molecules. Further developments are expected to have direct impact on applications outside separation science such as analyte controlled and modulated drug delivery, drug elimination, drug targeting, and biosensors. We have synthesized and characterized imprinted gels for the macromolecular recognition of D-glucose with emphasis on imprinted controlled release systems. Novel copolymer networks containing poly(ethyleneglycol) dimethacrylate (20-80% mole/mole monomers in feed) and functional monomers such as acrylic acid, methacrylic acid, and acrylamide were synthesized in polar, aprotic solvent (dimethyl sulfoxide) via UV-free radical polymerization. Monomers were selected to match corresponding glucose binding protein residues of aspartate, glutamate, and asparagine. Polymers were characterized by single and competitive equilibrium and kinetic binding studies, single and competitive fluorescent and confocal microscopy studies, dynamic network swelling studies, DPC, and SEM. Results qualitatively and quantitatively demonstrate effective glucose-binding polymers in aqueous solvent. For acrylamide and acrylic acid functionalized polymers, the imprinted to non-imprinted bound ratios were 4.3 and 5.8, respectively, demonstrating the memorization of glucose within the networks. The imprinting process resulted in a more macroporous structure with absorption of water occurring via non-fickian diffusion at a faster rate and with a higher equilibrium value. The processes and analytical techniques presented are applicable to other biologically significant molecules and recognitive networks, in which hydrogen bonding, hydrophobic, or ionic contributions will direct recognition.
This work is supported by NSF Grant DGE-99-72770.

9:00 AM N9.4
BIOMIMETIC COMPOSITES USING A POLYMER-INDUCED LIQUID-PRECURSOR (PILP) PROCESS. Matthew J. Olszta, Philip L. Varona, Yi-Yeoun Kim, Laurie B. Gower, Department of MS&E, University of Florida, Gainesville, FL.

We have put forth the hypothesis that the morphogenesis of biominerals may proceed via a Polymer-Induced Liquid-Precursor (PILP) process. In our in vitro studies, mimetic biopolymers are used to induce liquid-liquid phase separation in the crystallizing media of calcium-based minerals. Droplets of the minor component of this phase separation process contain a highly enriched ionic phase which undergoes dehydration and solidification to form the mineral products. Because this is a distinct phase which can be manipulated, and the crystals that result retain the shape of the minor phase, it can be considered a precursor phase. The PILP phase solidifies into a variety of non-equilibrium crystal morphologies (e.g. crystal drops, films, fibers, etc.). Non-equilibrium crystal morphologies are the hallmark of biologically-formed crystals, thus we have proposed that the PILP process could be a viable mechanism for generating the enviable microstructures found in biocomposite tissues. We are testing this concept by attempting to duplicate, in vitro, a variety of structures which mimic their biological counterparts, such as the nacre of mollusk shells, the intrafibrillar mineralization of collagen in bone, the fibrous mineral prisms of dental enamel, and the various micromolded and patterned crystals found in biological composites in general. By using a biomimetic approach, we anticipate being able to benefit from the two desirable aspects of biomineralization; (1) the ability to fabricate organic-inorganic composites with complex architectures; and (2) the ability to incorporate biological components (e.g. proteins, enzymes, cells) into ceramic biomaterials.

9:15 AM N9.5
SELF-ASSEMBLY OF SURFACTANT PEPTIDES INTO NANOSCALE SUPRAMOLECULAR STRUCTURES.
Steve S. Santoso, Sylvain Vauthey, Shuguang Zhang, Massachusetts Institute of Technology, Center for Biomedical Engineering, Cambridge, MA; Haiyan Gong, Boston University School of Medicine, Department of Ophthalmology, Boston, MA.

Surfactant peptide molecules undergo self-assembly in solution to form a variety of supra-molecular structures at the nanometer scale, such as micelles, vesicles, unilamellar membrane, and tubules. These assemblies can be engineered to perform a broad spectrum of functions, including delivery systems for therapeutics, and templates for nano-scale wires in the case of tubules. An active area of research is to explore many different types of amphiphilic systems, characterize the self-assembled structures, and elucidate the conditions that induce their formation. Here we report that short, surfactant-like peptides form the aforementioned structures in aqueous solution. Each peptide consists of a hydrophilic head group having at least one polar amino acid, and a hydrophobic tail containing a chain of nonpolar residues. At neutral pH and high concentration, the peptides assemble into nanotubes with a diameter in the range of 50 nm, as shown using dynamic light scattering and cryo-transmission electron microscopy. Preliminary studies show that at lower concentrations they form vesicles and closed-ended tubes. Understanding these surfactant peptide self-assembling behaviors at different conditions will allow us to create and manipulate different structures from the same peptide for many different nanomaterials and nanoengineering applications. Our system allows for rapid screening of different peptide amphiphiles and conditions. We will carry out molecular modeling and simulation studies of the self-assembly process using available molecular

dynamics software for proteins and engineer wires out of the nanotubes and delivery system from the vesicles.

