DOE Grant DE-SC0000611

Structure-Property Relationships in Biomineralized and Bio-mimetic Composites

Current world technologies and environments rely in large measure upon a growing need for the development of new structural materials with high strength and durability, toughness, light weight, low cost, and complete recyclability. Such materials may be derived synthetically, but Nature has evolved efficient strategies, exemplified in the mineralized tissues of numerous species, that have led to low-weight structural materials that exhibit exceptional strength, durability, and other unique and important properties. In these biomineralized systems, minerals and proteins or additional molecules exist in close proximity and at a various length scales, through their hierarchical structure from nano- to microscale dimensions. Interactions at these inorganic-organic interfaces are vital to the functions of a great variety of structural materials and biological tissues found in the biosphere, including, for example, the shells of mollusks and the bones and teeth of vertebrates.

An active and constantly growing area of research explores the structure-mechanical property relationships of these biomineralized composites with the intention of applying this body of knowledge to the study and mimicry of biomimetic composite materials that can play key roles in developing new materials and structures. The resulting materials will offer a new combination of low weight, high strength/toughness and multifunctionality to break traditional engineering paradigms in a radical manner, and they could benefit a wide spectrum of applications including those related to the automotive industry, energy, shipbuilding, defense, and civil and aerospace engineering. Moreover, studies of biological materials, such as bones and teeth, are also of great importance in the health care arena. The development of biomaterials and tissue-engineered materials is crucial for those instances in which tissues and biological materials need to be replaced, repaired or regenerated because of congenital defects of individuals, injury and other forms of external/internal damage, or disease.

The characterization and study of these materials require the use of modern tools such as electron and scanning probe microscopy, small-scale mechanical testing capabilities, and advanced multiscale numerical models and simulation techniques. At the same time, these investigations are best accomplished through multidisciplinary approaches and so require the close collaboration among material scientists, chemists, physicist, mechanicians, bioengineers, clinicians and biologists. The main purpose of Symposium KK held at the 2009 MRS Spring Meeting was to bring together hundreds of scientists and researchers working in the diverse and interdisciplinary areas of biomineralized and biomimetic composite materials. Over 90 oral and poster presentations were given over a period of four days, including those by 20 internationally recognized invited speakers from academia, national laboratories, industries, government, and international institutions. The topics discussed included modeling and simulation of mechanical properties in biomineralized and mimetic systems, experimental design, synthesis and properties in biomineralized and mimetic systems, experimental design, synthesis and properties of biomimetic composites, structure-function properties of biomineralized tissues, biomaterials for tissue engineering, investigation of biomineralized tissue ultrastructure (inorganic and organic), quantitation of the ultrastructural response of the organic and inorganic phases in biological composites to mechanical stresses, and use of high resolution *in situ* synchrotron and spectroscopic techniques. These specific subjects were categorized and detailed in ten sessions, each demonstrating the interdisciplinary and distinctive nature of this interesting and significant area of research. A collection of several representative papers is included in the Proceedings Volume 1187.

The organizers acknowledge that the success of Symposium KK depended critically on the full participation of excellent researchers and scientists as well as graduate students. The organizers are also extremely grateful to MRS for making this Symposium possible through its dedicated work before, during and following the Meeting and to the volunteer students who kindly assisted with audio and video help during the sessions. We also thank Gatan UK, the Journal of Materials Chemistry, Nanoforce Technology, Ltd, the Office of Naval Research and the U.S. Department of Energy for their generous and substantial financial support.

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Symposium KK: Structure-Property Relationships in Biomineralized and Biomimetic Composites

April 14 - 17, 2009

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* Invited paper

SESSION KK1: Structure-Function Relationships in Biomineralized Tissues I
Chairs: David Kisailus and Eli Sone
Tuesday Morning, April 14, 2009
Room 3024 (Moscone West)

8:30 AM *KK1.1
Brachiopod Shells Control the Material Properties of Calcite. Maggie Cusack[1], Alberto Perez-Huerta[1] and Wenzhong Zhu[2]; [1]Geographical & Earth Sciences, University of Glasgow, Glasgow, United Kingdom; [2]Scottish Centre for Nanotechnology in Construction Materials, University of the West of Scotland, Paisley, United Kingdom.

Mineral-producing organisms exert exquisite control on all aspects of biomineral production. Among shell-bearing organisms, there is a wide range of microstructures. Our knowledge of the relationship between mineral microstructure and material properties and how these relates to mode of life, is limited. Nanoindentation reveals that, in brachiopod shells, calcite semi-nacre is harder and less elastic (H = 3-6 GPa; E = 60-110/120 GPa) than calcite fibres (H = 0-3 GPa; E = 20-60/80 GPa). Brachiopod calcite fibres are composed of nanogranules, yet are effectively single crystals with each granule possessing the same crystallographic orientation. In addition to this level of biological control over crystallographic orientation, the biological influence succeeds in generating fibres that are less hard and more elastic towards the shell interior than the shell exterior, producing a shell with maximum protection at the exterior and sufficient flexibility in the interior.

9:00 AM KK1.2
Abstract Withdrawn

9:15 AM KK1.3
Nacre Evolution : A Proteomic Approach. Benjamin Marie[1], Gilles Luquet[1], Arul Marie[2], Lionel Dubost[2], Milet Christian[3], Laurent Bedouet[3], Michel Becchi[4], Isabelle Zanella-Cleon[4] and Frederic Marin[1]; [1]Umr 5561 Biogeosciences, University of Burgundy, Dijon, Burgundy, France; [2]Département RDDM, MNHN, Paris, France; [3]UMR CNRS 5178, BOME, MNHN, Paris, France; [4]UMR 5086 CNRS, IBCP, Lyon, France.

For several reasons, the molluscan nacre is one of the most studied shell microstructure. It is considered by many authors as the reference model, because of its apparent geometrical simplicity, and of its exceptional toughness. The building of nacre is controlled by an extracellular organic matrix, which remains embedded within the biomineral. So far, in spite of the resolution of several nacre protein sequences, the mechanism by which nacre tablets grow and coalesce is far from being elucidated. From an evolutionary viewpoint, nacre constitutes also a fascinating object. It appeared in the Cambrian period, about 500 million years ago, and since then, has been remarkably perennial throughout the Phanerozoic times. Nacre is restricted to the mollusc phylum, where it occurs in three main classes, bivalves, gastropods and cephalopods. It is then legitimate to wonder whether all nacres are built from the same “macromolecular tools”. To this end, we investigated two new nacre models, the cephalopod Nautilus macromphalus, and the freshwater bivalve Unio pictorum. We applied to their nacre matrices a biochemical and proteomic approach, i.e., fractionation of matrix components on 2D gels, spotting, tryptic digestion, and mass spectrometry analysis. We obtained numerous peptide sequences, which represent a sampling of the whole nacre protein set. These data were compared to those obtained from already characterized nacre models, including the abalone Haliotis sp. and the pearl oyster Pinctada sp. Strikingly, our finding suggests that nacre proteins may be less evolutionary constrained than expected, and/or that similar types of nacre may be constructed through different biochemical pathways. This may have important consequences for the in vitro design of nacre-like biomaterials.

9:30 AM KK1.4
Phenomenon of Multiphase Biomineralization: Silica-Chitin-Aragonite and Silica-Calcite Biocomposiites Within Skeletal Formations of Marine Sponges. Hermann Ehrlich and Eike Brunner; Institute of Bioanalytical Chemistry, Dresden University of Technology, Dresden, Germany.

The biochemical and biophysical processes leading to the formation of complex nano- and micro-scale structures are one major unresolved problem in biology. Marine sponges are “living fossils” and produce their skeletons either from silica or calcium carbonate. A sponge of Verongida family previously assumed to form only a stable organic skeleton is now shown to actually use two different mineral phases in addition to polysaccharide chitin: amorphous silica and aragonite as crystalline calcium carbonate thus forming a completely novel hybrid biomaterial. The structure of this unique biocomposite is determined by using an arsenal of state-of-the art analytical techniques including electron diffraction, HR-TEM, Raman- and IR-spectroscopy, photoemission and X-ray absorption spectroscopy. Seemingly, chitin may be vital in many biomineralization processes across the animal kingdom, acting as an evolutionary very old template for various inorganic crystals. A related observation was made on the mace-formed spicules of the poorly investigated glass sponge Caulophacus sp. It could be found that the mace-formed structures which are responsible for the mechanical coupling between different spicules contain calcite. This unique silica-calcite biocomposite is another novel observation. Weiner and co-workers as well as Williams and co-workers described previously two kinds of multiphase-based biocomposites: silica-chitin-goethite in limpet radula, and silica-chitin-hydroxyapatite in juvenile shells of brachiopod, respectively. Therefore, the discovery of nanostructured silica-chitin-aragonite and silica-calcite biocomposites in marine sponge skeletons suggests the presence of multiphase mineralization in nature, and opens many questions relating to biomineralization and development of novel biomaterials.

9:45 AM KK1.5
Mechanical Function of a Complex Three-dimensional Suture Joining the Bony Elements in the Shell of the Red-eared Slider Turtle. Ron Shahar[1], Stefanie Krauss[2], Efrat Monsonego[3] and Peter Fratzl[2]; [1]Koret school of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel; [2]Department of Biomaterials, Max-Planck-Institute of Colloids and Interfaces, Potsdam, Germany; [3]Institute of Biochemistry and Nutrition, The Hebrew University of Jerusalem, Rehovot, Israel.

Certain design strategies appear repeatedly in a variety of biological structures. One such motif consists of a soft and pliable interface joining much larger and stiffer elements. Examples include the craniofacial sutures between the bones of the skull, the sutures between the bony plates in shell of turtles and the periodontal ligament between teeth and their sockets. Yet the detailed mechanics of these systems are not fully understood. Turtles are believed to have existed already in the early Triassic, about 200 million years ago. They are thus one of the oldest non-extinct vertebrates. Their shell is therefore a particularly attractive subject for investigation since it has developed and conserved through such an extremely long evolutionary process and has achieved a highly optimized structure. The shell of turtles is a bony shield which encases the entire body and consists of vertebrae and ribs, fused together with dermal bones which span the distance between adjacent ribs. The shell is located exterior to the limbs in a totally unique anatomical fashion. It has a ‘sandwich’ structure typical of flat bones like the skull of vertebrates. It consists of two external, relatively thin sheets of dense bone (internal endocortical and external exocortical bone plates) which contain very few voids, and between them a thick and very porous spongy bone layer. At the mid-distance between adjacent ribs the dermal bones are separated by soft sutures which have a unique and complex 3-D shape. The primary function of the shell is to protect the turtle from external trauma, and therefore it has to be stiff. However excessive stiffness may result in microdamage accumulation as a result of everyday activities like minor impact, and decrease the efficiency of respiration and locomotion. We speculate that the structure and architecture of the sutures allow easy deformation of the shell at small loads but cause it to become considerably more rigid at larger loads, reminiscent of composite materials with interlocking elements. We hypothesize that this mechanical property is related to the putative function of the suture in the turtle shell. In order to examine this hypothesis we studied samples obtained from shells of the red eared slider turtle (Chrysemys scripta elegans). We used several imaging techniques (micro-computed tomography, scanning electron microscopy and light microscopy), histology and mechanical testing. Based on these observations we present a concept of the structure-mechanics relationship that explains how the shell withstands minor loads by low-stiffness deformation, and becomes much stiffer only when the external load increases beyond a certain threshold. We also present a simple mathematical model of the deformation pattern of the suture-containing samples in 3-point bending tests and compare its predictions to our experimental results.

10:30 AM *KK1.6

Glass in Sponges. Joanna Aizenberg[1], Peter Fratzl[2] and James C Weaver[3]; [1]Harvard University, Cambridge, Massachusetts; [2]MPI, Golm, Germany; [3]UC Riverside, Riverside, California.

