

SYMPOSIUM FF

Physical Characterization of Biological Materials and Systems

November 27 – 29, 2001

Chairs

Adrian B. Mann

Manchester Matls Sci Ctr
Univ of Manchester
Manchester, M1 7HS UK
44-161-200-3560

Deborah E. Leckband

Dept of Chemical Engr
Univ of Illinois-Urbana
Biochem, Biophysics & Comp Bio
Urbana, IL 61808
217-244-0793

Jan H. Hoh

Dept of Physiology
Johns Hopkins School of Medicine
203 Physiology
Baltimore, MD 21205
410-614-3795

Gang Bao

Dept of Biomedical Engr
Georgia Inst of Technology
Atlanta, GA 30332-0535
404-385-0373

David C. Martin

Materials Science and Engineering
Univ of Michigan-Ann Arbor
2022 H. H. Dow Building
Ann Arbor, MI 48109-2136
734-936-3161

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* Invited paper

SESSION FF1: SPM AND SURFACE
CHARACTERIZATION

Chairs: Jan H. Hoh and Timothy P. Weihs
Tuesday Morning, November 27, 2001
Hampton (Sheraton)

8:30 AM FF1.1

PROBING INTERFACIAL STRUCTURES AND REACTIVITIES OF CORE-SHELL NANOPARTICLE ASSEMBLIES USING ATOMIC FORCE MICROSCOPY. Chuan-Jian Zhong, Jin Luo, Mathew M. Maye, Li Han, Nancy Kariuki, Dept of Chemistry, State University of New York at Binghamton, Binghamton, NY; Lingling Chen, Biology Dept, Indiana University, Bloomington, IN.

Metallic nanocrystals encapsulated with organic monolayers, core-shell nanoparticles, 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 chaperonins using atomic force microscopy. Both non-covalent and covalent binding strategies are utilized for the interfacial immobilization at the core-shell nanoparticle assembled platforms. Hydrogen-bonded inter-shell linkages are exploited as a new route for immobilizing the biological molecules, which involves the conversion of hydrogen-bonded linkages to anhydride linkages and further conversion to amide or imide linkages. Importantly, scanning probe microscopic imaging of the interfacial nanostructures is facilitated by coupling monolayer patterning and selective assembling strategies. Surface infrared reflection spectroscopic and electrochemical techniques are employed to provide additional insights into the nanostructured interfacial reactivities. The implication of the results to developing biological nanomaterials and characterization capabilities will also be discussed.

8:45 AM FF1.2

PROBING NANOSCALE COOPERATIVITY NEAR THE GLASS TRANSITION. Koneswaran Sinnathamby, Nathan Israeloff, Northeastern University, Department of Physics, Boston, MA; Ezequiel Vidal Russell, Instituto Balseiro, Bariloche, ARGENTINA.

Using non-contact atomic force microscope techniques, we measure low frequency dielectric noise in a glassy polymer, polyvinyl-acetate (PVAc) near the glass transition (290K-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 RTS state occupancy time distribution. This analysis shows that individual cluster configurations exhibit stretched-exponential relaxation with various characteristic times, but with stretching exponent similar to bulk values.

9:00 AM FF1.3

MORPHOLOGY OF PHASE SEPARATED DOMAINS IN PLANAR CONSTRAINED LIPID BILAYERS. Adrian S. Muresan, University of Chicago, Dept of Physics, Chicago, IL; Haim Diamant, University of Chicago, James Franck Institute, Chicago, IL; Ka 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 distearoyl-phosphatidylcholine (DSPC)/dimyristoylphosphatidylcholine (DMPC) 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 enable membrane compartmentalization on a nanometer scale. 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 FF1.4

FORMATION AND PROPERTIES OF SUPPORTED MEMBRANES. John T. Woodward, John T. Elliott, Curtis W. Meuse, 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 FF1.5

CHEMICALLY-COUPLED UNFOLDING IN AFM-EXTENSION OF MULTIDOMAIN PROTEINS. Philippe Carl, David Speicher, and Dennis Discher, 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 folding forces of several multi-Ig-domain Ig-CAMs, including VCAM-1. We have exploited the disulfide as a conformational 'switch'. In the absence of reducing agent, a sawtooth pattern of unfolding peaks appears for IgCAMs 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 obligate intermediate to reduction and full unfolding. The results suggest that covalent chemistry on single protein molecules can be done by AFM.

9:45 AM FF1.6

PROBING MATERIAL PROPERTIES OF UNSTRUCTURED POLYPEPTIDES BY ATOMIC FORCE MICROSCOPY. Jan H. Hoh, Sanjay Kumar, and Rajendrani Mukhopadhyay, 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 intermolecular interactions. We are studying the material properties of proteins in two systems, neurofilaments 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 suggest that they be viewed as polyelectrolytes. These results explain the ability of these proteins to form gel like materials, and suggests that novel biomaterials might be derived from polypeptides that are wholly or mostly unstructured.

10:30 AM *FF1.7

EXPLOITING COMPLEMENTARY SURFACE ANALYTICAL AND MICROSCOPY TECHNIQUES TO DEVELOP BIOMIMETIC MATERIALS. Kevin E. Healy, Depts. of MS&E 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 cadre of microscopy techniques is essential to understand cell-materials interactions. The function of biomimetic materials that actively integrate 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 acrylamide 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), spectroscopic ellipsometry (SE) to confirm the surface chemistry and formation of the IPN. To exploit bimolecular engagement between

ligands of the extracellular matrix grafted to the IPN and cell-surface receptors, the aforementioned surface analytical techniques and fluorescence microscopy (FM) were used in a complementary manner to measure the ligand (e.g., peptide) density on the surface. The strength of cell adhesion to these ligand-modified surfaces was measured using a radial flow apparatus (RFA) that exposed cells to a hydrodynamic force capable of shearing them from the surface. The use of the RFA allowed us to identify which ligands on the surface promoted the greatest cell adhesion and the receptors on the cell surface involved. The IPN has now been successfully applied to numerous implant materials like titanium and its alloys, stainless steels, and PET, which supports the approach of surface characterization and development on model materials (e.g., quartz, silicon) for eventual conversion to actual implant materials. Thus, the combination of surface analytical tools, and fluorescence and optical microscopy has greatly enhanced our ability to synthesize biomimetic materials and control the behavior of mammalian cells in contact with these surfaces.

11:00 AM FF1.8

ANALYSIS OF ENZYME KINETICS USING MICRO-DIFFERENTIAL BIOREACTORS FABRICATED FROM MICROPATTERNED ARRAYS OF COLLOIDAL PARTICLES.
Nathaniel J. Gleason 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 microfluidic devices 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 present two fabrication strategies for these microreactors: one is based on the patterning of enzymes via directed laminar flow in micro-channels, 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

BIOCOMPATIBILITY COMPARISON OF STAINLESS STEEL, GOLD COATED, AND HEAT TREATED GOLD COATED ENDOVASCULAR STENTS. Elazer Edelman, Philip Seifert, Adam Groothuis, Alisa Morss, Danielle Bornstein, Campbell Rogers Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA.

As endovascular stents are altered to add functionality, for example by adding radiopaque coatings, biocompatibility 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 7-micron layer of gold. Physical and material effects were examined in four different gold-coated stent types, two at each length, that either had the coating applied to the standard strut, ie, gold coated thicker than controls, or had the coating applied to thinned struts, ie, gold coated of the same thickness as control struts. Simple gold coating exacerbated intimal hyperplastic and inflammatory reactions over 28 days, but postplating thermal processing negated the adverse tissue response to gold. Materials analysis using AFM, SEM with XPS, and Auger 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 base 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 stents, but not without effects on vascular repair. Material effects predominate and can be abrogated by heating coated stents to alter surface finish. Clinical results may suffer unless consideration is given to material and physical effects of gold.

