SYMPOSIUM L

Structure and Mechanical Behavior of Biological Materials

March 29 - 31, 2005

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Proceedings to be published online
(see *ONLINE PUBLICATIONS* at www.mrs.org)
as volume 874E
of the Materials Research Society
Symposium Proceedings Series.
This volume may be published in print format after the meeting.

* Invited paper

SESSION L1: Hard Tissues I
Chairs: Peter Fratzl and Rizhi Wang
Tuesday Morning, March 29, 2005
Room 3004 (Moscone West)

8:30 AM *L1.1
Mechanistic Aspects of The Failure of Hard Mineralized Tissue: Fracture, Fatigue and Aging in Bone and Dentin.
Robert O. Ritchie[1,2], Ravi K. Nalla[2], Jamie J. Kruzic[3] and John H. Kinney[4]; [1]Materials Science & Engineering, University of California, Berkeley, Berkeley, California; [2]Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California; [3]Mechanical Engineering, Oregon State University, Corvallis, Oregon; [4]Lawrence Livermore National Laboratories, Livermore, California.

Biomaterials comprising hard mineralized tissue, such as bone and dentin (the major constituent in teeth), have hierarchical nano/microstructures with characteristic length scales ranging from nanometers to hundreds of micrometers. In this presentation, the in vitro fracture toughness and fatigue properties of such materials, specifically human bone and dentin, are examined from a perspective of discerning how these properties depend upon such nano/microstructures. The motivation for this is that although there is substantial clinical interest in the fracture resistance of bone and teeth, only limited mechanistic information is available on how they derive their resistance to cracking and how this is specifically affected by cyclic loads. In the present talk, in vitro experiments are described that establish that the initiation of fracture in bone and dentin is locally strain-controlled and that subsequent crack growth (characterized by resistance-curve behavior) is associated with a variety of extrinsic toughening mechanisms, most importantly crack bridging (from individual collagen fibrils and especially uncracked ligaments), macroscopic crack deflection, and to a lesser extent diffuse microcracking. In particular, the role of aging, which is well known to cause a marked deterioration in the mechanical properties of bone and teeth, is examined in terms of how age-related biological changes affect these specific toughening mechanisms.

9:00 AM L1.2
High-Speed Photography of Compressed Human Trabecular Bone Correlates Whitening to High Local Strains.
Philipp Johannes Thurner[1], John D. Langan[2], Jeff Scott[2], Maria Zhao[2], Blake Erickson[1], Zachary Schriock[1], Georg Ernest Fantner[1] and Paul K. Hansma[1]; [1]Physics, University of California Santa Barbara, Santa Barbara, California; [2]Computational Sensors Corp., Santa Barbara, California.

The mechanical properties of healthy and diseased bony tissue are extensively studied in mechanical tests. Most of this research is motivated by the immense costs of health care and social impacts due to osteoporosis in post-menopausal women and the aged. Osteoporosis results in bone loss and change of trabecular architecture, causing a decrease in bone strength. Correlations between density and different mechanical properties of cancellous bone have been demonstrated for large populations using power-law regressions. However, changes in density can explain only 10% - 90% of the variation of trabecular bone strength on an individual basis, leaving 90% - 10% of the strength variation unexplained. Thus, accurate diagnosis is difficult, because the combination of density and structure, referred to as bone quality, is poorly understood. For this reason, research today increasingly drives toward also assessing bone microarchitecture, bone microcracks, cell distributions and organic matrix quality. Ideally, all bone quality parameters should be included into the investigation of failure analysis - a formidable task. To address the problem of assessing bone microarchitecture and concomitant microcracking behavior, we recently combined mechanical compression testing of trabecular bone samples with high-speed photography at a spatial resolution around 20 μm. In an exemplary study, we investigated healthy, osteoarthritic, and osteoporotic human vertebral trabecular bone. Cubic bone samples, 5 mm x 5 mm x 4 mm in size and devoid of bone marrow were loaded along their principal load-bearing axis immersed in a NaCl buffer at pH 7 at high strain rates simulating similar boundary conditions as experienced in individuals during falls. Even at small global strains huge local deformations could be seen in the recorded high-speed photography frames. Moreover, strained trabeculae were seen to whiten with increasing global and local strains. After applying a motion energy filter to the recorded movies, as well as a cross-correlation analysis of different frames, we conclude that the whitened areas can be mainly associated with areas of high deformation. We hypothesize that the effect seen is due to microcrack formation in these areas, similar to stress whitening seen in synthetic polymers. This hypothesis is currently tested applying the gold standard for the detection of microcracks: en bloc staining and histological investigation. Bone whitening is briefly mentioned in literature and so far could not be shown to be conclusive of microdamage. We believe that this is mainly due to the fact that up to our best knowledge no one has combined high-speed photography and mechanical testing of trabecular bone at high spatial resolution. We believe that this method will bring us to further insights into bone failure mechanisms, and help toward a thorough understanding of bone fracture.

9:15 AM L1.3
Non-Contact Determination of Surface Displacements of Mineralized Biological Tissues Tested in Water Using Speckle Interferometry.
Paul Zaslansky[1], Ron Shahar[3], John D. Currey[4], Asher A. Friesem[2] and Steve Weiner[1]; [1]Dept. of Structural Biology, Weizmann Institute of Science, Rehovot, Israel; [2]Dept. of Physics of Complex Systems, Weizmann Institute of Science, Rehovot, Israel; [3]School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel; [4]Dept. of Biology, University of York, York, United Kingdom.

In order to understand hierarchical biomaterials such as bones and teeth, it is necessary to measure their mechanical properties and relate them to the microstructure. Results of measurements of large specimens (mm to cm length scale) have provided rough estimates for these properties and more recently, micro and nano-indenters have provided new insights on the nanometer scale and have measured properties of very small features on such specimens. However, there is still a need to reliably determine, to high precision, the subtle variations in the deformation patterns of such specimens at an intermediate sub-millimeter length scale. This length scale, commonly termed the meso scale, is the length scale of biological adaptation where variations in structural motifs and tissue organization can be found. It is thus necessary to locally determine the extent of deformation of sub-sections of millimeter-sized specimens. Only few methods allow for direct measurements of deformation and surface displacement without physically contacting the measured specimen. Moreover, water can severely affect both the mechanical properties and the results of deformation measurement. Here we report on the use on a method that takes measurement under water using the speckle effect known to appear when coherent light illuminates optically rough surfaces. A field of interferometers is created and projected onto the surface of a specimen. Sampling many points of a CCD detector array, digital holographic images can be produced and used to determine the relative differences in the random phases of the light scattered from specimen surface. Because these phases depend on the surface architecture, they will vary linearly with any displacement. Automated measurements allow the detection of deformation on the order of $\lambda/30$ (< 30 nm) to be mapped over meso-scale regions on the specimen. Here we show that by highly controlling the extent of the mechanical loading and by repeated measurements a sufficient S/N ratio can be achieved allowing for a precise and repeatable determination of the surface displacements. Using such measurements, displacement and surface strains can be determined for small parallelepiped samples as small as 1x1x2mm. These have been used to determine elastic constants such as Young's modulus and Poisson ratios on samples of tooth dentin and bone, and they can be used to establish visco-elastic time constants for specimens loaded under small physiological loads. Furthermore, these displacement fields and gradients can be used to establish and compare the deformation and bending modes of whole complex 3D structures of bones and teeth loaded along various orientations. Such measurements allow the validation of theoretical models of deformation on the one hand, and can be used, on the other hand, for comparison with measurements of deformation of bones and teeth taken from genetically modified strains of mice.

9:30 AM L1.4
A Fracture Resisting Molecular Interaction in Trabecular Bone: Sacrificial Bonds and Hidden Length Dissipate Energy as Mineralized Fibrils Separate.
Georg Ernest Fantner[1], Tue Hassenkam[1], Johannes H. Kindt[1], James C. Weaver[4], Leonid Pechenik[1], Jacqueline A. Cutroni[1], Laura Golde[1], Marquesa M. Finch[1], Philipp Thurner[1], Geraldo A. G. Cidade[5], Galen D. Stucky[3], Danniel E. Morse[4] and Paul K. Hansma[1]; [1]Physics, University of California Santa Barbara, Santa Barbara, California; [2]Department of Chemistry, University of California Santa Barbara, Santa Barbara, California; [3]Department of Chemistry and Biochemistry, University of California Santa Barbara, Santa Barbara, California; [4]Institute for Collaborative Biotechnologies, University of California Santa Barbara, Santa Barbara, California; [5]Biophysics Institute Carlos Chargas Filho, University of Rio de Janeiro, Rio de Janeiro, Brazil.

Properties of the organic matrix of bone[1] as well as its function in the microstructure[2] are important contributors to the remarkable mechanical properties of bone[3]. A molecular energy dissipation mechanism in the form of sacrificial bonds and hidden length was previously found in bone constituent molecules[4] of which the efficiency increased with the presence of Ca2+ ions in the experimental solution. Here we present evidence for how this sacrificial bond-hidden length mechanism contributes to the mechanical properties of the bone composite. From investigations into

the nanoscale arrangement of the bone constituents[5-7] (by Atomic Force Microscopy and high resolution Scanning Electron Microscopy) in combination with pico-Newton adhesion force measurements between mineralized collagen fibrils, based on single molecule force spectroscopy, we find evidence that bone consists of mineralized collagen fibrils and a non fibrillar organic matrix[2] which acts as a glue that holds the mineralized fibrils together. We propose that this glue resists the separation of mineralized collagen fibrils. Like in the case of the sacrificial bonds in single molecules, the effectiveness of this glue increases with the presence of Ca2+ ions. We further investigate how this molecular scale strengthening mechanism increases the fracture toughness of the macroscopic material. Possible molecules that are involved in this sacrificial bond mechanism (sacrificial molecules) range from non fibrillar collagens, glycoproteins, osteopontin and other bone associated polymers (osteopontin, osteonectin, bone sialoprotein). We investigate the possible sacrificial bond function of these bone constituents by single molecule force spectroscopy and adhesion measurements to hydroxyapatite crystals. With this knowledge, we hope to better understand the molecular processes involved in bone fracture which might lead to new ways to assess bone quality and give new ideas for fracture prevention. 1. Burr, D. B. The contribution of the organic matrix to bone's material properties. Bone 31, 8-11 (2002). 2. Braidotti, P., Branca, F. P. & Stagni, L. Scanning electron microscopy of human cortical bone failure surfaces. Journal of Biomechanics 30, 155-162 (1997). 3. Rho, J. Y., Kuhn-Spearing, L. & Zioupos, P. Mechanical properties and the hierarchical structure of bone. Medical Engineering & Physics 20, 92-102 (1998). 4. Thompson, J. B. et al. Bone indentation recovery time correlates with bond reforming time. Nature 414, 773-776 (2001). 5. Hassenkam, T. et al. High-resolution AFM imaging of intact and fractured trabecular bone. Bone 35, 4-10 (2004). 6. Grynpas, M. D., Tupy, J. H. & Sodek, J. The Distribution of Soluble, Mineral-Bound, and Matrix-Bound Proteins in Osteoporotic and Normal Bones. Bone 15, 505-513 (1994). 7. Taton, T. A. Nanotechnology - Boning up on biology. Nature 412, 491-492 (2001).

9:45 AM L1.5
Molecular Response of Bone Matrix Collagen and Bone Mineral to Mechanical Loading. A Raman Spectroscopic Study. Michael D. Morris[1], Andrew Callender[1], Kurtulus Golcuk[1], David H. Kohn[2,3] and Nadder D. Sahar[2]; [1]Chemistry, University of Michigan, Ann Arbor, Michigan; [2]Biologic and Materials Sciences, University of Michigan, Ann Arbor, Michigan; [3]Biomedical Engineering, University of Michigan, Ann Arbor, Michigan.

Bone tissue responds to normal physiological and traumatic mechanical loading at every level of architecture from chemical structure to whole bone. Much is known about response at the levels of ultrastructure and above, but there is less information available about response at the chemical structure level. Using murine cortical bone, we show that Raman microspectroscopy is a powerful probe of chemical structure response to external loading. The matrix response to elastic loading is primarily distortion of pyridinoline cross-links and the response to plastic deformation is rupture of these cross-links. In both tension and compression changes in Raman shifts and intensities return to original positions when load is removed, indicating reversibility. The mineral response to elastic compressive and tensile loading includes movement of anions and cations within the crystallite lattice and movement of water into and out of the lattice and is observed as changes in shifts of band positions and band widths of the phosphate symmetric stretch envelope. Plastic deformation results in permanent rearrangement of the ions. Bone tissue spectra under load are observed using a miniaturized dynamic mechanical tester designed for operation on the stage of a Raman microprobe and constructed with provision for maintaining tissue hydration. Changes are correlated with results from other vibrational spectroscopic studies and from solid-state proton NMR.

10:30 AM *L1.6
The Role of Type I Collagen Molecular Structure in Tendon Elastic Energy Storage. Joseph Warren Freeman, Orthopaedics, University of Virginia, Charlottesville, Virginia.

In order to facilitate locomotion and limb movement many animals store energy elastically in their tendons. The formation of crosslinked collagen fibers results in the conversion of weak liquid-like embryonic tissues to tough elastic solids that can store energy and do work. Collagen fibers in the form of fascicles are the major structural units found in tendon. The purpose of this paper is to review the literature on collagen self-assembly and tendon development and to relate this information to the development of elastic energy storage in non-mineralizing and mineralizing tendons. Of particular interest is the mechanism by which energy is stored in tendons during locomotion. In the turkey, much of the force generated by the gastrocnemius muscle is stored as elastic energy during tendon deformation and not within the muscle. As limbs move, the tendons are strained causing the collagen fibers in the extracellular matrices to be strained. Through analyses of turkey tendons, collagen fibers, and a molecular model it is hypothesized elastic energy is stored in the flexible regions of the collagen molecule. Data from the molecular model, mineralized fibers, and turkey tendons show that the presence of calcium and phosphate ions cause an increase in elastic energy stored per unit strain. Based on the theoretical modeling studies the increase in stress with strain is a result of the initiation of stretching of the rigid regions.

11:00 AM L1.7
Nanoindentation and Finite Element Analysis of Resin-Embedded Bone Samples as a Three-Phase Composite Material. Michelle L. Oyen[1], Ching-Chang Ko[1], Amanpreet K. Bembey[2], Andrew Bushby[2] and Alan Boyde[3]; [1]University of Minnesota, Minneapolis, Minnesota; [2]Department of Materials, Queen Mary, University of London, London, United Kingdom; [3]School of Medicine and Dentistry, Queen Mary, University of London, London, United Kingdom.

The effective elastic modulus of composite materials results from a combination of elastic moduli of the component phases. Recent efforts to understand the mechanical behavior of calcified tissues, such as bones and teeth, require estimates of the component phase properties, which are difficult to establish independently. A three-phase system, based on naturally-occurring bone, is therefore examined by a combined nanoindentation and finite element modelling approach to better understand the proportions and properties of the component phases. Bone samples were prepared in four two- or three-phase composite configurations as follows: (1) as a dehydrated mineral-protein composite (with some void space); (2) similarly dehydrated mineral-protein composite but with polymethylmethacrylate (PMMA) resin filling the voids resulting in three solid phases; (3) as a PMMA-mineral composite following protein removal and replacement with PMMA, and (4) as a PMMA-protein composite following mineral removal and replacement with PMMA. Effective component volume fractions and elastic moduli for each phase in each system were computed based on the composite nanoindentation results. Finite element models of the two- and three-phase systems were constructed to explore the structural anisotropy of the composite systems, as demonstrated in the nanoindentation tests, and to examine the sensitivity of the composite results to changes in the assumed component properties.

11:15 AM L1.8
Nanomechanical Mapping of Remineralisation in Human Enamel Lesions. Michelle Emma Dickinson[1,3], Kurt V. Wolf[2] and Adrian B. Mann[1,3]; [1]Ceramics and Materials Department, Rutgers University, Piscataway, New Jersey; [2]Evans East, East Windsor, New Jersey; [3]Biomedical Engineering, Rutgers The State University Of New Jersey, Piscataway, New Jersey.

The complex nanoscale structure of human dental enamel consists of hydroxyapatite prisms arranged into clusters. These are closely packed into an organic matrix to give the enamel's macroscale structure. In the oral environment the enamel surface is exposed to continually cycled pH conditions due to acidic dietary and bacterial components. These are counteracted by the buffering properties of saliva. If the local acidic environment on the enamel surface is not counterbalanced then a "white spot" or "carious" lesion may form. This is where the enamel looses its constituent mineral, becomes demineralised and ultimately develops into a carie (cavity). With specialised care and remineralising treatments, the lesion can become arrested and not proceed to form a cavity, thus preventing the need for a restoration. Remineralisation treatments including fluoride and calcium phosphates have been used for years and are widely available in commercial toothpastes to aid in the prevention of cavities. However the actual mechanism for both the lesion formation and the remineralisation process is still poorly understood. This investigation uses a high resolution nano-mechanical mapping technique combined with ToF-SIMS maps to study the mechanical and chemical properties of the lesion cross section and the effects of applying topical remineralisation solutions. The nano-mechanical maps show the properties of the lesions to vary dramatically with depth below the enamel surface. The hardness (H) and elastic modulus (E) at the surface were found to be 0.68 $\pm$0.19GPa and 16.84 $\pm$3.06GPa respectively, however deeper into the lesion's interior the values were as low as 0.02GPa and 1.38GPa respectively. Upon treatment with a fluoride based remineralisation solution, the mechanical properties of the enamel surface did not increase significantly; however, at the base of the lesion there was a dramatic increase in H from 1.02GPa to 10.30GPa and Elastic Modulus from 25.77GPa to 187.88GPa. The increased mechanical properties are higher than those found for natural dental enamel hydroxyapatite suggesting that the remineralisation process results in a new phase, possible a fluorapatite based structure. Chemical surface analysis with ToF-SIMS correlated the chemical data with the mechanical data to relate the structural integrity of the enamel with the change in hardness and modulus.