Structural materials in nature exhibit remarkable designs with building blocks, often hierarchically arranged from the nanometer to the macroscopic length scales. We will discuss structural properties of biosilica in glass sponges. The highly-hierarchical design of the mineralized parts of sponges overcomes the brittleness of their constituent material, glass, and shows outstanding mechanical rigidity and stability. The structure-function relationships in this biogenic composite will be presented.

11:00 AM KK1.7

Functional Biomimetics - Structure-Property Correlations in Hybrid Biological Tissues Mehmet Sarikaya[1,2], Hanson Fong[1,2,4], Malcolm L Snead[4,1] and Martha Somerman[3]; [1]Genetically Engineered Materials Science and Engineering Center, University of Washington, Seattle, Washington; [2]Materials Science and Engineering, University of Washington, Seattle, Washington; [3]Dental School, University of Washington, Seattle, Washington; [4]Craniofacial Molecular Genetics, University of Southern California, Los Angeles, California.

The structure and hierarchical organization of materials dictate their physical properties including magnetic, optical, electronic, and mechanical. In synthetic systems, elemental and molecular compositions, lattice defects and their distributions, nano- and microstructures, interfaces and grain organizations including texturing all constitute structures. In engineering systems, one seeks to control one or more of these structural parameters towards achieving certain desired properties using traditional approaches including vacuum deposition (surface and interface chemistry), melting/casting (metallurgy), powder packing and sintering (ceramics and high-T superconductors), or atom-by-atom deposition (microelectronics) or the newly developed self-assembly (usually involving a linker molecule such as thiolates or silanes) and colloidal processes (nanoparticles and molecules). In traditional systems, therefore, trial and error approaches, although extremely slow and often energy inefficient (which also may lead to toxic by-products), lead to engineered materials for practical uses. Both thermodynamically and kinetically, achieving the desired structure requires energy, which traditionally has been in the form of heat. Biological hard tissues, such as magnetic nanoparticles in magnetotactic bacteria, mother-of-pearl in mollusk shells, spicules of sponges, and bone or dental tissues of mammalians, all have specific structures, often hierarchical, evolved to provide the desired functions, i.e., super paramagnetic, strength/toughness, optical transmission, and piezoelectric, respectively. In biological systems, through many millions (perhaps, billions) of years of evolution, the materials with controlled and organized structures have become multifunctional biological devices. The common denominator in all hard tissues is the presence of peptides and proteins which control ion transport, provide molecular scaffolds, carry out the enzymatic reactions, and, simultaneously, be integral part of the system with utility not achievable in engineered materials. Proteins, based on their molecular conformation and function, therefore provide the required energy for functional materials formations. In this presentation, we provide an overview of structure-property correlations of biological hard tissues at all dimensional scales of hierarchy from the molecular to the nano- and the micrometer, and draw lessons for genetic engineering design of materials systems both for technology and regenerative medicine. Supported by NSF MRSEC, NSF-BioMat, and NIH.

11:15 AM KK1.8

High Performance Impact-Tolerant and Abrasion-Resistant Materials: Lessons From Nature James C Weaver[1], Anthony Tantuccio[3,1], Jie Lian[2], Sabrina Louie[1], Junlan Wang[2] and David Kisailus[1]; [1]Chemical and Environmental Engineering, UC Riverside, Riverside, California; [2]Mechanical Engineering, UC Riverside, Riverside, California; [3]Chemical Engineering, The Cooper Union for the Advancement of Science and Art, New York, New York.

Current methods for synthesizing impact-tolerant and abrasion-resistant materials are traditionally inefficient and costly and often require the use of environmentally hazardous components and processes. In stark contrast to their industrial counterparts, however, biological systems are well known for their ability to synthesize a wide range of high performance composites at ambient temperatures and pressures, and near neutral pH without the use of caustic precursors of byproducts. One such example is found in the mineralized teeth of the chitons, a group of benthic marine invertebrates common along the North American Pacific Coast. The teeth are anchored to a flexible belt like structure, the radula that is used for scraping algae from rocks, on which the chitons feed. Because of their constant rasping motion, the teeth must be specifically adapted to persist under such harsh conditions. Elemental mapping via Energy Dispersive Spectroscopy (EDS) in conjunction with electron and X-ray diffraction have revealed that each tooth is composed of two dominant biominerals, (an apatitic core and a thick magnetite veneer) that are intimately associated with the tooth organic matrix. Backscattered electron microscopy has also been used to investigate the interfaces between these two mineral phases and their roles in ultimately affecting the mechanical properties of the teeth. High-resolution imaging of this interface reveals that the transition between the two mineral phases is not abrupt as one would typically encounter in synthetic multi-material composites. Following mechanical loading of the teeth, cracks propagating through the apatitic phase are deflected laterally as they encounter the harder magnetite phase, revealing the fact that not only is this architecture specifically adapted for abrasion resistance, but is also very effective in preventing the propagation of large cracks originating form contact-induced surface defects. Nanoindentation of the two mineral phases reveals that there is a gradual 4-fold increase in modulus from the apatite core to the magnetite periphery of each tooth, a design strategy that has been shown through both experimental and modeling approaches to be very effective in increasing fracture toughness of related composite materials via crack deflection, without the problems associated with delamination of the two phases via complications arising from modulus mismatch. The chiton radula thus represents an excellent model system for investigating the properties of mechanically graded materials and future investigation will be aimed at elucidating the various stages of tooth maturation and mineralization. It is hoped that in the not too distant future, this and related research into the structural complexities of biological systems may ultimately guide the fabrication of a new generation of high performance synthetic materials for a wide range of technologically relevant applications.

11:30 AM KK1.9

Effect of Alport Syndrome Mutations in Tropocollagen on Molecular and Microfibrillar Mechanical Properties. Maya Srinivasan[1,2], Sinan Keten[2], Alfonso Gautieri[3,2] and Markus J Buehler[2]; [1]Mechanical and Aerospace Engineering, Princeton University, Princeton, New Jersey; [2]Laboratory for Atomistic and Molecular Mechanics, Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [3]Cellular and Molecular Biomechanics Research Group, Department of Bioengineering, Politecnico di Milano, Milan, Italy.

Alport Syndrome is a genetic disease characterized by breakdown of the glomerular basement membrane (GBM) around blood vessels in the kidney that leads to kidney failure in most patients. It is the second most inherited kidney disease in the US, and many other symptoms are associated with the disease, including hearing loss and ocular lesions. The goal of this study was to probe the mechanisms by which the disease acts, at the molecular level, using a bottom-up molecular dynamics approach. Because the GBM is under constant mechanical loading from blood flow, changes in mechanical properties, due to the mutations, are suspected to dominate the symptomatic breakdown of the GBM in Alport Syndrome patients. Through full-atomistic simulations in explicit solvent, the effects of single-residue glycine substitution mutations of varying clinical severity are studied in short segments of Collagen IV tropocollagen molecules. The segments, built from actual protein sequences, were equilibrated and subjected to tensile loading at a physiologically relevant pulling velocity. Major changes were observed at the single molecule level of the mutated sequence, including a bent shape of the structures after equilibration (with the kink located at the mutation site), significant softening of the molecule (reduction of elastic modulus), and an increase in hydrogen bond breaking rate upon application of external force. Furthermore, using the APBS electrostatics approach, the adhesion energy of a system of two molecules is calculated. We find that the adhesion energy of the mutated system is lower than that of the reference system, and thus, is more susceptible to structural degeneration under mechanical load. These results suggest that localized structural changes at amino acid level induce changes in the molecular properties and changes in the interactions between molecules. Since collagen is a hierarchical structure, the changes in molecular interactions may eventually affect the supramolecular structural arrangement and larger-scale material properties. This larger effect may induce the breakdown of the GBM in kidneys in Alport Syndrome patients.

11:45 AM KK1.10

Influence of Scaffold Composition on Gene Expression and Cellular Organization in Tissue-engineered Middle Phalanx Models of Human Digits. William Joel Landis[1], Yoshitaka Wada[1,2], Robin Jacquet[1], Elizabeth Lowder[1] and Noritaka Isogai[2,1]; [1]Integrative Medical Sciences, Northeastern Ohio Universities Colleges of Medicine and Pharmacy, Rootstown, Ohio; [2]Plastic and Reconstructive Surgery, Kinki University Medical School, Osaka, Japan.

To augment or replace defective, diseased, or impaired human digits, design and development of tissue-engineered phalanges (reviewed in Landis et al., Orthodontics and Craniofacial Research 8:303-312, 2005) are important and include a middle phalanx model. This construct consists in part of two square-shaped biodegradable polyglycolic acid (PGA) scaffolds (1 x 1 x 0.2 cm in length, width and thickness, respectively) seeded with cartilage cells (chondrocytes) obtained from young calves. One such seeded scaffold is sutured to each end of a rectangular-shaped scaffold (~2 x 0.7 x 0.5 cm in length, width and thickness) serving as the midshaft of the model. To examine the biological regenerative capacity of these biomimetic composites, midshafts were left uncovered or wrapped with periosteum, a tissue from calves giving rise to cartilage and bone. Midshafts were composed of poly(L-lactide-ε-caprolactone) [P(LA-CL)] or one of two ceramics, hydroxyapatite (HA) or β-tricalcium phosphate (β-TCP), admixed with P(LA-CL). When engineered middle phalanx models were implanted and grown for up to 20 weeks under dorsal skin flaps of athymic (nude) mice, resulting constructs varied in their midshaft bone and end plate cartilage composition and structure. Harvested from mice at 20 weeks, constructs (n = 3) without periosteum developed viable end plate cartilage as determined by Safranin-O staining for chondrocyte-secreted proteoglycans but cells were not organized as in normal growth plate cartilage of human digits. Midshafts remained devoid of cells and mineral. Implanted for the same 20 week period, constructs comprised of P(LA-CL) (n = 3), HA-P(LA-CL) (n = 3), or β-TCP-P(LA-CL) (n = 3) and enclosed by periosteum each developed viable end plate cartilage whose chondrocytes were organized into columns resembling normal growth plate cartilage of digits. Midshafts mineralized through the normal process of endochondral ossification. While these features were common to all periosteum-wrapped composites, specific differences occurred between them, apparently depending on midshaft copolymer composition. In particular, gene expression of end plate chondrocytes varied in their levels of type II collagen, aggrecan (proteoglycan), or bone sialoprotein, all markers for development of normal cartilage extracellular matrix and mineralization. Further, the rate of mineral formation over 20 weeks of implantation varied in construct midshafts. These results indicate that the composition of midshaft scaffolds comprising middle phalanx models of human digits affects the composition and structure of both midshaft bone and end plate cartilage of constructs. Further studies are ongoing to define more completely relationships between the structure and composition of bone and cartilage tissues developed and the properties of their underlying copolymer scaffolds in these biomineralized models.

SESSION KK2: Structure-Function Relationships in Biomineralized Tissues II
Chairs: Lara Estroff and Christine Orme
Tuesday Afternoon, April 14, 2009
Room 3024 (Moscone West)

1:30 PM *KK2.1
Alternative to DOPA in a Mussel Adhesive Protein. Herbert Waite, Marine Science Institute, UCSB, Santa Barbara, California.

The 3, 4-dihydroxyphenylalanine (Dopa) containing proteins of mussel byssus play a critical role in wet adhesion and have inspired versatile new synthetic strategies for adhesives and coatings. Apparently, however, not all mussels are beholden to using Dopa in their byssal proteins. The cDNA-deduced sequence of pvfp-1, a highly aromatic and redox-active byssal coating protein in the green mussel Perna viridis indicates that Dopa has been replaced by a post-translational modification of tryptophan. Pvfp-1 contains 42 canonical consensus repeats in the "repeat domain". A small collagen domain (18 Gly-X-Y repeats) is also present. Tandem mass spectrometry of isolated tryptic decapeptides has detected both C2-hexosylated tryptophan and C2-hexosylated hydroxytryptophan of which the latter is redox active. These represent intriguing new themes for bioinspired opportunistic wet adhesion.