11:30 AM FF1.10

BIOACTIVE CALCIUM PHOSPHATE THIN FILMS ON IMPLANT GRADE METAL ALLOYS USING A SILICA BARRIER LAYER.
J. Reid, M. Sayer, Department of Physics, Queen's University, Kingston, Ontario, CANADA; T.J.N. Smith, Millenium Biologix Inc., Kingston, Ontario, CANADA.

Resorbable calcium phosphate (CaP) thin films have been transferred from an existing quartz substrate to a Ti-6Al-4V implant grade titanium alloy substrate. In order to preserve the characteristic phase composition and surface morphology of the CaP thin films, an intermediate silica barrier layer has been deposited on the titanium

alloy substrate via chemical vapour deposition using tetraethyl orthosilicate as the metal organic precursor. The CaP thin films are then deposited on the intermediate layers using sol gel methods, and sintered at over 800C. The final sintered films are a phase mixture of calcium hydroxyapatite and a silicon stabilized form of alpha tri-calcium phosphate. The silica barrier layers have been characterized for thickness, surface morphology, and conformal coverage using scanning electron microscopy in terms of the main CVD processing parameters, and these parameters have been optimized to best reproduce the characteristic CaP thin film properties under a variety of sintering conditions. The CaP thin films have been characterized using glancing angle X-ray diffraction and scanning electron microscopy to examine the phase composition and surface morphology respectively.

SESSION FF2: LIPIDS, PROTEINS AND DNA
Chairs: Deborah E. Leckband and Julie A. Kornfield
Tuesday Afternoon, November 27, 2001
Hampton (Sheraton)

1:30 PM *FF2.1

CHARACTERIZATION OF HEALTHY AND DISEASED MYELIN MEMBRANES BY COMPLEMENTARY TECHNIQUES.
Jacob Israelachvili, Yufang 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 is Multiple Sclerosis (MS), which is believed to be an auto-immune disease that is characterized by gross morphological changes such as swelling or 'demyelination' and vesiculation of the myelin sheath. The cause and effective 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, inter-membrane 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 surface force 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.
Ajaykumar Gopal, Ka Yee C. Lee, The University of Chicago, 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 μm in length. Above approximately 32°C, the monolayer prefers to collapse by forming vesicular 3D structures on compression. These micron scale vesicular structures appear globular or tubular in shape. Collapse occurs via both folding and vesiculation in the 30 \pm 2°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 CELLULAR INTEGRIN PROFILES. Jonathan A. Phillips^{1,2}, Kanaklata Roy², Anthony N. Imbalzano², Lawrence J. Bonassar^{1,2}, University of Massachusetts Medical School, ¹Graduate School of Biomedical Sciences, Center for Tissue Engineering, ²Department 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 cells to the ECM. Our 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 control 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 α_5 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 α_5 integrins were specifically involved in differential tissue contraction, these experiments were repeated where α_5 integrins were blocked with monoclonal antibodies. Cells expressing the higher levels of α_5 integrin were able to contract collagen gels to a significantly greater extent ($p < 0.01$) than normal NIH 3T3 fibroblasts. When mutant cells were blocked with anti- α_5 , 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 FF2.4

ANALYSIS OF RADIATION DAMAGE IN LYSOZYME CRYSTALS WITH HIGH RESOLUTION TRIPLE AXIS X-RAY DIFFRACTION. R.J. Matyi, National Institute of Standards and Technology, Gaithersburg, MD; H.M. Volz, 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 number of Bragg reflections 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 the structural quality of the lysozyme crystals 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 functional 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 FF2.5

MORPHOLOGY OF HYDROGELS CONSTRUCTED VIA DIBLOCK COPOLYPEPTIDE SELF-ASSEMBLY. Darrin Pochan, Lisa Pakstis, University of Delaware, Materials Science and Engineering and Delaware Biotechnology Institute, Newark, DE; Andy Nowak, Tim 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 add the intriguing potential of assembled 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 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 nano- and microscopic morphology and the ability to heal after significant application of stress. 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 and will be the focus of this presentation. Changes in gel morphology and strength were observed by varying the relative volume fraction of hydrophilic to hydrophobic block and salt concentration. The

morphology, self healing, and peptidic foundation of these hydrogels make them intriguing candidates for biomaterials applications.

3:30 PM FF2.6

SUPRAMOLECULAR ULTRATHIN FILM STRATEGIES FOR DNA ASSEMBLIES: SURFACE SENSITIVE ANALYSIS OF LAYER ORDERING AND DNA STRUCTURES. Rigoberto 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. This involves the use of the layer-by-layer alternate solution adsorption technique and "polymer brushes". The properties of DNA are intimately associated with their polyelectrolyte behavior in solution. Deposition at surfaces is governed by the conformation, orientation, and charge density of these biomolecules in relation to the physisorption phenomena in oppositely charged surfaces. Controlling the "texture" 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-IRRAS. By combining with the alternate assembly of dendrimers, azobenzene, and phthalocyanine dyes, we have been able to prepare optobioelectronic substrates where the phenomena 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 FF2.7

Abstract Withdrawn.

4:00 PM FF2.8

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 wave 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 FF2.9

SURFACE CHARACTERIZATION OF DNA MICROARRAY ON SILICON DIOXIDE AND COMPATIBLE SILICON MATERIALS IN THE IMMOBILIZATION PROCESS. Wen Xu, Mei Xue, Mansun Chan, The Hong Kong University of Science and Technology, Dept of Electrical and Electronic Engineering, HONG KONG; Maria Carles, Ralf Lenigk, Dieter Wilhelm Trau, Nancy Y. Ip, The Hong Kong University of Science and Technology, Department of Biochemistry, HONG KONG; Nikolaus J. Sucher, 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 can facilitate the adoption of the mature electronic micro-fabrication to obtain lab-on-a-chip and integrated detection system with much higher density. In this work, the surface property of a 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 less 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 modifications 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 of complementary 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 oxidizing property of ammonium hydroxide used in the process of immobilization, some commonly used top layer silicon materials in microelectronic devices such as polysilicon, aluminum, titanium are etched. Thus they have to be passivated by other material before the immobilization process. 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 signal from that of the photo-diode integrated detection array indicating the possible optimization methods for electronic detection.

4:30 PM **FF2.10**

THE POROUS SILICON LAYERS AS MATRIX FOR DNA.

Andrew Verba, Dept of Radiophysics, Kiev National Shevchenko Univ, Kiev, UKRAINE.

The porous silicon (PS) layers are considered for the use as the supported matrix for synthesis of DNA. The method of silanisation of PS and SiO₂ layers that is an important step of DNA-synthesis has been elaborated and tested. The SEM image shows developed surface of PS with circular geometry of pores. The average size of pores was 0.5 μm. This size is sufficient for carry out the process of DNA-synthesis. FTIR spectroscopy has been extensively used in the characterization of the species precipitated on PS and SiO₂ surfaces during silanisation. The absorption bands which corresponds to the film of silane were observed only in PS layer. The FTIR reflectance spectra of different samples of PS after silanisation shows the good reproducibility of silane layers. The impedance measurements displayed shift of capacity for SiO₂ film during consequence series of experiments. At the same time, the imaginary part of impedance was not changed for PS layers.

SESSION FF3: MECHANICAL PROPERTIES AND MINERALIZED TISSUES

Chairs: David C. Martin and George M. Pharr
Wednesday Morning, November 28, 2001
Hampton (Sheraton)

8:30 AM ***FF3.1**

PHYSICAL CHARACTERIZATION OF DENTIN: NEW INSIGHTS INTO THE BIOMECHANICAL PROPERTIES OF MINERALIZED TISSUES. J.H. Kinney, G.W. Marshall, S.J. Marshall, University of California-San Francisco, Dept of Preventive and Restorative Dental Sciences, San Francisco, CA.