9:30 AM *N9.6
BIORECOGNITION STRATEGIES FOR IMPROVED BIOMATERIALS THROUGH CONTROL OF PROTEIN ADSORPTION, MOLECULAR RECOGNITION AND SELF ASSEMBLY. Buddy D. Ratner, University of Washington, Department of Bioengineering, Seattle, WA.

Biomaterials and devices comprised of them make up an industry with yearly revenues greater than 100 billion and have an enviable record of savings lives and improving the quality of life for millions. However, todays biocompatible biomaterials are simply walled off from the body as foreign objects. This can impede performance and degrade outcomes in many applications. What strategies might be used to improve healing? Nature routinely heals wounds, so copying natures approaches seems appropriate. Normal healing is a biologically specific process. Thus, we must turn on specific reactions. In this lecture, surface modification approaches for polymers that can inhibit non-specific reactions and turn on specific reactions will be described. An RF-plasma-deposited thin poly(ethylene glycol)-like layer can prevent non-specific protein adsorption. Specificity can be conferred by immobilizing key proteins involved in healing (e.g. osteopontin), attracting to the implant surface healing molecules, inhibiting proteins that trigger the foreign body reaction (e.g. thrombospondin 2), or sending chemical signals important for healing. Polymeric approaches aimed toward all four strategies will be described. Also, novel porous structures seem to trigger vascularized healing with little encapsulation. These will be explored using a class of hydrogels made porous with soluble microsphere templates.

SESSION N10: MATERIALS FOR DRUG AND GENE DELIVERY
Chair: John A. Frangos
Friday Morning, April 5, 2002
Metropolitan I (Argent)

10:30 AM N10.1
ORGANOSILICATE-POLYMER DRUG DELIVERY SYSTEMS: CONTROLLED RELEASE AND ENHANCED MECHANICAL PROPERTIES. Stephen H. Cypes, W. Mark Saltzman, Emmanuel P. Giannelis, Cornell University, School of Chemical Engineering, Ithaca, NY.

Two organosilicate additives with different aspect ratios were studied at different weight percentages in a dexamethasone release system formed from poly(ethylene-co-vinyl acetate), EVAc. The silicates were incorporated into the EVAc matrix by solution casting from methylene chloride. The resulting nanocomposites were both intercalated, as revealed by x-ray diffraction analysis, but the two silicates dispersed to different extents in EVAc as revealed by TEM. Release studies over 60 days reveal that for a low aspect ratio silicate, there is negligible change in the diffusion of dexamethasone. For a higher aspect ratio silicate, there is a slight reduction in the diffusion of dexamethasone as a function of the weight fraction of silicate present in the system. A theoretical model predicting the change in diffusion as a function of both aspect ratio and weight fraction silicate is presented. Both intercalated systems, regardless of the aspect ratio of the silicate additive, show increased mechanical properties as compared to the pure EVAc system, as revealed by DMA. Organosilicate additives prove to be a safe, inexpensive approach to controlled drug delivery in polymer systems while simultaneously increasing the mechanical properties of the polymeric matrix.

10:45 AM N10.2
STRUCTURAL EFFECTS OF CARBOHYDRATE-CONTAINING POLYCATIONS ON GENE DELIVERY. Theresa M. Reineke and Mark E. Davis, Chemical Engineering, California Institute of Technology, Pasadena, CA.

A major challenge in gene therapy has been the development of suitable systems for delivering genetic material in an efficient and non-toxic manner. Polycationic materials have emerged as promising delivery agents but significant differences in the delivery efficiencies and toxicities of these systems have been noted. Recently, our group has reported that linear, cationic β-cyclodextrin-containing polymers are capable of delivering DNA into mammalian cells with low toxicity. It was observed that the distance between the cationic groups within these polymers had a significant effect on the delivery efficiency and toxicity, and the structure containing six methylene units between the charge centers delivered DNA in the most efficient manner with low toxicity. A series of cationic polymers with six methylene units between the charge centers have been prepared to probe the structure-function relationships for linear, polycationic materials. Several polymers were prepared by varying the structure of the carbohydrate monomer to be either β-cyclodextrin or trehalose. In addition, a linker between the sugars and the charge centers within the polymer backbone was either amine or cysteamine. Furthermore, the charge center was varied by using cationic imidate groups or quaternary ammonium charge centers. The effects that these variations have on DNA binding ability, and the in vitro delivery efficiency and toxicity will be presented.

