SYMPOSIUM FF
Physical Characterization of Biological Materials and Systems

November 27 – 29, 2001

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*Invited paper
SESSION F1: PM AND SURFACE CHARACTERIZATION
Chair: J. H. Hobl and Thomas P. Weis
Tuesday Morning, November 27, 2001
Hampton (Sheraton)

8:30 AM F1.1
PROBING INTRAMILLELLAR STRUCTURES AND REACTIVITIES OF CORNEAL NANOPISTON ASSEMBLIES USING ATOMIC FORCE MICROSCOPY. Chuan-Jin Zhong, Jin Liao, Mathew M. Maye, Li Han, Nancy Kurzicki, Dept. of Chemistry, Stony Brook University of New York at Binghamton, Binghamton, NY; Lingling Chen, Biology Dept., Indiana University, Bloomington, IN.

Metallic nanocrystals encapsulated with organic monolayers, corneal shell membranes, are intriguing building blocks towards chemically and biologically functional platforms because of their fine-tunable electronic and structural properties at the molecular level. Key to the exploitation of such platforms is the understanding of the interfacial structures and reactivities of chemical and biological molecules. This presentation describes an investigation of the nanostructured interfacial immobilization and reactivities of antibodies, antigens and molecular chaperones using atomic force microscopy. Both non-covalent and covalent binding strategies are utilized for the interfacial immobilization at the corneal shell nanoresonator assembly platforms. Inclusion of intermolecular links is exploited as a new route for immobilizing the biological molecules, which is realized by the conversion of hydrophobic/bound links to amide links and further conversion to amide or iodine amide. Importantly, scanning probe microscopic imaging of the interfacial nanostructures is facilitated by coupling monolayer patterning to the assembly platforms. The assembly platforms are formed using reflection spectrophotometry and spectroelectrochemical techniques are employed to provide additional insights into the nanostructured interfacial reactivities. The implication of the results to developing biological nanostructures and characterization capabilities will also be discussed.

8:45 AM F1.2
PROBING NANOSCALE COOPERATIVITY NEAR THE GLASS TRANSITION. Konstantinos Simos, Ari S. Iserholz, Northeastern University, Department of Physics, Boston, MA; Evangelos Vitalis Russell, Instituto Balseiro, Bariloche, ARGENTINA.

Using non-contact atomic force microscopy techniques, we measure low frequency dielectric noise in a glassy polymer, polyvinyl-nitrate (PVAc) near the glass transition (290°C-310K) on a 50 nm length scale. Using a capacitance measuring scheme, with a PVAc film acting as the dielectric, we measured time dependent changes in dielectric polarization of the sample. The time series of PVAc polarization fluctuations, showed random telegraph switching (RTS). We further observed that RTS have two to four states, associated with distinct molecular cluster configurations. By changing the applied electric field, we measured reproducible changes in RTS state occupancy probabilities, which was related to changes in cluster dipole moment in the z direction. We analyzed the RTS kinetics, by observing the cluster dipole moment time distribution. This analysis show that individual cluster configurations exhibit stretched exponential relaxation with various characteristic times, but with stretching exponent similar to bulk values.

9:00 AM F1.3
MORPHOLOGY OF PHASE SEPARATED DOMAINS IN PLANAR CONFINED LIPID BILAYERS. Adrian S. Metzner, University of Chicago, Dept of Physics, Chicago, IL; Hans Durrer, University of Chicago, James Franck Institute, Chicago, IL; Kw Yee Lee, University of Chicago, Dept of Chemistry and Institute for Biophysical Dynamics, Chicago, IL.

We explore the morphology and dynamics of domain growth resulting from lateral phase separation in phospholipid membranes. Planar supported films composed of mixtures of distearoylphosphatidylcholine (DSC) and dioleoylphosphatidylcholine (DOPG) have been imaged in fluid using temperature- and time-dependent atomic force microscopy (AFM). We find that the lipid domains length scale is in the micrometer range for multilayers (bilayer deposited on top of another bilayer), but in nanometer range for a single supported bilayer. For supported multilayers, where the interaction with the substrate is reduced, ripple phase is observed. For a supported bilayer, which experiences greater influence from the substrate, the phase-separated domains can be compact or branched depending on the relative lipid ratio. The compact structure leads to the formation of isolated domains, while the branched aggregates eventually give rise to a network. These structures result in membrane compartmentalization on a nanoscale. Our results suggest that the constraints imposed on the lipid bilayer can have an important effect on the size and morphology of the phase separated domains.

9:15 AM F1.4
FORMATION AND PROPERTIES OF SUPPORTED MEMBRANES. John T. Woodland, John T. Elliott, Curtis W. Messe, Anne L. Plant, Biotechnology Division, NIST, Gaithersburg, MD.

We have used a variety of techniques to form a supported lipid monolayer on an alkanethiol self-assembled monolayer (SAM). Vesicle solutions, ethanol painting and a Langmuir trough can all be used to form supported monolayers. By using atomic force microscopy (AFM) in combination with non-local probes we are able to learn more about the formation and properties of these supported monolayers than either the AFM or non-local probe can provide alone. This in turn can aid in the design of experiments involving membrane proteins or supported cell membranes.

9:30 AM F1.5
CHEMICALLY-COUPLED UNFOLDING IN AFM EXTENSION OF MULTIDOMAIN PROTEINS. Philippe Carle, David Speicher, and Dennis Daucher, University of Pennsylvania, Philadelphia, PA.

Proteins of various types experience tensile forces in their function, and the IgCAM superfamily of Immunoglobulin Cell Adhesion Molecules are typical in this. They have a diverse number of domains, overall length, etc but almost every domain contains an intra-domain disulfide (or two). We have employed single molecule atomic force microscopy (AFM) to assess the force fields of several multi-Ig domain Ig-CAMs, including VCAM-1. We have exploited the disulfide as a deformational ‘switch’. In the absence of reducing agents, a smooth-stitch pattern of unfolding peaks appears for Ig-CAMs with average period and total length appropriate to known primary sequences. With reducing agent present, the average period increases as does the total unfolded length. Additional data suggests that partial unfolding is an obligation intermediate to refolding and full unfolding. The results suggest that conformational change on single protein molecules can be done by AFM.

9:45 AM F1.6
PROBING MATERIAL PROPERTIES OF UNSTRUCTURED POLYPEPTIDES BY ATOMIC FORCE MICROSCOPY. Jin H. Hobl, Sanjay Kumar, and Rajendra Mihopadyay, Johns Hopkins School of Medicine, Department of Physiology, Baltimore, MD.

Proteins are generally thought to derive function and properties from specific three dimensional “folds” adopted by the polypeptide chain. However, in recent years there has been increasing interest in unstructured proteins and the possibility that such proteins have functionally important properties. One idea that has been advanced is based on the notion that unstructured polypeptides can act as molecular spacers that confer unique mechanical properties to the proteins and used to control intramolecular interactions. We are studying the material properties of proteins in two systems, nematocysts and microtubule-associated proteins. AFM force measurements show that these proteins, when grafted to a surface, give rise to long range repulsive forces consistent with entropic or steric repulsion. The sequence of the polypeptides, and their responsiveness to ionic conditions make these systems ideal for investigating polyelectrolytes. These results explain the ability of these proteins to form gel like materials, and suggest that novel biomaterials might be derived from polypeptides that are wholly or mostly unstructured.

10:30 AM F1.7
EXPLOITING COMPLEMENTARY SURFACE ANALYTICAL AND MICROSCOPY TECHNIQUES TO DEVELOP BIOMATERIALS. Kevin E. Healy, Dept. of MSE and Bioengineering, Univ. of California at Berkeley, Berkeley, CA.

Surface characterization of biomaterials and biomedical devices can be an important component of the design process, fabrication protocol, and performance evaluation. Multiple analytical techniques are often required to fully characterize a materials surface both prior to and after exposure to the biological environment. In addition, a full range of microscopy techniques is essential to understand cell-matrix interactions. The function of biomaterials that interacts with components of living cells is particularly dependent on a wide spectrum of physical and chemical characterization techniques, at the nanometer length scale, during synthesis and performance evaluation. The decision of when to employ these techniques depends on the type of information desired and the depth of analysis needed. For example, the synthesis of an ultra thin interpenetrating polymer network (IPN) containing naphthamide and ethylene glycol that resists protein adsorption and cell adhesion was critically dependent on surface analytical techniques such as x-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), and scanning electron microscopy (SEM) to confirm the chemistry and formation of the IPN. To exploit biomolecular engagement between
11:00 AM FF1.8
ANALYSIS OF ENZYME KINETICS USING MICRODIFFERENTIAL BIOREACTORS FABRICATED FROM MICRO-PATTERNED ARRAYS OF COLLOIDAL PARTICLES.
Nathaniel J. Glenson and Jeffrey D. Carbeck, Princeton University, Department of Chemical Engineering, Princeton, NJ.

This talk describes an approach to the measurement of enzyme kinetics using immobilized enzyme micro-differential reactors. Solutions of the coupled convection, diffusion and reaction equations in these microreactors allow the determination of enzyme kinetic parameters under steady-state conditions. A challenge to the fabrication of these reactors was the development of efficient, reproducible processes for the patterning of immobilized enzymes on surfaces. We describe two fabrication strategies for these microreactors: one is based on the patterning of enzymes via directed linear flow in microchannels, the other is based on micro-patterned arrays of enzyme-functionalized colloidal particles. Patterned arrays of particles are produced through a combination of self-assembly, soft lithography and photolithography. Microreactors produced in this way are able to determine detailed kinetic information for enzymatic reactions in a rapid manner, using minimal amounts of protein. These microreactors are easily scalable into an array format to permit the simultaneous screening of multiple materials under varying reaction conditions.

11:15 AM FF1.9
BIODEGRADABILITY COMPARISON OF STAINLESS STEEL, GOLD COATED, AND HEAT TREATED GOLD COATED ENDOVASCULAR STENTS.
Elizer Edelman, Philip Seifert, Adam Groothuis, Alan Myers, Danielle Bornstein, Campbell Rogers, Harvard-MIT Division of Health and Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA.

As endovascular stents are altered to add functionality, for example by adding radio-opaque coatings, biodegradability may suffer. We examined the vascular response in porcine coronary arteries to stainless steel gold-coated NIR stents (7-cell, Medinol, Inc.). Stents, 9 and 16 mm in length, were left bare or coated with a 2 micron layer of gold. Physical and mechanical effects were examined in four different states: (a) gold-coated stent types, two at each length, that either had the coating applied to the standard strut, i.e., gold coated thinner than controls, or had the coating applied to chilled struts, i.e., gold coated of the same control strut. Single gold coated stents exhibited intimal hyperplastic and inflammatory reactions over 28 days, but postimplantation thermal processing negated the adverse tissue response to gold. Materials analysis using AFM, SEM with XPS, and Anger spectroscopy showed that surface roughness was the main difference between the gold coated and heat treated gold coated stents. In addition, the relative amounts of basic steel and gold coating and their different resistances to expansion and collapse determined the extent of stent recoil. We conclude that gold coatings enhance the radiopacity of steel struts, but without effects on vascular repair. Material effects predominate and can be abrogated by heating coated stents to accelerated rates. Clinical results may suffer unless consideration is given to mechanical and physical effects of gold.