Dentin, the most abundant mineralized tissue in the tooth, is similar in composition to bone: type I collagen fibrils reinforced by a nanocrystalline apatite 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 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 Young's modulus of wet and dry dentin. Resonant ultrasound spectroscopy (RUS) was used to validate the AFM measurements, as well as to provide the entire stiffness tensor. Excellent agreement was obtained between the AFM-method and the Young's modulus determined from the RUS-obtained stiffness tensor. RUS, however, provided additional information: dentin is elastically 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 Young's and shear moduli increased by only 10-15%. We attributed the large decrease in bulk modulus with drying 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 succeeded both in lowering 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 **FF3.2**

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 Electronmeccanica, Badajoz, SPAIN; Antonia Pajares, 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 material 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 **FF3.3**

ENAMEL BIOMIMETICS 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. Lo, M.L. Snead, Center for Craniofacial Molecular Biology, USC, Los Angeles, CA.

Dental enamel is the protective layer providing the mammalian tooth with the strength, toughness, and wear resistance necessary to withstand the complex, masticatory stresses. Since matured 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 biomimetics 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 microscopies, 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 **FF3.4**

ELASTIC MODULUS MAPPING OF BAMBOO MICRO-STRUCTURE. J. Anderson, S. Yedla, and R.M. Winter, 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 rivals steel. Given these desirable attributes bamboo serves as one of Nature's model composite materials. In this study we utilize the Hysitron nanoindenter and Interfacial 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 (15%), fibers (65%) and sieve tubes (20%). The IFM is a scanning probe microscope which utilizes a unique self-balancing 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-scopic substructures. 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 the measured micro-mechanical properties to macro-mechanical properties (e.g. tensile strength). This work provides insight as to how Nature builds this cellulose based composite material.

10:15 AM ***FF3.5**

CHARACTERIZING LOCAL VARIATIONS IN THE MECHANICAL PROPERTIES OF HUMAN ENAMEL. Tim Weihs Johns Hopkins Univ, Dept of Materials Science and Engineering, Baltimore, MD.

Mastication imparts significant stresses and abrasions to human teeth that can lead to fracture, wear, and failure. The exact nature of these stresses and abrasions, and their variation throughout a given tooth, are determined in large part by the mechanical properties of 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 describes our efforts to identify and understand local variations in the mechanical properties of enamel in human molars. Using nanoindentation techniques, we have mapped variations in hardness and stiffness on mesial cross-sections of maxillary second 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 enamel stiffness. These sharp variations correlate best with changes in local chemistry as opposed to microstructure. A variety of mechanical, chemical, and microstructural characterizations will be described.

10:45 AM **FF3.6**

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 characterisation 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 untested 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 **FF3.7**

CHARACTERIZATION OF MINERALIZED TISSUES BY COMBINING SCANNING SMALL-ANGLE X-RAY SCATTERING, QUANTITATIVE BACKSCATTERED ELECTRON IMAGING AND NANOINDENTATION. W. Tesch^{1,2}, P. Roschger¹, I. Zizak², O. Paris², K. Klaushofer¹, P. Fratzl². ¹Ludwig Boltzmann Institute of Osteology, ^{4th} Medical Department, Hanusch Hospital, Vienna, AUSTRIA. ²Erich Schmid Institute of Materials Science, Austrian Academy of Sciences, and Metal 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 ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) 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 1D- and 2D-mapping of bone, cartilage and tooth sections. Correlations of these results obtained at the exact same sample positions revealed 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 nanocomposites like bone and tooth provide deeper insights into the construction processes of such structures with possible applications for novel materials.

- (1) S. Rinnerthaler et al. (1999) *Calcif Tiss Int* 64:422-9.
- (2) P. Roschger et al. (1998) *Bone* 23:319-26.
- (3) W. Tesch et al (2001) *Calcif Tiss Int*: in press.

11:15 AM **FF3.8**

BULK MODULUS AND DENSITY MEASUREMENTS OF SMALL COMPRESSIBLE AND INCOMPRESSIBLE SAMPLES. Jack C. Hay, 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 device 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 disk-shaped samples 3 mm in diameter and 0.4 mm thick. The weighing device has a repeatability of 10nN and sample densities can be determined to 0.5%. A significant feature of this device is the ability to measure buoyancy forces at a plurality of gas densities, which allows one to capture nonlinear behaviors associated with compressible media. Results are presented for a quasi-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 **FF3.9**

MICROSTRUCTURE AND MECHANICAL PROPERTIES OF SKELETAL BONE IN GENE-MUTATED STPSEL_{DTL28D} AND WILD-TYPE ZEBRAFISH (DANIO RERIO). Y. Zhang, F.Z. Cui, X.M. Wang, Q. Cai and Q.L. Feng, Biomaterials Laboratory, Dept of MS&E, Tsinghua University, Beijing, CHINA.

Zebrafish (*Danio rerio*), the only vertebrate organism now amenable to large scale of 'saturation genetic 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 *Stpsel_{dtl28d}* (*stp/stp*) and wild-type zebrafish were firstly investigated using scanning electron microscope (SEM), transmission electron microscope (TEM), X-ray diffractometer and atomic force microscopy (AFM)-based nanoindenter. Significant change of bone microstructure including both collagen matrix and minerals was observed in *stp/stp* bone comparing 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 precipitated 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 indenter 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 human osteogenesis imperfect (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

Chairs: John H. Kinney and Darrin J. Pochan
Wednesday Afternoon, November 28, 2001
Hampton (Sheraton)

1:30 PM ***FF4.1**

PROBING THE MECHANICAL PROPERTIES OF BONE WITH NANOINDENTATION. George M. Pharr, 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 Swadener, Los Alamos National Laboratory, Los Alamos, NM; Jae-Young Rho, University of Memphis, Dept of Biomedical Engineering, Memphis, TN.

Nanoindentation, 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 central to the development of micromechanical and microstructurally-based models for bone as a composite material.

2:00 PM **FF4.2**

ASSESSING STRUCTURE-PROPERTY RELATIONS OF DISEASED TISSUES USING NANOINDENTATION AND FTIR. Donna M. Ebenstein, Lisa A. Pruitt, 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

tissue engineered equivalents, and understanding disease progression. Many biological tissues remodel in response to mechanical loading in vivo, resulting in microstructural and compositional changes. Disease processes often interfere with the normal balance of tissue remodeling, resulting in tissues with abnormal composition. For example, in atherosclerosis lipid and calcification are often found in the artery wall, whereas in healthy arteries the tissue microstructure is dominated by collagen. In addition, in osteoporosis mineral content or composition may vary from healthy bone tissue. Such variations in tissue composition likely result in changes in the material properties of the tissue. Using a combination of nanoindentation and spectroscopic techniques, our goal is to assess how changes in tissue composition affect the tissue's mechanical properties. Fourier Transform Infrared Spectroscopy (FTIR) is used to assess the biochemical composition of tissue samples, such as the lipid and calcium content of fibrous tissues in diseased arteries and the degree of mineralization in bone tissue. Nanoindentation is used to measure the local mechanical properties of the same tissue samples, such as reduced modulus and hardness. This information is then correlated by position in the sample to assess the contributions of different constituents to the overall structure-property relations of the tissues. While FTIR is equally effective in the study of hard or soft tissues, nanoindentation theory and techniques were originally developed for testing hard, smooth materials. As a result, mechanical testing of bone and other hard tissues is relatively straightforward, but the study of soft tissues requires modifications to standard indentation techniques. A discussion of some of the adaptations necessary to generate useful quantitative data for soft tissues will be provided, using agarose gel as a model material.

2:15 PM FF4.3

STRUCTURAL CHANGES IN A COLLAGENOUS SOFT TISSUE DURING MECHANICAL AND THERMAL TREATMENT.