This understanding of a site specific process for remineralisation can lead to a more targeted approach for commercial dental treatments.

11:30 AM L1.9
A Stochastic Lattice Model for Bone Remodeling and Aging. Richard Weinkamer[1], Markus A. Hartmann[1], Yves Brechet[2] and Peter Fratzl[1]; [1]Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany; [2]LTPCM, ENSEEG, Grenoble, France.

Being continuously remodeled trabecular bone is able to adapt its microarchitecture in response to changes of its loading. Biologically this maintenance and adaptation process is performed by an interplay between bone removing cells (osteoclasts) and bone forming cells (osteoblasts). It was reasoned that adaptation is enabled by a coupling of the cell activity and number to a local mechanical stimulus in the way that bone is deposited wherever mechanically needed and resorbed where not needed (Wolff-Roux Law). Implementing such a mechanical principle in computer simulations successfully demonstrated the emergence of a mechanically favorable trabecular architecture, which is maintained and also able to adapt to varying loads [1,2]. In our model the trabecular structure is mapped on a cubic lattice. Remodeling is described by a removal or deposition of lattice elements from the surface. Assuming an uniaxial loading the deformation in each element is assessed using a fast algorithm [2]. We observed that while the bone volume fraction remained constant the emerging microstructure coarsened. Since trabeculae along the main loading axis thickened faster, an anisotropy favoring the loading direction became more pronounced with time. Additionally different mathematical relations between cell activity and local mechanical stimulus have been tested. A qualitative different outcome to scenarios which can be observed also in real bone (e.g., an increased turnover rate) allows to obtain a phenomenological characterization of the coupling between cells and mechanical stimulus. [1] R. Huiskes et al., Nature 405 (2000), 704. [2] R. Weinkamer et al., Phys. Rev. Lett. (2004), approved for publication.

11:45 AM L1.10
Modeling Elastic Properties and Strength of Cancellous Bone. Ming Y. He, Chris Mercer and Anthony G. Evans; Materials Department, University of California, Santa Barbara, California.

To understand the inelastic response of cortical and cancellous bone, the objective of the present article is to devise and assess a constitutive law that characterizes the elastic-plastic response. A constitutive law representative of the deformation of cortical bone has been developed. The law allows for different yield strengths in tension and compression, as well as the dilatation upon tensile straining. Parameterized orthotropic constitutive models are developed from finite element analysis. The calculations demonstrate that inelastic bending and buckling of the ligaments affect the stress evolution beyond yield. To assure that the loads on the ligaments are not overestimated, initial imperfections are incorporated, with shape dictated by an eigenvalue analysis of the buckling modes. Both compressive and tensile responses have been determined by the cell model calculations for various parameters. Issues associated with effects of slenderness ratio, anisotropy and the differences between tensile and compressive strength are addressed. Calculated Stress/strain curves are shown to be consistent with measurements reported in the literature. Some limitations of the models and potential refinements are described.

SESSION L2/K2: Joint Session: Functional Biomaterials and Biomimetics
Chairs: Trevor Douglas and William J. Landis
Tuesday Afternoon, March 29, 2005
Room 3002 (Moscone West)

1:30 PM *L2.1/K2.1
Lamellar Bone: Old and New Insights into Structure and Function. Steve Weiner[1], Eugenia Klein[1], Meir Barak[1], Paul Zaslansky[1] and Ron Shahar[2]; [1]Department of Structural Biology, Weizmann Institute, Rehovot, Israel; [2]Faculty of Agriculture, Hebrew University, Rehovot, Israel.

The lamellar structure of bone is widespread, especially among the mammals. It was first identified by van Leeuwenhoek in 1691, and is still not fully understood. The basic motif resembles that of plywood, with parallel arrays of mineralized collagen fibrils arranged in layers with different orientations in a two-dimensional plane. Within the collagen fibril, are layers of very small plate-shaped crystals of carbonated apatite. Adjacent fibrils tend to have their layers aligned, but there is a progressive rotation of the fibrils from one surface plane of an individual lamella to the next. Here we confirm and amplify aspects of this structure using a Schottky FEG SEM with in-lens SE detector, and also show that there is a third structural element with mineralized collagen fibrils aligning the canaliculi, and hence being aligned perpendicular to the main lamellar plane. These fibrils originate from the main lamellar structure, but describe a 90 degree rotation to align themselves orthogonally to the lamellar plane. They may well fulfill a "pinning" function, by firmly bonding adjacent lamellae. Lamellae are often initially deposited as parallel arrays, but as a result of remodeling, reform as cylindrical secondary osteons. The elastic properties of lamellar bone are for the most part due to the lamellar structure, whereas the fracture properties are profoundly influenced by the cylindrical osteonal structure. Careful comparisons of lamellar bone types measured in water under tension and compression using electronic speckle pattern interferometry (ESPI), reveal new insights into the structure-mechanical properties of lamellar bone. Supported by grant DE006954 from the NIDCR to SW.

2:00 PM *L2.2/K2.2
Mechanisms Governing the Inelastic Deformation of Bone. Anthony Evans, Materials, UCSB, Santa Barbara, California.

To understand the inelastic response of cortical and trabecular bone, a three-part investigation has been conducted. In the first, a flexural test protocol has been designed and implemented that monitors the axial and transverse strains on both the tensile and compressive surfaces of cortical bone. The results are used to assess the relative contributions of dilatation and shear to the inelastic deformation. Unload/reload tests have characterized the hysteresis and provided insight about the mechanisms causing the strain. In the second part, a constitutive law representative of the deformation is selected. It is implemented to illustrate the coupled buckling and bending of ligaments that occurs in osteoarthritic trabecular bone loaded in compression. The third part devises a model for the intrinsic stress/strain response of bone, based on a recent assessment of the nano-scale organization of the collagen fibrils and mineral platelets. The model is used to rationalize the inelastic deformation in tension, as well as the permanent strain and the hysteresis.

2:30 PM L2.3/K2.3
Evidence for a Possible Mechanical Role of Bone Matrix Proteoglycans and Glycoproteins. Paul Hansma, Georg Fantner, Johannes Kindt, Philipp Thurner, Leonid Pechenik, Marquesa Finch, Patricia Turner, Georg Schitter, Blake Erickson, Zachary Schriock, Laura Star Golde, Erik Strong and Simcha Frieda Udwin; Physics, University of California, Santa Barbara, Santa Barbara, California.

Evidence from Atomic Force Microscope indentation, pulling and imaging together with evidence from macroscopic testing and enzymatic digestion suggests that collagen fibrils and mineral plates are not the only components of bone with a mechanical role. There appears to be "glue" that binds mineralized collagen fibrils to other mineralized collagen fibrils. Order of magnitude calculations show that less than 1% by weight of this "glue" can have profound effects on the fracture resistance of bone, because it involves a remarkable natural toughening and strengthening system: sacrificial bonds and hidden length. The sacrificial bond-hidden length system can dissipate large amounts of work against entropic forces while stretching out the hidden length that is exposed when sacrificial bonds break. This appears to occur when mineralized collagen fibrils are torn apart or slid relative to each other during bone fracture. In bone, this system depends on the presence of multivalent positive ions such as calcium ions. This dependence allows us to follow the involvement of the sacrificial bond-hidden length system right up to macroscopic fracture testing. Many bone matrix proteoglycans and glycoproteins have negatively charged groups at physiological pHs that could be bound together into sacrificial bonds by multivalent positive ions, and are thus natural candidates for this "glue". We cannot, however, rule out a possible involvement of nonfibrillar collagen. Further research will be necessary to determine precisely which candidate or candidates are involved.

2:45 PM L2.4/K2.4
Contact-induced Deformation and Failure of Dental Multilayers: Effects of Loading Rate. Xinrui Niu, Min Huang, Jikou Zhou and Winston O. Soboyejo; Mechanical and Aerospace of Engineering, Princeton University, Princeton, New Jersey.

This paper presents the results of a combined experimental, analytical and computational study of contact deformation and cracking in dental multi-layers. Dental structures are idealized as layered composites (real teeth and dental restorations). The mechanisms of contact-induced deformation and cracking are then studied at different loading rates. A combination of viscous deformation and fracture mechanics models is used to predict the effects of loading rate on the failure conditions in the dental multilayers deformed under monotonic and cyclic loading. These employ rate dependent constitutive relationships and visco-elastic material properties that are obtained from joint and foundation materials subjected to compressive loading in air and in water. Analytical and finite element

predictions of loading rate effects are shown to be in good agreement with experimental measurements, when the combined effects of viscous deformation and sub-critical cracking are modeled within a mechanistically-based framework.

3:30 PM *L2.5/K2.5

High-Efficiency Fiber-Optical Network in a Glass Sponge. Joanna Aizenberg[1], Andrew D. Yablon[2], V. C. Sundar[1], James C. Weaver[3] and Micha Ilan[4]; [1]Bell Labs/Lucent, Murray Hill, New Jersey; [2]OFS Laboratories, Murray Hill, New Jersey; [3]UCSB, Santa Barbara, California; [4]Tel-Aviv University, Tel-Aviv, Israel.

Even the most advanced optical designs made by humans are often primitive relative to the optical systems that have evolved in Nature. I will describe natural fiber-optical systems produced by a deep-sea sponge *Euplectella*, whose hierarchical architecture and hybrid character offer outstanding optical and mechanical properties. We demonstrate that the sponge forms glass fibers that are remarkably similar to commercial silica optical fibers. We show the spicules to have a characteristic design that encompasses a high refractive index core composed of Na-doped silica, with the refractive index higher than that of vitreous silica; and a low refractive index cladding composed of an organic-containing glass cylinder wrapped in organically glued, multiple layers of hydrated silica. The presence of the lens-like structures at the end of these biofibers that improves the light-collecting efficiency, high fracture toughness arising from their composite structure, the presence of index-raising dopants and the absence of residual stress in these fibers suggest advantages of the ambient temperature synthesis favored in nature and provide new ideas for the fabrication of improved optical systems, constructed using a bottom-up approach.

4:00 PM *L2.6/K2.6

Biomimetic Materials Chemistry. Rajesh R. Naik, Ryan M. Kramer, Melanie M. Tomczak, Joseph M. Slocik, Laura A. Sowards, Sharon E. Jones and Morley O. Stone; US Air Force Research Laboratory, Dayton, Ohio.

The interface between biology, chemistry, and materials science has motivated biomimetic approaches to the formation of inorganic nanomaterials. Biomolecules (proteins, peptides) and biomolecular architectures are being used as templates for the synthesis of inorganic nanomaterials. Our research efforts have been directed at not only understanding how biological organisms control nucleation and growth of inorganic materials, but also how this activity can be controlled in vitro. Biomolecules or biomolecular architectures can be used as building blocks in the bottom-up fabrication of inorganic structures. I will cover our efforts using biomolecules for growing inorganic structures and to exploit self-assembling structures for material synthesis by engineering desired functionalities into the self-assembling biomolecules for bottom-up fabrication.

4:30 PM L2.7/K2.7

Chemically-Tailored Nanofibers Derived from Self-Assembled Natural Templates. Samuel Shian[1], Dori Landry[2], Ye Cai[1], Brian Palenik[2], Mark Hildebrand[2] and Ken H. Sandhage[1]; [1]Materials Science and Engineering, Georgia Institute of Technology, Atlanta, Georgia; [2]Scripps Institute of Oceanography, University of California, San Diego, La Jolla, California.

Spectacular feats of nanoparticle self-assembly can be found in nature. Intricate three-dimensional (3-D) microshells (frustules) comprised of amorphous silica nanoparticles are constructed by diatoms (aquatic micro-algae). Each of the tens of thousands of extant diatom species assembles a frustule with a unique shape and pattern of fine (sub-micron) features. The diatom *CorethronCriophilum* forms a frustule containing spines with a high aspect ratio (a few hundred in diameter, tens of microns in length). These natural "nanofibers" would be attractive for a variety of applications, if the silica-based chemistry could be altered to expand the range of properties without loss of the fiber shape. In this work, we demonstrate how the silica-based spines of the *CorethronCriophilum* diatom can be converted into titanium dioxide nanofibers via use of a halide gas/silica displacement reaction. The chemical conversion process was performed in two steps: i) a displacement reaction of TiF_4(g) with SiO_2(s) to yield $TiOF_2$(s) and then ii) conversion of the $TiOF_2$(s) into TiO_2(s) upon exposure to oxygen. The influence of processing parameters on the extent of reaction and on the resulting morphology will be discussed. With judicious choice of processing conditions, the *CorethronCriophilum* spines were successfully converted into nanocrystalline titania (anatase) nanofibers.

4:45 PM L2.8/K2.8

A Halogen Smile: Br and I in the Jaws of Nereis, a Marine Worm. Henrik Birkedal[1], Rashda Khan[2], Nelle Slack[3], Chris Broomell[4], Helga C. Lichtenegger[5], Frank W. Zok[3], Galen D. Stucky[2,3] and Herbert Waite[4]; [1]Department of Chemistry, University of Aarhus, Aarhus, Denmark; [2]Department of Chemistry and Biochemistry, University of California, Santa Barbara, Santa Barbara, California; [3]Materials Department, University of California, Santa Barbara, Santa Barbara, California; [4]Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, California; [5]Department of Materials Science and Testing, Vienna University of Technology, Vienna, Austria.

The marine worm Nereis has a pair of pincer-like jaws that it uses to collect food. They consists of a proteinaceous matrix reinforced by Zn, the concentration of which increases towards the tip of the jaw [1]. The jaws also contain three of the halogens: Cl, Br and I. Chlorine is in part co-localized with zinc [1] while the heavier halogens are not. Here we show that they are rather concentrated towards the jaw outer surface and that they are bound to the amino acids of the protein matrix by post translational modifications. Several of these modified amino acids have not previously been observed in Nature. We suggest that the exterior of the jaw is analogous to the sclerotized protein of insect cuticle and speculate on the possible roles of this outer coating. [1] H.C. Lichtenegger, T. Schöberl, J.T. Ruokolainen, J.O. Cross, S.M. Heald, H. Birkedal, J.H. Waite, and G.D. Stucky. Proc. Natl. Acad. Sci. USA, 2003, 100, 9144-9149.

SESSION L3/BB3: Joint Session: Nanostructured Biomaterials
Chairs: Greg Swadener and Rizhi Wang
Wednesday Morning, March 30, 2005
Room 2016 (Moscone West)

8:30 AM *L3.1/BB3.1

On the Origin of Stiffening in Biopolymers. Teun Koeman, Patrick R. Onck and Erik Van der Giessen; Materials Science Center, University of Groningen, Groningen, Netherlands.

The cytoskeleton of living cells consists of three types of polymer fibers, made from different proteins and with different diameters: actin microfilaments, intermediate filaments and microtubules. Like other biopolymers, the cytoskeletal fibers are semi-flexible polymeric chains which form low-density networks in the presence of appropriate cross-linking proteins. Rheological experiments on in-vitro networks reveal that their stiffness increases as they deform to large strains. It is generally accepted that this stiffening plays a key role in various cell functions. The conventional view on the origin of stiffening is the nonlinear, entropic-like response of individual filaments when subject to stretching. Using the worm-like chain model for filaments, supplemented with an affine deformation assumption for the behavior of the network, several authors have demonstrated that initially isotropic networks of, for instance, actin stiffen when thermal undulations have been stretched out. This paper presents an alternative explanation that is based on the highly non-affine distortions of actin networks and the enormous compliance for bending of individual actin filaments compared to stretching. A finite-element, periodic cell model is presented for a two-dimensional network of filaments that is subjected to shear strains of up to 25%, like in some rheological experiments. It is demonstrated that the overall response of an initially isotropic network is governed by bending of the filaments, and not by stretching. As the overall deformation increases, the network distorts and a fraction of filaments re-orients in the principal stretching direction. After a geometrical transition, the response of the network is governed by the stretching of filaments rather than bending, thus giving a significantly higher overall stiffness. It is demonstrated that the entropic-like behavior of the filaments act solely to delay the transition. It is also shown that this mechanism operates for actin as well as for microtubular networks.

9:00 AM L3.2/BB3.2

Simulation of Protein and Nanotube Interactions. Melik C. Demirel, Engineering Science and Mechanics, The Pennsylvania State University, University Park, Pennsylvania.

Carbon nanotubes are important elements at the intersection of bio-nano technology. Nanotubes can potentially be assembled using protein templates as building blocks for molecular electronic, optical, and magnetic devices. Carbon nanotubes have been functionalized with proteins with specific and nonspecific binding (e.g. triton and diamide) by several experimentalist groups. We focus on the dynamics and assembly of streptavidin on a carbon nanotube using molecular dynamics methods. We have simulated the monomeric form of the streptavidin which has 135 aminoacids. The van der Waals and hydrophobic forces play an important role in the dynamics of streptavidin on a carbon nanotube.

9:15 AM L3.3/BB3.3

Texture and Smart Anisotropy of Biological Nano-Composites. Helmut Klein[2] and Dierk Raabe[1]; [1]Microstructure Physics, Max-Planck-Institut, Duesseldorf, Germany; [2]Mineralogisch-Kristallographisches Institut, Georg-August-Universitaet Goettingen, 37077 Goettingen, Germany.