11:30 AM FF2.1
CHARACTERIZATION OF HEALTHY AND DISEASED MYELIN MEMBRANES BY COMPLEMENTARY TECHNIQUES.
Jacob Israelachvili, Yufung Hu, University of California at Santa Barbara, Department of Chemical Engineering, Santa Barbara, CA.

The stability of the stacked myelin membrane structure is a key to understanding the functioning of healthy and diseased membranes. A common disease that disrupts the structure of myelin in Multiple Sclerosis (MS), which is believed to be an autoimmune disease that is characterized by gross morphological changes such as swelling or ‘demyelination’ and vesiculation of the myelin sheath. The cause and effects of treatment of MS remain elusive. The focus of the current study is to measure the effect of lipid and protein compositional changes on lipid/protein interactions, membrane structure, intermembrane adhesion and stacking. To this end, we have constructed two model lipid bilayer systems termed the ‘control membrane’ and ‘diseased membrane’. Their compositions closely resemble those of healthy native and MS myelin membranes of humans. A number of techniques have been used to characterize the two membrane systems, including structural measurements between supported bilayers with the Surface Forces Apparatus (SFA), dynamic light scattering, fluorescent microscopy, and freeze-fracture microscopy to study the morphology and aggregation of vesicles in solution. These techniques help to visualize, distinguish and quantify the different interactions that are responsible for membrane structure and stacking in the control and diseased membrane systems. Experiments are currently in progress on both lipid and lipid-protein systems.

2:00 PM FF2.2
MORPHOLOGICAL DEPENDENCE OF 2D-3D TRANSITIONS IN BINARY PHOSPHOLIPID LANGMUIR MONOLAYERS.
Ajoy Kumar Gopal, Ka Vee C. Lee, Department of Chemistry and Institute for Biophysical Dynamics, Chicago, IL.

We have concurrently studied the microscopic phase behavior, morphology and surface pressure-area isotherms of Langmuir monolayers of a binary mixture of phospholipids at various temperatures between 20 and 40°C. It is observed that the mode of 2D-3D transition at monolayer collapse correlates strongly with the monolayer morphology prior to collapse. At temperatures below 28°C, the monolayer appears biphasic prior to collapse and undergoes 2D-3D transition upon compression by forming large-scale folds which reliably unfold upon expansion. These folded structures can be 5 to several hundred μm wide and several mm in length. Above approximately 32°C, the monolayer prefers to collapse by forming vesicular 3D structures on compression. These micrometer scale vesicular structures appear globular or toroidal in shape. Collapse occurs via both folding and vesiculation in the 30-32°C temperature range. Morphological insights into the structure of the collapse features have been obtained using atomic force microscopy.

2:15 PM FF2.3
REGULATION OF EXTRACELLULAR MATRIX CONTRACTION BY GENETIC MANIPULATION OF CELULAR INTEGRIN PROPERTIES. Jonathan A. Phillips, Kevin A. McCarty, Kimberly H. N. Imahama, Lawrence J. Bonassar, University of Massachusetts Medical School, and Graduate School of Biomedical Sciences, Center for Tissue Engineering, Department of Cell Biology, Worcester, MA.

The mechanisms that signal cells to remodel tissue structure and its extracellular matrix (ECM) are mostly unknown. It is intuitive, however, to consider mechanosensitive cell surface receptors as major players in these processes. Integrin proteins are more recently being
accepted as strong candidates for mediating mechanotransduction within the cell. Integrins are heterodimeric transmembrane proteins which attach to the ECM. Our working hypothesis is that cells which express increased levels of integrin subunits will demonstrate increased contraction of collagen matrices on which they are seeded. The objective of these experiments is to determine if altered expression of integrin subunits may contribute to collagen tissue remodeling. Here we will show evidence correlating levels of cellular integrin expression and the extent of collagen gel contraction. Fibroblasts were genetically engineered to differentially express the α5 integrin subunit and seeded into a three-dimensional collagen gel matrix. The extent of gel contraction was compared based on the cell seeding density, stiffness of the gels and expression of integrin subunits. To verify that α5 integrins were specifically involved in differential tissue contraction, these experiments were repeated where α5 integrins were blocked with monoclonal antibodies. Cells expressing higher levels of α5 integrins were able to contract collagen gels to a significantly greater extent (p<0.01) than normal NIH 3T3 fibroblast cells when not blocked. These data show that the difference in contraction from mutant blocked and unblocked cells versus the difference in contraction from control blocked and unblocked cells was also significantly different (p<0.05). The ability to control cellular behavior by modifying cell-matrix interactions via gene therapy has numerous applications. Being able to alter cellular contractility may have applications in fighting metastatic tumors or speed up wound healing. This technology would also benefit burn victims and most surgery candidates.

2:30 PM EE2.2
ANALYSIS OF RADIATION DAMAGE IN LYSOZYME CRYSTALS WITH HIGH RESOLUTION TRIPLE AXIS X-RAY DIFFRACTION. H.J. Minty, National Institute of Standards and Technology, Gaithersburg, MD; H.M. Vahl, Materials Science Program, University of Wisconsin, Madison, WI.

We have used high resolution triple axis x-ray diffraction to study the physical processes that occur during radiation damage of hen egg white lysozyme crystals. Specifically, the distribution of the off-peak scattered intensity in the vicinity of a Bragg reflection from various lysozyme crystals has been monitored as a function of x-ray irradiation time. At short times, we typically observe a narrowing of the angular extent of the Bragg peak, indicating an improvement in lateral resolution of lysozyme molecules under x-ray irradiation. At long times (greater than 60 hours of x-ray irradiation in this study) the extent of the off-peak scattering increased monotonically with irradiation time. Control samples that were not irradiated showed no significant changes in the distribution of diffracted intensity with time. The fundamental dependence of the off-peak scatter on a double logarithmic plot shows a distinct transition at these longer times that we model in terms of a characteristic size in the irradiated crystal. This characteristic size is on the order of micrometers and decreases to about 1 to 3 μm at the longest irradiation times. Possible physical mechanisms that account for the observed behavior and their relation to chemical aspects of protein crystal degradation induced by x-ray irradiation will be discussed.

2:45 PM EE2.3
MORPHOLOGY OF HYDROGELS CONSTRUCTED VIA DIBLOCK COPOLYMER SELF-ASSEMBLY. Darrin Pechan, Tapan Pasiak, University of Delaware, Materials Science and Engineering, Newark, DE; Andrew Noman, Tam Deming, University of California at Santa Barbara, Departments of Materials and Chemistry, Santa Barbara, CA.

Different secondary structural motifs (helix, sheet, and coil) and molecular architectures (diblock vs. triblock) can be designed into synthetic polypeptides as controllable parameters to define molecular self-assembly. Single molecule phase transitions between the different secondary structures of the triblock copolypeptide, Bicopoly[ε-caprolactone]–ε-caprolactam, will be demonstrated. Three-dimensional structures that are responsive or 'smart' to changes in their environment. Additional possibilities of biocompatibility and specific biological function are also available when designing macroscopic structures from the molecular, polypeptide level. Amphiphilic, diblock polypeptides of hydrophilic l-lysine (K) or glutamic acid (E) and hydrophobic leucine (L) or valine (V) have been found to self-assemble into morphologically unique hydrogels at very low volume percent polypeptide in aqueous solution. At neutral pH and less than 0.5 wt% polymer, these polypeptides form hydrogels with unique macro- and microstructural morphology and the ability to heal after significant application of stress. The novel hierarchical morphology of these polypeptide microgels, small-angle neutron scattering (SANS), and initial cryogenic transmission electron microscopy (cryoTEM) imaging will be the focus of this presentation. Changes in gel morphology and strength were observed as the volume fraction of hydrophilic to hydrophobic block and salt concentration. The morphology, self-healing, and peptide foundation of these hydrogels make them intriguing candidates for biomaterials applications.

3:30 PM EE2.6
SUPRAMOLECULAR ULTRATHIN FILM STRATEGIES FOR DNA ASSEMBLIES: SURFACE SENSITIVE ANALYSIS OF LAYER ORDERING AND DNA STRUCTURES. Roberta Advincula, Department of Chemistry, University of Alabama at Birmingham, Birmingham, AL.

We describe our strategies and results in the preparation of supramolecularly ordered ultrathin films of DNA assemblies. These involve the use of the layer-by-layer alternate solution adsorption technique and "polymer brushes." The properties of DNA are intrinsically associated with the polyelectrolyte adsorption. Deposition of surfaces is governed by the conformation, orientation, and charge density of these biomolecules in relation to the physicochemical phenomena in oppositely charged surfaces. Controlling the "structure" of surfaces is important in modifying the adsorption phenomena. A number of surface sensitive spectroscopic and microscopic techniques were used to probe the adsorption and multilayer assembly, e.g. AFM, ellipsometry, SPS, XPS, FT-IR, PM/HR/LS. By combining with the alternate assembly of dendrimers, azobenzene, and phthiocyanine dyes, we have been able to prepare optoelectronic substrates where the phenomenon of irradiation and electrochemistry can be used to probe the ordering and response of these films. This is important for future drug delivery and biosensor applications.

3:45 PM EE2.7
Abstract Withdrawn.

4:00 PM EE2.8
VARIABLE RANGE HOPPING AND ELECTRICAL CONDUCTIVITY ALONG DNA. Z.G. Yu, Univ of Iowa, Dept of Physics and Astronomy, Iowa City, IA; Xueyu Song, Iowa State Univ, Dept of Chemistry, Ames, IA.

We present a model to describe electrical conductivity along the DNA double helix. In this model, DNA is considered as a one-dimensional disordered system and electrons are transported via variable range hopping between localized states. Thermal structural fluctuations in DNA further localize electronic functions, giving rise to a temperature-dependent localization length. The model quantitatively explains the temperature dependence of the conductivity observed in the lambda phage DNA.

4:15 PM EE2.9
SURFACE CHARACTERIZATION OF DNA MICROARRAY ON SILICON DIOXIDE AND COMPATIBLE SILICON MATERIALS IN THE IMMUNOBLOT PROCESS. Wen Xu, Mei Xue, Masan Chan, The Hong Kong University of Science and Technology, Dept of Electronic and Electrical Engineering, HONG KONG; Martin Carles Roca, Lenigk, Dieter Wilcohs Tsao, Nancy Y. Ip, The Hong Kong University of Science and Technology, Department of Biochemistry, HONG KONG; Nikolai J. Sacher, The Hong Kong University of Science and Technology, Department of Biology, Clear Water Bay, HONG KONG.