Michelle Oyen-Tiesma, Robert F. Cook, Department of Chemical Engineering and Materials Science, and John C. Bischof, Department of Mechanical Engineering, University of Minnesota, Minneapolis, MN.

Collagen constructs derived from porcine intestinal collagen layer (ICL, Organogenesis Inc, Canton, MA) are candidate materials for use as structural tissue grafts. As such, the mechanical deformation and failure properties of these materials, and their relation to tissue microstructure, will be a factor in their success. A number of methods (both in situ and ex situ), including energy dissipation, light scattering, and infrared absorption, were used to characterize ICL damage during cyclic mechanical loading. A key feature of the loading was the development of light scattering, resulting in localized regions of opacity, in a pattern reflecting the regions of maximum shear strain. These changes occurred gradually during cycling and saturated prior to gross mechanical failure. Eventual tissue failure occurred by the accommodation of the imposed strain by mode-II (shear) sliding damage, rather than mode-I (tensile) crack propagation. Infrared absorption spectra of mechanically loaded specimens were similar to those of thermally treated tissues, and both were substantially different from controls. These results suggest a common mechanism of localized collagen structural changes in mechanically or thermally damaged tissues.

2:30 PM FF4.4

EFFECT OF CROSSLINKING ON THE VISCOELASTIC

PROPERTIES OF COLLAGEN-RICH TISSUES. Julia Kornfield, Giyoong Tae, Fred Tessier, California Institute of Technology, Dept of Chemical Engineering, Pasadena, CA; Mary Dickinson, Angelique Louie, California Institute of Technology, Div of Biology, Pasadena, CA; Robert Lambert, Hampar Karageozian, ISTA Pharmaceuticals, Irvine, CA; John Park, Kathryn Rich, private individuals; Vincent Monnier, Institute of Pathology, School of Medicine, Case Western Reserve University, Cleveland, OH.

Non-enzymatic crosslinking causes a variety of collagen-rich tissues to lose their elasticity with age (e.g., hardening of arteries and wrinkling of skin). We examine the effects of crosslinking on the viscoelastic properties of tissue using glycerinaldehyde as a model crosslinking agent and the cornea as a model tissue. Rabbit and human corneas are treated under culture conditions that maintain crosslinking agent and the cornea as a model tissue. Rabbit and human corneas are treated under culture conditions that maintain normal morphology and hydration of the cornea. Glycerinaldehyde is applied topically (drops of 3% wt. glycerinaldehyde in saline are applied 3 times a day for six days) and the shear modulus is characterized subsequently using a parallel plate geometry. Substantial increases in the shear modulus are observed: 3 fold in rabbit and 5 fold in human corneas. Studies of model collagen systems—collagen fibrils from rat-tail tendon and reconstituted collagen gels—indicate that interfibrillar cross-linking is responsible for the change in the mechanical properties of corneas. Analytical biochemistry is used to identify the molecular structure of chemical crosslinks, which form predominantly between the primary amines of lysine residues.

3:15 PM *FF4.5

PROBING THE LOCAL STRUCTURE AND MECHANICS OF BIOLOGICAL MATERIALS WITH THERMALLY-INDUCED MOTION. M.L. Gardel, M.T. Valentine, A. Bausch and D.A. Weitz, 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 probes the pore structures of their environment. 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 biomaterials will be discussed.

3:45 PM FF4.6

COMPARISON OF MATERIAL PROPERTIES OF RABBIT FASCIA LATA AND LUMBODORSAL FASCIA. A.N. Morse, UMass Memorial Health Care, Department of Obstetrics and Gynecology, Worcester, MA; D.R. Foley, A.K. Roy, C.A. Vacanti, L.J. Bonassar, Center for Tissue Engineering, University of Massachusetts, Worcester, MA.

BACKGROUND: Fascia lata (FL) and lumbodorsal 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 Dynastat testing stand and strained to material failure at 1%/second. 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) Glycosaminoglycan (GAG) and cell number were determined to investigate the correlation of biochemical 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±3.1MPa vs. 3.75±2.0MPa, 39±15% vs. 63.3±16%, 36.7±17.2MPa vs. 7.99±4.2MPa, and 1.84±1.24J/mm³ vs 1.19±0.78J/mm³. 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. 13.9±1.8 and 4.35 vs. 1.21±0.70 for FL and LD respectively. Both differences were statistically significant (p<0.002). Hypro, GAG and DNA content were (mean±SD) 0.729±0.38ug/mg, 6.59±4.2ug/mg, 1.08±.48E 6 cells/mg and 0.453±0.42ug/mg, 8.74±13ug/mg, 1.80±.34E 06 cells/mg for FL and lumbodorsal fascia respectively. There were no significant differences in any of these parameters although there was a trend towards greater cell number in the FL (p=0.003).

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 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 FF4.7

USING RHEOLOGY TO PROBE THE MECHANISM OF JOINT LUBRICATION: SYNOVIAL FLUID'S POLYELECTROLYTE/ PROTEIN INTERACTIONS. Katherine M.N. Oates, Wendy E. Krause, Ralph H. Colby, Dept of MS&E, 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 ($M_w = 1.6$ MD), and the plasma proteins. Rheological properties are reported for a model of synovial fluid, comprised of hyaluronic acid (3 mg/mL), and the albumin (11 mg/mL, $M_w = 66$ kD) and γ -globulin (7 mg/mL, $M_w = 150$ kD) proteins. Coupled with other characterization methods, such as scattering and membrane dialysis techniques, we hope to gain full understanding of the synovial fluid mechanism of lubrication. This insight may lead to advances in anti-inflammatory drug therapy treatment for diseased joints.

4:15 PM FF4.8

ONE- AND TWO-PARTICLE MICRORHEOLOGY IN ENTANGLED SOLUTIONS OF FD VIRUS. Karim M. Addas, Jay X. Tang, Dept. Physics & Indiana Mol. Biol. Institute, Indiana University, Bloomington, IN; Alex J. Levine, Dept. of Chem. Engin.,

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 microrheology, employing micron-sized embedded beads and laser interferometric displacement detection, to study the rheological properties of entangled solutions of the filamentous fd virus. Thermal fluctuations of the embedded probes were measured and the viscoelastic parameters of the embedding medium were derived. In two-particle microrheology the correlated motions of two identical particles separated by a varying distance in the medium are analyzed, which can avoid biased results due to surface depletion effects near the probes. Particular emphasis was placed on the comparison between the one- and two-particle results.

4:30 PM **FF4.9**

LIQUID CRYSTALLINE PROPERTIES OF F-ACTIN: A QUANTITATIVE STUDY. Jorge Viamontes and Jay X. Tang, 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 (anisotropic) 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 closely correlate with the local concentrations of actin as probed by fluorescence imaging. Such a direct spacial correlation between the birefringence and the local actin concentration is characteristic of a first order phase transition. We also observed different features between a concentrated actin solution and the conventional thermotropic or lyotropic nematogens. The thermodynamic behavior of F-actin may have important effects on properties of the cytoplasm in living cells.

4:45 PM **FF4.10**

TWO-STAGE SWELLING BEHAVIOR IN AMPHIPHILIC POLY(ETHYLENE OXIDE)/POLY(EPSILON CAPROLACTONE) MULTIBLOCK COPOLYMER GEL. Tae Chul Kim, Jong Ho Kim, You Han Bae, 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 x-ray scattering. In the initial stage of the swelling, hydrophilic PEO chains relax and the lamellae period increases. The swelling time is inversely proportional to the PEO block size. As the swelling, continues, however, the lamellae period saturates, but the density modulation caused by swelling continues to increase. The swollen PEO/PCL multiblock gel phase-separates into ordered PEO-rich liquid and PCL-rich liquid as the PCL 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 phase separation might be described in terms of the LCST (lower critical solution temperature) behavior.

SESSION FF5: POSTER SESSION

Chairs: Adrian B. Mann, Deborah E. Leckband, Jan H.