Many biological materials such as encountered in the exoskeleton structures of molusc and crustacea form biological nano-composites with highly directional mechanical and functional properties. Many of the basic structural ingredients in such structures, i.e. the various mineral components, the proteins, glycoproteins, polysaccharides, and lipids, may at least in part occur in crystalline form. These different compounds are not distributed randomly in orientation space but they typcially occur in a variety of preferred topological and crystallographic orientations which are optimized with respect to the external mechanical boundary conditions. By using x-ray wide angle analysis (laboratory-scale x-ray Bragg diffraction in conjunction with an areal detector, synchroton Bragg diffraction) we present corresponding texture investigations on different biological materials.

9:30 AM *L3.4/BB3.4

Normal and Lateral Nanomechanics of Cartilage Aggrecan Macromolecules. Christine Ortiz[1], Delphine Dean[2], Lin Han[1] and Alan Grodzinsky[2]; [1]Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, Massachusetts.

The nanomechanical behavior of cartilage extracellular matrix macromolecules has received increasing attention since their degradation causes loss of tissue and joint function with age and arthritis. For example, loss of the brush-like polyelectrolytic aggrecan macromolecules and its constituent highly charged polysaccharide glycosaminoglycan (GAG) chains significantly reduces cartilage's compressive stiffness. In this study, the conformation and compressibility of a chemically end-grafted bovine epiphyseal aggrecan brush as a function of ionic strength and normal compressive load were measured by combining the techniques of micro-contact printing and contact mode atomic force microscopy (AFM). Micro-contact printing enabled the creation of a patterned surface with densely packed, chemically end-grafted aggrecan (2590+/-90 aggrecan/μm$\hat{2}$) confined to well-defined micrometer-sized surface areas and a hydroxyl-terminated self-assembling monolayer (11-mercaptoundecanol on Au, OH-SAM) confined to the rest of the surface area. AFM imaging over aggrecan-OH-SAM boundaries enabled the measurement of the height, and hence deformation, of the aggrecan as a function of applied compressive load, solution ionic strength, and pH. With ~0 nN of applied compressive force, an uncompressed height ~350 nm at 0.001M solution ionic strength was observed to decrease nonlinearly with increasing applied compressive force down to ~60 nm at ~20 nN. Since the contour length, Lcontour, of a single aggrecan macromolecule is ~400 nm, at 0.001M the aggrecan molecules exist in a partially extended state (~0.88*Lcontour) due to intra- and intermolecular electrostatic double layer repulsion between the CS-GAG side chains. As the ionic strength of the solution was increased to 1M, salt screening of the CS-GAG electrostatic forces caused the aggrecan layer height to nonlinearly decrease to ~100 nm. Lateral force microscopy images showed that for a constant applied lateral displacement rate, the aggrecan layers exhibited a lower lateral force than the neighboring OH-terminated SAM under lower normal load (~3 nN), resulting from shearing stiffness of aggrecan layer, and reversed behavior under higher normal load (~20 nN) at 0.001M, due to the "stick-slip" mechanism between the OH-SAM tip and the aggrecan molecules on the substrate. Ongoing theoretical modeling of this data is working to simulate how the unique branched macromolecular architecture of aggrecan leads to novel electrostatic double layer repulsion profiles and conformational entropy changes depending on the solution conditions.

10:30 AM *L3.5/BB3.5

Flaw Tolerant Nanostructures of Biological Materials. Huajian Gao, Max Planck Institute for Metals Research, Stuttgart, Germany.

This work aims at developing a mechanistic theory of nanostructured materials in nature. Biological materials such as bone have achieved superior mechanical properties through hierarchical composite structures of mineral and protein. Gecko and many insects have evolved hierarchical surface structures to achieve extraordinary adhesion capabilities. We show that the unique nanostructure of these biological materials play a key role in their superior properties. We suggest that the principle of flaw tolerance, which can be related to the survivability of a living organism, may have had an overarching influence on the evolution in biology. We demonstrate that the nanoscale sizes allow the mineral nanoparticles in bone to achieve optimum fracture strength and the nanoprotrusions in Gecko to achieve optimal adhesion strength. In both systems, strength optimization is achieved by restricting the characteristic dimension of the basic structure components to nanometer scale so that crack-like flaws do not propagate to break the desired structural link. Application of the flaw tolerance principle at different hierarchical levels allows the stiffness and toughness of biological materials to be optimized up to macroscopic length scales.

11:00 AM L3.6/BB3.6

Quantitatively Studying Nano-mechanical Properties within the Prism and Organic Sheath of Enamel. Fuzhai Cui, Jun Ge and Xiumei Wang; Department of Materials Science & Engineering, Tsinghua University, Beijing, Beijing, China.

Enamel is made up of enamel prisms separated by thin layer of organic sheaths. The mechanical properties of the prisms and the organic sheaths are obviously different from each other due to different compositions and microstructures. However, quantitative measurements of such differences have been a challenge in the past. The objective of this study is to accurately study the mechanical properties in the isolated domains within single enamel prism. The technique of nanoindentation combined with Atomic Force Microscopy (AFM) was employed to test the enamel specimens from mature human maxillary third molar. It was revealed that the nanohardness and elastic modulus of the sheaths were about 73.6% and 52.7% lower than those of the prisms. AFM topographies of the residual indent impressions also visually confirmed the differences. In addition to nanoindentation tests, the microstructures of enamel were carefully investigated in terms of hierarchical levels of organization to understand the structural reasons of the mechanical differences. We found a close relation between the variations of mechanical properties of enamel and its hierarchical structure. The analysis of the mechanical properties within enamel upon hierarchy is not only helpful to understand its unique property, but may also inspire ideas for the design of novel synthetic materials.

11:15 AM L3.7/BB3.7

A Nanoindentation Method for the Determination of the Initial Contact and Adhesion of Soft Materials. Yifang Cao[1], Dehua Yang[2] and Wole Soboyejo[1]; [1]Princeton Institute of Materials Sciences and Engineering (PRISM); Department of Mechanical and Aerospace Engineering, Princeton University, Princeton, New Jersey; [2]Hysitron Inc., Minneapolis, Minnesota.

This paper presents a new method for determining the initial contact position and adhesion of soft polymeric surfaces that are relevant to microelectronics and biomedical systems. Unlike stiff/hard materials, for which depth-sensing indentation (DSI) devices (such as nanoindentation instruments) and scanning probe microscopes (particularly atomic force microscopes (AFM)) are capable of generating force versus displacement data, the deformation of soft materials is usually difficult to determine because it is strongly affected by specimen compliance and adhesion phenomena. These generally make it difficult to determine the initial contact position. In this paper, we propose a method for the determination of the initial contact point during nanoindentation. The method uses a combination of the Maugis-Dugdale adhesion theory and a non-linear least square fitting to obtain zero indentation offset distance, the transition parameter and contact radius at zero load directly. The work of adhesion and the reduced elastic modulus are then determined indirectly. The method is used to analyze the load-displacement characteristics of poly-di-methyl-siloxane (PDMS), which is a material that is being used increasingly in microelectronics and bio medical systems.

11:30 AM *L3.8/BB3.8

Strain-Stiffening in Semiflexible Biopolymer Gels. Paul Albert Janmey[1], Cornelis Storm[3], Fred C. MacKintosh[2] and Tom C. Lubensky[1]; [1]University of Pennsylvania, Philadelphia, Pennsylvania; [2]Vrije Universiteit, Amsterdam, Netherlands; [3]Institut Curie, Paris, France.

Unlike most synthetic materials, biological materials often stiffen as they are deformed. This nonlinear elastic response, critical for the physiological function of some tissues, has been documented since at least the 19th century, but the molecular structure and the design principles responsible for it are unknown. A structural feature of many biopolymer systems is that filamentous biopolymers tend to be much stiffer than synthetic polymers, with persistence lengths ranging from 50 nm for DNA to >400 nm for intracellular intermediate filaments and extracellular fibrin protofibrils to the much stiffer polymers like F-actin, collagen, and microtubules that have persistence lengths from 10 micron to > mm. Most biopolymers are also quite long, reaching lengths well over a micron both in vivo and in vitro. As a result, the networks formed by these polymers in

aqueous solutions can be very dilute and still exhibit large elastic moduli. In many cases found in vivo and reconstituted in vitro, the mesh size of a biopolymer network is of the same order as the persistence length of the polymer, and theories to relate molecular architecture to macroscopic rheology based on those developed for rubberlike systems no longer apply and cannot predict the strain-stiffening seen in experiments. A new model for macroscopic elasticity based on the non-linear force-extension relation of a semi-flexible chain has been developed and compared with experimental measurements of a number of strain-stiffening biological hydrogels. This model accounts for strain stiffening in a wide range of molecularly distinct biopolymer gels formed from purified cytoskeletal and extracellular proteins. The good agreement of theory with experiment shows that systems of semi-flexible chains, such as filamentous proteins arranged in an open crosslinked meshwork, invariably stiffen at low strains without the need for a specific architecture or multiple elements with different intrinsic stiffnesses.

SESSION L4: Soft Tissues I
Chairs: Michael D. Morris and Fred H. Silver
Wednesday Afternoon, March 30, 2005
Room 3004 (Moscone West)

1:30 PM *L4.1
AFM Imaging and Nanomechanical Testing of Cells and Tissues. Xiaodong Li, Department of Mechanical Engineering, University of South Carolina, Columbia, South Carolina.

Characterization of the structure and mechanical properties of cells and tissues at the nanoscale imposes a tremendous challenge to many existing imaging and mechanical testing techniques and instruments. AFM has been used to image dry and wet cells and tissues. Experimental and instrumentation difficulties in imaging are discussed. Nanomechanical testing techniques such as nanoindentation, axial tensile testing, and in-situ AFM tensile testing are presented. Viscoelastic property measurement techniques have been used to assess the storage and loss moduli and creep resistance. The technique for charactering fatigue properties is described. Instrumentation limitations and calibration techniques are discussed.

2:00 PM L4.2
Structural Mechanics of Mitochondria.
Prashant Kishore Purohit, Physics and Astronomy, University of Pennsylvania, Philadelphia, Pennsylvania.

Powerful techniques in electron microscopy have led to a need to re-assess our thinking of the structure of intracellular organelles. A compelling example is the structure of the inner mitochondrial membrane obtained using cryo-electron tomography. It has now been convincingly demonstrated that the classic text book view of mitochondrial structure which hypothesizes lamellar infoldings of the inner membrane is erroneous. The structure is much richer and it includes tubular, vesicular and lamellar cristae. In this presentation we explore the shapes of the inner mitochondrial membrane using the mechanics of lipid vesicles. We show that the observed shapes are minimisers of the Helfrich energy under appropriate geometrical constraints, such as fixed volume or confinement. Moreover, we also see how these shapes can change in response to the osmotic environment, lipid composition, respiratory state and the presence/absence of key proteins. In particular, our model explains the 'onion' like shapes seen in many experiments and myopathic mitochondria. Other predictions from the model are being tested in our lab through experiments on mitoplasts. Our model has helped garner a quantitative understanding of mitochondrial structure and we hope that it will have larger implications on the study of other intracellular organelles.

2:15 PM L4.3
Protein Forced Unfolding and its Effects to the Finite Deformation Stress-Strain Behavior of Biomacromolecular Membrane and Solids. H. Jerry Qi[1], Christine Ortiz[2] and Mary C. Boyce[3]; [1]Mechanical Engineering, University of Colorado, Boulder, Colorado; [2]Material Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [3]Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Many mechanical load-carrying proteins, such as titin and spectrin, have been experimentally observed to exhibit a characteristic repeating pattern of a nonlinear rise in force with imposed displacement to a peak, followed by a significant force drop upon reaching the peak (a saw-tooth pattern) in their force-extension behavior due to successive unfolding of modules during extension. This behavior is speculated to play a governing role in the biological and mechanical functions of materials constituted of such protein networks. In this paper, models for the mechanical behavior of single modular macromolecules and networks of such macromolecules are developed. A constitutive model for the force-extension behavior of a single modular macromolecule is presented using the Freely Jointed Chain (FJC) and Worm-Like Chain (WLC) models of statistical mechanics together with a new unfolding criterion based on the orientation distribution of folded modules with respect to the chain stretch. The single molecule behavior is then used together with a formal continuum mechanics approach to construct constitutive models of the finite deformation stress-strain behavior of two- and three-dimensional macromolecular membranes and solids containing folded modules. The model for two-dimensional networks has applicability to biological membrane skeletons, and the model for three-dimensional networks provides a constitutive model for cytoskeletal networks and solid biological tissues containing modular macromolecules. Simulations of these networks under different loading conditions illustrate features of the stress-strain behavior of these materials and the stretching conditions which activate unfolding in these microstructures. The proposed models are based on statistical mechanics where the material parameters are related to the structure of a single macromolecule, and therefore allow for parametric study of the influence of these structural features on the stress-stretch behavior and enable the understanding of the manner in which the characteristic single molecule behavior is translated to membrane and solid behavior. These constitutive models for the membrane and the solid thus are a starting point for understanding the role of modular protein mechanical behavior in issues of cell mechanics as well as in issues of protein-rich biological materials which may act as load transfer agents in biological composite structures such as spider silk and the adhesive layers of the nacre of abalone shell.

2:30 PM *L4.4
Probing Energy Landscapes of Single Bio-Molecules by Mechanical Forces. Matthias Rief, Technical Univ Munich, Garching, Germany.

The mechanical properties of cytoskeletal proteins and molecular motors are important for their function in vivo. However, this information has become accessible only recently through the invention of single molecule techniques like atomic force microscopy or optical tweezers. In the talk I will show how application of a mechanical force can be used to probe the energy landscape of protein molecules

3:30 PM *L4.5
Fiber Spinning in Nature - Models for Polymer Design, Assembly and Function. Cheryl Wong, Chunmei Li, Bruce Panilaitis, Guillermo Castro, Hania Dames, Peggy Cebe and David L. Kaplan; Biomedical Engineering, Chemical/Biological Eng., Chemistry, Physics, Tufts University, Medford, Massachusetts.

Fibrous proteins, including silks, provide an important model for the study of protein-protein interactions leading to fiber formation during spinning by insects and spiders. Silks provide fertile ground for fundamental and applied inquiry due to the novel structures and properties of these fibers. Silks represent some of the most hydrophobic biopolymers generated in Nature, yet these proteins are solubilized to 30 weight percent in water in storage glands prior to spinning into fibers. This control of solubility is accomplished while avoiding premature crystallization into beta-sheet structures that would result in premature precipitation and catastrophic consequences to the organism. Chain folding and supramolecular assembly proceed within the context of sequence chemistry and the limitations imposed by an all aqueous processing environment. Subsequently, the proteins are formed into fibers with remarkable mechanical properties, and post spinning, the proteins are no longer soluble in water. The novel mechanisms utilized to accomplish the above outcome will be described, which has led to new ways to think about how to marry polymer chemistry designs with aqueous processing environments, leading to control of protein folding and assembly into hierarchically ordered structures. A second example of fiber spinning in Nature comes from bacterial cellulose. These fibers are spun via membrane pores at a size scale orders of magnitude smaller than for silks. The design rules applicable in this system are interesting in terms of comparison with the protein spinning systems in insects and spiders. Despite differences in scale and polymer chemistry, some of the features are similar and in all cases, the outcomes are fibers with remarkable materials properties. With new insight into these processes opportunities now exist to expand the features of these materials, attainable via genetic manipulation, physiological controls or post spinning processing. Some of these studies will be described along with some of the new materials and outcomes derived from these systems.

4:00 PM L4.6
Raman Spectroscopy Evidence of Self-Assembly Associated Conformational Events of the Alanine Motif in Spider Dragline Silk. Xiaojun He[1], Jacqueline M. Palmer[2] and Michael S. Ellison[1]; [1]Sch of Mat Sci & Engr, Clemson University, Clemson, South Carolina; [2]Genetics, BioChem and Life Sciences, Clemson

University, Clemson, South Carolina.

In recent years, insights into the microstructure assumed by the sequence of protein motifs in spider dragline silk, as well as their associated functions, have been obtained using XRD, NMR, IR and Raman Spectroscopy [1-6]. The dragline silk used in our study was collected by forced silking of cold-anesthetized Nephila clavipes spiders at a speed of 6.1 cm/s at room temperature. A tensile test with a concurrent in-situ Raman spectroscopy study (polarized and non-polarized) was performed in our lab to investigate stress-induced morphology transitions in N clavipes dragline silk. Our results support an hypothesis of self-assembly in these transitions. Spider silk samples of 40mm length were stretched in a step-wise manner, at a rate of 15 mm/min. We collected polarized Raman spectra with a Renishaw System 100 imaging probe. Spectra were recorded both immediately after imposition of the strain and again after allowing 10 hours for stress relaxation. The polymorph assignment of the dragline silk spectrum was made through a comparative study of the silk data with data corresponding to the known morphology of poly-L-Alanine fibers prepared in our lab. From these data we inferred that there were two β-structure entities in dragline silk, which were represented by the 1094 cm-1 and 1065 cm-1 bands originating from the Cα-Cβ (C-CH3) stretch of β-polyalanine [7]. Furthermore, the β-strand structure associated with the 1094 cm-1 band was randomly oriented; however, the β-sheet structure, corresponding to the 1065 cm-1 band, was highly axially oriented. Significant spectral changes at selected strain levels after stress relaxation lead us to conclude that self-assembly was accomplished in two steps: the formation of β-sheet alanine precursors induced by the stress and the subsequent addition of β-strand to the precursors during the stress relaxation. Our results showed that a critical concentration of β-sheet alanine precursor ($0.378 < \text{Ccrit} < 0.45$) must be reached to effect the self-assembly. Parallel experiments on silks taken randomly along the silk strand confirmed that self-assembly is a hallmark in stress-induced morphology transitions in N clavipes dragline silk and the extent of self-assembly was strongly influenced by its initial morphology. 1. Thiel, B. L.; Guess, K. B.; Viney, C. Biopolymers 1997, 41, 703-719. 2. Riekel, C.; Branden, C. I.; Craig, C.; Ferrero, C.; Heidelbach, F.; Muller, M. International Journal of Biological Macromolecules 1999, 24, 179-186. 3. Simmons, A. H.; Ray, Ed.; Jelinski L. W. Macromolecules 1994, 27, 5235-5237. 4. Simmons, A. H.; Michal, C. A.; Jelinski, L. W. Science, 1996, 271, 84-87. 5. Shao, Z.; Vollrath, F.; Sirichaisit, J; Young, R. J. Polymer 1999, 40, 2493-2500. 6. Sirichaisit, J.; Brookes, V.; Young, R. J.; Vollroth, F. Biomacromolecules 2003, 4, 387-394. 7. Dwivedi, A. M.; Krimm, S. Macromolecules 1982, 15, 186-193.