The immobilization of DNA on solid supports is a critical step for applications of DNA microarrays. The use of silicon compatible materials in the solid support process can facilitate the adoption of the mature electronic micro-fabrication to obtain high-on-a-chip and integrated detection system with much higher density. In this work, the surface property of DNA microarray with integrated photo-diode detection system is studied at different steps during the hybridization process. Silicon dioxide is chosen as the solid support for the immobilization, due to process compatibility with the integrated detection system underneath. It also shows background fluorescence and less surface roughness. Among all the prevalent immobilization methods, the covalent immobilization of thiol-terminated DNA oligonucleotides on self-assembled layers of (3-mercaptopropyl) trimethoxysilane (MPTS) by disulfide bond formation is selected. Necessary process modification experiments are performed to optimize the immobilization. Contact angle measurement is used to monitor the bonding of MPTS on the surface. Fluorescence microscopy reveals the efficiency of immobilization by the oligonucleotides labeled by FITC and the success of hybridization process. The immobilized oligonucleotides labeled by Texas Red to the probe. Atomic force microscopy (AFM) shows a sharp increase in particle size before and after hybridization and illustrate the efficiency of hybridization. Due to the strong covalent property of amine linkage, the thiolated DNA probe used in the process of immobilization, some commonly used top layer silicon materials in microelectronic devices such as polysilicon, aluminium, titanium are etched. Thus they have to be protected by other materials to improve the volume of immobilization. Silicon nitride, gold and platinum can withstand the process of
immobilization and no DNA will be hybridized on this material on the top layer. By a combination of silicon dioxide (where immobilization takes place) and silicon nitride (where no immobilization takes place), immobilization can be conformed to the desired area to reduce cross-talk between different array elements. The AFM results of the DNA sample after hybridization are correlated with the electronic signals from those of the photo-diode integrated detection array indicating the possible optimization methods for electronic detection.

4:30 PM  **F2.10**  
THE POROUS SI LICON LAYERS AS MATRIX FOR DNA.  
Andrew Verba, Dept of Radiology, Kiev National Shevchenko Univ, Kiev, UKRANIA.

The porous silicon (PS) layers are considered for the use as the supported matrix for synthesis of DNA. The method of silanization of PS and SiO$_2$ layers that is an important step of DNA-synthesis has been elaborated and tested. The SEM image shows developed surface of PS with a circular shape of porosity size of about 0.5 μm. This size is sufficient for carry out the process of DNA-synthesis. The FTIR spectroscopy has been extensively used in the characterization of the species precipitated on PS and SiO$_2$ surfaces during silanization. The adsorption bands which corresponds to the film of silane were observed only in PS layer. The FTIR reflection spectra of different samples of PS after silanization shows the good reproducibility of silane layers. The impedance measurements displayed shift of capacity for SiO$_2$ film during consequence series of experiments. At the same time, the imaginary part of impedance was not changed for PS layers.

SESSION **F3**: MECHANICAL PROPERTIES AND MINERALIZED TISSUES  
Chair: Dana C. Martin and G. M. Psaraki  
Wednesday Morning, November 28, 2001  
Hampton (Sheraton)

8:30 AM **F3.1**  
PHYSICAL CHARACTERIZATION OF DENTIN: NEW INSIGHTS INTO THE BIOMECHANICAL PROPERTIES OF MINERALIZED TISSUES.  

Dentin, the most abundant mineralized tissue in the tooth, is similar in composition to bone: type I collagen fibrils reinforced by a non-crystalline inorganic mineral phase. Because it is structurally more uniform than bone, dentin should be an ideal substrate for gaining fundamental insights into the mechanical behavior of all mineralized tissues. Unfortunately, the study of dentin has yet to provide these insights. Perhaps because of the small amount of tissue available for traditional test methods, there are three-fold discrepancies in the reported magnitudes of the elastic constants, and four-fold discrepancies in the measurements of the strength. The study of dentin can benefit from the appropriate application of new methods of physical characterization that have been optimized for small specimens. We adapted AFM-indentation to measure the Youngs modulus of wet and dry dentin. Resonant ultrasound spectroscopy (RUS) was used to probe the AFM measured indentations, as well as to provide the entire stiffness tensor. Excellent agreement was obtained between the AFM-method and the Youngs modulus determined from the RUS-obtained stiffness tensor. RUS, however, provided additional information: dentin is chalcotrichic isotropic and the bulk modulus is extremely sensitive to the moisture and mineral content. The bulk modulus decreased from ~40 GPa to 20 GPa with drying, while the Youngs and shear moduli increased by only 10-15%. We attributed the large decrease in bulk modulus to the loss of incompressible fluid from micropores in the dentin. Indeed, attempts to prevent fluid access by sealing off porosity with a mineralizing solution decreased the hydrated bulk modulus and in eliminating its sensitivity to moisture content. The insights gained from the application of these new methods of physical characterization are relevant to pathologies in dental hard tissues and bone.

9:00 AM **F3.2**  
CHARACTERIZATION OF DAMAGE MODES IN DENTAL LAYERED STRUCTURES.  
Yan Deng, Univ of Maryland, Dept. of Materials and Nuclear Engineering, College Park, MD; Brian R. Lawn, National Inst of Standards and Technology, Materials Science and Engineering Lab; Pedro Miranda, Univ de Extremadura, Dept Electronica Ingenieria Electromecanica, Badajoz, SPAIN; Antonio Pujares, Univ de Extremadura, Dept de Fisica, Badajoz, SPAIN.

Bilayer and trilayer model crown-like structures fabricated from a variety of dental ceramics are characterized using spherical Hertzian indentation tests. Critical loads to produce different damage modes, cone cracking and quasiplasticity at the top surface and radial cracking at the subsurface, are measured as functions of ceramic layer thickness and basic mechanical properties. It is proposed that these damage modes, especially radial cracking, are directly relevant to the failure of all-ceramic dental crowns. The experimental results are analyzed using critical load relations from fracture mechanics. These relations provide a physical basis for material and engineering design of dental layered structures for optimal resistance to lifetime-threatening damage.

9:15 AM **F3.3**  
ENAMEL BIOMECHANICS VIA GENETICALLY ENGINEERED MOUSE.  
Hanson Fong, M. Sarikaya, Dept of Materials Sci & Eng, University of Washington, Seattle, WA; S.N. White, School of Dentistry, UCLA, Los Angeles, CA; M. Paine, W.M. Sheid, Center for Craniofacial Molecular Biology, USC, Los Angeles, CA.

Dental enamel is the protective layer providing the mammalian tooth with strength, toughness, wear resistance, and the ability to withstand the complex, masticatory stresses. Since mature human enamel is a non-growing tissue, its loss or damage due to diseases, bacterial decay, or accidental overload requires restoration with an implant material. Despite the improvements in the restorative materials, the lack of biocompatibility and inferior mechanical properties limit their long-term durability. To overcome the problems associated with the traditional approaches to restoration, we have developed a program towards regeneration of enamel to clinically replace restoration materials. Enamel biomechanics requires a thorough understanding of structure-function relations as well as the biomineralization process in which certain proteins play important roles. Recently, we, and others, have found that one of the major proteins, i.e., amelogenin, significantly affects structural evolution of enamel. Using genetically engineered mice, we have modified the amelogenin in two domains (designated as A and B) and studied their in vivo self-assembly properties. Furthermore, the effect of these modified amelogenin proteins on structural formations and the subsequent mechanical properties were investigated using transmission and scanning electron microscopes, atomic force microscopy, and nanoindentation. We found that the morphology, crystallography as well as the chemistry of the hydroxyapatite nanocrystals were significantly altered. As a result of these variations in the structure and chemistry, nanoscale mechanical properties were also altered. These results have direct consequences in providing better insights both in the understanding of common dental diseases (e.g., amelogenesis imperfecta) and potential enamel biomimetics.

9:30 AM **F3.4**  
ELASTIC MODULUS MAPPING OF BAMBOO MICROSTRUCTURE.  
J. Anderson, S. Yedla, and R.M. Water, South Dakota School of Mines and Technology, Department of Chemistry and Chemical Engineering, Rapid City, SD.

Bamboo has long been used as a construction material in Asia and Central and South America given its ability to reach maturity in six months and outstanding mechanical properties such as tensile strength which rivaled steel. Given these desirable attributes bamboo serves as one of Nature's model composite materials. In this study we utilize the electron nanoninder and Interferential Force Microscope (IFM) to investigate the mechanical properties of the microstructure of several bamboo species. The "trunk" of the bamboo is referred to as the culm which is comprised of parenchyma cells and vascular bundles (1 mm in diameter) which are formed from vessels (10%), fibers (60%) and sieve tubes (20%). The IFM is a scanning probe microscope which utilizes a unique self-balance capacitance force sensor. Force-displacement curves obtained with the IFM are analyzed using Hertzian contact mechanics to extract the Young's moduli of the various micro and nano-structural subunits. We will discuss the relationship between the bamboo's micro- and nano-structure and chemistry and the measured elastic moduli. We will present a relationship to correlate these measured micro-mechanical properties to the macro- mechanical properties (e.g. tensile strength). This work provides insight as to how Nature builds this cellulosic based composite material.

10:15 AM **F3.5**  
CHARACTERIZING LOCAL VARIATIONS IN THE MECHANICAL PROPERTIES OF HUMAN ENAMEL.  
Tim Weir, Johns Hopkins Univ, Dept of Materials Science and Engineering, Baltimore, MD.

Mastication imparts significant stresses and strains to human teeth that can lead to fracture, wear, and failure. The exact nature of these stresses and strains, and their variation throughout a given tooth, are determined in large part by the mechanical properties of the teeth. The hardness and stiffness (Young's modulus) of enamel plays a major role in determining the mechanical response from mastication, and must be characterized to predict the deformation, wear, and fracture of teeth. In addition, as enamel interacts with foreign chemicals and synthetic materials, significant changes in near-surface,
mechanical properties can occur. This talk discusses our efforts to identify and understand local variations in the mechanical properties of enamel in situ. Using a variety of techniques, we have mapped variations in hardness and stiffness on mesial cross-sections of secondary molars, showing that local variations can be far greater than previously reported. For example, as one moves from the lingual apex to the dentin-enamel junction, hardness drops by more than a factor of three. Similar trends were obtained for Young's Modulus, which quantifies elastic stiffness. These sharp variations correlate best with changes in local chemistry as opposed to crystallographic changes. A map of mechanical, chemical, and microstructural characteristics will be described.

10:45 AM EP3.6
CONVENTIONAL MECHANICAL TESTING TECHNIQUES APPLIED TO SMALL SAMPLES OF BIOLOGICAL MATERIALS
Chris Smith, Ken Evans, Dept of Engineering, University of Exeter, Exeter, UNITED KINGDOM

The authors have developed equipment and techniques for mechanical characterization of small samples of biological materials, below 1 mm in length. Forces may be applied to samples in tension, compression, flexure or many other configurations. This has advantages over other techniques which rely on assumptions to do with isotropy, strain rate and Poisson’s ratio. Non-contact optical strain measurement techniques are described, including edge following and novel surface strain mapping systems. Results for several previously untreated biological materials are presented and discussed, including insect wing cuticle and single trabeculae from human cancellous bone. Data for elastic parameters including Poisson’s ratio and some fracture parameters are given.

11:00 AM EP3.7
CHARACTERIZATION OF MINERALIZED TISSUES BY COMBINING SCANNING SMALL-ANGLE X-RAY SCATTERING, QUANTITATIVE BACKSCATTERED ELECTRON IMAGING AND NONINVASIVE TECHNIQUES
T. V. Zhulina1, P. Roach', I. Zemk2, O. Pfeiffer3, K. Glaser4, Ludwig Boltzmann Institute of Osteology, 4th Medical Department, University Hospital, Vienna, AUSTRIA; 2Erich Schmid Institute of Materials Science, Austrian Academy of Sciences, and 3Metal Physics Institute, University of Leoben, AUSTRIA.