Hoh, Gang Bao and David C. Martin

Wednesday Evening, November 28, 2001

8:00 PM

Exhibition Hall D (Hynes)

FF5.1

UV LASER INDUCED AMINO GROUP SUBSTITUTION ON PET LIGAMENT TO PROMOTE INHIBITION OF COLLAGEN. H. Omuro, M. Murahara, The Faculty of Engineering of Tokai University, Kanagawa, 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 an artificial ligament because of its strength and good immune reaction. However, when transplanted in human bodies, its compatibility 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 fiber into a mesh state and after the transplantation 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 tissue can be enhanced. Then we substituted NH₂ and OH, which has a high affinity for collagen on the PET surface by ArF laser. PET is highly hydrophobic and does not dissolve well in aqueous solutions. To avoid this

reaction we make a thin ammonia water layer on the PET surface with capillary phenomenon. Then ArF laser was irradiated vertically onto the sample. The result of this treatment shows that untreated sample having the contact angle of 80° with water and the bonding power of only 1.0 kg/cm² with collagen was improved to have the contact angle of 45° and the bonding power to be 6.0 kg/cm² after treating in ammonia water as a reaction solution. Moreover, when treated in water, the contact angle was improved to be 33° and the bonding power to be 7.5 kg/cm². When the treated sample had been implanted into the subcutaneous tissue of a rabbit's regiones dorsales, existence of leukocyte colonies that are sign point of histotropic was confirmed on the hydrophilic parts of the sample.

FF5.2

SORPTION INTERACTION OF CATALASE WITH POLY-ETHYLENE IMINE HYDROGEL. Gulnara A. Bektenova, Esen A. Bekturov, A.B. Bekturov, Institute of Chemical Sciences, Almaty, KAZAKHSTAN.

The interaction of catalase with cationic polymer hydrogel of polyethylene imine in dependence on pH and concentration of external solution has been investigated. Influence of the time factor on the obtained sorption dependencies on physic-chemical parameters of the process determining the selectivity of a sorption was theoretically considered. The results were compared with the experimental data. The time factor was shown to be taken into account when interpreting these sorption dependencies. Study of kinetics of a process has shown that the interaction of the enzyme with gel is developed in time and determined by diffusion factors. The maximal binding occurs at moderate concentrations (0,04-0,06 M) of an external solution in the region of anion exchange (pH 7,0). The second maximum of catalase sorption at pH 5,5 near to its isoelectric point can be explained both by formation of polymer complexes with hydrogen bonds between side functional groups of a protein and iminogroups of a polymer and by ion-dipole interactions between them or by their combination. A possibility of the complexes stabilisation by hydrophobic interactions between hydrophobic network of the hydrogel and hydrophobic sites of the protein macromolecules is not excluded. Thus several types of noncovalent forces participate in the sorption binding of a catalase with polymer hydrogel that provides the irreversibility of the process. The change of external conditions of medium (pH, ionic strength) results in partial desorption of a catalase activity that indicates the stability of the formed polymer complexes of enzyme.

FF5.3

HYDROPHILIC TREATMENT OF THE AQUEOUS HUMOR PRESSURE CONTROL MEMBRANE BY ARF EXCIMER LASER FOR INTRACTABLE GLAUCOMA IMPLANT DEVICES. Y. Sato, M. Murahara, The Faculty of Engineering of Tokai University, Kanagawa, JAPAN; Jean Marie Parel, Bascom Palmer Eye Institute, University of Miami, Miami, FL.

The inner porous PTFE was substituted with hydrophilic group using an ArF laser and water. This material was developed that automatically pump out water when the pressure is raised above the fixed value. The increased intraocular pressure with aqueous outflow failure causes the glaucoma. In healthy human intraocular pressure is maintained at normal physiological level between 8 to 18 mmHg. When the pressure exceeds 21mmHg, a diagnostic 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 hydrophobic group that exists inside the porous PTFE with the hydrophilic group. Also it enabled to change the differential osmotic pressure freely by setting up the density of the hydrophilic group. Then we tried the new method of forcing the water into the inner porous PTFE. The water was photo-dissociated by irradiating the ArF laser into the inner porous PTFE. In the meantime 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 onto the porous PTFE, the water is trapped up to the inner side of porous PTFE. Under these conditions ArF laser is irradiated. The result showed that untreated samples of 3μ m pore could not be penetrated by water even if the pressure was increased to 300mmHg, whereas the treated samples irradiated with laser fluence of 15mJ/cm² could be penetrated by the water with 15mmHg. In conclusion our study demonstrates that samples with hydrophilic property treatment using water (H₂O) as the reaction liquid can be used for intelligent materials such as the biocompatible membrane.

FF5.4

CHARACTERIZATION OF SUSPENDED LIPID BILAYERS ON POROUS ALUMINA. Kornelius Nielsch, Max-Planck-Inst für Mikrostrukturphysik, Halle, GERMANY; Janine Drexler, Christian Horn, Christian Hennesthal, 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 advantageous for biosensor applications. Our system of pore-spanning lipid bilayers is based on nanoporous alumina substrates with pore diameters of about 40-60 nm and a depth of a few microns. These were prepared by an anodic etch 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 positive bias to the alumina substrate. Forced by the applied potential, giant liposomes composed of a negatively charged lipid mixture were fused onto the porous surface. The other technique started with a prefunctionalization of the alumina surface with self-assembled monolayers bearing the needed countercharges. This was achieved by first sputtering titanium and gold onto the porous alumina surface. Subsequent chemisorption of small 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 attributes of the suspended bilayers were investigated using scanning-force-microscopy (SFM), interferometry, impedance-spectroscopy and measurements of the photocurrent generated by membrane-incorporated bacteriorhodopsin. The model system described is a promising approach for a new kind of artificial membranes. By additional physisorption of alternating charged polymer-layers upon the substrate surface we intend to further increase the degree of vesicle fusion and membrane stability.

[1] C. Hennesshal, C. Steinem; JACS 122/33, pp. 8085-8086; 2000.
[2] A.P. Li, F. Müller, A. Birner, K. Nielsch, U. Gösele; J. Appl. Phys. 84/11, pp. 6023-6026; 1998.

FF5.5

DEVELOPMENT OF AlN THIN FILM BASED PIEZOELECTRIC SENSORS FOR ULTRASONIC IMAGING OF BIOLOGICAL ELEMENTS. Marvie Nickola, Wayne State University, Department of Biomedical Engineering, Detroit, MI; Greg Auner, Changhe Huang, Wayne State University, Department of Electrical & Computer Engineering, Detroit, MI; Ratna Naik, Wayne State University, Department of Physics, Detroit, MI; Vaman Naik, U. Michigan-Dearborn, Dept. of Natural Science, Dearborn, MI.

The piezoelectric properties of AlN thin films have been of practical interest for many acoustic wave sensor applications. In the present study, AlN 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 AlN thin films. Excimer laser (KrF) micromachining has been used to fabricate (8x8) pixilated array of AlN 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.

FF5.6

LOCAL MECHANICAL PROPERTIES AND MICROSTRUCTURE OF LIVING CELLS. Megan T. Valentine, Andreas Bausch, Hallam Stevens, Heather Rose, D.A. Weitz, Harvard University, Cambridge, MA.

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 fibroblasts.

FF5.7

ONE- AND TWO-POINT MICRORHEOLOGY OF F-ACTIN NETWORKS. Margaret L. Gardel, Megan T. Valentine, Harvard Univ, Dept. of Physics, Cambridge, MA; John C. Crocker, California Institute of Technology, Dept. of Applied Physics, Pasadena, CA; Andreas R. Bausch, David A. Weitz, Harvard Univ, Dept. of Physics, Cambridge, MA.