2:00 PM *KK2.2
Plant Actuation by the Swelling of Cellulose Nanocomposite Architectures. Peter Fratzl and Ingo Burgert; Biomaterials, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany.

Plants have evolved the ability to actuate some of their organs by water swelling and de-swelling of the cell walls. This passive actuation allows for movements even in dead parts of the organism, just relying on cyclic changes in air humidity. It is quite interesting to note that this actuation functions without the need for an active metabolism, very unlike molecular motors in the mammalian muscle, which makes plant cell walls particularly interesting in the context of biomimetic materials research. The structural basis for the functionality of these natural devices is a composite of stiff cellulose fibrils embedded in a pliant and highly swellable matrix consisting of hemicelluloses and pectins or lignin which constitutes the cell wall. All organ movements due to moisture changes in cell walls are controlled by the architecture of the stiff cellulose fibrils and the matrix polymers. The swelling or shrinking of the matrix can cause a substantial bending of the organ as known for instance for wheat awns and pine cones. Depending on the details of the fibre architecture, which acts mechanically in analogy to a lever arm, the energy from swelling can be transformed either into comparably large movements (as required for seed dispersal) or larger stresses with minimal movement (as needed to keep organs straight or upright). With this versatility, fibre-reinforced swelling gels are promising materials for biomimetic actuation in a variety of applications.

2:30 PM KK2.3
Adhesive Structure of the Freshwater Zebra Mussel, Dreissena polymorpha. Nikrooz Farsad[1], Trevor W Gilbert[2] and Eli D Sone[1,2,3]; [1]Materials Science & Engineering, University of Toronto, Toronto, Ontario, Canada; [2]Institute of Biomaterials & Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada; [3]Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada.

The freshwater Zebra Mussel, Dreissena polymorpha, is notorious in the Great Lakes region for its ability to attach to hard surfaces such as boat hulls and water intake pipes. This adhesive facility has led to the rapid spread of the invasive species since its accidental release ~20 years ago, resulting in serious environmental and economic consequences. Understanding the mechanisms of Zebra Mussel adhesion could lead to targeted strategies for preventing biofouling, as well as providing lessons for overcoming the technical challenges of wet adhesion in dental and medical applications. Like the well-studied marine mussels, attachment of the Zebra Mussel is achieved by means of its byssus, a series of proteinaceous threads that connect the animal to adhesive plaques secreted onto the substrate. Here, as in biomineralized tissues, the structure of the organic-mineral interface is critical to the function of the system as a whole. While the byssus of the Zebra Mussel is superficially similar to those of its marine counterparts, significant structural and compositional differences suggest that further investigation of the adhesion mechanisms in freshwater species is warranted. We report here on our ultrastructural investigations of the Zebra Mussel byssus, with emphasis on the plaque-substrate interface. In particular, we examine for the first time the detailed distribution of DOPA (3,4-dihydroxyphenylalanine)-containing proteins in the Zebra Mussel plaque. These proteins are believed to play a central role in the attachment of the mussel's adhesive plaque to the substrate, as has been shown for marine mussels. In addition, we present our findings on the behavior of peptide mimics of Zebra Mussel byssal proteins in solution and on solid substrates, in an attempt to elucidate the functional role of the proteins themselves in the adhesive structure.

2:45 PM KK2.4
Nano-Porous Sucker Rings from Dosidicus gigas. Miserez Ali[2,3], James C Weaver[4], Peter B Pedersen[1], Todd Schneeberk[2], Roger Hanlon[5], David Kisailus[4] and Henrik Birkedal[1]; [1]Department of Chemistry & Interdisciplinary Nanoscince Center, Aarhus University, Aarhus, Denmark; [2]Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, California; [3]Materials Department, University of California, Santa Barbara, Santa Barbara, California; [4]Department of Chemical and Environmental Engineering, University of California, Riverside, Riverside, California; [5]Marine Biological Laboratory, Woods Hole, Massachusetts.

Nature presents a wealth of unique hierarchical materials designed for mechanical function. While many of these structures are mineralized, others exhibit only sparse mineralization or are wholly organic. Here we report on the structural characterization of one such example, the sucker rings from the Humboldt squid, Dosidicus gigas, a large, aggressive and predatory species commonly encountered throughout the Eastern Pacific. The sucker rings are rigid toothed ring-like structures within the suckers that provide additional gripping power during prey capture and handling. As revealed from these studies, the sucker rings from this species exhibit a unique set of characteristics not reported previously for any other biological structural material. They consist of an proteinaceous nanoscale network of densely packed parallel tubular elements that are presumably stabilized almost entirely by hydrogen bonding and hydrophobic interactions. The network of channels exhibits a distinctive organizational gradient, reducing is diameter and abundance from the tooth core to the periphery. The mechanical properties of the bulk composite can be explained through results obtained from both nanoindentation and modeling studies. Additional investigations into the mechanisms of molecular self assembly that result in the formation of these porous biological structures may ultimately reveal novel design strategies for the synthesis of robust, wholly organic structural composites.

3:30 PM *KK2.5

In-vitro Mechanical Testing of Single Collagen Nanofibrils Steven Eppell[1], Zhilei Liu Shen[1], Reza Daj[1], Harold Kahn[2] and Roberto Ballarini[3]; [1]Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio; [2]Materials Science and Engineering, Case Western Reserver University, Cleveland, Ohio; [3]Civil Engineering, University of Minnesota, Minneapolis, Minnesota.

Predicting mechanical behavior of biological materials like bone and tendon requires application of multiscale models. These models use as inputs constitutive parameters of the individual structures within the hierarchy of the material. Many structural biological materials contain fibrils that are a few hundred nanometers in diameter, ~100 um long, and contain only type I collagen molecules and water. We used a method based on Microelectromechanical Systems (MEMS) technology allowing in-fluid uniaxial tensile tests on such fibrils isolated from sea cucumber dermis (Eppell et al, J R Soc Interface. 2006, 3(6):117-21). Two types of tests were performed in 1X PBS buffer at room temperature: monotonic stretching to fracture and cyclic loading. Stress-strain curves during monotonic strain to fracture were generated from six fibrils (diameters of 240-690 nm). Four fibrils broke at smaller stresses (20-110 MPa) and strains (40%-60%) and displayed relatively linear behavior prior to fracture. Two fibrils broke at larger stresses (240-440 MPa) and strains (120%-180%) and showed yielding prior to fracture. Interestingly, these strengths are in the range of tendon (20-100 MPa) and whole bone (~200 MPa). To our knowledge, these are the first experimental data providing in-fluid fracture properties of single collagen fibrils. The Young’s modulus determined from the linear regions was 180 ± 140 MPa (mean ± SD; range, 60-440 MPa), which was significantly lower than that obtained from in-air experiment (860 ± 450 MPa, Shen et al, Biophys J. 2008 95(8):3956-63). For the cyclic loading tests, two fibrils were tested up to 50% strain at strain rates spanning two orders of magnitude (0.1%/sec, 1%/sec and 10%/sec). The stress-strain curves were similar at all strain rates showing little hysteresis indicating no significant strain-rate dependent effects. Collagenous tissues such as tendon are known to be viscoelastic. Our results suggest that the viscous behavior arises from structures other than the collagen fibril itself, such as ground substance (proteoglycans) or fibril/fibril interactions.

4:00 PM *KK2.6

Cement Lines and Bone Quality - A Lesson from Studies of Long-lasting Cement Lines in Osteopetrotic Bones of Aging c-src Deficient Mice. Yoshiro Takano, Biostructural Science, Dept. of Hard Tissue Engineering, Tokyo Medical and Dental School, Tokyo, Japan.

In normal bones, “Fatigued bone matrix” is constantly replaced by a newly formed matrix in the process of remodeling, in such a way that the integrity of internal structures and quality of bone is maintained. As a consequence of perpetual remodeling, bone eventually becomes full of osteons, each bounded by a thin layer of cement lines, which are the thin mineralized layers interposed between the resorbed bone surfaces and the newly deposited matrix. Since the cement line is not traversed by matrix collagen fibers, it has been regarded as an interface of weak mechanical strength and hence one of plausible factors relevant to compromised mechanical properties of bone. In this context, bone remodeling create a complex situation with respect to maintenance of mechanical integrity of bone because it eliminates damaged bone matrix but at the same time appears to create new internal failures. Since cement lines are the interface of osteons where the propagation of microcracks is reported to be arrested or diverged and hence accumulated, precise understanding of the properties of cement lines is highly relevant to elucidate practical roles of cement lines and significance of its replacement in bone remodeling. In osteopetrotic bones of c-src deficient mice, slow and inefficient remodeling due to hypofunctional osteoclasts continues throughout life, leaving numerous “old” cement lines and microfractures. Such cement lines progressively mineralize and become brittle with age, but show no spatial relation to microfractures. Contrary to the bones of young osteopetrotic animals, the slow-remodeling, old osteopetrotic bones display significantly elevated strength relative to the bones of age-matched wild type animals, suggesting that cement lines in these bones are not internal failure and further that long-lasting cement lines have no negative impacts on mechanical property of bone. Precise analyses of osteopetrotic bones of aging c-src deficient mice will be demonstrated.

4:30 PM KK2.7

Unraveling Pathways for Functional Remineralization of Dentin. Luiz Eduardo Bertassoni, Stefan Habelitz, Megan Pugach, Sally J Marshall and Grayson W Marshall; Preventive and Restorative Dental Sciences, UCSF, San Francisco, California.

Dentistry is currently undergoing major conceptual changes that emphasize minimally invasive treatments. Remineralization of dentin, due to the complexity of its structural composition, remains among the most difficult and critical steps needed to preserve the maximum amount of tooth structure. Objective: To gain insights into the biomechanical recovery of dentin by means of remineralization. Methods: Structural characterization was performed by SEM, AFM and AFM-nanoindentation. The elastic modulus and hardness of hydrated sound dentin was compared to simulated carious lesions after various remineralizing treatments. Demineralization experiments used dentin substrates (12mm2) treated with acetate buffer (pH=5.0, 0.05M CaPO4, 8hrs). Remineralization experiments used calcium and phosphate solutions (pH=7.4) and compared static with constant solution composition (CSC) approaches (5 days). Secondly, using the CSC approach (24hrs), we assessed the influence of high and low degrees of saturation (DS) on the tissue’s mechanical recovery. Data analysis used a mixed effects regression model. Results: Demineralization experiments revealed that different acids left different amounts of mineral attached within the collagen fibrils (intrafibrillar-mineral). CSC experiments recovered the properties of demineralized dentin up to 65% of normal dentin, whereas the static approach recovered only by 10%. Comparison at different DS's revealed that mechanical recovery of demineralized dentin did not significantly depend on degree of saturation, as long as solutions are metastable. Cross-section analyses revealed that demineralization decreased the properties of dentin gradually from the outer-most zone inward, and remineralization recovered the properties gradually from the inner-most zone outward, yielding nonhomogeneous zones of full mechanical recovery. Conclusion: We provide first evidence that functional remineralization of dentin is achievable and theorize that this process depends upon the presence of intrafibrillar-mineral within the matrix, which may act as nucleation sites for mineral re-growth. Support :NIH DE16849

4:45 PM KK2.8

Damage and Crack Evolution in Mammalian Enamel James Jin-Wu Lee[1,2], Brian R Lawn[1], Paul Constantino[2] and Peter Lucas[2]; [1]Ceramics Division, National Institute of Standards and Technology, Gaithersburg, Maryland; [2]George Washington University, Washington, District of Columbia.