Native mineralized collagenous tissues like bone and tooth have a varying arrangement of structures at many length scales which work in concert to perform diverse mechanical, biological and chemical functions. The basic building block of these tissues is the mineralized collagen fibril. The fibrous protein collagen constitutes the main component of a three-dimensional matrix into which the mineral hydroxyapatite (Ca10(PO4)6(OH)2) forms. The degree of mineralization of the collagenous matrix as well as the size, shape, and arrangement of the mineral nanocrystals are crucial parameters which influence the mechanical properties of the whole structure. Due to the complex hierarchical structure, complementary physical characterization techniques have to be applied locally on the same samples in order to investigate correlations between mineral density, crystal size and shape, hardness and elastic modulus.

We used scanning small-angle X-ray scattering (1), quantitative backscattered electron microscopy (2), as well as atomic force microscopy together with nanoindentation (3) to perform 3D and 2D-mapping of bone, cartilage and tooth sections. Correlations of these results and their dependence on specific positions in different tissues were observed, relating gradients of composition, particle size and mechanical properties. In the case of dentin, for example, it could be shown that hardness and stiffness of the matrix are not only determined by the mineral particle density, but even more by the particle size (3). Similar observations were also made for the interface between bone and mineralized cartilage.

The study of hierarchical building principles of particle reinforced composites in enamel and tooth provide new insights into the construction processes of such structures with possible applications for novel materials.


11:15 AM EP3.8
BULK MODULI AND DENSITY MEASUREMENTS OF SMALL COMPRRESSIBLE AND INCOMPRESSIBLE SAMPLES
Jack C. Huy, Barry N. Lucas, Fast Forward Devices, LLC, Knoxville, TN.

A novel system is described for determining physical properties, including bulk modulus and density, of small solid samples. The system consists of a chamber in which the sample weight is measured by a weighing scale that is immersed in a gas of controllable density. Thus, the method of measuring density is based on Archimedes’ principle where the weight of an object is reduced by the weight of the displaced fluid. This particular device has been designed for examining the density of enamel samples 3 mm in diameter and 0.4 mm thick. The weighing device has a repeatability of 1 gN and sample densities can be determined to 0.5%. A significant feature of this device is the ability to measure density forces at a plurality of gas densities, which allows the capture of nonlinear behavior associated with compressible media. Results are presented for a quas-closed cell foam that experiences volume reduction as the gas pressure is increased. Volumetric strains are determined as the difference between the observed behavior and the linear behavior of incompressible media. Plots of hydrostatic stress versus volumetric strain are initially linear, as described by the bulk modulus, and exhibit a “kink” at high pressures, presumably due to the complete compression of internal cells.

11:30 AM EP3.9
MICROSTRUCTURE AND MECHANICAL PROPERTIES OF SKELETAL BONE IN GENE MUTATED STIPSEL, OSL, and WILD-TYPE ZEBRAFISH (DANIO RERIO)
Y. Zhang, F. Z. Cui, X. M. Wang, Q. Cui and Q. L. Feng, Biomaterisl Research, Dept of MS&E, Tsinghau University, Beijing, CHINA

Zebrafish (Danio rerio), the only vertebrate organism now amenable to large scale of "in situ generation screens", provides a single opportunity to investigate the complex gene mechanisms for normally vertebrate-specific processes. Studies of bone formation and bone diseases utilizing zebrafish system are therefore of interest. In the present study, microstructure and mechanical properties of skeletal bone in gene-mutated Stipse1,2/2, Osl, and wild-type zebrafish were firstly investigated by scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffractometry and atomic force microscopy (AFM)-based nanoindentation. Significant change of bone microstructure including both collagen matrix and minerals was observed in stp/stp bone compared to the wild-type control. It involves both abnormality of diameter and organization of collagen fibrils and alteration of mineralization in terms of mineral crystallinity, mineral phase, Ca/P molar ratio, mineral size, and precipitation location of minerals. Nanoindentation measurement disclosed that there was a significant increase of elastic modulus in stp/stp bone comparing to that of the control. Furthermore, AFM examination of the residual indent impressions and SEM observation of the transverse fracture surfaces of both bones indicate that the bone of zebrafish becomes more brittle after stp gene mutation. These results show interesting similarity to those corresponding problems in osteogenesis imperfecta (OI), suggesting that zebrafish is not only a good model system for studying bone mineralization at up to gene level but might also be a promising model for finding solution to human OI.

SESSION FF4: MECHANICAL AND RHEOLOGICAL PROPERTIES
Chair: John H. Kinney and Devin J. Pochan
Wednesday, Afternoon, November 28, 2001
Hampton (Sheraton)

1:30 PM FF4.1
PROBING THE MECHANICAL PROPERTIES OF BONE WITH NONINVADENT. George M. Parker, The University of Tennessee, Dept of Materials Science & Engr, Knoxville, TN, and Oak Ridge National Laboratory, Metals and Ceramics Division, Oak Ridge, TN; J. Gregory Swedner, Los Alamos National Laboratory, Los Alamos, NM; Jin-Yong Rho, University of Memphis, Dept of Biomedical Engineering, Memphis, TN.

Noninvasant, a technique for measuring mechanical properties at the micron and sub-micron scales, is widely used to probe the properties of thin films, thin surface layers, and small microstructural features. The technique has been widely applied to metals, ceramics, and polymers, and more recently to biological materials. In this presentation, work conducted to adapt the technique to the measurement of hardness and elastic modulus of key microstructural features of bone will be highlighted. Knowledge of these properties is critical to the development of micro- and microstructurally-based models for bone as a composite material.

2:00 PM FF4.2
ASSESSING STRUCTURE-PROPERTY RELATIONS OF DISEASED TISSUES USING NONIANDENTATION FTRI. Donna M. Elbsten, Lisa A. Pratt, University of California at Berkeley, UCB/UCSF Bioengineering Graduate Group, Department of Bioengineering, Berkeley, CA.

An assessment of structure-property relations for healthy and diseased tissues is essential for accurate modeling of tissues, constructing viable
3:15 PM TF4.5
Dept. of Physics and DEAS, Harvard University, Cambridge, MA.

The thermally-induced motion of small probe particles can be used to determine the local properties of biological materials. If the particles are small compared to the characteristic length scales of local structure, their motion is used to extract information about processes that affect them generically. If the particles are large compared to the characteristic length scales, their motion probes the local viscoelastic properties of the material. Examples of measurements of both the rheology and the structure of several different biomedical materials will be discussed.

3:45 PM FP4.6

BACKGROUND: Fascia lata (FL) and lumbo-dorsal fascia (LDF) play an important role in the biomechanics of locomotion and postural support. FL is also used in reconstructive orthopedic, urologic and gynecologic surgery. Nonetheless, characterization of elastic and viscoelastic properties of these fascial tissues is somewhat limited in the literature.

METHODS: For determination of elastic properties, fresh strips of FL and LDF from female, New Zealand White Rabbits were mounted on a Dynamic testing stand and strained to mineral failure. For determination of viscoelastic properties, tissue strips were subjected to a stress-relaxation protocol consisting of 6 steps of 5% strain with 60 seconds of relaxation between steps. Hydroxyproline (Hypro) and Glicysaminoglycan (GAG) and cell number were determined to investigate the correlation of biomechanical and mechanical properties. Student’s t-test was used with Bonferroni correction for multiple pairwise comparisons.

RESULTS: For FL vs. LDF, the UTS, yield strain, modulus and elastic toughness were (mean ± SD): 8.12 ± 3.1 MPa vs. 3.72 ± 0.9 MPa, 39.15% ± 6.3% vs. 46.3% ± 6%, 36.74 ± 17.2 MPa vs. 7.99 ± 4.2 MPa, and 1.84 ± 3.41 J/m² vs. 1.19 ± 2.8 J/m². UTS, yield strain, and modulus were significantly greater (p<0.001) for FL. Viscoelastic time constant and calculated instantaneous stress were (mean ± SD): 9.26 ± 2.7 vs. 13.94 ± 1.8 and 4.35 ± 1.21 ± 0.70 for FL and LDF respectively. Both differences were statistically significant (p<0.002).

Hypro, GAG and DNA content were (mean ± SD): 0.729 ± 0.808 mg/g, 6.094 ± 3.52 mg/g, 0.88 ± 0.48 mg/g, 7.84 ± 3.13 μg/g, 1.80 ± 0.34 μg/g and 0.06 μg/ml for FL and lumbo-dorsal fascia respectively. There were no significant differences in any of these parameters although there was a trend towards greater cell number in the LDF (p=0.0003).

DISCUSSION: There are consistent and marked differences in the elastic and viscoelastic properties of FL and LDF in the rabbit despite no evidence of significant differences in the concentrations of key extracellular matrix components. These data also suggest that the viscoelastic effects are important in the mechanical properties of the tissues and should be considered when characterizing their biological and medical roles.

4:00 PM FP4.7
USING RHEOLOGY TO PROBE THE MECHANISM OF JOINT LUBRICATION: SYNOVIAL FLUID’S POLYELECTROLYTE/PROTEIN INTERACTIONS. Katherine M. Ones, Wendy E. Krause, Ralph H. Colly, Dept. of MSE, Pennsylvania State University, State College, PA.

Rheology is a useful tool that can probe polymer interactions in mixed polymer systems. The outstanding lubricating properties of synovial fluid, found in freely moving mammalian joints, may be due to a soluble complex formation between hyaluronic acid, an anionic polysaccharide (Mw = ~1.6 MD), and the plasma proteins. Rheological properties of the synovial fluid are reported for a model of synovial fluid, comprised of hyaluronic acid (5 mg/ml), and the albumin (11 mg/ml, Mw = 66kDa) and r-globulin (7 mg/ml, Mw = 15kDa) proteins, as well as polymer-protein complexes, obtained after various characterization methods, such as scintillation and membrane dialysis techniques. We hope to gain full understanding of the synovial fluid-mediated mechanism of lubrication. This insight may lead to advances in anti-inflammatory drug therapy treatment for diseased joints.

4:15 PM FP4.8
ONE AND TWO PARTICLE MICROELECTRODE INSTALLATIONS: SOLUTIONS TO HIV VIRUS. Kanpur, M. Addin, Jay X. Tong, Dept. Physics & Indiana Mol. Biol. Institute, Indiana University, Bloomington, IN; Alex J. Levine, Dept. of Chem. Engr.,
University of California, Santa Barbara, CA; Christoph F. Schmidt, Vrije Universiteit Amsterdam, Dept. Physics of Complex Systems, Amsterdam, THE NETHERLANDS.

We have used one and two-particle microtography, employing micron-sized embedded beads and laser interferometric displacement detection to confirm the ecological properties of a crystalline phase of the filamentous virus. Thermal fluctuations of the embedded probes were measured and the viscoelastic parameters of the embedding medium were derived. In two-particle microtography the correlated motion of two different particles in a liquid suspension in the medium was analyzed, which can avoid biased results due to surface depletion effects near the probe. Particular emphasis was placed on the comparison between the one- and two-particle results.