4:15 PM L4.7
Mechanically and Electrostatically Driven Patterns at Cell-Free Inter-Membrane Junctions. Raghuveer Parthasarathy[1] and Jay T. Groves[1,2]; [1]Chemistry, University of California at Berkeley, Berkeley, California; [2]Physical Biosciences and Materials Science Divisions, Lawrence Berkeley National Laboratory, Berkeley, California.

To explore the relations between the material properties of biological membranes and the spatial organization of membrane components, we have constructed a simple cell-free inter-membrane junction that exhibits a hierarchy of pattern formation. Mobile, membrane-bound proteins sandwiched between lipid bilayers are mechanically driven into micron-scale spatial patterns due to the coupling of their lateral mobility to the inter-membrane adhesion. Multiple complimentary optical tools, including fluorescence resonance energy transfer and fluorescence interference contrast microscopy, allow reconstruction of the nanometer-scale topography of the molecular patterns. These mechanically driven protein patterns can electrostatically generate patterns of charged membrane lipids. Measuring the magnitude of this electrostatic interaction as a function of lipid composition and ionic strength, and analyzing the interplay between thermodynamics and electrostatics via a Poisson-Boltzmann approach, we are able to determine the charge density and surface potential of the junction molecules. Surprisingly, we find that the electrostatic interactions are dominated by our experiments' supported lipid bilayer and its underlying glass support, rather than by the proteins' electrostatic properties; the proteins' role is to modulate the nanometer-scale topography of the junction. The mechanical and electrostatic interactions uncovered in these simple systems highlight the rich self-organizing capabilities of membrane materials.

4:30 PM L4.8
Rheological and Network Properties of β-Hairpin Molecules -The Effects of Ionic Strength and Strand Length.
Bulent Ozbas[1,3], Karthikan Rajagopal[2], Joel P. Schneider[2] and Darrin J. Pochan[1,3]; [1]Materials Science and Engineering, University of Delaware, Newark, Delaware; [2]Chemistry and Biochemistry, University of Delaware, Newark, Delaware; [3]Delaware Biotechnology Institute, Newark, Delaware.

In this work we present the formation of hydrogels via the intramolecular folding and consequent self-assembly of β-hairpin peptide molecules. The effect of ionic strength and strand length on the β-sheet formation and self-assembly are studied. The peptide molecules are locally amphiphilic with two linear strands of alternating hydrophobic valine and hydrophilic lysine amino acids flanking a central ^{D}Proline-^{L}Proline-Threonine turn sequence. Circular dichroism spectroscopy shows that at pH 7.4 molecules are unfolded in the absence of salt. By raising the ionic strength of the solution the electrostatic interactions between charged lysines are screened and the peptide arms are forced into a parallel β-sheet secondary structure by the turn sequence. These folded molecules supramolecularly assemble via hydrophobic collapse and hydrogen bonding into a three dimensional network. The network properties and the nanostructure of the hydrogels are studied by rheology, TEM and SANS. Rheological measurements demonstrate that the resultant supramolecular structure forms an elastic material, whose structure, and thus modulus, can be tuned by salt concentration and temperature. The hydrogel network is composed of semiflexible fibrillar assemblies with viscoelastic behavior that follows the theoretical prediction for heavily crosslinked, semi-flexible polymer networks. Neutron scattering results show that the cross-sectional diameter of the fibrils can be varied by changing the number of amino acids of the β-hairpin molecules. Dynamic oscillatory and transient rheological measurements reveal that rigidity of the hydrogels and their creep and relaxation behavior also vary with the strand length. Hydrogels recover quickly to the original viscoelastic state after cessation of high magnitude of shear. The effects of temperature, ionic strength and peptide chemistry on this recovery behavior are studied.

4:45 PM L4.9
Molecular Modeling and Characterization of the Amino Propeptide Domain of Type XI Collagen α1 Chain and its Role in the Regulation of the Collagen Framework.
Lisa Rose Warner[1,2] and Julia Thom Oxford[1]; [1]Biology, Boise State University, Boise, Idaho; [2]Materials Science and Engineering, Boise State University, Boise, Idaho.

Collagen containing tissues are composite biomaterials that contain water, collagen, proteoglycans and proteins. Like any composite material, the components themselves and their interactions and assembly dictate the materials properties of the final product. Fibrillar collagens are the principal structural molecules of the connective tissues and require regulated assembly and growth in order to build an extracellular matrix (ECM) that is characteristic of a specific tissue. Assembly of the ECM determines the mechanical function of connective tissues such as cartilage, tendon, skin, bone, and arteries. In these different tissues, there is a stark difference in percent composition of collagen types in the fibril and the assembly of the framework collagen network. In comparing young and mature cartilages, fine fibrils of young growth cartilages contain $\geq$ 10% collagen IX, $\geq$ 10% collagen XI, and $\leq$ 80% collagen II, while the thicker and more varied fibril diameters of mature articular cartilage contain $\sim$1% collagen IX, $\sim$3% collagen XI, and $\geq$ 90% collagen II. The organization of the collagen fibrils is also an important variable in influencing the mechanical properties of a tissue. The tension-resisting property of fibril-forming collagens is the principal means of limiting the range of motion of joints, distributing forces generated by muscles, tranferring tensile strength to the bony skeleton, and resisting extension by the surface layers of articular cartilage. The arrangement and alignment of the collagen fibrils reflect the mechanical stresses acting on the tissues. Thickness of fibrils is an important structure/function regulator of collagen containing tissues. The thickness of collagen fibrils in the vitreous gel of the eye for example, are thin and disperse. As a normal consequence of aging and in some diseases, the collagen fibrils thicken and the vitreous gel undergoes liquefaction, which has been linked to retinal and vitreal detachment as well as nuclear cataracts. In cartilage the fibrils are necessarily thicker so as to bear greater loads; however in osteoarthritic cartilage it has been shown that collagen fibrils are abnormally thick, to the detriment of the tissue. In normal collagen fibrils, the amino propeptide (Npp) domain of collagen type XI α1 chain regulates fibril diameter growth. Npp is a globular domain which is thought to sterically hinder the dense packing assembly of collagen molecules in fibrils. This mechanism of regulating collagen fibril assembly may be more complex than steric hindrance. It is our hypothesis is that the Npp domain has a more dynamic role in the structure/function of collagen fibrils in connective tissues. The 3D structure of Npp was predicted by molecular modeling. Docking studies showed putative binding sites for heparan sulfate and divalent cations. These predicted binding sites were evaluated empirically by fluorescence spectroscopy and surface plasmon resonance.

SESSION L5: Poster Session: Biological Materials
Chairs: Peter Fratzl, William J. Landis, Fred H. Silver and Rizhi Wang
Wednesday Evening, March 30, 2005
8:00 PM
Salons 8-15 (Marriott)

L5.1
Viscoelastic Models Describing Stress Relaxation and Creep in Soft Tissues. Alexandr V. Kobelev[1,2], Yuri L. Protsenko[2], Irina V. Berman[3], Rimma M. Kobeleva[2] and Oleg Alex Kobelev[4]; [1]Inst of Metal Physics, Yekaterinburg, Russian Federation; [2]Inst of Immunology and Physiology, Yekaterinburg, Russian Federation; [3]Physical Department, San Jose Univ, San Jose, California; [4]Urals State Technical Univ, Yekaterinburg, Russian Federation.

One of the physiological functions of viscosity in living tissues is to prevent their breaks at fast deformations. Practically all biological tissues possess viscosity, however its nature in muscle tissues is unclear till present time. The most prominent features in which this friction appears in the experiments are the stress relaxation and the creep. Up till the present time, they are modeled within one-dimensional (1D) rheological models similar to four-element Burgers-Frenkel model. These models are tracing back to classical ones by Kelvin and Maxwell, in which the elastic (spring) and viscose (damp) elements are connected, consequently, in parallel, or in series. Recently it was pointed out by us that the non-linear steady-state stress-strain curves and quasi-static hysteretic force - length loops may be reproduced in the 2D models by a simple geometric factor involved in strained biological tissues, the so called unfolding effect of transversal vs. inclined elastic elements. An adequate description for papillary muscles was achieved using classical linear elastic elements combined into the 2D structure similar to the morphological functional unit of myocardium. The transversal elastic spring in 2D models plays an essential role hampering the unfolding and restoring the initial configuration after removal of the load. The viscosity has been taken into account using Kelvin blocks and it was found that the shape of hysteretic stress-deformation loop may indicate which elements viscosity prevails in the sample tissue. We use most primitive 2D models according to the principle of primary elements minimal number introduced in order to simulate observed rheological features. The models with parallel and series blocks combined in various topological structures show quite good possibility to describe the most characteristic steady state, hysteretic and stress relaxation features in living soft tissues. It is so, even in the models with Kelvin blocks, that can not give in 1D the stress relaxation itself. They do so if included in 2D models, due to an extra degree of freedom provided by two-dimensionality. This way, the relation between the structure and the function manifests itself in various models corresponding to different type of the tissue morphology. The most reliable model, with respect to experimental data, may reflect the most probable and working structure of the preparation. The non-linearity of stress-deformation relation in the case of stress relaxation leads to the dependence of the spikes amplitude at the equal-step deformation on the working length, determined by the step number. Moreover, the relaxation rate depends on the step number for the same reason. In other words, that means that if the soft element with low viscosity works first, the relaxation rate will be small at the first step. Then, when the hard elements with greater viscosity predominantly start working, the relaxation rate raises at the next deformation steps.

L5.2
Mesostructure, Microstructure and Anisotropy of the Lobster Cuticle. Patricia Romano[1], Ali Al-Sawalmih[1], Christoph Sachs[1], Dierk Raabe[1] and H.-G. Brokmeier[2]; [1]Microstructure Physics, Max-Planck-Institut, Duesseldorf, Germany; [2]GKSS-Forschungszentrum, Geesthacht, Germany.

The crustacean shell of lobster (homarus americanus) is an excellent example of a bio-nano-composite with variable properties and substantial structural and mechanical anisotropy which manifests itself both, at the microscopic and at the macroscopic scale. As in all the mineralized tissues, the mineral components of the mollusk shell are associated with complex organic matrices. The mollusk shells are composed of highly organized nanocomposites of CaCO3 crystals (aragonite, calcite) and organic macromolecules, which include proteins, glycoproteins, polysaccharides and lipids. The most important polysaccharide is the chitin, a cellulose-like biopolymer, one of the most abundant in nature that acts as a framework for the inorganic material. This study is concerned with experiments for a better understanding of the mesostructure and microstructure in such materials. We concentrate the investigtaions on the lobster claw. For the structure and microstructure characterization experiments we use light optical transmission microscopy, laboratory-scale x-ray diffraction in conjunction with an areal detector, synchroton radiation (DESY), high resolution scanning electron microscopy, and transmission electron microscopy. In order to identify all the different components in the material, some of them were gradually removed by tailored chemical etching. The microstructure of the lobster claw could then be observed after the subsequent removal of certain parts of the organic matrix, mainly of the proteins, and after the removal of the mineral phase. We will discuss the structures observed at the different scales and give details about the intricate procedures required for sample preparation.

L5.3
Electromagnetic Properties of the Lobster Cuticula. Ali Al-Sawalmih and Dierk Raabe; Microstructure Physics, Max-Planck-Institut, Duesseldorf, Germany.

Lobster shell is a natural chitin-based bio-nano-composite, where chitin fibers are covered by a protein matrix, associated with an inorganic fraction of biomineralization (calcite CaCO3). These materials are not only textured entailing crystallographic and topological anisotropy, but also are its properties site-dependent within the same lobster shell owing to the local variation in the calcite density. The objective of the present study is to investigate the relationship between the electrical properties and the structure of the lobster cuticula. Since the shell is a porous composite material with strong structural and mechanical anisotropy its electrical characteristics are expected to be complex. DC and AC measurements were carried out for different samples taken from different positions within the lobster. They were investigated in different current-flow directions in order to study the anisotropy. The measurements were conducted using digital multimeters designed for high resistance measurements. We present results on the DC resistivity, dielectric constants, relative permittivity, and loss factors. The data are correlated with the observed microstructures.

L5.4
Influence of Cross-Linking and Oxidation on the Microstructural Mechanical Properties of UHMWPE. Marcel Roy, Biomedical Engineering, Saint Louis University, St. Louis, Missouri.

Background This study examined the effects of cross-linking and oxidation on the microstructural mechanical properties of ultra-high molecular weight polyethylene (UHMWPE). UHMWPE, commonly used as a bearing surface in total joint implants, exhibits improved wear resistance when cross-linked [1]. Cross-linking followed by re-melting quenches radiation-induced free radicals, decreasing yield strength slightly but improving long-term resistance to oxidation while keeping the improved wear properties [2]. The degradation of the mechanical properties of UHMWPE due to an oxidation peak, due to free radical accumulation 0.5-2 mm below the surface, coincides with the extremely brittle subsurface "white banding" region observed in cross sections of aged and retrieved implants [3-5]. Methods Nanoindentation tests (TriboIndenter; Hysitron) were performed using a Berkovich diamond indenter, with tip geometry calibrated via fused silica. Following each test, the reduced modulus was calculated from the initial part of the unloading curve [6]. Small slices of the articulating surfaces of two machined GUR 1050 UHMWPE acetabular inserts (Wright Medical Technology), one of which was cross-linked by an unspecified method, were cut and glued to metal stubs without polishing. Tests were also performed along a freshly cut cross-section of a UHMWPE tibial insert (Exactech) that had been sealed in its original packaging and shelf-aged at least five years. One corner of the insert was sectioned for embedding in epoxy resin and polishing. Results and Discussion The reduced modulus of cross-linked UHMWPE (0.75 MPa) was significantly lower than native UHMWPE (0.88 MPa; two-sided t-test, $p < 0.0001$). The rough as-machined surfaces contributed to the large standard deviations, but the data generally agree with the literature [7-10]. Data from two experiments performed in adjacent areas on the same shelf-aged specimen cross-section revealed a peak in reduced modulus ($\sim$1.5 MPa) centered $\sim$1 mm below the surface. Although there was considerable scatter, the reduced modulus plot was similar to the oxidation profiles of aged UHMWPE observed by others [5], and a similar stiffness profile distribution has been reported [11]. Acknowledgments Instrumentation used in this study was supported by the NSF (DMR 0076497). The author also gratefully acknowledges the late Jae-Young Rho, Ph.D. for use of this instrument. References 1. Goldman M and Pruitt L. J Biomed Mater Res 40:378, 1998. 2. McKellop HA et al. J Orthop Res 17:157, 1999. 3. Sutula LC et al. Clin Orthop 319:28, 1995. 4. Daly BM and Yin J. J Biomed Mater Res 42:523, 1998. 5. Jacob RJ et al. J Biomed Mater Res 56:168, 2001. 6. Oliver WC and Pharr GM. J Mater Res 7:1564, 1992. 7. Schmidt MA et al. Trans 6th Biomat Congr, 1255, 2000. 8. Klapperich C et al. J Tribol 123:624, 2001. 9. Klapperich C et al. J Mater Res 17:423, 2002. 10. Zhou et al. J Tribol 126:386, 2004. 11. Woodard S et al. Trans 25th Amer Soc Biomech, 2001.

L5.5
Three-Dimensional PEG Hydrogel Construct Fabrication

using Stereolithography. Karina Arcaute[1,2], Luis Ochoa[1,2], Francisco Medina[1,2], Christopher Elkins[3] and Ryan Wicker[1,2]; [1]Mechanical & Industrial Engineering, University of Texas at El Paso, El Paso, Texas; [2]W.M. Keck Border Biomedical Manufacturing and Engineering Lab, El Paso, Texas; [3]Mechanical Engineering and Radiology, Stanford University, Stanford, California.

Rapid prototyping (RP) is generally becoming accepted as the most capable manufacturing method for tissue engineering (TE), since by its layered manufacturing (LM) nature, control over scaffold characteristics as well as placement of cells and bioactive agents within the scaffold are possible. However, the ideal RP process has yet to be determined and virtually all commercially available technologies have been explored with varying vigor and degrees of success. Surprisingly, although stereolithography (SL) was the first commercial RP technology and has remained one of the most accurate technologies available today, SL is underutilized in TE. One potential reason may be a lack of implantable polymers available for use in SL, although photocrosslinked hydrogel polymers may represent a solution as these materials are widely used in TE. To demonstrate the capabilities of SL in fabricating complex constructs and expand its use in TE, the present work employs hydrogels based on poly (ethylene glycol) (PEG) in a modified commercial SL system with a 325 nm, 40 mW He-Cd ultra-violet laser. The modified SL system includes a custom platform and hydrogel receptacle to accomodate an automated fill and remove pump system. For the experiments, two photoinitiators (PIs) were used, including 2-hydroxy-2-methyl-1-phenyl propanone or HMPP and 1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one or IRGACURE 2959 placed in 30%W PEG-dma (PEG dimethacrylate 1000MW) in distilled water solution. Cure depth curves were developed as a function of PI concentration with concentrations ranging from 0.04-5%. These curves determine the depth of penetration for a given laser power and scan speed, and allow for prescribing layer thicknesses in the LM approach. Results show that the cure depth increases and peaks at an intermediate value before decreasing asymptotically to a non-zero value. The maximum cure depths measured for a given laser energy and both HMPP and IRGACURE 2959 were ~17mm and ~1.95 mm, respectively, corresponding to PI concentrations of 0.04% and 0.1%, respectively. Using these curves, multiple complex gels were fabricated in single layers using both PIs. HMPP allowed for significantly greater layer thicknesses as a result of its curing characteristics. However, complex 3D constructs with internal channels were more readily fabricated using IRGACURE 2959. The successful fabrication of tailored 3D TE constructs with specific properties and chemical sequences appears feasible and may require incorporation of multiple PIs with varying concentrations, multiple PEGs and other materials, and effective strategies for fabrication using SL and other potentially integrated manufacturing technologies for controlling placement of bioactive agents and cells.