Mammalian teeth are inherently weak structures consisting of a protective brittle enamel coat on a soft dentin interior. However, teeth are also remarkably damage-tolerant and resilient, and can survive through the lifetime of the animal. In this work we report the result of fracture experiments on extracted human molars, in which crack evolution to failure is observed in situ during simulated occlusal loading. We describe how these cracks run longitudinally around the tooth walls at increasing load, ultimately linking the occlusal surface and cervical margins to cause 'failure', all the time remaining confined within the enamel. Critical loads to attain this failure condition are measured and compared with predictions from simple fracture mechanics models. Important intrinsic factors are enamel thickness and tooth size, as well as enamel modulus and toughness. Size and modulus of the object in actual occlusal contact (opposing dentition or intervening food) are important extrinsic factors. Detailed sectioning of failed teeth is performed to determine the role of enamel microstructure on the crack evolution. It is shown that inherent defects within the enamel provide weak pathways for fracture, yet act to inhibit prolonged crack propagation by deflecting and bridging the cracks or by self healing. This work has implications that extend beyond dentistry to anthropology. Some comparative experimental data on sea otters, which have a similar tooth configuration to that of humans but with different dimensions, are presented in support of this contention.

SESSION KK3: Structure-Property Relationships in Biomimetic Composites I
Chairs: Laurie Gower and Pablo Zavattieri
Wednesday Morning, April 15, 2009
Room 3024 (Moscone West)

8:30 AM *KK3.1

Spider Silk as a Novel High Performance Biomimetic Muscle Driven by Humidity. Ingi Agnarsson[2], Ali Dhinojwala[1], Vasav Sahni[1] and Todd Blackledge[2]; [1]Polymer Science, The University of Akron, Akron, Ohio; [2]Intergrated Bioscience Program, The University of Akron, Akron, Ohio.

The abrupt halt of a bumble bee's flight when it impacts the almost invisible threads of an orb web provides an elegant example of spider silk's amazing strength and toughness. Spiders depend upon these properties for survival, yet silk's impressive performance isn't limited solely to tensile mechanics. Here, we show that spider silk also exhibits powerful cyclic contractions that allow silk to act as a high performance mimic of biological muscles. These contractions in spider silk are actuated by changes in humidity alone and can repeatedly generate work 50x greater than the equivalent mass of human muscle. The simplicity of using wet or dry air to drive the biomimetic silk muscle fibers and the incredible power generated by the silk offer unique possibilities in designing light weight and compact actuators for robots and micro-machines, new sensors, and green energy production.

9:00 AM KK3.2

Silk/Silica Biomaterials for Bone Remodeling Aneta Joanna Mieszawska[1], Carole C Perry[2] and David L Kaplan[1]; [1]Biomedical Engineering, Tufts University, Medford, Massachusetts; [2]School of Science and Technology, Nottingham Trent University, Nottingham, United Kingdom.

The hypothesis for the proposed study is that novel biomaterials for bone reconstruction can be designed with a precise control over the organic and mineral parts that mimic, to the great extent, the natural constituents of bone. Our approach is based on using biodegradable silk from Bombyx mori silkworm as an organic scaffold with impressive mechanical properties. The self assembly of silk material into highly stable beta-sheet structures generates a strong composite matrix that can be processed into versatile morphologies such as fibers, hydrogels or films offering a wide range of applications. Additional silk modifications with silica mineral particles generate the final biomaterial where the degree of mineralization is controlled by silica content and variations in nanoparticles sizes. In a related approach we use a chemical modification of silk fibroin with silica precipitating peptides to generate biomaterials mineralized by chemical reaction. The overall goal of this study is to elucidate how alterations in the material design will lead to predictable changes in composite material properties and also how the material mineralization conditions influence material morphology and performance. The studies to date indicate successful formation of such nanocomposites with remarkable mechanical properties, biocompatibility, and a variety of morphologies. The following in vitro studies towards osteogenic outcomes determine the bone remodeling properties of the material, biodegradability, and replacement with a functional tissue.

9:15 AM KK3.3

Genetically Engineered Chimeric silk/ Metal Binding Proteins. Heather Currie[1], Rajesh R Naik[2], Carole C Perry[3] and David L Kaplan[1]; [1]Department of Biomedical Engineering, Tufts University, Medford, Massachusetts; [2]Materials and Manufacturing

Directorate, Air Force Research Laborartory, Wright-Patterson Air Force Base, Dayton, Ohio; [3]School of Science and Technology, Notingham Trent University, Nottingham, United Kingdom.

The growing interest in proteins capable of interacting and nucleating ions into unique, and often ornate, structures, such as the distinctive silica structures of marine diatoms and siliceous plants or the calcium deposition in bone and nacre, is the source of inspiration for this work. In Nature these composite or hybrid materials are limited in the ions nucleated by organic templates, the ability to utilize additional ions would offer options to generate materials with an array of properties which are physically, electrically, optically or magnetic in nature. Therefore, we are generating bioengineered fusion proteins which incorporate both metal binding domains, determined by biopanning experiments, and an organic phase based on the consensus repeat of Nephila clavipes spider silk. The silk self assembles to form highly stable beta-sheet structures imparting this material with impressive strength and toughness. A series of chimeric silk proteins containing metal binding peptides is being studied for the above purposes. Purification tag free systems usable in E. coli are exploited in the work to examine protein assembly and metal binding functions without interference from his tags. The studies yield fusion proteins examined for their ability to nucleate and influence metal ions and analysis of their composite structural features.

9:30 AM KK3.4

Effect of CaSiO3 structure and texture on the in vitro behavior of Human Mesenchymal Stem Cells Nianli Zhang[1], Nita Sahai[1,3], Jim Molenda[2] and William Murphy[2]; [1]Department of Geology and Geophysics, University of Wisconsin - Madison, Wisconsin; [2]Department of Biomedical Engineering, University of Wisconsin - Madison, Madison, Wisconsin; [3]Department of Chemistry, University of Wisconsin - Madison, Wisconsin.

We hypothesize that silicate bioceramic structure controls dissolution kinetics of the bioceramic, thus, Ca and Si ion release rates, ultimately affecting human mesenchymal stem cell (hMSC) viability and differentiation into osteoblasts. Wollastonite and pseudowollastonite are two polymorphs of CaSiO3. Wollastonite is made up of silicate tetrahedra linked into silicate chains that are held together by Ca ions, whereas in pseudowollastonite, three silicate tetrahedra are linked in the form of highly-strained rings that are connected by Ca ions. These differences in silicate structure control hydroxyapatite layer formation on the surfaces of these two materials in Simulated Body Fluid (SBF), suggesting they may be good candidates for bone tissue engineering. However, few studies have compared the in vitro effects of these two materials on the activities of hMSCs. We tested our hypothesis by using wollastonite and pseudowollastonite bioceramic pellets, of similar roughness and surface texture, for in vitro cell culture experiments. The Ca, Si, and P concentrations in the media were analyzed by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy). Fluorescence images and TOT DNA kits were used to investigate cell viability and proliferation, and alkaline phosphatase (ALP) production was monitored as an indicator of cell proliferation. Results indicated that initial dissolution of pseudowollastonite is faster leading to higher Si concentration (~120 ppm) than that of wollastonite with lower Si concentration (~15 ppm) at day 1. The fluorescence results indicated that the attachment and viability of hMSC on wollastonite are better than on pseudowollastontie at day 1. After day 1, cells were able to proliferate on both substrates, which is consistent with total DNA data. DNA data also suggest cell number increased at higher rate on wollastonite substrate than on pseudowollastonite. Interestingly, however, we found higher ALP/DNA ratios from pseudowollastonite than wollastonite cell culture, which suggests that pseudowollastonite promote cell differentiation better than wollastonite. The combined data suggest that, for bioceramic surfaces of similar roughness and texture, and identical chemical composition, silicate crystal structure controls dissolution rate and, thus, Si and Ca release rates. High Si concentrations are cytotoxic to hMSCs, but when the surviving cells reach confluence, Si promotes differentiation to osteoblasts. Thus, bioceramic structure (and texture) can control cell attachment, viability, and differentiation.

9:45 AM KK3.5

Modeling the Mechanical Properties of a Soft Matrix in Biological Composites. Markus A. Hartmann and Peter Fratzl; Biomaterials, Max-Planck-Institute of Colloids and Interfaces, Potsdam, Germany.

Biological materials such as bone obtain their outstanding mechanical properties, a high stiffness together with an elevated toughness, through the hierarchical arrangement of materials with opposing properties. Collagen, the organic matrix of bone, is a soft, but tough material, whereas the inorganic reinforcing mineral platelets of hydroxyapatite are very stiff, but brittle. According to recent models, these two constituents are arranged in such a way, that during deformation the mineral particles are predominantly loaded in tension, while the soft matrix experiences shear deformation [1]. Thus, understanding the shear behaviour of the soft matrix is of great interest and importance to understand the mechanical properties of bone as a whole. Recent experiments show evidence that the mechanical performance of the matrix may be governed by electrostatic interactions between negatively charged proteins and positively charged divalent ions, most probable calcium [2]. Motivated by these findings we investigated the shear behaviour of a simple model system solely governed by electrostatics by means of Monte Carlo simulations. Our model consists of two parallel plates, which both carry negative charges resembling negatively charged proteins that are found in bone. Charge neutrality is ensured by divalent counterions that can freely move between the two plates. While one of the two plates is held fixed, the other can move freely. Two extreme scenarios were investigated: (1) the negative charges on the two plates were arranged ordered according to a triangular lattice and (2) the same number of negative charges was distributed randomly. The shear behaviour of the two arrangements was completely different. While in the ordered case the material showed elastic and ideally brittle behaviour, in the non-ordered case pronounced plastic deformation was observed. In the ordered case all formed bonds were loaded in exactly the same way, leading to a high shear modulus, but to a low ultimate strain. On the contrary for the disordered system the ultimate loads were strongly reduced, while the ultimate strain increased significantly. The process behind this behaviour is a stick-slip mechanism. When in one part of the system the load was too high and led to local failure, after a period of plastic deformation the system eventually found another (more stable) configuration that prevented it from failing. Although the disordered system could carry only much lower loads than the ordered one, due to the much higher ultimate strain the energy dissipation (comparable to the toughness) of the two systems was comparable. Considerations on how bone achieves its high stiffness together with elevated toughness, shows that such a mechanical behaviour of the soft matrix is highly desirable [3]. [1] I. Jäger & P. Fratzl, Biophys. J 79, 1737 (2000) [2] H. Gupta et al., J. R. Soc. Interface 4, 277 (2007) [3] P. Fratzl & R. Weinkamer, Prog. Mat. Sci. 52, 1263 (2007)

10:30 AM *KK3.6
Biologically Inspired Strategies for Interfacial Control in Polymer Nanocomposites. Phillip Messersmith, Biomedical Engineering, Northwestern University, Evanston, Illinois.

Marine and freshwater mussels are famous for their ability to permanently adhere to a wide variety of wet surfaces, such as rocks, metal and polymer ship hulls, and wood structures. To accomplish this they secrete a series of byssal threads which serve to tether the mussel onto substrates. Located at the distal end of each thread is an adhesive pad containing specialized proteins collectively referred to as mussel adhesive proteins (MAPs). Of great interest to us as well as other groups, is to enhance our understanding of the molecular aspects of biointerfacial adhesion and the translation of this knowledge into new strategies for control of interfacial adhesion in practical materials systems. In this talk the composition, properties, and adhesive mechanisms of MAPs will be described. Of primary interest will be the interfacial role of 3,4-dihydroxy-L-alanine (DOPA), an unusual amino acid found in high concentration in MAPs. Previous observations that DOPA is highly enriched in proteins found near the interface between adhesive pad and substrate, have led to speculation that DOPA plays an important interfacial role. Single molecule force spectroscopy has begun to shed light on the adhesive roles of DOPA and other key amino acids found in these proteins. Specifically, we have shown that DOPA interacts remarkably strongly with both organic and inorganic surfaces, in some cases in a reversible manner. Interactions between DOPA and surfaces appears unaffected by the presence of excess water, suggesting that similar chemical functional groups could form the basis of new water resistant interfacial bonding agents for composites designed for wet or humid environments. Cooperativity among DOPA residues is also being studied, with implications for design of self-healing and energy dissipating interfaces. Finally, the biophysical studies of mussel adhesive proteins are providing new ideas for control of interfaces between materials and biological systems, and for design of interfaces in composites. Due to their extremely high interfacial surface areas, nanocomposites represent an interesting platform for testing some of these ideas. For example, polymer mimics of mussel adhesive proteins are showing early promise for constructing high strength polymer/clay nanocomposites and polyelectrolyte multilayer films, wherein the DOPA functional groups appear to play a crucial role in enhancing mechanical properties.