4:30 PM FP4.0
LIQUID CRYSTALLINE PROPERTIES OF F-ACIN:
A QUANTITATIVE STUDY. Jorge Viamonte and Jay X. Yang, Indiana University, Physics Department, Bloomington, IN.

Actin filaments found in the cytoplasm are important for cell shape, division, and motility. Solutions of filamentous actin (F-actin) undergo a phase transition, from an isotropic to a nematic liquid crystalline (mesomorphic) phase. Measurements of optical birefringence with either no shear applied, or with up/down inversions by centrifugation, were performed at different actin concentrations and filament lengths. The measured birefringence at various locations was found to correlate well with the local concentrations of actin as probed by fluorescence imaging. Such a direct spatial correlation between the birefringence and the local actin concentration is characteristic of the order phase transition. We also observed different features between a concentrated actin solution and the conventional thermotropic or lyotropic nematic phase. The thermodynamic behavior of F-actin may have important effects on the properties of the cytoplasm in living cells.

4:45 PM FP4.10
TWO-STAGE SWELLING BEHAVIOR IN AMPHIPELIC.
POLY(ETHYLENE IMINE)/POLY(ETHYLENE CAPROLACTONE) MULTIBLOCK COPOLYMER GEL. The Chul Kim, Jong Ho Lee, You Hun Bo, Do Young Noh, Kwangju Institute of Science and Technology, Kwangju, KOREA (SOUTH)

We studied the swelling behavior of amphiphilic PEO/PCL multiblock copolymer of various block sizes using small-angle wide-angle x-ray scattering. In the initial stage of the swelling, hydrophilic PEO chains relax and the lamellar period increases. The swelling time is inversely proportional to the PEO block size. As the swelling continues, however, the lamellar period saturates, but the density modulation caused by swelling continues to increase. The swollen PEO/PCL multiblock gel phase-separated into ordered PEO-rich liquid and PCL-rich liquid as the PEO melts with increasing temperature. At 336K, the hydrogen bonding associations in the swollen gel network are broken by the increased hydrophobic interaction, and the gel collapses. The observation might be described in terms of the LCST (lower critical solution temperature) behavior.

SESSION FE5 POSTER SESSION
Chairs: Adrián B. Mann, Deborah E. Lockwood, Jan H. Hoh, Gang Bao and David C. Martin
Wednesday, November 28, 2001
8-10 PM
Exhibition Hall D (Hynes)

FP5.1
UV-LASER INDUCED AMINO GROUP SUBSTITUTION ON PET LIGAMENT TO PROMOTE INHIBITION OF COLLAGEN.
H. Omura, M. Murakami, The Faculty of Engineering of Tokai University, Kamo, Ibaraki, JAPAN.

Amino functional group was substituted on PET film surface for the purpose of making the implantation of collagen readily. The PET has been widely used for medical materials such as artificial ligament because of its strength and good immune reaction. However, when transplanted in human bodies, its biocompatibility is not good enough to adapt to the collagen, which grows from living body tissues. To avoid this reaction medicine has been used clinically which makes the PET film into a more biocompatibility status. After the transplanted into a human body, makes the tissue intrude in the PET fiber. However, this method has not shown satisfactory enough results to promote rehabilitation. If the living body compatibility of materials is improved the initial adapting power with the final level between 8 to 10 mmHg. When the pressure exceeds 21mmHg, a diagnostically of glaucoma may be predicted. Then, we designed new type of membrane to pump the aqueous humor and regulate its outflow was created. The membrane has gained characteristics of aqueous humor penetration by substituting a part of hydrophilic group that exists inside the porous PTFE with hydrogel group. Also it enabled to change the differential osmotic pressure freely by setting up the density of the hydrogel group. Then we tried the new method of forcing the water into the inner porous PTFE. The water was photo dissociated by irradiating the Ar laser into the inner porous PTFE. In the membrane the hydrophilic group was substituted there, by photo-exciting the inner pore setting of PTFE. Then the ethyl alcohol is dropped on the porous PTFE, it penetrates into inner porous PTFE easily. Then the water is dropped on the porous PTFE, the water is transferred up to the inner side of porous PTFE. Under these conditions Ar laser is irradiated. The result showed that untreated samples of 3µm pore could not be penetrated by water even if the pressure was increased to 300mmHg, whereas the treated samples irradiated with irradiance of 15mW/cm² by the Ar laser with 1mmHg. In conclusion our study demonstrates that these samples with hydrophilic property treatment using water (H₂O) as the reaction liquid can be used for intelligent materials such as the biocompatible membrane.

FP5.4
CHARACTERIZATION OF SUSPENDED LIQUID BILAYERS ON POROUS ALUMINA. Komishia Nischel, Christian Horn, Christian Heinestahl, Claudia Steinem, Institut für Analytische
Recently, a new model system for biological membranes has been invented [1] combining the merits of black lipid and solid supported bilayers. While black lipid membranes allow an easy incorporation of fully functional transmembrane proteins, solid supported membranes exhibit long-term stability advantages for bioanalytical applications. Our system of pore-spanning lipid bilayers is based on nanoperforated alumina substrates with pore diameters of about 40-60 nm and a depth of a few microns. These were prepared by anodic etching utilizing the technique of Li et al. [2]. To attach lipid bilayers onto these substrates, we used electrostatic interactions between charged lipids and the alumina substrate. One method is based on directly applying a protein solution to the alumina substrate. Coupling molecules were linked to the lipid bilayer using the technique of Li et al. [3]. This was achieved by first spotting titanium and gold onto the porous surface. The other technique started with a prefunctionalization of the alumina surface with self-assembled monolayers bearing the desired functionalities. This was followed by a second treatment with titanium and gold. Subsequent chemisorption of negatively charged thiol compounds led either to negatively or positively charged surfaces. Correspondingly, the functionalized surfaces were incubated in solutions of positively and negatively charged vesicles, respectively. The properties of the substrate, the degree of vesicle fusion and the content of the surface-supported vesicles and the supported bilayers were investigated using scanning-force microscopy (SFM), interferometry, impedence spectroscopy and measurements of the photocurrent generated by membrane incorporated bacteriorhodopsin. The model system described is suitable for the further studies of the structure and function of biomembranes. By additional physicochemical analysis of alternating charged polymers-layered upon the substrate surface we intend to further increase the degree of vesicle fusion and membrane stability.


F5F.5 DEVELOPMENT OF AIN THIN FILM BASED PIEZOELECTRIC SENSORS FOR ULTRASONIC IMAGING OF BIOLOGICAL ELEMENTS. Marvin Nickels, Wayne State University, Department of Biomedical Engineering, Detroit, MI; Greg Aumer, Chonghy Heung, Wayne State University, Department of Electrical & Computer Engineering, Detroit, MI; Ruta Nak, Wayne State University, Department of Physics, Detroit, MI; Vaan Nak, U. Michigan-Dearborn, Dept. of Natural Science, Dearborn, MI.

The piezoelectric properties of AIN thin films have been of practical interest for many acoustic wave sensor applications. In the present study, AIN thin films have been grown by plasma source molecular beam epitaxy (PSMBE) on Si substrates. Both x-ray diffraction and optical studies confirm high quality of AIN thin films. Excimer laser (KrF) micromachining has been used to fabricate (80μm) pixelated array of piezoelectric sensors. The results of ultrasonic wave imaging using a 5 MHz ultrasonic wave will be presented. A study has been conducted using mice that have been injected with human breast cancer for non-invasive biological detection.


We present a multiple particle tracking technique for making precise, localized measurements of the mechanical microenvironments of inhomogeneous materials. Using video microscopy, we simultaneously measure the thermally activated dynamics of fluorescent tracer particles embedded in a complex medium, and interpret their motions in terms of local viscoelasticity and microstructure. This technique is particularly well suited to studying biological materials where small sample volumes and complex, heterogeneous structures necessitate localized measurements. We will present our work on the local mechanical response of living fishrods.

F5F.7 ONE- AND TWO-POINT MICROPHYSIOLOGY OF F-ACTIN NETWORKS. Margaret L. Gaudenz, Megan T. Valentine, Harvard Univ, Dept. of Physics, Cambridge, MA; John C. Crocker, California Institute of Technology, Dept. of Applied Physics, Pasadena, CA; Andrea R. Bousch, David A. Weitz, Harvard Univ, Dept. of Physics, Cambridge, MA.

The technique of microphysiology is now widely applied to study the microscopic viscoelastic properties of complex fluids, including polymer networks. The technique of single micro-sized beads embedded in the polymer is used to extract viscoelastic moduli. However, in networks of the semi-flexible polymer F-actin, comparison of bulk and microscopic results exhibit discrepancies, which are thought to be due to the heterogeneous nature of the probe or the coupling of the probe particles to the medium. We are able to eliminate the differences between the micro- and macro-rheology by extracting moduli from the pairwise correlated movements of the probe instead of single probe motility. We use a multiparticle tracking approach to observe the motion of several hundred probes embedded in the actin network. Here we present results studying actin networks varying both probe size and surface chemistry to explore the interpretation of both single and correlated probe motion in Actin networks.

F5F.8 MECHANICAL STRESS AFFECTS ON BIOLOGICAL ACTIVITY. Scott Mickey, J.C. Poler, University of North Carolina at Charlotte, Dept. of Chemistry, Charlotte, NC.

The purpose of this study is to examine the effects of mechanical strain at the molecular level. Chemical reactivity dependence on strained molecular bonds is well understood. However, the effect of molecular strain in a substrate during biological processes has not previously been studied at length. We hypothesize that the observed affect on activity in this model system has implications to genetic manipulations within living cells and possibly related to disease. Enzymatic activity was monitored as a function of mechanical strain using a lambda DNA restriction endonuclease model. One end of modified DNA substrate was bound to an immobile gold surface while the other free end was attached to a paramagnetic bead. Modified DNA fragments were stained with YOYO-1 for single molecule visualization via epifluorescence microscopy. A magnetic field was employed to effectively lengthen DNA strands as EcoRI activity was monitored in order to develop a relation between DNA length and enzymatic activity. Single- and room-temperature photolysis techniques were used to process the gold anchor points for the DNA.

Photolithography was also used to define a single flow and visualization cell for this system under study. PDMS molding was used to fabricate the microfluidic chamber. Gold functionalization was studied with contact angle measurements and PAMAM to control site-specific binding of the DNA and localize its position to within a micron.

F5F.9 CONTROLLED DELIVERY OF VANCOMYCIN FOR TREATING THE CHRONIC OTITIS MEDIA BY INHIBITION OF MRSABIOGROWTH: APPLICATION OF A TEMPERATURE RESPONSIVE BIODEGRADABLE POLYMERIC. Jeong Ok Lim, Medical Research Institute, Kyungpook National Univ., Taeju, KOREA; Yui Mi Kim, Dept of Biomedical Engineering, Kyungpook National Univ., Taeju, KOREA; Jeong Min Soh, Medical Research Institute, Kyungpook National Univ., Taeju, KOREA; Yeon Yi Baek, Dept. of Anesthesiology, Kyungpook National University, Taeju, KOREA; Sung Hee Lee, Dept. of Otolaryngology, Kyungpook National Univ., Taeju, KOREA.