11:00 AM N10.3
CHITOSAN DERIVATIVES AS POTENTIAL GENE CARRIERS: CHARACTERIZATION, TRANSFECTION MECHANISM AND EFFICIENCY. Hong Shen, Alaina Steck, W. Mark Saltzman, Cornell University, School of Chemical and Biomolecular Engineering, Ithaca, NY.

Non-viral gene delivery systems are suggested as safer alternatives to viral vectors for DNA vaccines and gene therapy, yet they have low efficiency. Chitosan, a biodegradable chitin derivative, has many good attributes as a non-viral DNA delivery system, especially its low toxicity, excellent biocompatibility, and susceptibility to chemical modification. Here, DNA-chitosan complexes were formulated via two methods, yielding micron-scale aggregates and homogenous nanoparticles, respectively. The factors affecting uptake of these complexes and subsequent plasmid expression in different cell lines were investigated, including chitosan molecular weight, amine group:phosphate group (N:P) ratio of the complex, and size and charge of particles. The transfection efficiency was low in both CHO cells and Hela cells. To examine the possible causes of low transfection efficiency, YOYO-1-labeled plasmid coupled to chitosan was used to track transport of particles towards and within the cell. DNA-chitosan either condensed to cell surface or accumulated in endosomes. We hypothesize that more effective cell uptake and endosomal escape could enhance the transfection efficiency. To test this hypothesis, endosomal escape moieties, imidazole groups, were conjugated with chitosan amine groups. The solubility and buffering capacities of imidazole-containing chitosan were increased.
Imidazole-chitosan/DNA complex was observed to accumulate in the nucleus. Protein expression was also elevated, but still cell-type dependent.

11:15 AM N10.4
USE OF COMPLEXATION HYDROGELS FOR ORAL DELIVERY OF CANCER THERAPEUTICS. James Blanchette, Kinam Park, Nicholas Peppas, Purdue University, Dept of Biomedical Engineering, West Lafayette, IN.

The development of carriers to deliver a variety of cancer therapeutics orally would represent a significant advance in the treatment of this disease. Methacrylic acid (MAA) and ethylene glycol (EG) combined in a 1:1 molar ratio were reacted to form P(MAA-g-EG) nanospheres by UV-initiated free radical polymerization. MAA was vacuum distilled. Poly(ethylene glycol) 1000 monomethyl ether monomethacrylate was used to form the EG grafts. Tetraethylene glycol dimethacrylate was added as a crosslinking agent in mole percents ranging from 0.5 - 2.5%. Bleomycin, chosen as a model cancer therapeutic, was added to the monomer mixture prior to polymerization in concentrations ranging from 0.01 - 0.05 mg/ml to allow loading of drug by in situ polymerization. Photon correlation spectroscopy studies showed a narrow size distribution around 375 nm for the diameter of these hydrogel carriers. MAA and EG were chosen to give the nanospheres pH-responsive swelling behavior and mucoadhesive properties as has been shown by previous work in our lab with oral delivery of proteins. The current work expands the scope of this carrier to include delivery of cancer therapeutics. Bleomycin was loaded into these carriers with an efficiency of 76% (+/- 9% n=3). Release studies were carried out in conditions to model the environment of the stomach and small intestine. Results showed that bleomycin is preferentially released at a higher pH due to the increased mesh size of the swollen hydrogel carrier. The potential cytotoxicity of bleomycin on the small intestine was investigated with the use of Caco-2 cells (human colon adenocarcinoma). Studies done with bleomycin concentrations ranging from 0.01 - 1.0 mg/ml showed maintenance of both viability and proliferation in treated cells compared to control cells.
This research is supported by NSF Grant DGE-99-72770.

11:30 AM *N10.5
POLYANHYDRIDE AND POLY(ANHYDRIDE-ESTERS) FOR TISSUE SCAFFOLDING. Kathryn Uhrich, Rutgers University, Dept of Chemistry & Chemical Biology, Piscataway, NJ.