11:00 AM KK3.7
A New Tool for Determining Intra- versus Inter-fibrillar Mineral Content in Biomimetic Bone Composites. Sang Soo Jee, Taili Thula, Elliot P Douglas and Laurie B. Gower; Materials Science & Engineering, University of Florida, Gainesville, Florida.

Bone is a hierarchically-structured composite which at the nanostructural level consists of an interpenetrating network of platelets of hydroxyapatite embedded within a collagen matrix. The interpenetrating nature of the organic-inorganic phase has been qualitatively demonstrated using selective etching techniques, and there have been various estimates of the degree of intra- versus inter- or extra-fibrillar mineral in bone. Now that the nanostructured architecture of bone has been successfully mimicked in the lab (by our group and others), it is conceivable that the next generation of bone-graft substitutes will have mechanical properties and bioresorptive potential similar to bone. As we begin to test the mechanical properties of such biomimetic composites, a means for quantitatively determining the degree of intrafibrillar mineral will contribute toward understanding and mimicking bone's mechanical behavior. We have found that thermal analysis may be a useful tool in this regard because collagen exhibits distinctly different calorimetry peaks when it is in the pure state versus in bone. Our biomimetic composite with bone-like nanostructure shows very similar behavior, where the high temperature peak that is found for pure collagen shifts to lower temperature as it is progressively mineralized, and matches that of bone at high degrees of mineralization. This shift in the exotherm peak to lower temperature has been attributed to a disruption of crosslinks between tropocollagen molecules as the crystals form between them. Based on infrared analysis, we propose a different explanation for the high temperature peak, and demonstrate that thermal analysis can provide a quantitative tool for characterizing the organic-inorganic interactions in bone and related biomimetic composites.

11:15 AM KK3.8
Controlled Magnetite Formation by Mimic Peptides from the Mms6 Protein of Magnetotactic Bacteria. Atsushi Arakaki, Fukashi Masuda, Yosuke Amemiya and Tadashi Matsunaga; Life Science and Biotechnology, Tokyo university of Agriculture and Technology, Tokyo, Japan.

Magnetite particles produced by magnetotactic bacteria are dependent on bacterial species or strains, suggesting presences of biologically controlled mechanism in each organism. To elucidate the molecular mechanism of bacterial magnetite biomineralization, proteome analyses of the magnetite surface proteins have been recently conducted. Mms6 is a small acidic protein which is tightly associated with bacterial magnetite surface in *Magnetospirillum magneticum* AMB-1. The amino acid sequence of this protein is amphiphilic, and consists of an N-terminal LG-rich hydrophobic region and a C-terminal hydrophilic region containing multiplets of acidic amino acids. Following competitive iron binding analysis with other inorganic cations, it has been suggested that the acidic region is an iron binding site. Furthermore, magnetite has been formed by co-precipitation of ferrous and ferric ions in the presence of Mms6 producing uniformed crystals with sizes ranging from 20 to 30 nm, while the absence of this protein resulted in the formation of magnetic particles of irregular shapes and sizes. However, the exact role of Mms6 in the magnetite synthesis process still remains unknown. In this study, we designed short peptides by mimicking characteristic amino acid sequences of Mms6, and utilized them for the *in vitro* magnetite synthesis. Magnetite synthesis was performed by partial oxidation of ferrous hydroxide in the presence of the peptides, and the crystallographic characteristics of the synthesized magnetites were analyzed and compared. The magnetite synthesis using the peptides containing C-terminal acidic region of Mms6 resulted in the formation of a uniformed size and narrow size distribution with a cubo-octahedral morphology. In contrast, rectangular particles with sharp corners were obtained when other proteins or peptides were used. Size distribution and circularity of the synthesized magnetite particles were also statistically analyzed from transmission electron micrographs. These results indicated that the magnetite particles synthesized by the *in vitro* chemical synthetic method with the Mms6 peptides revealed similar features that of biogenic magnetites and the method presents an alternative route for controlling the size and shape of magnetite crystals without the use of organic solvent and high temperatures.

11:30 AM KK3.9
Abstract Withdrawn

SESSION KK4: Structure-Property Relationships in Biomimetic Composites II
Chairs: Himadri Gupta and Nico Sommerdijk
Wednesday Afternoon, April 15, 2009
Room 3024 (Moscone West)

1:30 PM *KK4.1
Inorganic interaction Protein Sequences: Lifetime Members of the Unique ``Intrinsically Disordered Protein" Sequence Club. John Spencer Evans, Fairland Amos and Irit Katash; New York University, New York, New York.

he unstructured state, common to all living organisms, is essential for basic cellular functions, and has now become recognized as a separate functional and structural category within the protein kingdom. These proteins are characterized by an almost complete lack of folded structure and an extended conformation with high intramolecular flexibility and little secondary structure. IDPs fall into five broad functional classes based on their mode of action: entropic chains, effectors, scavengers, assemblers, and display sites. The major functional asset of IDPs is their disorder - order chain transition when the protein binds or interacts with its molecular target. This transition is accompanied by a large decrease in conformational entropy, which uncouples binding strength from specificity and renders highly specific interactions reversible. We now believe that many biomineralization proteins belong to this interesting class of proteins. To support this hypothesis, we marshall experimental data that shows that the protein sequences found in mollusk shell and tooth enamel matrices are not only unfolded or partially folded in solution and consist of disorder-promoting amino acids, but that these sequences are functionally active in this state. We also believe that inorganic interaction bacterial and phage display sequences directed against inorganic materials in the laboratory are also drawing from the IDP family. We provide an overview of the unfolded structural features of known inorganic binding sequences and describe their similarities and differences. Our conclusion is that the sequence and structural features that are encoded within the IDP proteomes for biomineralization may serve as a potential source of sequences for inorganic interaction and materials/nanotechnological applications.

2:00 PM *KK4.2
Biologically Inspired Organic-Inorganic Nanocomposites. Ilhan Aksay, Chemical Engineering, Princeton University, Princeton, New Jersey.

Biologically produced materials are multifunctional, adaptive, and have properties (e.g., sensing and actuation, self-replication, self-healing) that we are yet to introduce into man-made materials. The objective of this presentation will be to provide an understanding of the biological processes for controlling materials properties through nano- and microstructural design and processing with the goal of attaining multifunctional and adaptive materials. "Self-assembly" and "pixelation" are the prevailing mechanisms used in the construction of biological structures. However, the current methods suffer from drawbacks that limit their application: (i) Self-assembled structures have uncontrolled multiple domains at micrometer and larger length scales due to the statistical nature of domain nucleation and growth. (ii) The location of individual structures cannot be controlled, making integration into macroscopic structures difficult. (iii) The small size and high density of self-assembled units require new methods of addressing, differentiating, and interconnecting. To address these drawbacks, I will demonstrate examples of the integration of self-assembled nm- to µm-scale building blocks into spatially defined ("pixelated") macroscopic structures. As an alternative approach, I will also demonstrate how some of the structural design principles utilized in biological nanocomposites can be used with nanofillers such as molecular sheets of graphene to produce polymer matrix composites that display multifunctional properties.

2:30 PM KK4.3
Single Crystals with Incorporated Polymer Networks: Hierarchical Structures and Improved Mechanical Properties. Hanying Li[1], Huolin L Xin[2], David A Muller[3] and Lara A Estroff[1]; [1]Material Science and Engineering, Cornell Univeristy, Ithaca, New York; [2]Department of Physics, Cornell University, Ithaca, New York; [3]School of Applied and Engineering Physics, Cornell University, Ithaca, New York.

One of the interesting characteristics of biominerals is that they often incorporate a biomacromolecular matrix while still diffracting X-rays or electron beams as single crystals. We examine, in detail, the internal structure of synthetic calcite crystals grown in an agarose hydrogel and show that the gel-grown calcite crystals, like biogenic calcite crystals, incorporate the polymer network without disrupting their single crystal nature. Annular dark field scanning transmission electron microscopy (ADF-STEM) and electron tomography reveals a 3-dimensional random networks of agarose fibers (diameters ranging from several nanometers to 20 nm) penetrating throughout the entire calcite crystals. Both selected area electron diffraction (SAED) and ADF-STEM lattice images demonstrate that even in the presence of large amounts of organic fibers, the crystals still retain their single crystal nature. The incorporation of the polymer network gives the calcite single crystals a hierarchical structure. Macroscopically, the crystals maintain the characteristic rhombohedral morphology of calcite, expressed by six {104} faces. At a length scale of 100's nm, the interfaces between crystals and fibers are curved and thus are defined by the contours of the agarose fibers. At shorter length scales (10's nm), however, the interfaces are faceted. In addition to the low energy {104} facets, high energy facets such as {012} facets are also observed by ADF-STEM. When we remove (by burning) the polymer phase, we observe that the crystalline structure collapses into a multi-crystalline aggregate, at the temperature at which the polymer was completely removed. The presence of the polymer inside of the crystals also improves the toughness of the calcite crystals, as evidenced by non-angular features that were observed on fractured surfaces. In conclusion, the gel-grown calcite crystals provide an excellent platform for characterizing the structure-property relationships in polymer-reinforced single crystals.

2:45 PM KK4.4

Crystallisation of Calcium Carbonate within Controlled Microenvironments Yi-Yeoun Kim[1], Chris Stephens[2], Nicola B Hetherington[1], Ana-Paula Ramos[1], Hugo K Christenson[2] and Fiona C Meldrum[1]; [1]Chemistry, University of Bristol, Bristol, United Kingdom; [2]Physics, University of Leeds, Leeds, Yorkshire, United Kingdom.

A key feature of biologically-controlled mineralisation is that mineral formation occurs within well-defined microenvironments. Further, there is growing evidence that crystal nucleation and growth processes are often modified in confined volumes as compared with bulk solutions. The work described here provides a systematic study of the precipitation of calcium carbonate within well-defined volumes using two contrasting systems. In the first system, calcium carbonate nano-rods were precipitated within the pores of track-etch membranes which provided defined, cylindrical compartments with diameters ranging from 30 nm to 5 micron in size. The influence of the pore size, membrane surface chemistry and the reaction conditions was investigated, and both single crystal calcite nanorods with aspect ratios of up to 200 and hollow cylinders were produced on control of these parameters. The second system comprises a confined volume created between two crossed cylinders. The crossed-cylinder configuration allows the nucleation and growth of calcium carbonate crystals to be studied at varying surface separations Z, increasing radially from zero (contact) to hundreds of microns. Calcium carbonate precipitation within the confines of the track-etch membrane pores was carried out using ion-by-ion growth and via an amorphous precursor phase. While the former was achieved by combination of Ca2+ and CO32- ions at room temperature in a double diffusion set-up, amorphous calcium carbonate (ACC) was precipitated in the membrane pores in the presence or absence of the soluble additive poly(acrylic acid) (PAA). All of these methods yielded calcium carbonate rods which were single crystals of calcite. Interestingly, the mechanism of particle formation appeared to be different in the two cases. The ion-by-ion pathway appeared to generate crystals precipitate by a “fill-in” mechanism, where a single nucleus is established and grows to fill the pore volume. In contrast, on forming particles via an amorphous precursor phase, the ACC particles initially coat the pore walls, sometimes generating hollow tube structures, according to the pore size. The surface chemistries of the pores were also modified using a layer-by-layer (LBL) technique and was shown to influence the crystallisation process. Crystallisation of calcium carbonate between the crossed cylinders was achieved using double-decomposition or diffusion methods and the nucleation rate, crystal size and morphology was influenced by the solute concentration, growth time and temperature. While large surface separations supported the growth of regular, rhombohedral calcite crystals, changes in crystal morphologies was observed around the contact zone. At small separations, Z<1 micron, flattened, highly irregular structures were which corresponded to amorphous calcium carbonate. These results demonstrate that confinement can have a significant effect on the structure and morphology of calcium carbonate.