L5.6

Nanoscale Microstructure and Mechanical/Tribological Behavior of Cross-Linked UHMWPE. Jikou Zhou[1], Xinrui Niu[1], Dele Popoola[3], Nan Yao[2] and Wole O. Soboyejo[1]; [1]Mechanical and Aerospace Engineering, Princeton University, Princeton, New Jersey; [2]Princeton Institute for Science and Technology of Materials, Princeton University, Princeton, New Jersey; [3]Zimmer, Inc., Zimmer, Inc., Iowa.

This paper presents the results of a study of the nanoscale microstructure and mechanical/tribological properties of cross-linked ultra-high molecular weight polyethylene (UHMWPE) that is being used increasingly in biomedical applications. The structure of the cross-linked UHMWPE is elucidated via transmission electron microscope and atomic force microscopy. The local variations in mechanical and tribological properties of the lamellar crystalline and amorphous regions are then studied using nanoindentation and nano-scratching techniques. The paper concludes by explaining the influence of in situ deformation on the orientation of lamellar crystalline regions within the cross-linked structure.

L5.7

Functional Gradients and Mechanical Properties of the *Mytiluscalifornianus* Byssal Attachment Plaque. Scott A. Jewhurst[1] and J. Herbert Waite[1,2]; [1]Marine Science Institute, UC Santa Barbara, Santa Barbara, California; [2]Department of Molecular, Cell and Developmental Biology, University of California, Santa Barbara, California.

The marine mussel Mytilus californianus inhabits the nutrient-rich, but severely wave-swept intertidal zone, living as a sessile organism attached by an adhesive tether (byssus) to a substratum (rocks, pilings, other mussels, etc.). The byssus, consisting of an adhesive anchor plaque, a thread, and the stem which links the byssus to the organisms retractor muscles, is a sophisticated protein composite well designed for its task. Previous research has shown that the byssal thread is a functionally graded material, using tailored proteins to produce a gradient in the elastic modulus and yield behavior, of which one possible function is to mediate the stresses caused by the elastic modulus mismatch between the substratum (Ey ~ 1-200 GPa) and the organisms retractor muscles (Ey = 0.2 MPa). Large, abrupt transitions in the elastic modulus would result in catastrophic stress concentrations when a load is placed on the byssus, but may be mitigated by having a more gradual transition in the modulus, dispersing the stress over a larger volume. Recent research has discovered that the adhesive anchor plaque, consisting primarily of polymeric protein foam with seawater filled pores, is also a functionally graded material which exhibits a porosity dependent stiffness (effective modulus) gradient between the substratum and the distal portion of the byssal thread, which should reduce the interfacial stress between the byssal thread and the substratum by dispersing it over the volume of the plaque. The porosity gradient of the plaque has been determined to be tailored to the stiffness of the substratum to which it is attached, within the limits of the organism to do so. The plaque porosity is multifunctional, also serving as a hydration reservoir and as a hydraulic shock-absorber, dissipating energy from tension applied to the byssus through capillary drag and osmotic pressure on the pore fluid. The role of the plaque-substratum interfacial chemistry on the plaque properties are also investigated, as the chemistry of this interface may affect the post-translational modifications of the plaque proteins by the organism in order to optimize the plaque properties for a specific substratum.

L5.8

Study of Elasticity and Viscoelasticity of Human Cortical Bone by Nanoindentation. Leandro de Macedo Soares Silva[1], Vincent Ebacher[1], Danmei Liu[2], Heather McKay[2], Thomas R. Oxland[2] and Rizhi Wang[1]; [1]Department of Materials Engineering, University of British Columbia, Vancouver, British Columbia, Canada; [2]Department of Orthopaedic Surgery and Mechanical Engineering, University of British Columbia, Vancouver, British Columbia, Canada.

Bone is a composite material combining a fibrous matrix (predominantly type I collagen) and hard mineral plates (dahllite nanocrystals) in its primary level of organization. The degree of mineralization varies from bone to bone and also in different locations of an individual bone. Bone is a viscoelastic material. The mechanical properties strongly depend on orientation, degree of mineralization, as well as water content. Nanoindentation is a valuable technique in determining the mechanical properties of bone. Due to its impressive capability of making indentations in the submicron dimension, it becomes possible to investigate the mechanical properties locally in the bone, for example, the Haversian system and to evaluate the effect of bone mineral density, and water content in the bone as a material. Compared with conventional indentation technique, nanoindentation with dynamic loading function could test both elastic modulus and viscoelastic properties. In this study, depth sensing indentation methods (MTS Nano Indenter XP) were applied to test human cortical bone. A small oscillatory load is superposed on the primary loading signal, allowing measurements of the mechanical properties as a continuous function of surface penetration (Continuous Stiffness Measurement −CSM). The data collected include elastic modulus, loss angle, hardness and their depth dependence. Up to 500 indentations were carried out on the transversal sections of three human tibias in both dry and wet conditions. The indentations cover both Haversian bone and interstitial bone from periosteal site to endosteal site. The results showed no statistical differences in bone properties across the cortex. Hydration of bone dramatically decreases elastic modulus and hardness, but significantly increases viscoelasticity. Detailed statistics will be presented along with preliminary bone mineral density results by peripheral quantitative computed tomography (pQCT).

L5.9

Probing Electromechanical Properties of Biological Systems Down to the Nanoscale. Alexei Gruverman[1], Brian J. Rodriguez[2] and Sergei V. Kalinin[3]; [1]Materials Science and Engineering, North Carolina State University, Raleigh, North Carolina; [2]Physics, North Carolina State University, Raleigh, North Carolina; [3]Condensed Matter Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

There is currently a paradigm shift in nanotechnology from silicon-based devices to molecular and biological systems. To design functional bionanodevices and biomaterials, the physical properties of biological systems need to be characterized with the highest possible resolution. In this paper, a combination of scanning probe microscopy (SPM) methods has been applied to study nanoscale elastic and electromechanical properties of biological systems including abalone shell, hair, and human tooth. Strong electromechanical coupling is a universal property of biological systems. For example, piezoelectric properties were found in number of proteins, such as collagen and keratin. Here, we demonstrate the use of Piezoresponse Force

Microscopy (PFM) to obtain a 3-dimensional map of electromechanical activity in various biological systems with nanoscale resolution. One of the examples includes electromechanical characterization of enamel and dentine layers in human tooth. These studies are complemented by Ultrasonic Force Microscopy (UFM) measurements, which provide information on local elastic properties. It is emphasized that the SPM techniques allow local electromechanical and elastic properties to be measured in systems that are inaccessible by macroscopic techniques. As an example, 3D piezoelectric properties and local elasticity of the butterfly wing are measured with nanoscale resolution and interpreted in terms of the relative orientation of chitin molecules in the wing scales. Furthermore, the 3D electromechanical response of a bundle of collagen molecules in a human tooth has been visualized with nanoscale resolution. The future prospects of SPM for electromechanical characterization of complex biological systems are discussed. AG acknowledges financial support of the National Science Foundation (Grant No. DMR02-35632). Research performed as a Eugene P. Wigner Fellow (SVK).

L5.10
Synthesis and Nanomechanical Studies of Biomimetic Modular Multidomain Polymers. Jason T. Roland, Dora Guzman, Jane Z. Bai and Zhibin Guan; Chemistry, University of California, Irvine, California.

A specific challenge in biomaterials research is to design polymers that have a combination of mechanical strength, fracture toughness, and elasticity - three fundamental mechanical properties that are highly desired but usually exclusive to each other in biomaterials. Many structural biopolymers, such as cell adhesion proteins and muscle protein titin, employ modular domain structures to achieve the combination of these three fundamental mechanical properties in one system. Single molecule nanomechanical studies on titin and other modular proteins suggest that these exceptional properties arise from a modular elongation mechanism. The sequential unfolding allows modular biopolymers to sustain a large force over the whole extension of the chain, which makes the polymer strong, along with a large area under the force-extension curve, making it tough as well. In addition, when the external force is removed, the unfolded domains of modular proteins will refold automatically, making them elastic. To mimic nature, our laboratory has developed a number of synthetic polymers having modular multidomain structures with the aim to design biomaterials having a combination of mechanical strength, toughness, and elasticity. The mechanical properties of the synthetic modular polymers are studied both at single-molecule level using atomic force microscopy (AFM) and bulk level using Instron. Single-molecule force-extension experiments revealed similar sawtooth patterns as observed in titin. This suggests the modules on a polymer chain sequentially unfold as the chain is stretched. Systematic studies are currently underway to understand the fundamental relationship between polymer nanostructures and polymer physical properties. Biomaterials having combined mechanical strength, toughness, and elasticity should find many biomedical applications such as implants and tissue replacements for both soft and hard tissues.

L5.11
Combining Nanoindentation and qBEI for a Better Understanding Bone Strength. Markus Weber[1,2], Paul Roschger[2], Thomas Schoeberl[1], Klaus Klaushofer[2] and Peter Fratzl[3]; [1]Erich Schmid Institute of Material Sciences, Austrian Academy of Sciences and University of Leoben, Leoben, Austria; [2]Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 4th Medical Department, Hanusch Hospital, Vienna, Austria; [3]Department of Biomaterials, Max-Planck-Institute of Colloids and Interfaces, Potsdam, Germany.

Bone is a composite material that has to bear static and dynamic mechanical loads applied by the body weight and locomotion. The self-assembly mechanism of bone reacts on mechanical stresses like tension, compression or shearing and warranties an optimum in terms of stability and weight. Bone strength is influenced by a number of factors, depending on the particular hierarchical levels. The high stiffness of bone material is mainly achieved by the reinforcement by mineral platelets. A model for this elementary structure level consists of an arrangement of staggered mineral bricks, embedded in collagen matrix, which provides both stiffness and toughness [(1)]. The mechanical properties depend not only on the amount, shape and arrangement of the mineral particles but on the properties of the collagen as well. Thus, investigations on the nanoscale level become more meaningful by a combination of two complementary methods, e.g. quantitative backscattered electron imaging (qBEI) and nanoindentation. The local Ca-content, representing the degree of the mineralization, is extracted from the qBEI measurements [(2)], whereas the local mechanical properties, elastic modulus and hardness, are measured by nanoindentation, a miniaturised hardness testing using a small diamond tip [(3)]. The measured correlations between local Ca-content and mechanical strength helps to verify biomechanical models based on the nanocomposite structure of bone. Samples of bone with diseases like osteoporosis and osteogenesis imperfecta (brittle bone disease) where the local variation of bone strength have an influence on fracture are presented as examples for our investigations. Our studies show, that there does not exist one generally valid correlation between Ca-content and local E-modulus and hardness of the bone material, but moreover, this relationship seems to be modulated by changes in the organic component of the composite.[(4)] Acknowledgment: This work was supported in part by the FWF Project #P16880-B13 1 H. Gao et al., Proc. Natl. Acad. Sci., 2003, 100 (10), 5597-5600. 2 P. Roschger et al., Bone, 1998, 23(4), 319-326. 3 W.C. Oliver et al., J. Mater. Res., 1992, 4, 1564. 4 P. Fratzl et al., J. Mater. Chem., 2004, 14, 2115-2123.

L5.12
In-Situ Atomic Force Microscopy of Mineralized Collagen Fibrils on Fracture Surfaces of Bovine Trabecular Bone Before and After Demineralization. Johannes Kindt, Philipp Thurner, Georg Fantner and Paul Hansma; Physics, University of California, Santa Barbara, Santa Barbara, California.

High resolution AFM images of bovine trabecular bone fracture surfaces reveal individual fibrils. Treatment with EDTA removes a mineral coating and reveals the underlying collagen fibrils in images taken at the same location. The mineral coating is distinctly different in different regions. In some regions it is in the form of mineral plates with average dimensions of 70 +/- 35 nm parallel to the fibrils and about half that perpendicular to the fibrils. In other regions it is in the form of mineral particles are smaller and rounder, of order 25 +/- 15 nm both parallel and perpendicular to the fibrils, with more rounded top surfaces. Significantly, we never observe bare collagen fibrils in fracture surfaces before EDTA treatment. This implies that fractures always propagate between the mineral particles associated with one collagen fibril, and the mineral particles associated with another collagen fibril. Thus, to understand the mechanics of fracture on the molecular scale it will be crucial to understand the molecular nature of the adhesion between the mineral plates on one collagen fibril and the mineral plates on the adjoining collagen fibrils, because this is the weak interface that fails during fracture.

L5.13
Precise Control of Genetically Engineered Self-Assembling Polypeptide for Nanoscale Device Integration. Seiichiro Higashiya[1], Natalya I. Topilina[1], Vladimir V. Ermolenkov[1], Ludmila A. Popova[1], Christopher C. Wells[1], Narender Rana[2], Autumn Carlsen[2], Christopher Kossow[2], Eric T. Eisenbraun[2], Alain E. Kaloyeros[2], Robert E. Geer[2], Igor K. Lednev[1] and John T. Welch[1]; [1]Department of Chemistry, University at Albany, Albany, New York; [2]College of Nanoscale Science and Engineering, University at Albany, Albany, New York.

Nanoscale device self-assembly with control of size, structure, and functionality is a challenging and intriguing research area. Development of nanoscale scaffolds for assembling nanoscale devices with atomic precision up to 30 nm is particularly important , as this represents a regime where the performance and fabrication of materials for nanoelectronic applications is limited by fundamental quantum mechanical principles. Most surface-patterning studies have concerned on the assembly of relatively small units with difficulty to control size and complex functionality. Genetically engineered polypeptides are precisely programmable by appropriately coded DNA sequences and can be generated in monodisperse size, sequence, and configuration using a biological system. The same degree of precision is difficult to achieve by chemical methods. We have been focused on characterization of β-sheet forming repetitive polypeptides as nanoscale building blocks and templates for assembly of functionality. The repetitive and block-copolymerized β-sheet forming polypeptides have been prepared by a generalized strategy for libraries of artificial repetitive DNA sequences that were prepared based on unidirectional head-to-tail polymerization. Constructed DNAs were expressed in standard and commercially available *E.coli* expression systems. STM images of 32KYEY polypeptides, repeats of a 32-amino acid unit $[(GA)_3\mathbf{K}(GA)_3G\mathbf{Y}(GA)_3G\mathbf{E}(GA)_3G\mathbf{Y}]$, showed highly ordered structure on graphite. The effects of changing the length of GA repeats, turn moieties, and physico-chemical conditions, such as pH, temperature, and ionic strength, were evaluated by spectroscopic and probe/electron microscopic methods, such as deep UV resonance Raman spectroscopy and STM/TEM, respectively.

L5.14
Characterizing and Modeling of Calcium Phosphate and Calcium Oxalate Systems to Gain a Better Understanding of the Complex Kidney Stone Formation. Hemangi Sheetal Bhalsod, Amos Fairland and Laurie Gower; Material Science Engineering, University of Florida, Gainesville, Florida.

By understanding the system that makes up kidney stones the main purpose of this experiment is to isolate a segment of protein rich in polyaspardic acid which will then be used to study the crystal growth relationship of calcium phosphate and calcium oxalate. The frequency of kidney stones found in the population currently has caused some attention to be placed on the calcium phosphate and calcium oxalate systems which make up most of the composition. About 10% of the population will have a kidney stone attack each year which is caused by stones getting stuck in the kidney or ureter and therefore blocking the flow. Kidney stones can be split into two major components: minerals (calcium phosphate and calcium oxalate) and organic matrix (lipids, proteins, and carbohydrates). The study being conducted focuses on both components and in past experiments amorphous calcium phosphate was studied based on kidney stone systems. Since, kidney stones are considered to be calcium phosphate cores with calcium oxalate layers, research in the past have been conducted introducing each component in the order they are thought to have formed. However, since it is important to understand kidney stone formation with both minerals simultaneously in this experiment they both will be introduced into the system at the same time. In the system being studied polyaspardic acid is focused on due to it being the most potent part of the protein in kidney stones and since it affects the changes in organic material the most. Since a simple system is being studied arachidic acid is used first so that the calcium may bind to the anionic fatty acid. If this simple system provides beneficial results then a more complex system with lipids can be studied.

L5.15

Elastic Modulus and Mineral Density of Dentine and Enamel in Natural Caries Lesions. Amanpreet K. Bembey[1], Michelle L. Oyen[2], Ching-Chang Ko[2], Andrew Bushby[1] and Alan Boyde[3]; [1]Department of Materials, Queen Mary, University of London, London, United Kingdom; [2]University of Minnesota, Minneapolis, Minnesota; [3]School of Medicine and Dentistry, Queen Mary, University of London, London, United Kingdom.