The technique of microrheology is now widely applied to study the microscopic viscoelastic properties of complex fluids, including polymer networks. The thermal motion of single micron-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 micro-rheological results exhibit discrepancies, which are thought to be either due to the heterogeneous nature of the network 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 probes instead of single probe motion. 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.

FF5.8

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 affects of mechanical strain at the molecular level. Chemical reactivity dependence on strained molecular bonds is well understood. However, the affect 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 epi-fluorescence 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. Standard clean-room photolithographic techniques were used to process the gold anchor points for the DNA. Photolithography was also used to define a special 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 PM-IRRAS to control site-specific binding of the DNA and localize its position to within a micron.

FF5.9

CONTROLLED DELIVERY OF VANCOMYCIN FOR TREATING THE CHRONIC OTITIS MEDIA BY INHIBITION OF MRSA GROWTH: APPLICATION OF A TEMPERATURE RESPONSIVE BIODEGRADABLE POLYMER. Jeong Ok Lim, Medical Research Institute, Kyungpook National Univ, Taegu, KOREA; Yu Mi Kim, Dept of Biomedical Engineering, Kyungpook National Univ, Taegu, KOREA; Jeong Min Suh, Medical Research Institute, Kyungpook National Univ, Taegu, KOREA; Woon Yi Baek, Department of Anesthesiology, Kyungpook National University, Taegu, KOREA; Sang Heun Lee, Dept of Otolaryngology, Kyungpook National Univ, Taegu, KOREA.

Vancomycin 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 MRSA are required hospitalization for regular oral intake and intravenous injection of vancomycin. In order to improve the patients compliance, vancomycin was incorporated with biodegradable polymers and the efficacy of the system was evaluated using human MRSA collected from patients ears. A various type of temperature responsive biodegradable polymer containing vancomycin has been prepared to conveniently administer 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-vancomycin complex, the systems were found to be suitable for injection to the body and the MRSA growth was well inhibited from the slow release of the vancomycin. The degradability of the polymer was also characterized.

FF5.10

NEUTRON SPIN ECHO SPECTROSCOPY STUDIES OF BIOMOLECULES IN SOLUTION. Zimei Bu, Amos M. Tsai, Nicholas Rosoy, NIST Center for Neutron Research, Gaithersburg, MD.

Neutron Spin Echo (NSE) spectroscopy probes the intermediate scattering function (Q, τ) (the cosine transform of $(S(Q, \omega))$), over the range ($0.01, \text{\AA}^{-1} < Q < 1.6, \text{\AA}^{-1}$) with times ranging from 50,ps to 100,ns for the NSE spectrometer located at the NIST Center for Neutron Research. NSE is especially suited for the investigation of relaxation-type motions, which are of interest in polymer and glass studies, among others. To illustrate the information which may be extracted from such an experiment, 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 solvent decreases 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 α -lactalbumin 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 extended this work to coherent motions on longer time scales with NSE spectroscopy, studying both the intermolecular correlated dynamics and the intramolecular backbone motions.

FF5.11

MORPHOLOGY AND BIOCOMPATIBILITY OF HYDROGELS CONSTRUCTED VIA DIBLOCK POLYPEPTIDE SELF-ASSEMBLY. Lisa Pakstis, 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 (~ 20 k g/mol), amphiphilic, diblock polypeptides of hydrophilic lysine (K) or glutamic acid (E) and hydrophobic 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 nano- and microscopic 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 hydrophilic to hydrophobic blocks and salt concentration. The morphology, high modulus (as observed rheologically), and peptidic 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 AND ABSENCE OF PROTEIN ORGANIC NANO-SUBSTRATES: A MOESSBAUER SPECTROSCOPIC CHARACTERIZATION. Georgia C. Papaefthymiou, Villanova University, Dept. of Physics, Villanova, PA; Guanghua Zhao and N. Dennis Chasteen, University of New Hampshire, Dept. of Chemistry, Durham, NH.

Biomimetic iron oxide nanophases are becoming increasingly important in biotechnology as magnetic resonance imaging contrast agents and drug delivery systems [1]. These nanophases 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 nanophases 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 nanophases 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. De-convolution of the Moessbauer spectra into core vs. surface atom contributions allowed physical characterization of the organic/inorganic interface of these composites. The electronic properties, superparamagnetic behavior and magnetic anisotropy energies of these nanophases will be presented. Their potential use as NMR imaging contrast agents will be discussed.

[1] U. Hafeli et al. Eds., Scientific and Clinical Applications of Magnetic Carriers, Plenum Press, New York, London, (1997).

[2] S. Mann, Ed., Biomimetic Materials Chemistry, VCH Publishers, Inc., New York, Weinheim, Cambridge (1996).

[3] G.J. Long and F. Grandjean, Eds., Moessbauer Spectroscopy Applied to Magnetism and Materials Science, Plenum Press, New York (1993).

FF5.13

MECHANICAL PROPERTIES AND BIOACTIVITY OF LAMELLAR DIOPSIDE SUBSTRATE. Noriyuki Y. Iwata, Yuhki K. Nakamura, Hironari Y. Ishizuka and Norimichi Kawashima, Toin 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 tetraethyl orthosilicate (TEOS). Powders composed of only diopside were synthesized adding ammonia to the starting solution and firing the precipitates at 1373K for 12-24h. The hydrolyzed 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 1095K. Diopside substrates were fabricated by hot-pressing at 1473K under a pressure at 40 MPa for 2-2.5h. 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 1373K for 24h and then 1473K for 2.5h gave a bending strength and vickers hardness higher than those in literatures. The maximum bending strength and vickers hardness were 338 MPa and 722 Hv, 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 sol-solution and heating at 1123K for 30 min. The pore size on this film depended on immersing time or the thickness before crystallizing 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. Gewaily, New Hyde Park Memorial High School; Y.S. Seo, V.A. Samuilov, J. Sokolov, M. Rafailovich, 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 ($\sim 2 \mu$) 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 air/water/Si interface, forming a ring-like morphology on the surface. The DNA c chain bundles are organized in a aligned 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 2800 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 electrophoresis. This work is supported by NSF-MRSEC program.

SESSION FF6: ELECTRON MICROSCOPY AND NOVEL TECHNIQUES

Chairs: Adrian B. Mann and Robert 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, and in the presence of water vapour 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, along with 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

ASSEMBLIES OF POLYMERIZED PHOSPHOLIPID VESICLE STRUCTURES CHEMISORBED ON Au FILMS VISUALIZED IN SITU BY ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY. Ivan Stanish, Alok Singh, Center for Biomolecular Science and Engineering, Naval Research Laboratory, Washington, DC.

Surfactant vesicles are spheroidal capsules with diameters that range from 20 nm to 100 nm and with a shell thickness of ~5 nm composed 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, cosmetics, wastewater treatment, nanoparticle synthesis, 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 vesicle instabilities and a lack of appropriate tools necessary to characterize these soft materials. In the early 1980s, vesicle structural instability was addressed by incorporating polymeric functionality into monomeric surfactants; and the first *in situ* AFM characterizations of immobilized nonpolymerized and polymerized vesicles were reported in 1996 (T. Shibata-Seki et al.) and in 2001 (I. Stanish et al.), respectively. Although *in situ* AFM is a powerful structural (i.e., topographical) characterization tool, we will demonstrate for the first time a technique, environmental scanning electron microscopy (ESEM), that enables *in situ* measurement of immobilized vesicle size, shape, dispersity, 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 two-layer vesicle film formed through controlled, covalent, vesicle stacking.

9:15 AM FF6.3

ELECTRON MICROSCOPY CHARACTERIZATION OF BONE-BONDING TO HYDROXYAPATITE AND Ti SURFACES OF HUMAN FEMORAL STEMS RETRIEVED AT AUTOPSY.

Alexandra E. Porter^{a,b,c}, Punam Taak^{a,b}, Linn W. Hobbs^a, Gordon W. Blunn^d, Melanie J. Coathup^d and Myron Spector^e.