3:30 PM *KK4.5

Overview of Biomimetic Composites for Morphing Wing Skins Christopher Henry and Geoff McKnight; Active Materials and Adaptive Structures, HRL Laboratories LLC, Malibu, California.

Structural enhancement is one of the first and most obvious lessons we can learn from biomimicry. As such, high mechanical performance structures from low performance biomineralized materials illustrate the elegance and sophistication of nature’s evolution. The exemplary structure is nacre from which tough and durable mollusk shells are made from calcium carbonate under low temperature processing conditions. While the form and function of narce’s mineralized platelets are becoming evident, the low volume fraction matrix also imparts unique mechanical properties to the bulk composite. Thus, the promise of combining not only enhanced mechanical properties but also biology’s processing advantages may enable a new class of structural composites with high strength, durability, light weight, and low cost. Here we review our area of interest, morphing wing skins, and its cross-section with biomimicry. The realization of this concept requires structures, which can accommodate the large deformations necessary with modest weight and strength penalties. Other studies suggest morphing structures need new materials to realize the benefits that morphing may provide. To help meet this need, we have developed novel composite materials based on platelet reinforcements and shape memory polymer matrices that provide unique combinations of deformation and stiffness properties. To tailor and optimize the design and fabrication of these materials for particular structural applications, one must understand the envelope of morphing material properties as a function of microstructural architecture and constituent properties. We will point out the differences and similarities between these architectures and nacre materials through 3D simulations and micromechanical models, such as by Gao. We will also show design, fabrication and properties of these platelet composites and illustrate how their performance needs to be improved. Thus, morphing biomimetic composites may provide new functionalities and significant improvement in platform performance by permitting optimization over broad operating conditions

4:00 PM *KK4.6

Wood and Paper as Materials for the 21st Century. Philip Jones[1] and Theodore H Wegner[2]; [1]Imerys, Roswell, Georgia; [2]Thodore Wegner, USDA Forest Sevice, Forest Products Laboratory, Madison, Wisconsin.

Wood and paper are ubiquitous in societies around the world and are largely taken for granted as part of traditional industries with no new science to learn. Much of the technologies used in these industries have been gained empirically through experience. Their complexities are now yielding to newer tools and we can see how much of the mechanical properties of wood are related to hierarchical structures based on 2 to 10 nm diameter several hundred nm long fibers of nanocrystalline cellulose (NCC). The liberation of these NCC’s is allowing their re-assembly into remarkably strong structures. Examples will be given of the nature of these building blocks and structures assembled from them. Examples will include nanocomposites as well as very high strength “paper”. Paper is another example of a process whereby nanofibrils are released and then re-assembled with the use of “retention, drainage and formation aides” to make substrates we call paper with remarkable strength to weight performance. Other disciplines call this process “self-assembly” and the “aids” as necessary surfactants and additives to control structure and perormance. Glossy magazine papers, for example, have approximately 10 micron thick coatings of white minerals and latex binders which are increasingly of nano dimensions, assembled in structures to provide optical barrier performance (opacity) as well as controlled ink interaction with the necessary strength to survive printing and handling. These coatings are frequently similar in structure to seashells and, from these learnings, progress has been made in understanding the mechanisms at play in achieving higher strength coatings. More recently kaolin clays have been introduced with mean crystal thicknesses in the range 20 to 40 nm instead of the usual 100 to 140 nm. These clays show useful strength performance and represent what may be called pragmatic nanoclays. Novel chemistries based on biomimetic learnings are emerging to displace the conventional starch or latex binders. Examples will be given of protocols for moving toward higher strength systems.

4:30 PM KK4.7
Mechanical Analysis of Macromodels of Biomimetic Composite Materials using Rapid Prototyping Techniques Mark Cooper[1], Katherine Frank[1], Phillip Russel[1], Michael Waters[1], Alejandro H Strachan[1] and Pablo D. Zavattieri[2]; [1]School of Materials Engineering, Purdue University, West Lafayette, Indiana; [2]General Motors Research and Development Center, Warren, Michigan.

In this work we design, fabricate and mechanically test “large-scale” biomimetic composite materials to identify and characterize the mechanisms that govern the exceptional mechanical properties of nacre, a biocomposite material found in mollusk shells. In particular, the main objective of this work is to demonstrate key conceptual ideas of the micromechanical behavior of this materials leading to a better understanding of its strengthening mechanisms and, eventually, concepts adaptable for future designing and fabrication of synthetic micro-composites. As in nacre, our specimens are composed of stiff tablets interconnected by a very compliant polymer with a characteristic size in the order of millimeters (much larger than the characteristic lengths in naturally-occurring nacre). We use rapid prototyping to explore geometrical features and combination of materials with different properties not possible with other conventional techniques. Our mechanical tests show that increasing the angle of waviness in the sample increases the role the tablet plays in the strengthening the sample. Furthermore, in-situ observations of the specimens during the tensile tests revealed some of the important mechanisms for damage tolerance. Particle-based computer simulations of these composites enable further characterization of the strengthening mechanisms as well as the development of scaling laws that apply when the characteristic size and material properties of their constituents are changed.

4:45 PM KK4.8
Structure and Stability of Bio-Inspired Calcite Crystals Studied by X-Ray Techniques Anna Sophia Schenk[1], Barbara Aichmayer[1], Oskar Paris[1], Helmut Coelfen[2], Christine Lausser[2] and Peter Fratzl[1]; [1]Department of Biomaterials, Max-Planck-Institute of Colloids and Interfaces, Potsdam, Brandenburg, Germany; [2]Department of Colloid Chemistry, Max-Planck-Institute of Colloids and Interfaces, Potsdam, Brandenburg, Germany.

Numerous mineralized tissues with complex morphologies on different hierarchical levels such as bone, dentin and mollusc shells have been evolved by nature. These materials often show fascinating microstructures and superior properties compared to synthetic ceramics. In order to get a clearer picture of how organic additives influence the crystallization of an inorganic component, we investigate the biomimetic formation of calcium carbonate particles in the presence of the polyelectrolyte poly (sodium styrene sulfonate). We are especially interested in evaluating the effects that organic inclusions exert on the internal structure of the mineral. A very important question in this context is how the organic macromolecules are arranged within the crystalline lattice. For that reason we perform structural studies by means of (synchrotron)- small- and wide-angle X-ray scattering (SAXS and WAXS) complemented by electron microscopy (SEM, TEM) and atomic force microscopy (AFM). The samples we analyzed had been prepared by a gas diffusion method implementing different [Ca2+]/[polymer] ratios. Orientation correlations between the nm-sized organic inclusions and the mineral lattice were derived from single particle measurements carried out at the synchrotron facility BESSY (μ-Spot beamline) utilizing a very small X-ray beam defined by a 30 μm pinhole. A setup allowing for simultaneous SAXS and WAXS detection enabled us to observe an interrelation between the preferential direction of the anisotropic SAXS signal and the position of specific planes in the crystalline lattice. In addition measurements on a SAXS laboratory instrument (Nanostar, Bruker AXS) were carried out in order to obtain a small-angle signal of high resolution averaged over several particles of one type. Furthermore the thermal stability of the polymer-mineral composites was analyzed by analogous investigations on annealed samples. Our results should help to get a better understanding how organic macromolecules affect the structure and properties of biogenic and biomimetic minerals.

SESSION KK5: Poster Session: Structure-Property Relationships in Biomineralized and Biomimetic Composites
Chairs: Lara Estroff, Himadri Gupta, David Kisailus, William Landis and Pablo Zavattieri
Wednesday Evening, April 15, 2009
8:00 PM
Salon Level (Marriott)

KK5.1
Microstructural and Biochemical Characterization of the Nano-porous Sucker Rings from Dosidicus gigas James C. Weaver[1], Ali Miserez[2], Peter B Pedersen[3], Todd Schneeberk[2], Roger T Hanlon[4], Henrik Birkedal[3] and David Kisailus[1]; [1]Department of Chemical and Environmental Engineering, University of California, Riverside, Riverside, California; [2]Materials Department, University of California, Santa Barbara, Stanta Barbara, California; [3]Department of Chemistry, University of Aarhus, Aarhus, Denmark; [4]Marine Biological Laboratory, Woods Hole, Massachusetts.

Nature presents a wealth of unique hierarchical materials designed for mechanical function. While many of these structures are mineralized, others exhibit only sparse mineralization or are wholly organic. Here we report on the structural characterization of one such example, the sucker rings from the Humboldt squid, Dosidicus gigas, a large, aggressive and predatory species commonly encountered throughout the Eastern Pacific. The sucker rings are rigid toothed ring-like structures within the suckers that provide additional gripping power during prey capture and handling. As revealed from these studies, the sucker rings from this species exhibit a unique set of characteristics not reported previously for any other biological structural material. They consist of an proteinaceous nanoscale network of densely packed parallel tubular elements that are presumably stabilized almost entirely by hydrogen bonding and hydrophobic interactions. The network of channels exhibits a distinctive organizational gradient, reducing is diameter and abundance from the tooth core to the periphery. The mechanical properties of the bulk composite can be explained through results obtained from both nanoindentation and modeling studies. Additional investigations into the mechanisms of molecular self assembly that result in the formation of these porous biological structures may ultimately reveal novel design strategies for the synthesis of robust, wholly organic structural composites.

KK5.2

Osteogenesis Imperfecta Mutations in Tropocollagen Protein Domains Lead to Molecular Softening and Reduced Intermolecular Adhesion. Alfonso Gautieri[3,2], Maya Srinivasan[1,2], Sinan Keten[2] and Markus J Buehler[2]; [1]Mechanical and Aerospace Engineering, Princeton University, Princeton, New Jersey; [2]Laboratory for Atomistic and Molecular Mechanics, Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [3]Cellular and Molecular Biomechanics Research Group, Department of Bioengineering, Politecnico di Milano, Milan, Italy.

Osteogenesis Imperfecta (OI) is a genetic disease characterized by fragile bones, skeletal deformities and in severe cases, prenatal death that affects more than 1 in 10,000 individuals. Here we show by full atomistic simulation in explicit solvent that OI mutations have a significant influence on the mechanical properties of single tropocollagen molecules, and that the severity of different forms of OI is directly correlated with the reduction of the mechanical stiffness of individual tropocollagen molecules. The reduction of molecular stiffness provides insight into the molecular-scale mechanisms of the disease. The analysis of the molecular mechanisms reveals that physical parameters of side chain volume and hydropathy index of the mutated residue control the loss of mechanical stiffness of individual tropocollagen molecules. We propose a model that enables us to predict the loss of stiffness based on these physical characteristics of mutations. This finding provides an atomistic-level mechanistic understanding of the role of Osteogenesis Imperfecta mutations in defining the properties of the basic protein constituents, which could eventually lead to new strategies for diagnosis and treatment the disease. The focus on material properties and their role in genetic diseases is an important yet so far only little explored aspect in studying the mechanisms that lead to pathological conditions. The consideration of how material properties change in diseases could lead to a new paradigm that may expand beyond the focus on biochemical readings alone and include a characterization of material properties in diagnosis and treatment.

KK5.3

Characterization of Crustacyanin A2 Subunit as a Component of the Organic Matrix of the Cherax quadricarinatus Gastroliths. Gilles Luquet[1], Nathalie Le Roy[1], Sergio Bucarey[2], Isabelle Zanella-Cleon[3], Michel Becchi[3], Maria Soledad Fernandez[2], Jose Luis Arias[2], Nathalie Guichard[1], Benjamin Marie[1] and Frederic Marin[1]; [1]UMR 5561 CNRS-Université de Bourgogne, Laboratoire de Biogéosciences, Dijon, France; [2]Faculty of Veterinary and Animal Sciences, University of Chile, and Centre for Advanced Interdisciplinary Research in Materials (CIMAT), Santiago, Chile; [3]Institut de Biologie et Chimie des Protéines, UMR 5086 CNRS-Université Lyon 1, Laboratoire de Spectrométrie de Masse, Lyon, France.