Dental tissues have been reported to show a considerable decrease in both their mineral content and mechanical properties in caries lesions. The changed properties of dentine and enamel have been shown to be dependant on crystal size and not only mineral content (Angker et al., Arch. Oral. Bio., 2004), although the connectivity between the mineral crystals has been overlooked. Teeth with carious lesions were sectioned, embedded in polymethylmethacrylate and diamond micromilled. Nanoindentation and quantitative backscattered electron imaging were used to determine relationships between the elastic modulus and mineral density of sound and carious regions within dentine and enamel. The changes in elastic modulus with decreased mineralisation for dentine and enamel could not be explained by simple composite mechanics expressions relating elastic modulus and mineral volume fraction. Finite element modelling of dentine and enamel as a two-phase composite material at the ultrastructure level were used to demonstrate how changes in the mineral phase connectivity can produce changes in the elastic modulus. Tissue models for enamel, in which the mineral phase is both the major component of the structure ($\sim$ 90% by volume) and highly interconnected, were consistent with the modulus of sound enamel. The drastic change in enamel modulus with a relatively small change in mineral volume fraction could be modelled as a decrease in mineral phase connectivity at nearly constant volume fraction. The more gradual trend in the dentine data was also consistent with a structure that is initially highly connected in the mineral phase, consistent with the known structure of dentine, and for which the change in modulus is more directly related to changes in mineral content than mineral connectivity.

L5.16

Calcium Phosphate Mineralization Using the Cowpea Chlorotic Mottle Virus (CCMV) Viral Protein Cage. Masaki Uchida[1,3,4], Deborah Willits[2,3], Mark Young[2,3] and Trevor Douglas[1,3]; [1]Department of Chemistry and Biochemistry, Montana State University, Bozeman, Montana; [2]Department of Plant Science, Montana State University, Bozeman, Montana; [3]Center for Bio-Inspired Nanomaterials, Montana State University, Bozeman, Montana; [4]Institute for Human Science and Biomedical Engineering, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan.

Calcium phosphate is one of the most important biominerals for vertebrates. Therefore, it is interesting to understand the mineralization process in biological systems. Although acidic non-collagenous proteins are believed to play an essential role in the mineralization process, the details of how proteins induce mineral nucleation is still poorly understood. In the present study, calcium phosphate mineralization using a genetically engineered protein cage architecture (Cowpea chlorotic mottle virus-CCMV) has been investigated. CCMV is a plant virus composed of 180 identical subunits that self-assemble into an icosahedral protein cage. In the wild type CCMV, the positively charged N-termimus of each subunit projects into the interior of the viral capsid and the cationic interior interacts strongly with the polyanion of RNA. A mutant of CCMV has been engineered (subE), in which the eight basic residues on each subunit have been replaced by glutamic acid residues. This mutant assembles into a cage structure indistinguishable from the wild type but the interior of the cage is highly negatively charged. The protein construct provides a good model to investigate the role of acidic non-collagenous proteins in the calcium phosphate mineralization process. Under defined conditions bulk precipitation occurs rapidly from a calcium/phosphate solution, but in the presence of subE this homogeneous nucleation of calcium phosphate is suppressed. Analysis using transmission electron microscopy revealed the presence of a mineral phase that should be a calcium phosphate within the cage-like architecture of subE. From this result, it is assumed that the negatively charged interior surface of subE can induce heterogeneous nucleation of an initially amorphous calcium phosphate.

L5.17

Microstructure and Adhesive Properties of Copal Composite in Dental Incrustations. Lauro Bucio[1], Irma Araceli Belio[2], Jacqueline Rodriguez Chavez[3], Minerva Orta Amaro[1], Jesus Arenas[1], Jose Luis Espinoza[4] and Ma. Carmen Flores-Grajeda[2]; [1]Estado Solido, Instituto de Fisica, UNAM, Mexico, Distrito Federal, Mexico; [2]Facultad de Odontologia, Universidad Autonoma de Sinaloa, Culiacan, Sinaloa, Mexico; [3]Universidad Autonoma de Guadalajara, Guadalajara, Jalisco, Mexico; [4]Coordinacion Estatal de Salud Bucal, Secretaria de Salud del Estado de Sinaloa, Culiacan, Sinaloa, Mexico.

There are evidences that in ancient cultures in Mexico, copal was probably used for gluing precious stones in teeth cavities and for dental restorations as well. This is an important reason for making experiments using copal and copal-based composites in order to have more information for the potential applications of this material in modern dentistry. In our experiments concerning with dental incrustations of turquoise, we have practiced round cavities (about 1 mm depth) in the middle of incisive teeth by using a low-speed air turbine. Turquoise was cut in such a way that fits exactly on the tooth cavity. Copal and powdered apatite were used to glue the stone into the cavity. With Scanning electron microscopy we have observed that the composite copal-powdered apatite, penetrates the dentin tubules in the tooth tissue (depth of penetration from 5.6 to 41 micrometers), suggesting the existence of a micromechanical adherence. Concerning to the characterization of the adhesive properties, we have applied the ASTM D2095-72 test considering the adhesion of two cylinders of bone, and the copal composite as the adhesive. A maximum cohesive tensile strength around of 0.1 MPa for elastic response was measured corresponding to a strain below of 0.2 mm. We will discuss these results considering the reports of dental incrustations in ancient Mexico by several authors as well as the possibilities for its applications in modern Dentistry.

L5.18

Comparison of Murine and Human Dentin. Stefan Habelitz[1], Shabnam Zartoshtimanesh[1], Sally J. Marshall[1], Grayson W. Marshall[1] and Pamela K. DenBesten[2]; [1]Restorative Dentistry, University of California, San Francisco, California; [2]Growth and Development, UCSF, San Francisco, California.

The mouse model is a widely accepted model for comparative research on human biology. This study tests the hypothesis that sound murine and human dentin are comparable in regards to structure, composition and mechanical properties. Methods: Murine (age: 8 weeks,, n = 5) and human (age: 19 to 30 years, n = 5) dentin samples were prepared from 1st and 3rd molars, respectively, by cutting and polishing (up to 0.1 ?m). Atomic force microscopy (AFM) was used to study the microstructure of dentin, including the size of collagen fibers. Mechanical properties of the intertubular dentin were determined by nanoindentation and dynamic stiffness mapping (DSM). Chemical composition was determined using micro-Raman spectroscopy. Results: AFM and DSM revealed the presence of peritubular dentin surrounding the tubules in mouse and human dentin. Collagen fibrils showed an average diameter at around 100 nm with a d-spacing of approximately 67 nm. The hydrated dentin of mice showed an average modulus of 15.1 GPa and hardness of 0.68 GPa and was thus slightly but significantly lower than human dentin (E = 20.1GPa, H = 0.83 GPa). DSM showed further an increased heterogeneity of moduli in murine dentin. Raman spectroscopy revealed no significant differences in mineral and organic phases in the two dentins. Conclusion: The large correlation in structure, chemistry and properties of murine and human dentin suggest the suitability of the mouse model. Comparative studies on the mechanical properties, however, need to consider the decreased properties of murine dentin suggesting slight differences in dentin biomineralization. Funding: NIH-NIDCR P01-DE09859

L5.19
Biological Mechanism of Mechanical Behavior of Nacre.
H. Jerry Qi[1], Christine Ortiz[2] and Mary C. Boyce[3]; [1]Mechanical Engineering, University of Colorado, Boulder, Colorado; [2]Material Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [3]Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Many hierarchically structured natural materials exhibit unique combinations of superior mechanical properties. In particular, natural armor materials such as the nacre of abalone shells possess a microstructure with important features and properties at a variety of lengthscales, from the structure and mechanical behavior of the various constituent materials to the overall integrated, synergistic behavior of the complex brick and mortar microstructure. To understand how the nanostructure and constituent materials work in concert to provide the observed superior macroscopic mechanical behavior, three primary features of these materials require quantification – (1) the structure and the mechanical properties of the aragonite tablets or bricks; (2) the structure and stress-strain behavior of the organic matrix or mortar; and (3) the geometrical arrangements and lengthscales of these constituents that ultimately provide the mechanics that produces superior performance under impact and penetration loading conditions. These three features of the nanostructured nacre are under experimental and modeling characterization in a collaborative research effort of the Ortiz and the Boyce groups at MIT. In this paper, the influences of the stress-strain behavior of organic matrix and the geometrical arrangements of the constituents to the overall mechanical behavior of nacre are investigated. Literature data has revealed the axial force-extension behavior of the organic matrix strands to exhibit a saw-tooth character, whereupon numerous distinct load drops are found to occur over the course of large axial extension. This saw-tooth character is due to unfolding the folded modules or breaking the sacrifice bonds in the protein macromolecule and is speculated to be responsible for the excellence mechanical properties of nacre. Micromechanical modeling considering the complex brick and mortar microstructure, together with a newly developed finite deformation constitutive model accounting for the unfolding behavior of single protein molecule in the organic matrix, is used to study how the single molecular level unfolding is translated into the mechanical property improvements and the biological mechanism of superior mechanical behavior of nacre. Numerical simulations on tensile tests show that during the increased deformation, the folded modules in the organic matrix are progressively unfolded, offering a softening mechanism in the organic materials and resulting in the reduction of the magnitudes of stresses in the aragonite tablets as well as the magnitude of limiting stretch ratio of macromolecular chain in the organic matrix. This softening mechanism also allows larger deformation of nacre without creating damage in the aragonite mineral and organic matrix, and therefore offers an effective avenue for energy dissipation, which is critical in improving the overall performance of nacre, such as the impact resistance and fracture toughness.

L5.20
Biodegradable Polyurethane Artificial Periosteum for the Treatment of Articular Cartilage Defects.
Katarzyna Gorna[1] and Sylwester Gogolewski[1]; [1]Polymer Research, AO Research Institute, Davos, Switzerland; [2]Polymer Research, AO Research Institute, Davos, Switzerland.

Permanent pain, disability and joint dysfunction are the typical consequences of articular cartilage injuries. The surgical treatment of articular cartilage defects almost never results in the formation of hyaline cartilage. The defects are filled with fibrocartilage, which does not take on the functions of a normal articular surface. These problems call for new procedures that might allow the regeneration of functional hyaline cartilage tissue. One such procedure involves transplantation of cultured human autogenous chondrocytes in combination with a periosteal flap. A tissue engineering approach exploits the possibility of using constructs consisting of autogenic chondrocytes cultured on suitable matrix which might take the function of periosteal flap. When implanted into a cartilage defect the construct may potentially induce the regeneration of functional hyaline cartilage. In the present study new biodegradable polyurethane elastomers were used to design an artificial periosteum. The polymers were based on hexamethylene diisocyanate, poly(e-caprolactone) 530 diol and dianhydro-D-sorbitol. The membranous porous scaffold was produced using a phase-inverse (precipitation) process. Solvents were DMSO and DMF and water was a precipitant. The membrane had a porous structure with interconnected pores. The size and geometry of pores on the membrane surface was determined by the polymer concentration in solution and the interface between the substrate and/or the coagulant. The membrane surface in contact with the substrate showed the presence of regular hexagon-like pores with an average size of 20-80 mm. The membrane surface in contact with the coagulation bath had patchy structure. For the membranes formed from the polymer solution with a lower concentration, the porosity of this surface was better developed than for the membranes cast from the solution with a higher concentration. The tensile strength of the membranes was in the range of 0.06 to 0.3 MPa and the modulus of elasticity in the range of 0.2 to 0.7 MPa, respectively. The membranes with controlled elasticity withstand suturing. The membranes obtained in the study supported attachment and proliferation of human articular chondrocytes and may be promising candidates for the repair of articular cartilage defects using a tissue engineering approach.

L5.21
Structural and Mechanical Characterization of Nanoclay-Reinforced Nacre-Like Polymer Composites.
Xiaodong Li[1], Hongsheng Gao[1], Wally A. Scrivens[2], Dongling Fei[2], Michael A. Sutton[1], Anthony P. Reynolds[1] and Michael L. Myrick[2]; [1]Department of Mechanical Engineering, University of South Carolina, Columbia, South Carolina; [2]Department of Chemistry and Biochemistry, University of South Carolina, Columbia, South Carolina.

Nanoclay-reinforced nacre-like polymer composites with varying weight percentage ranging from 0 to 80% of nanoclays were prepared and characterized by transmission electron microscope (TEM), micro/nanoindentation, microtensile loading and bending tests. Elastic modulus and tensile strength increase with increasing nanoclay loading. At 60% nanoclay loading, elastic modulus increases eight times compared to polymer matrix. TEM results showed that nanoclays were laminated in the polymer matrix imitating nacre architecture. Micro/nanoindentation tests were carried out to investigate indentation damage mechanisms. Fracture surface was examined using a scanning electron microscope (SEM). The reinforcing mechanisms are discussed with reference to the nanoclay dispersion, interfacial bonding, and load transfer in the nanoclay-reinforced polymer composites.

L5.22
Structure-Function Relationships in Biological Glass Fibers.
James C. Weaver[1], Michael J. Porter[1], David J. Kisailus[1], Georg E. Fantner[2], Johannes H. Kindt[2], Almut Rapp[3], Mark Najarian[1], Yannicke Dauphin[4], Joanna Aizenberg[5], Peter Fratzl[6], Bradley F. Chmelka[3], Paul K. Hansma[2], and Daniel E. Morse[1]; [1]Materials Research Laboratory and the Institute for Collaborative Biotechnologies, University of California, Santa Barbara, Santa Barbara, CA; [2]Department of Physics, University of California, Santa Barbara, CA; [3]Department of Chemical Engineering, University of California, Santa Barbara, CA; [4]Universite de Paris XI, Orsay, France; [5]Lucent Technologies, Murray Hill, NJ; [6]Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany.

Recent interest in the optical and mechanical properties of silica skeletal structures (spicules) made by living sponges, and the possibility of harnessing these mechanisms for the synthesis of advanced materials and devices, motivate our investigation of the micro- and nanoscale architecture of these remarkable biological materials. High resolution scanning electron and atomic force microscopic analyses of spicules isolated from several different sponge species reveals an unanticipated diversity of structural complexity characteristic of these unique skeletal systems. All spicules measuring greater than a few mm in total length exhibit a laminated architecture consisting of alternating layers of hydrated amorphous silica and organic that effectively resists and retards crack propagation through these materials. In spicules that experience stresses not confined to a single axis (e.g. the anchor spicules from *Euplectella aspergillum*), there is a reduction in silica layer thickness from the spicule core to the periphery. In contrast, spicules that experience uniaxial loading exhibit a discrete graded architecture with the thickest silica layers found in regions of maximum compression and the thinnest layers in regions of maximum tension. Basic design principles learned from these studies are presented and may prove useful in a wide range of technologically important applications including the design of more fracture-resistant optical fibers.

SESSION L6: Soft Tissues II
Chairs: Paul Hansma and Fred H. Silver
Thursday Morning, March 31, 2005
Room 3004 (Moscone West)

8:30 AM *L6.1
Fibroblast Contraction of a Collagen-GAG Scaffold.
Lorna Gibson[1], T. M. Freyman[2], B. A. Harley[3] and I. V. Yannas[3]; [1]Materials Science and Engineering, MIT, Cambridge, Massachusetts; [2]Boston Scientific, Natick, Massachusetts; [3]Mechanical Engineering, MIT, Cambridge, Massachusetts.

As a wound in the skin heals, fibroblasts migrate into the wound bed and pull the edges of the wound together. Wound contraction is associated with the formation of scar tissue which is stiffer than normal dermis and can limit the range of motion of a joint. Normal dermis can be regenerated if wound contraction and the formation of scar tissue is inhibited. Our group has been studying the mechanics of fibroblast contraction of a collagen-glycosaminoglycan scaffold used in for the regeneration of skin in burn patients as a model for the contractile process. We have built a cell force monitor to measure the contractile deflection of the scaffold by a population of fibroblasts over time. We have found that the force increases with time according to a 1-exp curve, reaching an asymptotic value after about 16 hours. The force per cell is roughly 1 nN/cell: since not all of the cells actively contract, this is an underestimate of the force per cell. The contractile deflection of the scaffold by the fibroblasts decreases as the stiffness of the system increases; the contractile force is found to be independent of the system stiffness. Fibroblasts seeded onto the scaffold are initially rounded; over time they spread and become more elongated. Measurement of cell aspect ratio with time indicates that the aspect ratio, too can be described by a 1-exp curve with the same time constant as the force versus time curve. Histology and video optical microscopy show that as cells spread, the attachment points move to the periphery of the cell and that struts within the scaffold buckle or bend. Actin filaments within the cytoskeleton are tensile elements that are bound to a scaffold strut at focal adhesion complexes. The tensile forces in the actin filaments are resisted by compression in the strut. As cell spreads, and adhesion sites move to the periphery of the cell, the buckling load of the strut that the cell is attached to decreases. Buckling, and in some cases, bending of the struts results in overall contraction of the scaffold by the fibroblasts. Currently we are measuring the stress-strain curve of individual struts in the scaffold using optical tweezers to estimate the strut buckling force and the force that an individual cell can impose. We are also measuring the compressive stress-strain curve for the scaffold to estimate the contractile force of a population of cells in free-floating contraction experiments in the literature, for comparison with our cell force monitor results. In the future, we plan to observe the evolution of cytoskeletal elements (e.g. actin filaments) and adhesion sites on live cells during contraction using cell staining techniques and confocal microscopy. Our ultimate goal is to develop a micromechanical model of the contractile process.

9:00 AM L6.2
Multi-scale Mechanics of Lung Parenchyma.
Andrew Gouldstone[1] and Mary D. Frame[2]; [1]Department of Materials Science and Engineering, Stony Brook University, Stony Brook, New York; [2]Department of Biomedical Engineering, Stony Brook University, Stony Brook, New York.