Vancocycin is a potent therapeutic agent being used for treating MRSA(methicillin resistant staphylococcus aureus), however it induces systemic toxicity by repeated usage and patients with severe MRSA are required hospitalization for regular oral intake and intravenous injection of vancocycin. In order to improve the patients condition, vancocycin was incorporated with biodegradable polymers and the effects of the system was evaluated using human MRSA infected from patients ears. A various type of temperature responsive biodegradable polymer containing vancocycin has been prepared to conveniently deliver the antibiotic to the target site and to localize the drug delivery for chronic otitis media. The phase transition phenomena of the prepared systems were characterized. From in vitro and in vivo study of the polymer-vancocycin complex, the systems were found to be suitable for injection to the body and the MRSA growth was well reduced in the slow release of the antibiotic. The degradability of the polymer was also characterized.

F5F.10 NEUTRON SPIN ECHO SPECTROSCOPY STUDIES OF BIOMOLECULES IN SOLUTION. Zimei Bu, Amos M. Tsui, Nicholas Rosov, NIST Center for Neutron Research, Gaithersburg, MD.

Neutron Spin Echo (NSE) spectroscopy probes the intermediate scattering function $g(\Gamma, Q, \tau) = \int g(Q, \omega) \delta(\omega) d\omega$ with times ranging from 50 ps to 1.0 ns for the NSE spectrometer located at the NIST Center for Neutron Research. NSE is especially suited for investigating relaxation-type motions, which are of interest in polymer and glass studies, among others. To illustrate the information which may be extracted from such data, we will present results from solutions of two different biomolecules: lysozyme and α-lactalbumin.
SANS studies of lysozyme have shown that at high concentrations (50 mg/ml) these concentrations there is a liquid-like ordering of the lysozyme molecules. The addition of glycerol to the solution reduces the characteristic interaction length scale. We have investigated how the correlated dynamics between lysozyme molecules is affected by the addition of a glass-former (glycerol) to the water solvent. The local dynamics of d-alaethionine has previously been studied in the picosecond time range by neutron time-of-flight and triple-axis spectroscopy where the native and molten globule states differ in that the side-chain protons remain in their potential wells for much less time in the molten globule state. We have used complementary methods to study both the intermolecular correlated dynamics and the intramolecular backbone motions.

**FF5.11 MORPHOLOGY AND BIOCOMPATIBILITY OF HYDROGELS: CONSTRIC H0L BLOCK COPOLYMERS**

**SELF-ASSEMBLY**

Lisa Falaknaz, Darrin Pochan, University of Delaware, Materials Science and Engineering, Newark, DE; Clifford Robinson, Delaware Biotechnology Institute, Chemistry and Biochemistry, Newark, DE; Andrew Nowak, Timothy Deming, University of California, Department of Materials, Santa Barbara, CA.

The self-assembly of low molecular weight (=20k g/mol), amphiphilic, diblock polypeptides of hydrophobic lysine (K) or glutamic acid (E) and hydrophilic leucine (L) or valine (V) has been studied in aqueous solution. At neutral pH and very low volume fraction of polymer (vol. fraction polypeptide > 0.5 wt%), these polypeptides form hydrogels with unique morphologic morphology. The novel hierarchical morphology of these gels has been studied using laser confocal microscopy (LCM), small angle neutron scattering (SANS), and initial cryogenic transmission electron microscopy (cryoTEM) imaging. Changes in gel morphology and strength were observed by varying the relative volume fraction of hydrophobic to hydrophilic blocks and salt concentration. The morphology, high modulus (as observed rheologically), and peptide foundation of these hydrogels make them intriguing candidates for biomaterials applications. Therefore, biocompatibility properties of these peptide-based materials are currently being assessed with cytotoxicity measurements utilizing mammalian cells. Antimicrobial properties have also been observed with E. coli.

**FF5.12 IRON-oxide FORMATION IN THE PRESENCE OF ABSENCE OF PROTEIN ORGANIC NANO-SUBSTRATES: A MOESBAUER SPECTROSCOPIC CHARACTERIZATION**

George C. Papageorgiou, Villanova University, Dept. of Physics, Villanova, PA; Guanghui Zhao and N. Dennis Chasteen, University of New Hampshire, Dept. of Chemistry, Durham, NH.

Biomimetic iron oxide nanoparticles are becoming increasingly important in biotechnology as magnetic resonance imaging contrast agents and drug delivery systems [1]. These nanoparticles are self-assembled within the confines of protein organic cages or micelles [2]. We present comparative Moessbauer [3] spectroscopic studies of the physical characterization of iron oxide nanoparticles self-assembled in aqueous solutions in the presence and absence of the protein nano-substrate for iron biomineralization, apoferritin. Under similar synthesis conditions, in the absence of apoferritin nanoparticles of a mixture of maghemite (30%) and hematite (70%), with a blocking temperature above 200 K, are produced. In contrast, the presence of apoferritin, which provides an interior cavity of ca. 8 nm diameter as substrate for iron deposition and accumulation, leads to the promotion and stabilization of a single phase, that of iron oxide hydroxide (100%), with a blocking temperature of ca. 40 K. Dis-convolution of the Moessbauer spectra into core-shell surface spin contributions allowed physical characterization of the core shell/magnetic interface of these composites. The electronic properties, superparamagnetic behavior and magnetic energy of these nanoparticles will be presented. Their potential use as NMR imaging contrast agents will be discussed.


**FF5.13 MECHANICAL PROPERTIES AND BIOACTIVITY OF LAMELLAR DIOPSID SUBSTRATE**

Noriyuki Y. Inoue, Yuki K. Nakamura, Hiroshi Y. Ishizuka and Norimichi Kawanishi, Toh University of Yokohama, Center for Advanced Research in Biomedical Engineering, Yokohama, JAPAN.

Diopside, calcium magnesium silicate was investigated as a biomaterial having both high mechanical strength and biocompatibility. Starting powders were prepared by a coprecipitation method using ethanol solution of calcium nitrate, magnesium nitrate and tetrathyl orthosilicate (TEOS). Powders composed of only diopside were synthesized adding ammonia to the starting solution and firing the precipitates at 1375 K for 1230 h. The hydroformed product was characterized by means of DTA and XRD measurements. The dry powders having different particle sizes were X-ray amorphous and crystallized into diopside at 1095 K. Diopside substrates were fabricated by hot-pressing method at 1427 K under a pressure of 40 MPa for 2-2.5 h. Bending strength, fracture toughness, vickers hardness and relative density were determined. Both sintering ability and strength improvement of diopside substrate were observed with decreasing holding time in the thermal process. The sintered body heat-treated at 1375 K for 24 h and then 1475 K for 2.5 h gave a bending strength and vickers hardness higher than those in literatures. The maximum bending strengths were 328 MPa and 722 Knoop respectively. A detailed study on the dependence of the final particle size on the sintered body is in progress. Lamellar diopside substrates were fabricated by the dip-coating method. The lamellar substrates have the structure of porous dense porous layers with respective thickness. Porous diopside films could be formed on the sintered body by the dip-coating method. The films were obtained by dipping the sintered body into the solution and heating at 1125 K for 30 min. The pore size on this film depended on immersing time or the thickness before crystalizing treatment. After seven days the bone like apatite layer formed on all over the diopside surface in the simulated body fluid. EPMA spectral diagram showed a change of composition across the junction diopside to the precipitated apatite layer.

**FF5.14 STUDY OF DNA MORPHOLOGY IN A SMALL DROPLET DURING EVAPORATION**

D. Gemel, N. Byers, Park Memorial High School, Y.S. Seco, Y.A. Smolgov, J. Sokolov, M. Rafaev, Department of Materials Science and Engineering, SUNY at Stony Brook, NY; B. Chu, Department of Chemistry, SUNY at Stony Brook, NY.

DNA interaction and adsorption kinematics in a small droplet (~2 μl) of DNA-buffer solution on a flat silicon substrates are reported. Kinematic studies during evaporation were performed by measuring contact angle and volume changes of the droplet along with measurements of the DNA distribution by confocal microscopy and AFM. The results show that the DNA strongly adsorbs to the silicon/air interface, forming a ring-like morphology on the surface. The DNA end in the droplet is arranged in a helically perpendicular to the perimeter of the ring. The results show that addition of DNA (0.05 mg/ml) increases the evaporation time of the droplet by from 1200 s to 3000 s while decreasing the contact angle from 110 to 10°. The perimeter of the ring is fixed and hence we show that this method can be used to deposit DNA for high resolution surface lithography. This work is supported by NSF-MRSEC program.

**SESSION FF6: ELECTRON MICROSCOPY AND NOVEL TECHNIQUES**

Chair: Adriaan Van Heel and Richard J. Young

Thursday Morning, November 29, 2001

Hampton (Sheraton)

**8:30 AM FF6.1 NO NEED TO DRY: ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY OF HYDRATED SYSTEMS**

Athene M. Donald, Cavendish Laboratory, University of Cambridge, Cambridge, UNITED KINGDOM.

Environmental Scanning Electron Microscopy (ESEM) is ideally suited for the study of many biological systems. Its ability to image insulating samples without coating, in and the presence of water vapor so that the natural state of hydration can be maintained, means that many samples which previously could only be imaged following severe preparation routes can now be readily observed in their native state. An overview of the advantages of ESEM for studying a range of biological systems will be presented, alongside some of the difficulties. The possibility of carrying out dynamic experiments (such as in situ hydration and dehydration) will also be considered.

**9:00 AM FF6.2 ASSEMBLY OF POLYMERIZED PHOSPHOLIPID VESICLE STRUCTURES ON FILMS PREPARATION IN SITU BY ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY**

J. Schirme, Alex Singh, Center for Biomolecular Science and Engineering, Naval Research Laboratory, Washington, DC.
Surface vesicles are spherical capsules with diameters that range from 200 nm to 1000 nm and with a shell thickness of ~5 nm comprised of noncovalently self-assembled amphiphiles arranged into a bilayer structure. Vesicles serve as useful model membranes for studying biomolecular structure and function with technological utility in drug delivery, cancer treatment, water treatment, mesoporous structure synthesis, and artificial photosynthesis. Not until rather recently, several unresolved fundamental scientific issues have delayed progress in this area, primarily as a result of the instability and biodegradability of the lipids used to prepare these soft materials. In the early 1980s, vesicular structural instability was addressed by incorporating polymeric functionality into monomeric surfactants, and the first in situ AFM characterizations of immobilized polymerized and polymeric vesicles were reported in 1996 (T. Shibata, Seki et al.) and in 2001 (I. Stainier et al.), respectively. Although in situ AFM is a powerful structural (i.e., topographical) characterization tool, we demonstrate for the first time in real-time the development of [poly(dimethylsiloxane)] (PDMS)-based [acrylate ester] (ESEM), that enables in situ measurement of immobilized vesicle size, shape, density, number density, and spatial organization; and further, generates a three-dimensional image for elucidation of relevant structure-function relationships. We show, using ESEM complemented with in situ AFM, that vesicle morphology (i.e., size, shape, etc.) is indeed retained upon surface immobilization. We also provide visual evidence for an immobilized vesicle superstructure, a twolayer vesicle film formed through controlled, covalent, vesicle stacking.