^aMassachusetts Institute of Technology, Department of Materials Science & Engineering, Cambridge, MA. ^bUniversity of Cambridge, Department of Materials Science & Metallurgy, Cambridge, UNITED KINGDOM. ^cImperial College of Science, Technology & Medicine, London, UNITED KINGDOM. ^dRoyal National Orthopaedic Hospital, Stanmore, Middx, UNITED KINGDOM. ^eBrigham & 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 (Biomet, UK) hemi-arthroplasty. The average patient age was 86 years, and the stems remained implanted for times between 40 and 1094 days before being collected together with surrounding bone. Sawn and polished cross sections were imaged by environmental SEM operating in backscattered electron mode, and both stained and unstained microtomed cross sections were imaged in TEM. While the HA coating was observed to significantly improve initial bone bonding, the percentage of implant surface apposed by bone decreased from 100% at 173 days to 60% at 660 days, the decrease attributable to loss of the HA coating with increasing implant time. Bone apposition to 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 platelets intimately associated with collagen fibrils adjacent to implant surfaces. In some areas, globular clusters of apatite ribbons and less organized nanocrystalline mineral phase were identified. All three mineralization features have been previously observed for shorter time (3-10 day) implants in canine models.

9:30 AM FF6.4

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 (LVEM) for microstructural investigations of thin polymer and organic molecular films. The LVEM was developed by DeLong Instruments in Brno, Czech Republic (www.dicomps.com), and is equipped with a Schottky field-emission source, permanent magnet condenser and objective lenses, and electrostatic projector lenses. The LVEM can be operated in TEM, ED, STEM, and SEM modes. High contrast micrographs of polymer and organic films have demonstrated the ability of the low energy electrons to penetrate films up to 40 nm thick with an estimated 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 electrospun fibers of bioactive protein polymers.

9:45 AM FF6.5

CHARACTERISATION OF BIOMEDICAL MATERIALS, CELLS AND INTERFACES USING ENVIRONMENTAL SEM (ESEM). Debbie Stokes, Cavendish Lab, University of Cambridge, UNITED KINGDOM; Susan Rea, 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 bioactivity of these materials is dependent upon such factors as phase composition, chemical composition, surface activity, crystallinity and microstructure. Using ESEM it is possible to obtain surface-sensitive, specimen-dependent secondary electron images (in the absence of specimen coating), yielding potentially new perspectives on microstructure to complement information derived from other techniques. To this end, we have recently begun using ESEM to study the apposition of bone on HA-based biomedical materials, from both *in vitro* and *in vivo* investigations. The emphasis of this initial work is on establishing the most appropriate criteria for handling and imaging these types of specimen with ESEM whilst demonstrating the potential usefulness of this technique. Another use of ESEM in this field is x-ray microanalysis and elemental mapping of, for example, bone grafts, metal alloy implants and hip replacement joints. 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 bioactivity or implant failure.

10:30 AM FF6.6

HIGH RESOLUTION WATER MAPPING IN HYDRATED TISSUE AND POLYMERS. A. Aitouchen, V. Johnson, M. Libera, Stevens Institute of Technology, Hoboken, NJ; M. Misra, S.L. Shi, Unilever Research, Edgewater, NJ.

Most biological tissues have an average hydration level of order 70 vol%. 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 integumentary 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 distinguish differences in inter- and intra-cellular distributions of water. This paper describes the application of spatially resolved electron energy-loss spectroscopy (EELS) in a scanning transmission electron microscope (STEM/ENFINA (1)) to map water in frozen hydrated sections of porcine tissue as well as in various synthetic polymer systems. The method builds on the technique developed by Leapman et al. (2) using multiple least squares fitting of reference spectra. Water can be distinguished from protein and from most synthetic polymers based on characteristic differences in valence structure measurable in low-loss energy-loss spectra. The quantitative accuracy of this approach has been confirmed using frozen-hydrated sections of aqueous solutions of bovine serum albumin (BSA). A spatial resolution of approximately 75 nm has been achieved in studies of frozen hydrated porcine tissue with dramatic image contrast based simply on the local water concentration. Analysis of these same sections after 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 soap has been achieved with a spatial resolution of 15 nm and shows that the water is nonuniformly distributed throughout the structure. [1] Brink, H.A. et al. in Proc. Ann. MSA Meeting (Philadelphia, Pennsylvania), 2000, 212. [2] Sun, S; Leapman, R; et al., Ultramicroscopy, 1993, 50, 127.

10:45 AM FF6.7

SELF-ASSEMBLY OF DYNAMIN: A CRYO-ELECTRON MICROSCOPY STUDY. Dganit Danino, Jenny E. Hinshaw, Laboratory of Cell Biochemistry and Biology, NIDDK, NIH, Bethesda, MD.

Dynamain is a 120kDa GTPase implicated in numerous fundamental intracellular processes, including synaptic vesicle recycling, caveolae internalization and trafficking into and out of the Golgi. In

receptor-mediated endocytosis dynamin is believed to localize to clathrin coated pits, and to redistribute to the necks and possibly participate in vesicle fission upon GTP binding and hydrolysis. Here we studied the effects of nucleotide binding and hydrolysis, without the effect of an underlying lipid model system. Cryogenic transmission electron microscopy (cryo-TEM) is a powerful direct non-perturbing method, devoid of staining and drying artifacts, used for studying macromolecular assemblies in the 2-500 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 recently developed digital-imaging 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. Dynamin self-assembles into rings and spirals in low salt. A mutant lacking the c-terminal proline rich domain (Δ PRD) also assembles into rings and spirals, and constricts in the presence of non-hydrolyzed GTP analogues such as GMP-PCP and GTP γ S. The constricted spirals are long, straight and well ordered. Images show that the rings (edge-on views of short spirals) consist 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, Δ PRD and wild-type dynamin were dialyzed into GDP BeF. Small, less-ordered spirals formed, suggesting a different conformation for these assemblies as a result of partial hydrolysis. Preliminary measurements show these spirals are constricted as well.

11:00 AM FF6.8

FIRST-PRINCIPLES DETERMINATION OF BIOMIMETIC MEMBRANE STRUCTURE BY PHASE-SENSITIVE SPECULAR NEUTRON REFLECTOMETRY. C.F. Majkrzak, N.F. Berk, S. Krueger, J.A. Dura, U. Perez-Salas, NIST Center for Neutron Research, Gaithersburg, MD; V. Silin, C.W. Meuse, J. Woodward, A.L. Plant, J. Elliott, NIST Biotechnology Division, Gaithersburg, MD; M. Tarek, Univ. of Pennsylvania, Dept. of Chemistry, Philadelphia, PA; D.Tobias, Univ. of California, Irvine, Dept. of Chemistry, Irvine, CA.

Biomimetic films that serve as models of cell membranes are of importance in understanding such fundamental biological processes 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) depth profiles of biomimetic membranes by first-principles inversion, without fitting or adjustable parameters. The SLD profile so obtained is unique and can be directly compared with a corresponding chemical compositional profile of the film, predicted, for example, by an ab initio molecular dynamics calculation at a spatial resolution currently approaching subnanometer detail. 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 electrochemical cells, are presented to illustrate the practicality and power of this new technique.