Several biodegradable polyanhydrides have been prepared for short-term medical and dental therapies. The function of these polymers is two-fold: the polymer itself provides a physical barrier between tissues, whereas the polymers' degradation products locally influence the inflammatory process. We are evaluating these

degradable polymers for controlling periodontal disease as well as bone formation. The relative degradation rates and mechanical properties of several new poly(anhydride-esters) and polyanhydrides will be discussed. The aim of this project is to design degradable polymers that actively participate in the wound healing process.

SESSION N11: TISSUE ENGINEERING
Chair: Kathryn E. Uhrich
Friday Afternoon, April 5, 2002
Metropolitan I (Argent)

1:30 PM N11.1
A NOVEL BIODEGRADABLE ELASTOMER. Yadong Wang, Guillermo A. Ameer, Robert Langer, Massachusetts Institute of Technology, Department of Chemical Engineering, Cambridge, MA; Barbara J. Sheppard, Massachusetts Institute of Technology, Division of Comparative Medicine, Cambridge, MA.

Biodegradable polymers have significant potential in biotechnology and bioengineering. However, for some applications, they are limited by their inferior mechanical properties and unsatisfactory compatibility with cells and tissues. A strong, biodegradable, and biocompatible elastomer could be useful for fields such as tissue engineering, drug delivery, and in vivo sensing. Inspired by the structure of collagen and elastin, the main fibrous protein components of extracellular matrices, we designed biorubber, a tough and biodegradable elastomer. This elastomer forms a three-dimensional network of random coils with degradable covalent crosslinks and hydroxyl groups attached to its backbone. In vitro and in vivo studies show the polymer has good biocompatibility. Polymer implants under animal skin are absorbed completely in 60 days with total restoration of the implantation sites to their normal architecture.

1:45 PM N11.2
MICROFABRICATION OF A BASAL LAMINA ANALOG: APPLICATIONS FOR TISSUE ENGINEERING. George D. Pins, Worcester Polytechnic Institute, Biomedical Engineering Department, Worcester, MA; Mehmet Toner and Jeffrey R. Morgan, Center for Engineering in Medicine and Surgical Services, Massachusetts General Hospital, Harvard Medical School and Shriners Hospital for Children, Boston, MA.

A microfabrication approach was used to produce novel analogs of the basal lamina with complex topographic features. A test pattern of ridges and channels with length scales (40 μm to 310 μm) similar to the invaginations found in a native basal lamina was laser machined into the surface of a polyimide master chip. Negative replicates of the chip were produced using polydimethylsiloxane silicone elastomer and these replicates were used as templates for the production of thin ($\sim$21 μm) membranes of collagen or gelatin. The resulting membranes had a complex topography of ridges and channels that recapitulated the features of the master chip. To demonstrate their utility, these complex membranes were laminated to type I collagen sponges and their surfaces seeded with cultured human epidermal keratinocytes to form a skin equivalent. The keratinocytes formed a differentiated and stratified epidermis that conformed to the features of the microfabricated membrane. Interestingly, the topography of the membrane influenced the differentiation of the keratinocytes because stratification was enhanced in the deeper channels. Membrane topography also controlled the gross surface features of the skin equivalent, infolds of the epidermis increased as channel depth increased. These novel microfabricated analogs of the basal lamina will help to elucidate the influence of topography on epithelial cell proliferation and differentiation and should have applications in the tissue engineering of skin equivalents as well as other basal lamina containing tissues.

2:00 PM *N11.3
NEURAL TISSUE ENGINEERING FOR SPINAL CORD INJURY REPAIR. Molly Shoichet, Paul Dalton, Derek Shaw, Samar Saneinejad, Lauren Flynn, University of Toronto, Dept of Chemical Engineering & Applied Chemistry, Dept of Chemistry, Institute of Bioimaterials and Biomedical Engineering, Toronto, ON, CANADA.

Spinal cord injury is a devastating disorder of the central nervous system for which there is no clinical therapy. We are designing a multi-component device to enhance nerve regeneration after injury which consists of a hollow fiber membrane (HFM) that is filled with contact-mediated and diffusible cues of regeneration. This seminar will focus on creating the haptotactic cues of regeneration. In a 2-dimensional model system, we patterned poly(chlorotrifluoro-ethylene) (PCTFE) film surfaces with alternating regions of cell adhesion and non-adhesion. The cell adhesive regions consisted of self-assembled monolayers of cysteine-terminated laminin peptides on gold stripes while the non-adhesive regions were poly(ethylene glycol)-modified PCTFE, taking advantage of a metal-halogen exchange reaction between chlorine of PCTFE and lithium of PEG-Li. Primary neurons adhered to the adhesive areas alone and nerve fibers (neurites) were true to the adhesive regions for an extended time. Building on these experiments, we modified ePTFE fibers with the same peptide sequences and found that both fiber diameter and chemistry are important determinants of neurite length and guidance. To translate these model systems to a device, we are using a newly developed, "centrifugal spinning" technique to make HFM and scaffolds. By orienting and modifying the scaffold within the HFM, we are investigating guided regeneration both in vitro and in vivo.