The lung is a dynamic organ that undergoes high volume/pressure changes during each respiration. As such strains are part of its function, healthy operation is directly linked to its mechanical behavior. The mechanics of inflation and deflation (i.e., bulk behavior) of the lung have been studied in the physiological and clinical literature. The mechanical behavior of the lung parenchymal tissue when exposed to repeated shear is less well-understood. The elastic and visco-elastic shear properties of lung parenchyma have been probed in a number of investigations, including indentation experiments on excised mammalian lungs. A key finding is a residual imprint after low-strain deformation that persists until high inflation pressure is applied. This residual, or 'plastic', imprint is ostensibly due to atelectasis (localized collapse of the gas filled alveoli) that occurs when applied shear strains are sufficient to bring alveolar surfaces into contact, at which point surface tension prevents their separation during the next inhalation cycle. Beyond this phenomenological explanation, there has been little investigation into the macroscopic or microscopic mechanical criteria for plastic deformation of the lung in shear. For example, it is unknown whether this plastic deformation is linked to inflammation affecting the connective tissue mechanics or composition. A quantitative, multi-scale understanding of this phenomenon would be useful for physiological understanding, and importantly, for prediction of chronic and acute lung pathologies, including asthma and lung injury. In addition, a micromechanical understanding of alveolar collapse would be applied to future design of safe and conservative ventilation strategies for its reversal. In this work, we describe methods to investigate this, by recourse to instrumented Hertzian indentation and sub-surface observation on excised small mammalian lungs. The effect of loading rate, loading history and ventilation strategies on propensity for atelectasis will be addressed.

9:15 AM L6.3
Mechanical Behavior of Human Stratum Corneum.
Kenneth S. Wu[1] and Reinhold H. Dauskardt[2]; [1]Mechanical Engineering, Stanford University, Stanford, California; [2]Materials Science & Engineering, Stanford University, Stanford, California.

The outermost layer of skin, the stratum corneum (SC), provides mechanical protection and a controlled permeable barrier to the external environment while subject to highly variable conditions including natural changes in local temperature and humidity as well as potentially damaging acute and chronic chemical exposure. Temperature, hydration, and the application of topical agents can influence the mechanical properties of SC. We present mechanics-based techniques to study both the in-plane and the out-of-plane mechanical behavior of human SC tissue as a function of temperature, hydration, and chemical treatment. In order to modify the tissue structure, behavior is reported after treatments using selected surfactant and pH solutions. In addition to basic mechanical properties and fracture behavior, we report on the viscoelastic nature of SC which has been long recognized. However, a more detailed understanding of the underlying viscoelastic and molecular relaxation processes is currently unknown. These processes are systematically explored using dynamic and transient mechanical tests involving creep-recovery and stress-relaxation experiments to probe retardation and relaxation processes. Stretched exponential modeling of the behavior provides a measure of the distribution of relaxation time scales and their dependence on temperature, hydration, and other salient conditioning and loading parameters. Implications for tissue treatments and emerging technologies that interface with skin are considered.

9:30 AM L6.4
A Micromechanical Material Model for the Numerical Simulation of the Degenerated Human Cornea. Giorgio Fotia[2], Federico Manganiello[1] and Anna Pandolfi[1]; [1]Dipartimento di Ingegneria Strutturale, Politecnico di Milano, Milano, Italy; [2]Department of Computational Methods for Engineering, CRS4, Pula, Cagliari, Italy.

The human cornea is a spherical layered thin shell with the double function of protecting the interior part of the eye and deviating the light rays onto the retina. The main layer of the cornea, the stroma, is made by collagen tissue sheets (lamellae) aligned along the shell middle surface. Lamellae result from the organization of parallel collagen micro-fibrils of uniform diameter. Each micro-fibril results from the assembly of basic (triple helix) fibrillar collagen micro-molecules. The lamellae are organized into two interwoven structures: in the central part of the cornea, the lamellae are oriented in two orthogonal directions; along the boundaries the lamellae lie in the circumferential direction. This micro-organization confers to the healthy cornea the appropriate shape, stiffness, and transparency. An alteration of such multi-level organization (possibly due or accompanied by a chemical induced variation in the material properties) can explain the change of the corneal shape observed in keratoconus, a degenerative illness responsible of serious refractive deficiencies. According to recent developments in refractive surgery, the best way to treat keratoconus is to rehabilitate, both structurally and visually, the cornea functionality with the use of micro-implants (intra-ocular lenses and rings). An anisotropic finite deformation material model of the structured stromal tissue, able to describe the normal and degenerated behavior of the cornea, is here proposed. In view of its use in surgical planning of implants placements, the proposed material model is employed to numerically evaluate the stress state of the cornea. The micro-mechanical model may play a novel role not only in understanding and evaluating the effects of the placement of micro-prothesis, but also in developing newer modalities to achieve predictable results in problematic cases.

9:45 AM L6.5
Study of the Morphology and Adhesion Properties of Collagen Fibers in the Bruch Membrane. Albena Ivanisevic[1] and Shrestha Basu Mallick[2]; [1]Purdue University, West Lafayette, Indiana; [2]Physics, Purdue University, West Lafayette, Indiana.

Atomic force microscopy and force-volume images were used to probe the chemical and mechanical properties of the Bruch membrane. The membrane is a thin layer of fibers and was extracted from pig eyes. In this study we report the morphological properties (e.g. d-periodicity and packing) of the collagen fibers from the Bruch membrane. We compare the properties of these fibers with the ones extracted from other portions of the eye. Furthermore, we map the adhesion properties of the fibers using a chemical force microscopy methodology. The results of our studies are essential when trying to engineer better scaffolds for transplant strategies of retinal pigment epithelial cells.

10:30 AM *L6.6
Cell Organization in Compliant Environments due to Active Mechanosensing. Ilka Bettina Bischofs and Ulrich Sebastian Schwarz; Theory Division, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany.

The functional properties of tissues are determined by an intricate

interrelation between cell organization and extracellular matrix. Cell organization in tissues is determined by a variety of guiding principles, including chemotaxis, haptotaxis, contact guidance and durotaxis. Due to active mechanosensing through integrin-based cell-matrix contacts, tissue cells tend to move and orient in favor of maximal effective stiffness in their environment. This behaviour can be investigated experimentally by culturing mechanically active cells on elastic substrates. It also can be modeled theoretically as an extremum principle in linear elasticity theory. For single cells, we predict that cells orient in the direction of external tensile strain. Cells are attracted by clamped boundaries, where they orient perpendicularly; this behaviour is reversed for free boundaries. For intermediate cell densities, we predict that cells align in strings. Due to screening of the cellular force patterns in the strings, little correlation exists between them. For high cell densities, we predict isotropic ring-like structures for incompressible media and nematic string-like structures for compressible media. Our model contributes to a better understanding of physiological situations like tissue maintenance and wound healing. Moreover it can be used for rational design of artificial tissues.

11:00 AM L6.7
A Newtonian Fluid Meets an Elastic Solid: Coupling Lattice Boltzmann and Lattice Spring Models. Gavin A. Buxton, Rolf Verberg, David Jasnow and Anna C. Balazs; University of Pittsburgh, Pittsburgh, Pennsylvania.

We present a novel algorithm that captures the coupling between a compliant bounding surface and the hydrodynamic response of an enclosed fluid. The fluid is simulated with the lattice Boltzmann model, an efficient solver of the Navier-Stokes equations. The solid walls are modeled by the lattice spring model, which simulates the dynamics of a continuum elastic material. We implemented solid-fluid interactions that give stick boundary conditions for the fluid and allow for a dynamic interaction between the elastic walls and the confined fluid. Here, the fluid and the solid are coupled through pressure and shear forces that are excerted across the interface. The algorithm is validated for the so-called "breathing mode" oscillations of an elastic spherical shell filled with a Newtonian fluid, i.e., the dynamic responds of the system after an initially uniform expansion of the shell. We then apply the model to study the impact of a fluid-filled, elastic shell on a hard wall and on an adhesive surface. Understanding the dynamics of fluid-filled shells, especially near adhesive surfaces, can be particularly important in the design of microcapsules for pharmaceutical and other technological applications. Our studies reveal that the binding of these capsules to specific surfaces can be sensitive to the physical properties of both the outer shell and the enclosed fluid. The integrated LBM/LSM model opens up the possibility of accurately and efficiently capturing the dynamic coupling between fluid flow and a compliant bounding surface in a broad variety of systems, for example, in the biomechanics of blood flow in the cardiovascular system, or air flow in the respiratory system.

11:15 AM L6.8
Constitutive Modeling of the Stress-Stretch Behavior of Membranes Possessing a Triangulated Network Microstructure. Melis Arslan[1], Mary C. Boyce[1] and Jerry H. Qi[2,1]; [1]Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]Mechanical Engineering, University of Colorado, Boulder, Colorado.

The mechanical behavior of the membrane of the red blood cell is governed by two primary microstructural features: the lipid bilayer and the underlying spectrin network. The lipid bilayer is analogous to a 2D fluid in that it resists changes to its planar area, yet poses little resistance to planar shear. A skeletal network of spectrin molecules is crosslinked to the lipid bilayer and provides the shear stiffness of the membrane. Here, a continuum level constitutive model of the large stretch behavior of the red blood cell membrane that directly incorporates the microstructure of the spectrin network is developed. The resemblance of the spectrin network to a triangulated network is used to identify a representative volume element (RVE) for the model. A strain energy density function in terms of an arbitrary planar deformation field is proposed using the RVE. Differentiation of the strain energy density function provides expressions for the stress-stretch behavior of the model. The stress-strain behavior of the membrane when subjected to different loading conditions is given, showing the capabilities of the proposed microstructurally-detailed constitutive modeling approach.

11:30 AM L6.9
Elasto-Mammography: Elastic Property Reconstruction in Breast Tissues. Z. G. Wang, Y. Liu, Lizhi Sun and G. Wang; University of Iowa, Iowa City, Iowa.

Mammography is the primary method for screening and detecting breast cancer. However, it frequently fails to detect small tumors and is not quite specific in terms of tumor benignity and malignancy. The objective of this presentation is to develop a new imaging modality called elasto-mammography that generates the modulus elastograms based conventional mammographs. A new elastic reconstruction technique is described based on elastography and mammography for breast tissues. This technique involves the application of compression to breast tissues. Elastic distribution can be reconstructed through the measurement of displacement provided by mammographic projection. It is shown that the proposed elasto-mammography provides higher sensitivity and specificity than the conventional mammography on its own for breast cancer diagnosis. The rationale for this novel development is that the elastic response of tumors is significantly different from that of the surrounding tissues. Using our proposed imaging modality, benign/malignant tumors would be better differentiated in an earlier stage with synergistic utilization of modulus elastograms.

11:45 AM L6.10
Mechanical Properties of Honeybee Waxes. Robert Buchwald[1] and Alan Greenberg[2]; [1]Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado; [2]Mechanical Engineering, University of Colorado, Boulder, Colorado.

We investigated the mechanical and thermal properties of six species of honeybee waxes. Beeswax is a multicomponent viscoelastic material used by bees to store resources and brood. The wax of the Western honeybee (Apis mellifera) is also an important agricultural resource used in cosmetics, dentistry, medicine, and food processing. Wax was collected from nests of six species of honeybees, A. mellifera, A. cerana cerana, A. cerana japonica, A. florea, A. andreniformis and A. dorsata. After removing any contaminants, we melted and cast the waxes in to right, circular cylinders and tested in an electromechanical tester. The thermal properties of these samples were also examined with differential scanning calorimetry (DSC). Wax of the six species differed significantly in their yield strengths and stiffnesses, although not among their measures of resilience. This study not only identifies important physical information about a widely used biomaterial but also illustrates how studies of a relatively simple biomaterial can reveal how evolution has optimized these materials over time.

SESSION L7: Hard Tissues II
Chairs: Peter Fratzl and William J. Landis
Thursday Afternoon, March 31, 2005
Room 3004 (Moscone West)

1:30 PM L7.1
Structural Distinctions Between Biogenic and Geological Aragonite. Boaz Pokroy[1], John P. Quintana[2], El'ad N. Caspi[3], Andy Fitch[4] and Emil Zolotoyabko[1]; [1]Department of Materials Engineering, Technion, Israel Institute of Technology, Haifa, Israel; [2]DND-CAT Synchrotron Research Center, Northwestern University, Argonne, Illinois; [3]Physics Department, Nuclear Centre - Negev, Beer-Sheva, Israel; [4]European Synchrotron Radiation Facility, Grenoble, France.

Biogenic crystals, i.e. the crystals produced by organisms, often reveal superior material characteristics due to the presence of a small amount of organic phase within a ceramic matrix. In this paper, we attempt to address the general question, *viz.* whether or not the structure of biogenic crystals comprising organic macromolecules is exactly the same as that one of their non-biogenic counterparts. For this purpose, we performed high-resolution synchrotron powder diffraction measurements of geological aragonite and different aragonite crystals of biogenic origin by using the dedicated beam lines at the Advanced Photon Source (APS) of Argonne National Laboratory (IL, USA) and European Synchrotron Radiation Facility (ESRF, Grenoble, France). Applying the Rietveld refinement procedure to the high-resolution diffraction spectra, we were able to extract the aragonite lattice parameters with an accuracy of 10 ppm. As a result, we found anisotropic lattice distortions in the *AcanthocardiaTuberculata* aragonitic seashell relative to geological aragonite [1]. Comparison between atomic positions in biogenic and geological aragonite allowed us to conclude that lattice distortions most probably are induced by intra-crystalline proteins. Examination of a variety of shells belonging to different classes (bivalvia, gastropoda and cephalopoda) and taken from different habitat origins (sea, fresh water and land) showed that anisotropic lattice distortions is a widespread phenomenon in the aragonitic shells. This finding contributes to better understanding the biomineralization process and the development of bio-inspired "smart" materials. [1] B. Pokroy, J. P. Quintana, E. N. Caspi, A. Berner and E. Zolotoyabko. Anisotropic lattice distortions in biogenic aragonite. *NatureMaterials*, doi:10.1038/nmat1263.

1:45 PM L7.2
Structural and Microstructural Characterization of Barnacle Shell. Jose Ignacio Arias[1], Maria Soledad Fernandez[1], Alejandro Rodriguez-Navarro[2] and Jose Luis Arias[1]; [1]Veterinary Science, Universidad de Chile, Santiago, Chile; [2]Mineralogia y Petrologia,

Universidad de Granada, Granada, Spain.

Austromegabalanus psittacus is a large barnacle from the coast of Chile. Its hard shell is a biocomposite ceramic consisting of an organic matrix and a crystalline calcium carbonate (calcite) filler. The organic matrix consists mainly of chitin and sulfated macromolecules referred to as proteoglycans. The shell shows different anatomical region where an specific distribution of the organic matrix has been reported. By using X-ray diffraction, SEM and TEM electron microscopy, immuno-histochemistry and biochemical analysis, we have studied the distribution of specific macromolecules within the shell and correlated the nature and localization of these macromolecules with the orientation of the calcite crystals. The inner part of the shell wall is formed by concentric cylinders of chitin closely associated to a keratan sulfate proteoglycan. Between these cylinders there are other proteoglycans such as dermatan sulfate and chondroitin 6 sulfate. X-ray diffraction pattern of the inner side of the shell showed the ocurrence of highly oriented calcite cystals with their c-axes aligned perpendicular to the shell surface. The organic matrix architecture of the outer side of the shell is more intricate, and coincidently, the calcite crystals in the outer surface are randomly oriented. In summary, barnacle shell is a calcitic structure composed of an outer part rich in organic matter and an inner part with less but highly negatively charged organic matter. The outer part is constituted of randomly oriented calcite crystals, while the inner part is formed by preferentially oriented crystals. FONDAP 11980002.

2:00 PM L7.3
Structure and Mechanical Properties of the Cuticle of Lobster. Christoph Sachs, Patricia Romano and Dierk Raabe; Microstructure Physics, Max-Planck-Institut, Duesseldorf, Germany.

The cuticle of lobster (homarus americanus) is a multilayer chitin-protein-based biological composite containing variable amounts of nanoscopic biominerals. Basically, the cuticle consists of three layers: Epicuticle, exocuticle and endocuticle. The epicuticle is an outer thin waxy layer providing a permeability barrier to water and gas. The following exocuticle and endocuticle are made up of chitin fibers arranged in lamellas of different thickness. Local variations in composition and structure of the material provide a wide range of mechanical properties. It can be either rigid serving as a highly protective exoskeleton or it can be flexible serving as a constructional element as in articular membranes at joints. Consequently, the lobster cuticle is an excellent example of a highly versatile and efficient solution to structural and functional challenges for arthropods. We present a systematic study on the correlation between the microscopic structure and composition of the lobster cuticle on the one hand and the resulting local mechanical properties on the other. The mechanical experiments are conducted using micro-tensile tests, micro-compression tests, micro-indentation, and nano-indentation. Structure investigations are conducted by using electron microscopy. Particular attention is placed on the effect of the local grade of biomineralization via embedded calcium salts and the resulting mechanical properties of the cuticle.

2:15 PM *L7.4
Microcracking and Microdamage in Bone Deformation. John Currey, Biology, University of York, York, United Kingdom.