Like many invertebrates, the arthropods possess an outer rigid exoskeleton, also called cuticle that they have to renew regularly to grow. As a consequence, all the physiology of these animals is tightly linked to molting cycle. In crustaceans, the cuticle is not only hardened by sclerotization but also by calcification. Thereof these animals have to find cyclically a source of calcium ions to mineralize each new brand skeleton. Some terrestrial crustaceans (amphipods, isopods, decapods) but also some aquatic species, cyclically store calcium ions for undertaking a quick vital postmolt calcification. For example, some terrestrial crabs, lobsters and crayfishes store calcium in their stomach wall as one or two pairs of gastroliths, mainly constituted of amorphous calcium carbonate precipitated within an organic network. For understanding the cyclic elaboration and stabilization state of these calcified structures, we studied the components of the organic matrix (OM) of the 2 gastroliths elaborated by the Australian red claw crayfish, Cherax quadricarinatus. After decalcification with acetic acid, we analysed on SDS-PAGE the proteinaceous components of the OM. After performing a 2D electrophoretic separation, we extracted and 9 polypeptidic spots submitted them to mass spectrometry (MALDI-TOF) analysis. We obtained around 90 peptidic sequences, which were compared to sequences previously registered in the databases. Among the OM Cherax polypeptides, one migrates at around 25 kDa and presents strong homology with the crustacyanin A2 fragment of Homarus gammarus (Swiss-Prot accession number CRA2-HOMGA). For obtaining the complete sequence of this protein, we performed RT-PCR after designing specific primers from the MALDI-TOF sequences. The sequence obtained revealed 80% homology with the Homarus gammarus similar A2 subunit. Crustacyanin is an octamer of a heterodimer (A2-C1), involved in the binding of the carotenoid astaxanthin (one molecule bound by monomer) resulting in the blue color of the carapace of the crustacean. If the finding of this molecule within the gastrolith is in agreement with the blue coloration (more or less accentuated) of these storage structures, the reason of its presence among the components of the organic matrix remains enigmatic.

KK5.4

Mechanical Properties and Surface Characterization of Calcium Carbonate Platelet Extracted from Freshwater Pearl Shell. Xinqi Chen, NUANCE Center, Northwestern University, Evanston, Illinois.

Freshwater pearl shell is a nature biocomposite material which is made up of calcium carbonate platelets as the building blocks and protein as the matrix. The composite exhibits structural toughness despite the brittle nature of the inorganic platelets. A thorough understanding of the mechanism of the stiffness could inspire new ideas in material design and synthesis. This paper reports a study on the mechanical properties of individual platelets with nanoindentation. The individual platelets have been exfoliated from the raw shell materials through mechanical and chemical methods. The intact surface of the platelets has been characterized using ToF-SIMS, XPS, FT-IR, and SEM. The analysis results of the protein layer will be discussed.

KK5.5

Extreme Mechanical Anisotropy in Bone at the Mesoscale. Jong Seto[1], Himadri S Gupta[1], Paul Zaslansky[1], H. D Wagner[2] and Peter Fratzl[1]; [1]Department of Biomaterials, Max-Planck-Institute of Colloids and Interfaces, Potsdam, Germany; [2]Materials and Interfaces, Weizmann Institute of Science, Rehovot, Israel.

Bone is mechanically and structurally anisotropic with oriented collagen fibrils and nano-sized mineral particles aggregating into lamellar or woven bone. This hierarchical architecture makes direct measurement of mechanical properties such as strength and modulus of sub-lamellar tissue constituents difficult. Nanoindentation provides insight from effective modulus values; however, such measurements represent near-surface volumes and are partially averaged over fibril orientations. We find a modulus anisotropy ratio (Etransverse/Eaxial) of 1:1.5 from nanoindentation data—although significant, this ratio is likely to be conservative since triangular pyramid Berkovich tips are only partially sensitive to structural anisotropy. We circumvent the limitations of the nanoindentation approach by individually isolating and measuring parallel-fibered units of bovine bone in tension under controlled humidity conditions. Surprisingly, we find a ratio as large as 1:20 in elastic modulus and 1:15 in tensile strength between orientations perpendicular and parallel to the main collagen fiber orientation in native wet bone, reducing to 1:8 and 1:7 respectively, when dry. This extreme anisotropy has never been reported, most likely because mechanical measurements at this length scale in bone have never been performed to date.

KK5.6
Effects of fetuin-A Deficiency on the Material Bone.

Jong Seto[1], Himadri S Gupta[1], Stefanie Krauss[1], John Dunlop[1], Admir Masic[1], Willi Jahnen-Dechent[2] and Peter Fratzl[1]; [1]Department of Biomaterials, Max-Planck-Institute of Colloids and Interfaces, Potsdam, Germany; [2]Biomedical Engineering, Biointerface Group, RWTH Aachen University, Aachen, Germany.

Non-collagenous proteins (NCP) in bone are implicated in several critical processes in maintaining tissue integrity—from assembling the organic bone matrix to apatite nucleation, factors that can modulate the tissue’s mechanical properties. One such NCP is fetuin-A (fetA), a glycoprotein that is abundantly found in vertebrates as a blood plasma protein, has been linked as a mineral chaperone. FetA deficiencies lead to pathological mineralization and has been correlated to a systematic dysfunction in controlling mineralization in organs ranging from the cardio-pulmonary to excretory systems. Despite its role in inhibiting soft tissue mineralization, fetA function in the skeletal system is not entirely understood. Through the use of in-situ micromechanical tensile measurements coupled with synchrotron small-angle X-ray scattering, the mechanical behaviors of murine cortical bone samples from 1 year old fetA (-/-) mutants and normal fetA (+/+) were characterized from the nano- to micro- meter length-scales. We report that negligible differences were found in the mechanical behaviors and material properties of mutant and normal bone samples—suggesting fetA has no structural role in bone and is not involved in bone mineralization. Complemented by results from Raman spectroscopy and nanoindenation, the degree mineralization in mutant and normal fetA bones were found to be indistinguishable. These results indicate bone mineral size and mineralization in vertebrates is highly regulated as well as the existence of a possible vascular-bone interface.

KK5.7
Abstract Withdrawn

KK5.8
In Vitro Human Osteoblast Responses to Titanium Oxide-Based Surfaces with Varying Topology and Composition.

Charles Andrew Collier[1], Helen J Griffiths[1], Athina E Markaki[2], James A Curran[1] and T. W Clyne[1]; [1]Materials Science and Metallurgy, University of Cambridge, Cambridge, Cambridgeshire, United Kingdom; [2]Department of Engineering, University of Cambridge, Cambridge, Cambridgeshire, United Kingdom.

The topology and composition of prosthetic implant material surfaces affect cell responses and are therefore important design features. Plasma electrolytic oxidation (PEO) is a surface modification technique that can be used to produce oxidised surfaces with various surface properties. In this work, a commonly used biomedical alloy, Ti-6Al-4V, was PEO processed to give a number of surfaces with different roughness and constitution. The characteristics of the surfaces were assessed using X-ray diffraction, SEM/EDS, optical profilometry and contact angle measurement. In vitro culture of human foetal osteoblasts was performed on the surfaces, in order to examine cell responses to various surface characteristics. Cellular proliferation, morphology and differentiation were examined, using the AlamarBlue assay, SEM imaging and an alkaline phosphatase assay respectively. Additionally, the individual effects of the various oxides present in the PEO processed surfaces (including rutile, anatase, aluminium oxide and aluminium titanate) on the cells were observed, by binding them in powder form to produce surfaces with similar morphology, but differing compositions. Changes in the topology and chemistry of the oxide surfaces affected osteoblast response. Observing these allows conclusions to be made about the effects of different surface characteristics on human osteoblast behaviour, providing information for future implant material design.

KK5.9
Phases, Composition and Microstrain in the Mineralized Byssus of Anomia.

Henrik Birkedal and Jakob R Eltzholtz; Department of Chemistry & Interdisciplinary Nanoscince Center, Aarhus University, Aarhus, Denmark.

The bivalve Anomia and a few related species have a unique solution to support their sedentary lifestyle: they adhere through a mineralized byssus. The byssus is calcified with both aragonite and calcite. Here we present the results of position resolved X-ray diffraction data and SEM/EDX analyses that show that 1. There are distinct distributions in Mg-content in the calcite part as evidenced by large variations in lattice constants and EDX signals. 2. There are very clear signatures of anisotropic local strain, which could be successfully mapped by Rietveld analysis. The results show that Anomia controls polymorph selection and chemical composition and that local crystal strains are anisotropic and vary significantly with position.

KK5.10
Shell Recovery Process in the Clam Ruditapes Phillippinarum, Affected by the Brown Ring Disease (BRD): a Biochemical Study.

Nolwenn Trinkler[1], Frederic Marin[2], Nathalie Guichard[2], Maylis Labonne[1], Christine Paillard[1] and Jean Francois Bardeau[1]; [1]LEMAR UMR CNRS 6539, IUEM UBO, Plouzané, France; [2]Laboratoire de Biogéoscience UMR 5561, UB,

Dijon, France.

In 1987, mass mortalities of the cultured manila clam Ruditapes phillipinarum were recorded in Landeda (North Finistère, Brittany), which was the first production site in France (500t in 1987). In 1989, Paillard and Maes showed that mortalities were associated with the presence of a brown deposit on the inner surface of the valves. This disease, named Brown Ring Disease (BRD) is caused by a bacterium Vibrio tapetis. Vibrio tapetis colonizes the periostracum and inhibits the normal process of shell biomineralization. The response of the clam to the bacterium attack consists in the production and accumulation of a brown organic matrix on the inner face of the shell, which results in the death of the infested clam. However, in some cases, clams recover by secreting a mineralized white layer, which covers the brown organic matrix. Physical investigations with scanning electron microscopy, Raman spectrometry and Wave-length Dispersive Spectrometry microprobe have shown the quantitative importance of the organic matrix before and during the recovery process: first, the brown deposit is almost totally organic. Then, in the mineralized repaired zone, the level of organic matrix remains high. The aim of this study was to characterize and compare the organic matrix extracted from healthy and repaired zones in the shell of the manila clam. Different batches of repaired clams were used, for which the organic matrix was extracted and quantified from healthy inner layer and from repair layer. Afterwards, 1D and 2D electrophoreses, Elisa tests, Western Blots and in vivo CaCO3 crystallizations were performed. Quantifications showed that the soluble/insoluble ratio varies between repair and healthy zones. Electrophoretic profiles as well as serological comparisons with ELISA showed that the matrix associated with the repair zone exhibits certain variability, from very similar to that of the healthy zone to very different. This study is now being pursued, in order to find molecular markers for shell recovery process. To this end, we are developing a proteomic approach combined with immunological investigations with antibodies raised against purified protein fractions.

KK5.11
Abstract Withdrawn

KK5.12
Abstract Withdrawn

KK5.13
Novel Dental Restorative Composites having Excellent Mechanical Properties and Reduced Volumetric Shrinkage during Polymerization. Sun H Yoo, You S Chung and Chang Keun Kim; School of Chemical Engineering & Materials Science, Chung-Ang University, Seoul, Korea, South.

Dental composites are composed of a soft organic matrix and hard inorganic fillers. Restorative dentistry is fraught with problems brought about by the inherent volumetric shrinkage that accompanies polymerization of composite resin matrices. Various problems such as postoperative sensitivity, secondary caries, and pulpal pathoses still occur as a result of the inherent volumetric shrinkage resulting from the polymerization of the composite resin matrix. The organic matrix containing the classical monomers, i.e., 2,2-bis [4-(2-hydroxy-3-methacryloyloxy propoxy) phenyl] propane (Bis-GMA) as a base resin and triethylene glycol dimethacrylate (TEGDMA) as a diluent, has been used in the past. spiro orthocarbonates (SOCs) have been widely investigated because they show significant volume expansion during polymerization. Even though SOCs exhibited volumetric expansion during polymerization, several drawbacks such as poor mechanical properties prevented their incorporation into the resin matrix of dental composites. In this study, to overcome drawbacks of SOCs and the original resin matrix, new monomers containing bisphenol-A unit and spiro units were synthesized and then incorporated into the resin matrix of dental composites. Enhancement in the mechanical strength stemmed from bisphenol-A unit in the Bis-GMA and reduction in the volumetric shrinkage stemmed from spiro units might be expected. Dental composites containing new monomers exhibited excellent mechanical properties with reduction in the volumetric shrinkage.