11:15 AM FF6.9

NUCLEAR MICROPROBE ANALYSIS OF TRANSMEMBRANE ION FLUX IN RAT BRAIN. Karen P. Briski, College of Pharmacy, University of Louisiana at Monroe, Monroe, LA; William A. Hollerman, and Gary A. Glass, Acadiana Research 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 electrophysiological and/or 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/ionic mechanisms by which local sensor cells transduce energetic distributions into neural signals remain unresolved. Microbeam particle-induced X-ray emission spectrometry (μ PIXE) is a novel investigative technique offering multielemental quantitation at ppm sensitivities, with high accuracy at spatial resolutions less than cellular 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 refinement of nuclear microprobe analytical techniques for use with established neuro-anatomical and pharmacological approaches in a novel strategy to characterize electrolytic indices of neuronal function, in defined cell populations in the brain, in response to in vivo manipulation of glucose availability. This presentation will describe efforts to map for

effects of glucose deficits on transmembrane flux of ions, e.g. sodium, potassium, chlorine, and calcium, 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 a Research Award from the American Diabetes Association and the Louisiana Education Quality Support Fund (LEQSF) under grants LEQSF (2000-2003)-39, DOE/LEQSF (1993-95)-03, and DE-FC02-91ER75669.

11:30 AM FF6.10

MINIATURIZED PIEZOELECTRIC UNIMORPH CANTILEVERS FOR IN-SITU AQUEOUS BIOSENSING. J. W. Yi, W. Y. Shih, Raj Mutharasan, and W.-H. Shih, Drexel University, Philadelphia, PA.

Miniaturized piezoelectric cantilevers offer the advantage of simple electrical detection and the potential for a wide range of sensing applications. Addition of a small mass can be detected from 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 bioagents or microbes. Immobilizing receptors at the cantilever tip, binding of the bioagents to the receptors can be detected by monitoring the cantilever's resonance frequency shift. The concentration of the bioagent 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 FF6.11

A QUARTZ CRYSTAL MICROBALANCE CELL BIOSENSOR DETECTING DRUGS VIA ALTERATIONS IN CYTOSKELETON. Kenneth A. Marx, Tian Zhou, Anne Montrone, Susan Braunhut, 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 line the interior surface of blood vessels. ECs were used to form stable monolayers on the gold covered quartz surface of a Quartz Crystal Microbalance (QCM) device, creating an EC QCM biosensor. We studied a small drug, nocodazole, which binds and disrupts microtubules-a major part of the cellular cytoskeleton. Nocodazole can be sensed by the EC QCM biosensor down into the nM concentration range and its kinetics measured over a 6 hr period via a significant decrease in the measured crystal oscillation frequency f and increase in interfacial motional resistance R (1-3). The f and R shift magnitudes vary in a sigmoid shaped dose dependent fashion with [nocodazole], with a midpoint of 900 nM. The nocodazole effects on the EC QCM biosensor 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 330 nM nocodazole. With increasing [nocodazole], 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 μ m. These results indicate that the EC QCM biosensor 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 small and large molecule therapeutics' effects on adherent cells' cytoskeleton-the membrane bound integrins, or the ECM, regardless of the mechanism of action. Supported by NIH R21 GM58583 and UML Res. Fdn. Seed Grant.

1. Zhou, et.al. Biotechnology Progress, 15, 522-528 (1999).
2. Marx, et.al. Biotechnology Progress, 16, 268-277 (2000).
3. Marx, et.al. Biosensors & Bioelectronics, in press (2001).

SESSION FF7: LASER AND OPTICAL CHARACTERIZATION

Chairs: Gang Bao and David A. Weitz
Thursday Afternoon, November 29, 2001
Hampton (Sheraton)

1:30 PM *FF7.1

DEFORMATION MECHANISMS IN NATURAL POLYMER FIBERS AND COMPOSITES. Robert J. Young, Stephen J Eichhorn, Victoria L. Brookes, 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 fluorescence-free spectra can be obtained from the major amputate

(MA) dragline silks reeled from different species of spiders: *Araneus diadematus*, *Nephila edulis*, *Latrodectus mactans* and *Euprosthenois* sp. Information upon the chain conformations can be obtained from the spectra and it has been demonstrated that compared to silk obtained from the silkworm *Bombyx mori*, all spider silks have less beta-sheet material. Stress-induced Raman bands shifts were also obtained for all the silk fibres implying that mechanical deformation leads to stretching of the polypeptide chains in the fibres. It has also been possible to obtain well-defined Raman spectra from natural cellulose fibres such as Flax, Hemp and Ramie though the use of an IR laser as the excitation source. Again stress-induced Raman band shifts can be obtained and differences in the behaviour of the different materials is observed. It has also been found that similar behaviour is observed from the cellulosic material in wood during deformation implying that, in all cases, direct deformation of the cellulose molecules takes place. The deformation behaviour of the different types of natural fibres has been analysed and it has been demonstrated that the deformation follows a uniform-stress series model. This can be contrasted with the behaviour of natural composites such as wood where there is generally uniform strain deformation. The implications of these observations upon our understanding of the deformation of natural polymeric materials and in the design of new materials will be discussed.

2:00 PM FF7.2

NANOSHAPING OF POLYMERS BY ELECTROSPINNING TECHNIQUES: "REAL TIME" RAMAN STUDIES.

Jean S. Stephens, Simon Frisk, Silke Megelski, John F. Rabolt, 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 micro- and nanofibers, have been used to control the shape, orientation, and texture of polymer fibers. Raman spectra of as-spun fibers produced through electrospinning have shown that high S/N data can be obtained on 50 mm 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 'online' 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 FF7.3

Abstract Withdrawn.

2:30 PM FF7.4

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. Sotak, Worcester Polytechnic Institute, Department of Biomedical Engineering, Worcester, MA; Anuj Bellare, 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 to the technique. Elongation in one direction (cylinders) and elongation in two directions (lamellae) 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 vacuum nor dyeing, therefore more accurately depicting 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 articular 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 FF7.5

VISCOELASTIC SCALING IN COLLOIDAL SYSTEMS.

Maria L. Kilfoil, 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 background fluid is viscoelastic. We investigate whether universal viscoelastic behaviour can be found for these complex gels,

via optical microscopy, light scattering and rheology. Other questions we address, of practical importance, are how do these complex gels age, what are the kinetics of the colloidal aggregation in this environment, and how important is the elasticity of the viscoelastic background fluid compared to that of the colloidal network.

3:30 PM FF7.6

SMALL ANGLE LIGHT SCATTERING FOR THE STUDY OF RHEUMATOID ARTHRITIS IN HUMAN CARTILAGE.

Karen M. McNamara, Eric Dahlgren, Worcester Polytechnic Institute, Department of Chemical Engineering, Worcester, MA; Anuj Bellare, 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 displaying varying degrees of degradation 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 FF7.7

ELECTROPHORETIC DETECTION OF CHARGE REVERSAL OF FILAMENTOUS PHAGES UNDER HIGH CONCENTRATIONS OF MULTIVALENT COUNTERIONS. Karim Addas and 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 resolubilize at much higher concentrations of counterions. The mean-field Poisson-Boltzmann theory of macro-ions 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 resolubilization, by predicting that the overall charge of the linear polyelectrolyte is reversed in sign with the presence of very high concentrations of polyvalent counterions. An electrophoresis instrument (Coulter Delsa), 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 aggregation and after filament bundles were resolubilized, where charge reversal is expected. At high concentrations of magnesium acetate or spermidine, the average value of mobility does change sign, 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 FF7.8

NEAR-FIELD SCANNING OPTICAL IMAGES OF BACTERIA.

Ana M. de Paula, Juliana A. Toledo, Haroldo B. Silva, Gerald Weber, Laboratório de Nano-Espectroscopia Óptica, Universidade São Francisco, Bragança 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 the Gram method and we measured the absorption of the laser light at 488 nm by the dye (Safranin) fixed at the bacteria membrane. To obtain good images we had to improve 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 details of the absorption of the laser 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 could be an important tool for instance in the studies of the effects of antibiotics on the cell membrane.

4:15 PM FF7.9

OPTICAL CHARACTERIZATION OF BIOLOGICAL AND OTHER SYSTEMS. Alexandra G. Bezrukova, St. Petersburg State Technical Univ, St. Petersburg, RUSSIA.

Static and dynamic light scattering can provide further progress in on-line control of complex 3D disperse 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, lipoproteids, 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.