2:30 PM N11.4
QUANTIFICATION OF FIBROBLAST GENE EXPRESSION AT THE BIOMATERIAL INTERFACE. Catherine Klapperich, Lawrence Berkeley National Laboratory, Berkeley, CA; Carolyn Bertozzi, Department of Chemistry, Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, CA.

Biomaterials-tissue interactions have long been at the center of research involving short and long term implants, diagnostic devices and drug delivery techniques. The application of a widely accepted definition of biocompatibility is still not a reality in the biomaterials community. Considerable research has been carried out that suggests the protein layer that forms non-specifically on a biomaterial surface soon after implantation is the primary factor in the direction of the subsequent immune response to that biomaterial. Little is known about the global response of the cell in terms of gene expression patterns to engineered surfaces and environments. In this work we begin the process of quantifying, via gene expression patterns, the biomaterials-tissue interaction from the perspective of the cell. Rather than relying on a secondary measure of gene expression, we have isolated the mRNA of human fetal lung fibroblasts (IMR-90) after controlled exposure to a variety of biomedically relevant materials. Polymer materials studied include tissue culture polystyrene, ultra-high molecular weight polyethylene, and medical grade polyurethane. These materials represent a range of polymers used in diagnostics and short-term implants. Also included are silicon, quartz and glass surfaces, which are currently being investigated in the microfluidics field. The metal material investigated was the CoCrMo alloy most commonly used in total joint replacements. Young, adherent fibroblasts were cultured and exposed to the clean, sterilized surfaces for 15 min., 1 hr., 12 hrs, 24 hrs, and three days. The cells were then lysed and the total RNA was isolated. The mRNA was then hybridized to form cDNA, which was labeled and exposed to a human genome microarray (GeneChip Human Genome U95, Affymetrix, Mountain View, CA). We are particularly interested in identifying any changes in gene expression that correlate with the structure of the underlying material. Clustering analysis of the data is being used to identify genes that appear to take part in the response of these cells to foreign surfaces. In addition to adherent cells on surfaces, we plan to extend this technique to study cells seeded onto three-dimensional scaffolds for tissue engineering as well as specific ligand-receptor interactions.

3:00 PM *N11.5
BIOMIMETIC MATERIALS FOR TISSUE ENGINEERING. Kevin E. Healy, UC Berkeley, Dept of Bioengineering and Materials Science and Engineering, Berkeley, CA.

A central limitation in the performance of materials used in the medical device and pharmaceutical industries is that they lack the ability to integrate with biological systems through either a molecular or cellular pathway. A current theme in tissue engineering is to design and synthesize materials that actively regulate the response of mammalian cells and ultimately control tissue development. For example, we have created both interpenetrating polymer network (IPN) coatings ($\sim$20 nm thick) and hydrogels to test hypotheses regarding cell-materials interactions in two and three dimensions, respectively. The IPN is based on polyacrylamide and poly(ethylene glycol) and characterization by contact angle goniometry, spectroscopic ellipsometry, XPS, and static SIMS has confirmed the formation of an interfacial IPN on various model and implant surfaces. Grafting peptides, containing the cell-binding (-RGD-) and heparin-binding (-FHRRIKA-) domains present in bone sialoprotein, to the IPN has affected cell adhesion, proliferation, matrix mineralization, and enhanced bone formation surrounding metallic implants in vivo. We have also embarked on a long-term project to create artificial polymeric extracellular matrices (ECMs), or scaffolds, that are environmentally responsive and tunable with respect to mechanical properties (e.g., G*), incorporation of biological ligands (e.g., RGD, FHRRIKA), and degradation by proteases. To achieve this goal, we have exploited the phase behavior of poly(N-iso-propylacrylamide) [p(NIPAAm)] in aqueous media to synthesize injectable hydrogels. When heated from room to body temperature (i.e., 37C), the p(NIPAAm)-based hydrogels demonstrated a

significant increase in G* (i.e., rigidity) without exhibiting a significant change in either volume or water content. Ideally these artificial ECMs can be designed from first principles and be used for tissue formation ex vivo, tissue regeneration in vivo, drug or chemotherapy agent delivery, cell transplantation, and gene therapy.