9:15 AM FF6.5
ENHANCED MICROSCOPY CHARACTERIZATION OF BONE-BONDING TO HYDROXYAPATITE AND Ti SURFACES OF HUMAN FEMORAL STEMs RETRIEVED AT AUTOPSY
*Massachusetts Institute of Technology, Department of Materials Science & Engineering, Cambridge, MA. **University of Cambridge, Department of Materials Science & Metallurgy, Cambridge, UNITED KINGDOM. **Imperial College of Science, Technology & Medicine, London, UNITED KINGDOM. **Royal National Orthopaedic Hospital, Stanmore, Middlesex, UNITED KINGDOM. "Brigham & Women's Hospital, Harvard Medical School, Boston, MA.

The need to optimize adhesion between femoral stems and surrounding bone tissue has led to the development of plasma-sprayed hydroxyapatite (PSHA) coatings as attachment facilitation vehicles for hip prostheses. The efficacy of HA for bone attachment and the bonding mechanisms associated with PSHA coatings have been explored clinically using implanted femoral stems coated with a plasma-sprayed porous Ti layer, overlain in some of the implants by a 50-micrometer >85% crystalline PSHA coating. These stems were retrieved at autopsy from human subjects who had been treated for a fractured femoral neck with a Bimetric (Bionet, UK) hemi-arthroplasty. The average patient age was 86 years, and the stems remained in place, in vivo, for 263 ± 21 days before being collected together with surrounding bone. Saw and polished cross sections were imaged by environmental SEM operating in backscattered electron mode, and both stained and unstained microcrystalline phosphate cores were imaged. While higher HA coating was observed to significantly improve initial bone bonding, the percentage of implant surface apposed by bone decreased from 100% at 1.73 days to 10% at 600 days, the decrease attributable to loss of the HA coating with implanting bone. Little difference was apparent between the uncoated porous Ti stems increased concomitantly and proceeded comparably to that experienced by Ti regions of the HA-coated stems exposed by loss of HA. TEM revealed aligned apatite phaeostructures, likely associated with collagen fibrils adjacent to implant surfaces. In some areas, globular clusters of apatite blocks and less organized microcrystalline mineral phase were identified. Three additional mineralization features have been previously observed for shorter time (1-10 days) in implants can mice models.

9:30 AM FF6.6
LOW VOLTAGE TABLE-TOP ELECTRON MICROSCOPY OF POLYMER AND ORGANIC MOLECULAR THIN FILMS
David C. Martin, The University of Michigan, Ann Arbor, MI.

We have been evaluating the utility of a new low voltage (<5 kV) table-top electron microscope (IVEM) for microstructural investigations of thin polymer and organic molecular films. The IVEM was developed by Delong Instruments in Bremo, Czech Republic (www.dicomps.com), and is equipped with a Schottky field-emission source, permanently scanning condenser lenses, and an electrostatic projector lenses. The IVEM can be operated in TEM, ED, STEM, and SE modes. High contrast micrographs of polymer and organic films have demonstrated the ability of the low energy electrons to produce films up to 400 nm thick with an enhanced lateral resolution of 2 nm. Images have been obtained from a variety of samples including semiconducting molecular crystals, polymer single crystals, inorganic oxide nanocrystals, polyolefin blends, and electrograft films of bioactive protein polymers.

9:45 AM FF6.7
CHARACTERISATION OF BIOMEDICAL MATERIALS, CELLS AND INTERFACES USING ENVIRONMENTAL SEM (ESEM)
Debbie Stokes, Cwmdihl Lab, University of Cambridge, UNITED KINGDOM; Susan Ren, Alexandra Porter, Serena Best and William Bonfield, Dept of Materials Science and Metallurgy, University of Cambridge, UNITED KINGDOM.

An important field of research to which ESEM can be applied is that of materials for biomedical applications, such as hydroxyapatite (HA) ceramics and HA/polymer composites that are being developed for use as synthetic scaffolds in bone tissue engineering. The biocompatibility of these materials is dependent upon such factors as phase composition, chemical composition, surface activity, crystallinity and morphology. Using ESEM in conjunction with secondary electron imaging, this type of analysis permits assessment of the distribution of species at grain boundaries, interfaces and in surrounding tissues, thereby assisting in the determination of mechanisms underlying biocompatibility or implant failure.

10:30 AM FF6.8
HIGH RESOLUTION WATER MAPPING IN HYDRATED TISSUE AND POLYMERS
A. A. Koch, N. V. Johnson, V. J. Andronick, M. L. Schertzer, Institute of Technology, Hoboken, NJ; M. Misek, SLASH, Unilever Research, Edgewater, NJ.

Most biological tissues have a mean hydration level of order 70%. There can, however, be substantial variations around this mean both between and within cells. One can also expect changes in water concentration in response to various stimuli. Among these are the inflammatory and higher-order responses to the in vivo introduction of a synthetic implant or tissue-engineering construct as well as the effects of various transdermal drug-delivery schemes on the interstitial system. The water distribution of non-tissue samples is also of interest as it can provide an assessment of manufacturing and may allow for some prediction of performance. Despite this significance, there has been little work involving direct experimental methods to quantitatively determine water concentrations with a spatial resolution sufficient to detect most synthetic polymers of a synthetic implant or tissue-engineering construct as well as the effects of various transdermal drug-delivery schemes on the interstitial system. The water distribution of non-tissue samples is also of interest as it can provide an assessment of manufacturing and may allow for some prediction of performance. Despite this significance, there has been little work involving direct experimental methods to quantitatively determine water concentrations with a spatial resolution sufficient to detect most synthetic polymers. The application of spatially resolved electron energy-loss spectroscopy (EELS) and scanning transmission electron microscopy (STEM) to water in frozen hydrated sections of porcine tissue as well as in various synthetic polymer systems. The method built on the technique developed by Legman et al. (2) using multiple low-angles of detection. Spectra can be distinguished from protein and lipid inclusions, and the method is characterized in this work. In contrast to previous characterizations, this technique provides a high-resolution image of the high-resolution sle of bone serum albumin (BSA). A spatial resolution of approximately 50 nm has been achieved in studies of frozen hydrated porcine tissue with dramatic contrast based simply on the local water concentration. Analysis of these sections removing the hydration by sublimation shows that in situ drying introduces dramatic and unnecessary distortion. Application of this same approach to hydrophilic materials such as polycarbonate has been achieved with a spatial resolution of 5.5 nm and shows that the water is nonuniformly distributed throughout the structure.

receptors mediated endocytosis dynamin is believed to localize to clathrin-coated pits, and to redistribute to the necks and possibly participate in maintaining its function upon GTP hydrolysis. Here we studied the effects of nucleotide binding and hydrolysis, without the effect of an underlying lipid model system. Cryo-translation electron microscopy (cryo-TEM) is a powerful direct non-perturbing method, devoid of staining and drying artifacts, used for studying macromolecular assemblies in the 2.50 - 1 nm range. In classical cryo-TEM, imaging is done on photographic plates without looking at the area imaged to avoid radiation damage. To overcome this difficulty we developed high-resolution cryo-TEM that allows a real-time feedback on specimen and image quality and a preview of the exact area to be recorded at low. This technique is especially advantageous for studying morphology and specific assembly details. Dynamics of assembly into rings and spirals, and constrictions in the presence of non- hydrolyzed GTP analogues such as GDP-β-S and GTP-γ-S. The constricted states of the rings are more stable and well ordered. Images show that the rings (edge-on views of short spirals) consists of inner and outer layers each made of 13-15 electron dense globular domains. The diameter of the rings and spirals, reflecting the ultimate constriction potential of dynamin, is 35 ± 1 nm, 3-4 nm less than the diameter of non-constricted spirals. To mimic the transition state, ΔPR1 and wild-type dynamin were dabeled with signal traps and analyzed in cryo-TEM. Experiments described in this paper show these spirals are constructed as well.

11:00 AM Ff 6.8

Biomimetic films that serve as models of cell membrane are of increasing interest in the study of biomimetic processes such as phospholipid self-assembly, molecular recognition, and cell-protein interactions. Newly developed phase-sensitive specular neutron reflectometry makes it possible to determine the scattering length density (SLD) of deep layers of biomimetic membranes by first-principles inversion, without fitting or adjustable parameters. The SLD profile of the film is obtained and can be directly compared with a corresponding chemical composition through the thickness of the film, predicted, for example, by the ab initio molecular dynamics calculations in a spatial resolution currently approaching 100 square nanometers. Recent measurements, performed on hybrid bilayer membrane systems in intimate contact with aqueous reservoirs, and in some cases with the films being components of functioning biochemical systems, are presented to illustrate the practicality and power of this new technique.

11:15 AM Ff 6.9
NUCLEAR MICROPROBE ANALYSIS OF TRANSMEMBRANE ION FLUX IN RAT BRAIN. Karen P. Braski, College of Pharmacy, University of Louisiana at Monroe, Monroe, LA; William A. Hollerman, and Gay A. Ghas, Acadiana Regional Laboratory, University of Louisiana at Lafayette, Lafayette, LA.

Maintenance of euglycemia is crucial because glucose is the sole nutrient that can be utilized by the brain in sufficient quantities to provide required levels of energy, and carbohydrate reserves in neural tissue are limited. Neurons located in select brain sites, including the hindbrain nucleus tractus solitarius/area postrema complex (NTS/AP), exhibit uniquely sensitive neurochemical and for genomic responses to glucopenia, suggesting that regulatory signaling of this substrate fuel deficit originates within discrete loci. Fundamental questions concerning the identification of monitored metabolic variables and the molecular and cellular mechanisms by which local sensor cells transude energetic distributions into neural signals remain unresolved. Microbeam particle-induced X-ray emission spectrometry (µPIXE) is a novel investigative technique offering milli-elevental quantification at ppm sensitivities, with high accuracy at spatial resolutions less than cell dimensions, and thus offers the possibility that quantitative spatial imaging of intracellular ion concentrations in 'glucose-sensitive' brain sites can be generated. Current collaborative efforts involve the use of microprobe analytical techniques for use with established neuroanatomical and pharmacological approaches in novel strategies to characterize electrolytic indices of neuronal function, in defined cell populations in vivo, in response to microinjections of glucose and glucose analogues. This presentation will describe efforts to map for effects of glucose deficits on transmembrane flux of ions, e.g. sodium, potassium, calcium, and chloride, that regulate neuronal plasma membrane function and neuronal signaling. This research is expected to significantly advance current understanding of the cellular and molecular bases linking neuronal energetics with homeostatic regulation of glucose availability. This research was funded by the American Diabetes Association and the Louisiana Education Quality Support Fund (LEQSF) under grants LEQSF (2006-2008) 3.39, DOE/LEQSF (1995-98), and DOD-FC35-91ER75690.

11:30 AM Ff 6.10

Miniaturized piezoelectric unimorph cantilevers offer the advantage of simple electrical detection and the potential for a wide range of sensing applications. Addition of moving parts to the cantilever’s resonance frequency shift. In addition, a piezoelectric unimorph cantilever can better withstand damping, making it particularly suitable for in-situ aqueous quantification of biomolecules such as dielectric receptors at the cantilever tip. Binding of the bioreceptors to the cantilevers can be detected by monitoring the cantilever’s resonance frequency shift. The concentration of the bioreceptors can be quantified from the time dependence of the resonance frequency shift. Results of model study on yeast cell detection will be presented. In addition, we will show both theoretically and experimentally that a cantilever’s mass sensing sensitivity increases inversely with the fourth power of the cantilever size.