When bone deforms it will sooner or later develop microdamage. This is clearly associated with the damage (shown as a decline in Young's modulus) that is known to occur post-yield, but the relationship is by no means clear. There are semantic difficulties about what to include in the term 'microdamage'; certainly the word means very different things to different workers. Many workers think that because it is difficult to examine very small microcracks (say less than 10 microns long) they have to be ignored. Others think that they are so important they cannot be ignored. Certain facts are clear, however: Microdamage looks very different in tension and compression; The pattern of microcracking differs according to whether the bone is adapted to loading habitually in tension or compression. Microdamage has a clear spatial relationship with the microscopic structure of bone (but its relationship with the fine structure is unknown); Increasing microdamage is associated with increased compliance of the bone material; Tensile microdamage develops mainly when bone is loaded into the post-yield region; Microdamage is reduced by high strain rates, high mineral contents, and treatments such as ionizing radiation that also make the bone brittle. In general, reduction in the post-yield strain is accompanied by a reduction in the amount of microdamage. Much less clear are the following: The three-dimensional structure of microcracks; The nature of the diffuse microdamage which everyone reports and no- one has a clue about; The role that microdamage may play in initiating or directing modelling and, more probably, remodelling (the formation of secondary osteones); The role, if any, that microcracking plays in aiding or inhibiting fracture. The last is probably the most important unknown about microdamage. The main reason that this role is unclear is because we know so little about the three-dimensional structure, and the mathematics and physics of so-called microcrack toughening is very difficult and still inchoate. The most difficult problem about analysing microdamage is that it is not yet possible to quantify the mount of microdamage in a piece of bone, unless extremely arbitrary decisions are made as to what must be left out. Crude attempts have been made by measuring the light reflected from specimens, but they do not deal with the total area of microdamage, if it is in the form of many microcracks, or volume, if it is truly (whatever that may mean) diffuse. Although microdamage was recognised in the 1970s, it is only in the last 15 years or so that serious attempts have been made to work on it. These attempts started when scanning confocal microscopes became readily available. It is, therefore, not surprising that there is a great deal still to find out. When we truly understand microdamage, we shall be well down the road to understanding what goes on when bone fractures.

2:45 PM L7.5
Collagen Content and Organization Relate to Bone Nanomechanical Properties. Eve Donnelly[1], Rebecca M. Williams[2], Shefford P. Baker[3] and Marjolein C. H. van der Meulen[1,4]; [1]Mechanical Engineering, Cornell University, Ithaca, New York; [2]Applied and Engineering Physics, Cornell University, Ithaca, New York; [3]Materials Science and Engineering, Cornell University, Ithaca, New York; [4]Musculoskeletal Integrity Program, Hospital for Special Surgery, New York, New York.

Cancellous bone plays an important load-bearing role in the skeleton, yet relatively little is known about the microstructure-property relationships of the tissue at the sub-10 μm level. Cancellous tissue is characterized by a layered microstructure with variable proportions of collagen and mineral. The lamellar material is substantially stiffer than the interlamellar material at the nanomechanical level. However, the microstructural origin of the observed differences in mechanical properties of these structures has not been investigated. In this study, second harmonic generation microscopy was used to examine collagen in human vertebral cancellous bone. Nanoindentation was used to assess the indentation modulus of lamellar and interlamellar bone at the same location in the tissue. The stiff lamellae corresponded to areas of highly ordered, collagen-rich material, while the compliant interlamellar regions corresponded to areas of unoriented or collagen-poor material. The lamellar bone was approximately 30% stiffer and contained approximately 50% more oriented collagen than the interlamellar bone. These observed differences in the mechanical properties and collagen content and organization of lamellar and interlamellar tissue are consistent with previous scanning electron microscopy studies showing greater mineral and collagen content and organization in lamellar bone. Given the well-known coupling between collagen and mineral in bone tissue, these results may also be indicative of the effects of mineral content on the mechanical properties; however, similar measurements of local variations in mineral content may provide additional insights into the nanomechanical behavior.

3:30 PM *L7.6
The Effect of Injury Mechanism on Spinal Cord Injury. Anthony Choo[1,2], Carolyn Sparrey[1,2], Carolyn Greaves[1,2], Jie Liu[2], Wolfram Tetzlaff[2], Marcel Dvorak[1,2] and Thomas R. Oxland[1,2]; [1]Orthopaedics, University of British Columbia, Vancouver, British Columbia, Canada; [2]ICORD, University of British Columbia, Vancouver, British Columbia, Canada.

There are many clinical descriptions of spinal cord injury (SCI) mechanisms and these are mostly hypothetical. Our overall research program aims to determine a connection between the mechanism of spinal column injury and the specific damage to the spinal cord tissue. The specific objectives of this presentation are to explore the effect of impact velocity and three column injury mechanisms (contusion, distraction, dislocation) on SCI. Sprague-Dawley rats (n=31) were subjected to a 1mm contusion at the T10 level at velocities of 3mm/s or 300mm/s. The animals were sacrificed immediately to observe the mechanical injury. Cord sections were stained with H&E to evaluate haemorrhage volume and SMI32 antibody to evaluate neurofilament damage. The results showed that the volume of haemorrhage in the white matter was a function of impact velocity while the total haemorrhage volume showed no difference. SMI32 reactivity demonstrated a significant relationship between impact velocity and axonal damage and grey matter injury. We conclude that impact velocity has an effect on the magnitude of injury within the white matter and an effect on the degree of neuronal damage in the grey matter. A novel device was developed using an electromagnetic actuator to produce SCI in contusion, distraction and dislocation at 1m/s in a rat model. Fluorescein-dextran (10kD) was used to visualize increases in membrane permeability. A range of displacements was used in this pilot series. Animals were sacrificed immediately to analyse the primary injury. At the site of injury, grey matter damage particularly in the ventral horn was common to all injury mechanisms; although the extent of dextran entry in neuronal somata appeared

greatest for dislocation and least severe in distraction. Ventral axolemma perturbations were most severe in dislocation with dextran entry extending several millimeters further rostrally than caudally to the apparent epicenter. These preliminary observations suggest that distinct injury mechanisms result in regional differences in the primary damage of spinal cord grey and white matter. A finite element model of the cervical spine was used to compare strain distributions in the cord for compression, distraction, and dislocation mechanisms. The model was based upon geometry from the Visible Human Project and included three vertebrae (C4-6), the spinal cord, dura mater, ligaments and discs. Results indicate that fractures with dislocations may lead to more extensive shear strains than those without dislocation. Comparisons with experimental data suggest that axial tension correlates with axonal damage and that spinal cord grey matter has a reduced tolerance to mechanical strain. Overall, these three studies suggest a strong link between spinal column injury mechanism and the tissue damage in the spinal cord. We anticipate that further research in this area may ultimately enable us to classify SCI by the mechanism of primary injury.

4:00 PM L7.7
Effects of Aging and Osteopontin on Hardness, Elastic Modulus and Viscoelasticity of Mouse Bone.
Beril Kavukcuoglu[1], Adrian Mann[1,2] and David Denhardt[3]; [1]Ceramic and Materials Engineering, Rutgers University, Piscataway, New Jersey; [2]Biomedical Engineering, Rutgers University, Piscataway, New Jersey; [3]Cell Biology and Neuroscience, Rutgers University, Piscataway, New Jersey.

The mechanical properties of bone are not fixed but change during its lifetime. In very young bones growth does not occur under mechanical loading, but during maturation, the bone properties change significantly as a result of changes in mineralization. Osteopontin (OPN), a phosphorylated glycoprotein, is among the most abundant non-collageneous bone matrix proteins. OPN has been implicated in bone formation, resorption and remodeling. This study has used nanoindentation to identify the effects of aging on hardness and elastic modulus and the variations of these properties across the radial axis of cortical femurs of osteopontin deficient (OPN -/-) and wild-type (OPN+/+) control mouse bones. The mechanical properties have been correlated with the changes in local structure and chemistry of the bone as observed with SEM and micro-Raman. Because of the viscoelastic nature of the collagen fibers in the bone matrix, bone itself has remarkable viscoelasticity. Nano-DMA and Modulus Mapping techniques have been used to study the viscoelastic properties (storage and loss characteristics) of bone. Those methods also enable comparison of local variations in the mechanical properties of OPN -/- and OPN +/+ mice bone. The tests were conducted on bone from mice aged from 3 weeks to 58 weeks. The preliminary results suggest that the mechanical properties of mouse bones decrease substantially with maturity. It has been found that there are large abrupt variations in mechanical properties across the femur radial section for young mouse bone. On the other hand, for adult mouse bone the mechanical properties are quite homogeneous along the radial axis. But the viscoelasticity tests suggest that the magnitude of the mechanical variation appears to depend not only on the age, but also the presence or absence of OPN. The mechanical variations also correlate with changes in the degree of mineralization and crystallinity of the bone as shown by the structural analysis. That is, high mineral content and high crystallinity corresponds to regions of increased hardness and stiffness. The results for OPN-/- and OPN+/+ mouse bones are particularly important as control of OPN activity has been postulated as a potential treatment for bone pathologies that exhibit a change in the bone mineralization, such as osteoporosis, osteopetrosis and Paget's disease. Understanding the effects of OPN on bone mechanics is a vital step in the development of the new treatments for these conditions.

4:15 PM L7.8
Novel Tissue Engineered Chitosan Based Three Dimensional Sintered Microspheres Matrices: Design, Mechanical Properties and Cellular Responses. Tao Jiang[1] and Cato Laurencin[2,1,3]; [1]Chemical Engineering, University of Virginia, Charlottesville, Virginia; [2]Orthopaedic Surgery, University of Virginia, Charlottesville, Virginia; [3]Biomedical Engineering, University of Virginia, Charlottesville, Virginia.

The natural biopolymer chitosan is biocompatible and shows antimicrobial and antifungic activities, making it a favorable option for biomedical applications. Three dimensional (3-D) matrices for bone regeneration require porous structures with relatively high mechanical properties. The current study presents methods of fabrication for novel high osteocompatible, 3-D chitosan/poly (lactide-co-glycolide) (PLAGA) matrices based on microspheres with high mechanical properties. Chitosan/PLAGA matrices were fabricated using a sintered microsphere technique developed in our laboratory1. Briefly, chitosan particles with size less than 75 microns were dispersed in 20% (w/v) PLAGA methylene chloride solution, which was poured to 1% poly (vinyl alcohol) (PVA) solution and was stirred at 250 rpm for 24 hours. Chitosan/PLAGA microspheres with diameters between 500 $\sim$ 710 microns were collected, packed in an appropriate mold and heated above the glass transition temperature of PLAGA. As a control, chitosan matrices were also fabricated2. Mechanical tests on matrices were performed using an Instron 5544 mechanical tester. Matrices were sterilized with 70% ethanol and seeded with MC3T3-E1 osteoblast-like cells at a density of 50,000 cells/ml and maintained under standard cell culture conditions. Cell proliferation on matrices was quantified using MTS assay. Results showed that 3-D chitosan matrices in a dry state possessed high compressive modulus of 655.3±101.4 MPa, however, due to high swelling of chitosan in water, the wet state compressive modulus decreased dramatically to 1.76±0.47 MPa. Two kinds of chitosan particles with different size distribution were used in chitosan/PLAGA matrices (Type I: 64±35 μm; Type II: 25±20 μm, sizes determined by laser diffraction). Type I composite matrices (sintering temperature at least 95 °C) required higher sintering temperature than type II matrices (sintering temperature 75 °C to 80 °C). Dry and wet state compressive moduli of type I matrices were 230.35+42.2 MPa and 127.25±17.37 MPa, respectively. Dry state compressive modulus of type II matrices was 272.68±12.94 MPa. The composite matrices underwent no obvious swelling in water and had much improved wet state mechanical properties. MTS assay showed significantly higher cell proliferation rate on type II matrices than that on type I matrices and both were significantly higher than that on chitosan matrices after 7 and 14 days. A more moderate processing temperature for type II matrices and high cell proliferation on type II matrices were attributed to the much smoother microspheres surfaces as evidenced from SEM. The present study demonstrates the feasibility of developing porous 3-D chitosan/PLAGA matrices with high mechanical properties in both dry and wet states and osteocompatibility for bone tissue engineering. This work is supported by NSF INT-0115595. References 1. M.D. Borden et al. Biomaterials 23: 551-559, 2002 2. T. Jiang et al. MRS 2004 fall meeting.

4:30 PM L7.9
Cellular Bone Replacement Materials with Designed Architecture. Alexander Woesz[1], Monika Rumpler[1], Inderchand Manjubala[1], Christine Pilz[1], Franz Varga[2], Nadja Fratzl-Zelman[2], Paul Roschger[2], Klaus Klaushofer[2], Juergen Stampfl[3] and Peter Fratzl[1]; [1]Dept. of Biomaterials, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany; [2]Ludwig Boltzmann Institute of Osteology, Vienna, Austria; [3]Institute of Materials Science and Testing, Vienna University of Technology, Vienna, Austria.

The material bone is a natural composite consisting of a biopolymer matrix (collagen) reinforced with mineral nanoparticles (hydroxylapatite), building up a dense shell on the exterior and a network of struts with a mean diameter of 200μm in the core of many bones. The architecture of the foamy inner part of bones (spongiosa) is determined by loading conditions [1]. The architecture strongly influences the mechanical properties of cellular solids together with the apparent density and the material it consists of [2]. In addition, the ingrowth of bone cells into porous implants depends on pore size, size distribution and interconnectivity. From this it is clear that the possibility to design the architecture of a bone replacement material is beneficial from a biological as well as a mechanical point of view. Our approach uses rapid prototyping methods, ceramic gelcasting and sintering to produce cellular structures with designed architecture from hydroxylapatite and other bioceramics [3]. The influence of sintering temperature and atmosphere on the physical properties of these scaffolds was investigated with x-ray diffraction, scanning electron microscopy, microtomography and scanning force microscopy. Furthermore, the cell ingrowth behaviour was determined in cell culture experiments, using the preosteoblastic cell line MC3T3-E1, derived from mouse calvariae. The cell ingrowth behaviour was evaluated during a culture period of two and three weeks respectively, by light microscopy and afterwards by histology after embedding and Giemsa-staining. The phase composition of the material was found to change with increasing sintering temperature and its surface characteristics seemed to be influenced by the sintering atmosphere. These changes also affected the cell ingrowth behaviour. In some experiments, the osteoblasts-like cells were found to cover the whole external and internal surface of the scaffold. The cells produced extracellular matrix consisting of collagen, which eventually filled nearly all the pores. In particular, the cells had the tendency to fill any crack or opening in the scaffolds, and to generally smooth the surfaces. In conclusion, rapid prototyping and ceramic gelcasting allows the freeform fabrication of porous bioceramics with controlled architecture. Such structures made of hydroxylapatit were found to support the growth of mouse osteoblasts. [1] Wolff, J., Archiv fuer pathologische Anatomie und Physiologie und fuer klinische Medicin, 50 (1870), 389 [2] Woesz, A., Stampfl, J. and Fratzl, P., Advanced Engineering Materials, 6 (3), (2004), 134-138 [3] Woesz, A., Rumpler, M., Stampfl, J., Varga, F., Fratzl-Zelman, N., Roschger, P.,

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4:45 PM L7.10
Indentation Micromechanics of Cell-Populated Fibrin/Collagen Constructs. C. Costales[1], R. G. Mooney[2], J. Curtin[1], W. Garner[3], B. Tawil[4,1], T.-L. Tuan[3] and M. C. Shaw[1]; [1]Bioengineering, California Lutheran University, Thousand Oaks, California; [2]University of Colorado, Boulder, Colorado; [3]Keck School of Medicine, University of Southern California, Los Angeles, California; [4]Baxter Healthcare Corporation, Westlake Village, California; [5]Biomedical Engineering, University of California, Los Angeles, California.

Skin is the largest organ in the body forming a critical protective barrier to the external environment. During normal wound healing, a fibrin clot is formed within the first few minutes and is replaced, over several days, by collagen and other extracellular matrix components which are populated with fibroblasts and other cells. This process leads to the rebuilding of dermal tissue on which the epidermal tissue is slowly rebuilt. In severe acute and chronic wounds dermal replacement materials are needed that mimic these processes. Current trends are to use scaffolds as structural substitutes as well as carriers for growth factors and cells for wound treatment. Fibrin-based sealants have been used over the last 30 years in hemostasis and tissue sealing applications and in the last 5 years as a scaffold, and consist of two primary components, fibrinogen and thrombin which form a fibrin clot when mixed. However, a basic understanding of the microstructure/property relationships of fibrin/collagen constructs is not yet fully established. Furthermore, while previous studies showed that cells proliferate and differentiate within 3D fibrin clots, their effect on structural mechanics of the fibrin clot has not been examined. Here, an indentation protocol was established to determine the effects of fibrin/collagen biochemistry and time-dependent cellular response on the elastic parameters of the constructs. Specifically, 4 ml fibrin/collagen constructs were prepared with varying compositions of human-derived fibrinogen (5 to 33 mg/ml), thrombin (1 or 2 U/ml) and bovine collagen (0, 25, 50, 75, 100 weight % of 2.4 mg/ml collagen). Constructs were prepared with/without the presence of human foreskin fibroblasts (ATCC NIH3T3) seeded at a density of 100K cells/ml. Using a 3-mm diameter punch indenter, the indentation load-displacement response was measured after 1, 5 and 10 days of incubation at 37C in a humidified air/5%CO2 atmosphere. Four replicates per experimental condition were prepared, and three indentations per replicate were performed. Gene expression of Type-I collagen (COL1) and matrix metallop'roteinase-1 (MMP-1) levels were determined at the same time points by RT-PCR. For the unpopulated fibrin, there was a linear (R=0.983) relationship between the indentation stiffness and fibrinogen concentration. Also, there was a nonlinear relationship between indentation stiffness and concentration of collagen in the fibrin/collagen constructs. Finally, although in most cases there was no measurable change in the stiffness of the cell-populated tissue constructs with incubation time, in one of the cell-populated formulations, however, the indentation stiffness decreased steadily with increasing incubation time. These results are correlated with the gene expression results and analyzed within the context of existing analytical micromechanics models for the relationships between scaffold structure, e.g., porosity / fibril diameter, and stiffness.