3:30 PM N11.6
FORMATION AND PROPERTIES OF LIPOSOME BASED MULTICOMPARTMENTS. Cecile Boyer, Cara Evans, Joseph Zasadzinski, Department of Chemical Engineering, University of California, Santa Barbara, CA.

Nature uses lipid bilayer membranes to define cells and control transport through their walls. Mimicking cellular structures has led to the development of unilamellar vesicles as drug delivery vehicles. However, this promising technology has limitations including the encapsulation of supramolecular objects. Moreover, it is difficult to optimize a variety of tasks using a single membrane. These include efficient encapsulation, controlled release, biocompatibility, stability *in vivo*, and specific targeting. Living organisms evolved from primitive forms as prokaryotic cells, with very little internal organization within the cell, to the larger and more complex architecture of eukaryotic cells. The latter contain a variety of compartments such as the nucleus, where the genetic material (DNA) is packaged, and discrete organelles, which perform specific chemical and physical tasks. The organelles of eukaryotes allow them to exhibit much higher levels of intracellular division of labor than is possible in prokaryotic cells. This inspired our group to develop the concept of bilayer-encapsulated vesicles in order to distribute the tasks among different compartments surrounded by different membranes; these may eventually become a very versatile drug delivery vehicle. Equally important, these vesicle within vesicle structures are produced by a series of simple self-assembly steps using the unique phase behavior of metastable interdigitated lipid bilayer sheets. Various lipids can be used to do the encapsulation; this offers a way to tune the release of molecules contained within the inner vesicles. Fluorescence and separation techniques are used to follow the release of model molecules entrapped by the inner vesicles. We believe that this encapsulation will modify the release profile from these organized structures when compared to that from single vesicles. Moreover, using this technique, potentially any colloidal suspension (DNA-lipid complexes, titanium dioxide particles') could be efficiently encapsulated within a lipid membrane, with no apparent adverse effect on the encapsulated material.

3:45 PM N11.7
HONEYCOMB FILMS OF BIODEGRADABLE POLYMERS FOR TISSUE ENGINEERING. Takehiro Nishikawa, Keiko Arai, Junko Hayashi, Masahiko Hara, Masatsugu Shimomura, The Institute of Physical and Chemical Research, Wako, JAPAN; Michiaki Matsushita, Satoru Todo, Hokkaido Univ. School of Medicine, Sapporo, JAPAN.

We report that microporous films (honeycomb films) (pore size: 5 μm, thickness: 2 μm) can lead hepatocytes and cardiac myocytes to tissue formation. The honeycomb films were fabricated by applying a moist air to a spread polymer solution containing biodegradable polymers (poly(L-lactic acid) (PLLA) and poly(ϵ-caprolactone) (PCL)) and an amphiphilic polymer on water surface. By the method, self-supporting honeycomb films were obtained. Hepatocytes were cultured on a self-supporting honeycomb film of PLLA. The hepatocytes formed a single layer of columnar shape cells with a thickness of 20 μm. The tissue formation of hepatocytes specifically occurred on the honeycomb film of PLLA, not on a flat film of PLLA. The artificial tissue of hepatocytes secreted albumin several times more than the hepatocytes cultured on the flat film. A honeycomb film of an elastic polymer, PCL could be stretched uniaxially. The honeycomb pores were deformed into elongated hexagons and rectangles. The arrays of the elongated hexagons exhibit anisotropic patterns, which are applicable to guiding cell alignment. We utilized a stretched honeycomb film of PCL as a culture substrate for cardiac myocytes. Cardiac myocytes were not aligned in a specific direction on a self-supporting honeycomb film. On the other hand, cardiac myocytes were aligned along the long axis of stretched micropores on the stretched honeycomb film. Three dimensional tissue structures were formed, when cells were cultured on both sides of the self-supporting honeycomb film. An artificial cardiac tissue was fabricated by culturing cardiac cells on both sides of the self-supporting honeycomb film. Synchronized cardiac contraction was observed on the artificial cardiac tissue. This indicates that cardiac cells can interact with each other laterally on each sides of the film and also vertically through the micropores. Thus the honeycomb films can control cell adhesion, cell alignment, and cell-cell interaction.

4:00 PM CONCLUDING REMARKS