11:45 AM Ff 6.11
A QUARTZ CRYSTAL MICROBALANCE CELL BIOSENSOR DETECTING DRUGS VIA ALTERATIONS IN CYTOSKELETON. Kenneth A. Marx, Tiemin Zhou, Anne Montrone, Susan Brunham, Center for Intelligent Biomaterials, Depts. of Chemistry and Biological Sciences, University of Massachusetts, Lowell, MA.

Endothelial cells (ECs), in vivo, stably attach to their underlying extracellular matrix (ECM) and thus line the interior surface of blood vessels. ECs were used to form stable monolayers on the gold covered quartz crystal microbalance (QCM). This was used as a surface to create an EC QCM bio sensor. We studied a small drug, nocardazole, which binds and disrupts microtubules-a major part of the cellular cytoskeleton. Nocardazole can be sensed by the EC QCM bio sensor because the drug concentration range and its kinetic behavior is measured over a 0.6 period via a significant change in the measured crystal oscillation frequency and increase in interfacial motional resistance R (1-3). The f and R shift magnitudes vary in a sigmoid shaped dose dependent fashion with [nocadazole], with a midpoint of 900 μM. The nocardazole effects on the EC QCM bio sensor are fully reversible. Fluorescence microscopy of the ECs fixed on the gold QCM surface and stained for actin demonstrated that the shape and cytoskeleton were affected by as little as 300 μM nocardazole. With increasing [nocadazole], ECs gradually occupied a smaller area, lost cell-cell contact, exhibited stress fibres at the cell periphery and acquired a rounded cell shape before beginning to detach at 15 μM. These results indicate that the EC QCM bio sensor can be used for the study of EC attachment and to detect EC cytoskeletal alterations. In addition to basic research, this novel cell QCM biosensor may be formed from a wide variety of cell types that adhere to surfaces, and may be used for the screening of novel therapeutic’s effects on adherent cells’ cytoskeleton-the membrane bound integrins, or the ECM, regardless of the mechanism of action. Supported by NIH R21 GM56803 and UML Res. Fdn. Seed Grant.


SESSION FF7: LASER AND OPTICAL CHARACTERIZATION
Chair: Guang Bao and David A. W. Zitz
Thursday Afternoon, November 1, 2001
Hampton (Sheraton)

1:30 PM FF7.A
DEFORMATION MECHANISMS IN NATURAL POLYMER FIBERS AND COMPOSITES. Robert J. Young, Stephen J. Eichhorn, Victoria L. Brooks, Manchester Materials Science Centre, UMIST/University of Manchester, Manchester, UNITED KINGDOM.

The presentation will demonstrate the extent to which Raman spectroscopy can be employed in the analysis of structure/property relationships in natural polymer fibres and composites. Well-defined fluorescent-free spectra can be obtained from the major amorphous
2:00 PM EP7.2
NANOSPINNING OF POLYMERS BY ELECTROSPINNING TECHNIQUES: "REAL TIME" RAY Mak stehen, Simon Krawitz, Silke Męgól, John F. Radke, University of Delaware, Dept. of Materials Science and Engineering, Newark, DE; D. Bruce Chase, Central Research and Development, DuPont, Wilmington, DE.

Electrospinning techniques, incorporating high voltage, high molecular weight polymers, and relatively high viscosity polymer solution in order to produce nanofibers, are used to control the shape, orientation, and texture of polymer fibers. Polymer spectra of as-spun fibers produced through electrospinning have shown that high N/SI can be obtained on 50 nm diameter fibers in relatively short collection times (25 sec). Using this same instrumental approach, "real time" Raman spectra of the electrospinning liquid fiber jet at the origin of the jet and 1 cm downstream have been obtained. The results show that "real time" analysis of the solvent/polymer ratio and spectroscopic measurements of polymer orientation are possible and will lead to a more quantitative understanding of the development of the polymer microstructure during electrospinning process.

2:15 PM EP7.3
Abstract Withdrawn.

2:30 PM EP7.4
THE STUDY OF CONNECTIVE TISSUE BY SMALL ANGLE LIGHT SCATTERING Karen M. McNamara, Eric D. Dahlgren, Worcester Polytechnic Institute, Department of Chemical Engineering, Worcester, MA; Christopher H. Sauck, Worcester Polytechnic Institute, Department of Biomedical Engineering, Worcester, MA; Amaj Bellure, Harvard Medical School, Department of Orthopedic Surgery, Cambridge, MA.

Small angle light scattering (SALS) can be used to probe structure on the micron and sub-micron length scales. Isotropic structures can be modeled as spheres, but this is not limited of the technique. Elongation in one direction (cylinder) and elongation in two directions (ellipsoids) are also valid models. Each geometry has significant impact on the form of the data observed. Collagen fibers in connective tissue are considered to be elongated in one direction; hence, they can be modeled as cylinders. SALS allows the quantitative determination of both fiber diameter and degree of orientation. The advantage of this technique over scanning electron and light microscopies lies primarily in sample preparation. SALS requires neither freeze-drying nor destroying tissue, and hence more accurately depicts in vivo conditions. One goal of this work is to relate the collagen fiber structure to the anisotropic mechanical properties of tissue such as ligament and Achilles cartilage. Initial light scattering results from the study of rabbit tendon under varying tensile loads will be presented and are compared with the results of conventional microscopy. Observed changes in fiber diameter and orientation or crimping demonstrate the quantitative usefulness of this technique.

3:15 PM EP7.5
VISCOElastIC SCALING IN COLLOIDAL SYSTEMS. Maria L. Kiffel, David A. Weitz, Harvard University, Division of Engineering and Applied Sciences/Physics, Cambridge, MA.

The universal scaling behavior which successfully describes colloidal networks in simple viscous fluids may no longer apply to colloidal gels when the backbone is viscoelastic. We investigate whether universal viscoelastic behavior can be found for these complex gels, vinyl optical microscopy, light scattering and rheology. Other questions we address, of practical importance, are how do these complex gels age, while are the kinetic of the colloid aggregation in this environment, and how important is the viscoelastic background fluid compared to that of the colloidal network.

3:30 PM EP7.6
SMALL ANGLE LIGHT SCATTERING FOR THE STUDY OF RHEUMATOID ARTHRITIS IN HUMAN CARTILAGE Karen M. McNamara, Eric D. Dahlgren, Worcester Polytechnic Institute, Department of Chemical Engineering, Worcester, MA; Amaj Bellure, Harvard Medical School, Boston, MA.

Small angle light scattering (SALS) has been successful in identifying changes in fiber diameter and orientation in healthy rabbit tendon under varying tensile loads. This study extends the application of small angle light scattering to the study of both human and diseased tissue, specifically human cartilage from patients with rheumatoid arthritis. Cartilage samples were characterized with varying degrees of degeneration were studied by small angle light scattering to examine for systematic variations in the collagen morphology of these tissues. The results of these measurements will be presented and discussed. In addition, the level of patient to patient variations was investigated in an attempt to assess reproducibility.

3:45 PM EP7.7
ELECTROPHORETIC DETECTION OF CHARGE REVERSAL OF FILAMENTOUS PHAGES UNDER HIGH CONCENTRATIONS OF MULTIVALENT COUNTERIONS. Karim Adineh, Jay X. Tang, Indiana University, Physics Department, Bloomington, IN.

Counterions condense in the surrounding of highly charged linear polyelectrolytes, which are known to cause lateral aggregation of DNA and charged protein filaments. These aggregates are found to be reversibly resolved at much higher counterion concentrations than used in the present study. The mean-field Poisson-Boltzmann theory of microsions predicts a reduced repulsion, but no attraction. The recent theoretical approach predicts the attractive interaction observed by taking into account ion-ion correlation and fluctuations. The theoretical analysis explains the phenomenon of resolvability, by predicting that the overall charge of the linear polyelectrolyte is reversed in sign with the presence of very high concentrations of polyelectrolyte counterions. The electrophoretic instrument (Gouter Deles), which uses the Doppler shift in scattered light to detect the speed of particles was used to study the mobility of fd viruses. Measurements were performed by adding magnesium acetate or spermidine, covering regions before the onset of resolvability, and after filament bundles were resolvability, where charge reversal is expected. At high concentrations of magnesium acetate or spermidine, the mean value of mobility decreases, although some interpretation is needed to account for the wide peaks obtained in the data. In addition, we are performing both agarose gel electrophoresis and direct microscopic observations by applying an electric field to a solution of fluorescently labeled viruses in order to confirm the reversal of charge.

4:00 PM EP7.8
NEAR-FIELD SCANNING OPTICAL IMAGES OF BACTERIA Ana M. de Paola, Julieta A. Takig, Haroldo B. Silva, Gerald Weber, Laboratorio de Nano-Espectroscopía Óptica, Universidade de São Francisco, Bragantia Paulista SP, BRAZIL.

Near-field scanning optical microscopy (NSOM) and spectroscopy techniques were used to study the shape and the cell membrane details in bacteria. We present transmission and topographic images for the bacteria Pseudomonas aeruginosa using the Aurora NSOM from ThermoMicroscopes. The P. aeruginosa has been widely studied due to its clinical importance in many infection diseases. The samples were stained by Gram method and we measured the absorption of the laser light at 488 nm by the dye (Safranin) fixed at the bacterial membrane. To obtain good images we had to perform the sample preparation in order to obtain isolated bacteria at the microscope slab. This was achieved using individual colonies, from a dry growth procedure, diluted in physiologic solution. Comparison of the topographic and transmission images give information on the shape and detail of the absorption of the inner light by the cell membrane. The results show patterns that depends on the thickness and shape of the membrane, thus revealing details of the cell membrane with nanometer resolution. These results were used to improve a model for instance in the studies of the effects of antibiotics on the cell membrane.

4:15 PM EP7.9
OPTICAL CHARACTERIZATION OF BIOLOGICAL AND OTHER SYSTEMS. Alexandra G. Bezrukov, St. Petersburg State Technical Univ. St. Petersburg, RUSSIA.

Static and dynamic light scattering can provide further progress in on-line control of complex 3D dispersion systems such as liposomes.
carrying various substances (enzymes, viruses, etc.), blood substitutes and others bioengineering nanostructures. These methods are also compatible with the nondestructive analysis of disperse systems by other optical methods: refractometry, absorbancy and fluorescence.

Our research has investigated different disperse systems: liposomes, blood substitutes, proteins, nucleoproteins, viruses, lipoproteins, lipid emulsions, etc. and mixtures. liposomes and viruses, blood substitutes with blood serum, etc. by static light scattering (integral and differential, unpolarised and polarised) and dynamic light scattering. For the solution of inverse physical problem of static light scattering the fitting method with approximation of particles as homogeneous spheres, core-shell structured spheres, oblate and prolate ellipsoids of rotation and regularization procedure for inverse problem of dynamic light scattering have been applied. By optical methods it is possible to determine parameters of disperse systems state (mean equivalent diameter and number of particles, mean refractive index and mass of disperse phase, number and mass distributions) and parameters of particles structure: form and thickness of shell.