KK5.14
Molecular Recognition at the Organo-Mineral Interface between Protein and Bone. Jonathan James Phillips[1,2] and Seung-Wuk Lee[1,2]; [1]Bioengineering, UC, Berkeley, Berkeley, California; [2]Physical Biosciences, Lawrence Berkeley National Laboratory, Berkeley, California.

Hydroxyapatite is a hard tissue widely employed in nature as the mineralized component of vertebrate skeletons, tooth dentin, enamel and fish scales. Because of the highly significant impact of hard tissue diseases, such as osteoporosis and dental caries, hydroxyapatite is a major focus in medical therapeutics. The mineral is remodeled in bone tissue, enabling regenerative prosthetic bone grafting under surgery. Dental enamel is naturally acellular, facilitating grafting without fear of immune rejection. Thus there is a need for understanding the interaction at the organo-mineral interface and a need for development of hydroxyapatite materials that are biocompatible at the nanoscale. Hydroxyapatite structures are both synthesized (such as in unerupted teeth) and resorbed (such as in the formation of dental caries) at the surface of the crystal under conditions imposed by the local solution environment (such as acidic saliva). The morphology of the growing hexagonal bipyramidal crystal and the stability of the dissolving crystal are directed by specific molecular interactions between secreted proteins and the solvated calcium phosphate mineral surface. Neither the termination structure, nor the solvation state of the surfaces of hydroxyapatite is known, owing to the high degree of technical challenge in probing the surface of a non-conductive mineral crystal. Therefore, we have sought to gain atomic level structures of proteins that specifically bind to one geometric surface, as determined by phage display, then to dock these structures to putative surface models of the crystal. The atomic resolution structure of protein bound to calcium and phosphate ions was investigated by nuclear magnetic resonance (NMR) and by transmission cryoelectron microscopy (cryoEM) of engineered virus particles. Through docking simulations, the resulting 3-dimensional protein structures inform the atomic arrangement of the mineral surface. We are then able to build a model for the interaction between native proteins and hydroxyapatite crystal in bone and enamel.

KK5.15
Abstract Withdrawn

KK5.16
Nanosilica Formation at Lipid Membranes Induced by Silaffin Peptides. Michael Kent[1], Jaclyn Murton[1], Frank Zendejas[2], Huu Tran[2], Blake Simmons[2], Sushil Satija[3] and Ivan Kuzmenko[4]; [1]Sandia National Labs, Albuquerque, New Mexico; [2]Sandia National Labs, Livermore, California; [3]National Institute of Standards and Technology, Gaithersburg, Maryland; [4]Argonne National Labs, Argonne, Illinois.

Diatoms are unicellular eukaryotic algae found in fresh and marine water. Each cell is surrounded by an outer shell called a frustule that is composed of highly structured amorphous silica. Diatoms are able to transform silicic acid into these sturdy intricate structures at ambient temperatures and pressures, whereas the chemical synthesis of silica-based materials typically requires extremes of temperature and pH. Cationic polypeptides, termed silica affinity proteins (or silaffins), recently identified from dissolved frustules of specific species of diatoms, are clearly involved and have been shown to initiate the formation of silica in solution. The relationship between the local environment of catalytic sites on these peptides, which can be influenced by the amino acid sequence and the extent of aggregation, and the observed structure of the silica is not understood. Moreover, the activity of these peptides in promoting silicification at lipid membranes has not yet been clarified. In this work we developed a model system to address some of these questions. We studied peptide adsorption to Langmuir monolayers and subsequent silicification using X_ray reflectivity and grazing incidence X-ray diffraction. The results demonstrate the lipid affinity of the parent sequences of several silaffin peptides. Further, the results show that the membrane-bound peptides promote the formation of interfacial nanoscale layers of amorphous silica at the lipid-water interface that vary in structure according to the peptide sequence.

KK5.17
Synthesis Of Porous Calcium Phosphate Nanotubes. Deepa Khushalani, Dept. of Chemical Sciences, TIFR, Mumbai, MH, India.

For the production of useful biomaterials, Calcium Phosphate has been known to be an integral inorganic component. Calcium Phosphate exists in different phases, namely Octa-Calcium Phosphate, Tri-Calcium Phosphate, Brushite and Hydroxyapatite (HAp). These phases are known to differ in both their physical and chemical properties. Due to their excellent biocompatibility and bone-repair properties Calcium Phosphate based bioceramics have attracted much attention among researchers in medicine and dentistry (e.g. matrices for controlled drug release and tooth paste additives). Owing to the importance of Calcium Phosphate based bioceramics, there has been a great challenge to prepare the pure phase of Calcium Phosphate by different methods such as co-precipitation, emulsion, template and sol-gel techniques. Herein, we report the synthesis of hollow Calcium Phosphate nanotubes by template assisted method. In this study, we investigated the crystallization of Calcium Phosphate by controlling various synthetic parameters such as temperature, concentration of starting precursors of both Calcium and Phosphorous, stirring rate, addition rate of the precursors etc in the presence of a solid porous template. The representative length and width of the resulting Calcium Phosphate hollow nanotubes was found to be 15-20 μm and 100-240 nm respectively. The compositional and structural changes were characterized using various experimental techniques such as scanning electron microscope (SEM), transmission electron microscope (TEM), X-ray diffraction (XRD) and energy dispersive X-ray analysis (EDAX). Preliminary bioactive studies have also been performed and these will be detailed in the presentation.

KK5.18
The Effect of Silk Fibroin Hydrogels, Peptides, and β-chitin on Calcium Carbonate Crystallization: A Synthetic Model for Nacre Formation. Ellen C Keene[1], John S Evans[2] and Lara A Estroff[1]; [1]Material Science & Engineering, Cornell University, Ithaca, New York; [2]Laboratory of Chemical Physics, New York University, New York City, New York.

A silk fibroin-like hydrogel, coupled with occluded glyco-proteins and a functionalized surface, is the current model for the organic matrix involved in the formation of the nacreous layer of mollusk shells (model developed by Falini et al.). Based on this proposed model, we created a biomimetic setup using silk fibroin (from silkworm cocoons) hydrogels combined with functionalized surfaces to study the effect of proteins and peptides on calcium carbonate crystal growth. The occluded peptides include poly-glutamic acid and a nacre-specific peptide fragment, n16N, from the Japanese pearl oyster (Pinctada fucata). Crystals grown in a silk fibroin hydrogel alone produce poly-crystalline calcite on glass or Self Assembled Monolayers (SAMs), while those grown on β-chitin (from the squid pen of the Loligo species) are flat, with distinct angled terraces. Crystallization results using n16N (an aragonite facilitator) shows that there is little affect on aqueous grown calcite crystals when grown on bare gold or SAMs of various functionalities, but when grown on β-chitin polycrystalline aragonite forms. When poly-glutamic acid is grown on chitin (with or without the silk the hydrogel) calcite crystals (as determined by Raman) start to penetrate and grow into the chitin substrate, visually appearing to align perpendicular to the fibers. A synthetic chitin SAM (acetyl amide functionality) is being used to investigate the possibility of peptide-chitin interactions and more specifically possible chitin binding domains in the n16N peptide.

KK5.19
Abstract Withdrawn

KK5.20
Electrical Characterization of Functionalized Diatom Pinnularia sp. Biosilica. Timothy Gutu[1], Clayton Jeffryes[2], Gregory L Rorrer[2] and Jun Jiao[1]; [1]Department of Physics, Portland State University, Portland, Oregon; [2]Department of Chemical Engineering, Oregon State University, Corvallis, Oregon.

Diatoms are unicellular and photosynthetic microalgae that live in marine and freshwater environments. The cell walls of diatoms are composed of biosilica and have exceedingly hierarchical ornate nanostructures. Consequently, these nanostructures have long been regarded as the paradigm for future silica nanotechnology. We have functionalized diatom Pinnularia sp. biosilica by coating the structure with a thin film of CdS using a chemical bath deposition technique. Possible uses for these functionalized diatoms include the development of new nanodevice fabrication techniques and optoelectronic applications. Electron microscopy techniques were utilized to study their morphologies. Their electrical characteristics were investigated using an Agilent 4156C precision semiconductor parameter analyzer and a Cascade probe station. The CdS coating was found to be dense, adherent and nanostructured. The functionalized diatoms exhibited both metallic and semiconductor diode behavior.

KK5.21

Numerical Studies and Dimensional Analysis for Designing Bio-inspired Composite Materials Jee E Rim[1], Pablo D. Zavattieri[2] and Horacio D Espinosa[1]; [1]Department of Mechanical Engineering, Northwestern University, Evanston, Illinois; [2]General Motors Research and Development Center, Warren, Michigan.

The attractive mechanical properties of some hard biological materials, in particular the combination of strength and toughness, have inspired a large class of biomimetic materials and organic/inorganic composites. However, there still is a lack of quantitative and comprehensive analysis of the design parameters that allow the mechanisms to operate. Moreover, the creation of a synthetic biological material with its intricate microstructure is a challenge that requires both the design of optimum microstructures and the development of fabrication procedures to implement these designs. The main purpose of this work is to use novel numerical techniques with state-of-the-art models under the framework of dimensional analysis to guide the design and fabrication efforts. The analysis of the effect of the various geometrical and material parameters involved in the mechanics could be very exhaustive if not done systematically. A multi-objective optimization scheme could also be prohibitive if the analyst does not have an initial insight into the importance of each material and geometrical parameter. In our numerical effort, we interrogate this function numerically by looking at suitable projections involving relationships between selected parameters in order to better understand how this can be used for the design of nacre-like microstructures in response the applied loads.

KK5.22

Abstract Withdrawn

KK5.23

Composite Biomaterial of Ceramic-polymer: Development and Characterization. Miriam Estevez, Rogelio Rodriguez, Angel M Escamilla and Ana Leonor Rivera; Centro de Física Aplicada y Tecnología Avanzada, Universidad Nacional Autonoma de México, Queretaro, Qro, Queretaro, Mexico.

In this work is developed a new hybrid composite biomaterial of Al2O3 and SiO3 inside a cylindrical polyurethane matrix. It is described the material synthesis and its characterization through SEM and mechanical tests. Our goal is the development of a new biocompatible material for a bone substitute that has similar chemical and mechanical properties to osseous tissue. The strength analysis is reduced to compression due to the medical troubles with transplants in patients. The composite has a reinforced phase (a ceramic of alumina and silica) and a continuous phase (a monocomponent polyurethane matrix). The matrix assures the structural cohesion and the strength transmission. To find the best composite, we analyze different formulations of the ceramic varying the combinations of alumina (Al2O3) and silica (SiO3). The nanoparticles of SiO3 have a typical size of 17 nm, while the micrometric Al2O3 has 5μm. To assure the biocompatibility of our composite, we add hydroxidepatite (Ca10(PO4)6 (OH)2). The mechanical test follows the norm ASTM D-695-02a. This test gives us the Young modulus of our material and the maximum strength that the composite can support without broking. The results of this test for the different formulations are shown in table I. In this case the best formulation are the H0A50S50 because it supports the biggest force before broken and the H0A60S40 because it has the largest Young modulus. The porosity of the different formulations was verified through their SEM images. This characteristic is very important to assure that blood vessels can pass through the porous of the osseous tissue. For leg bones, for example, the porous are typically of μm. SEM images of the different formulations have various porous sizes. The best formulation is H0A75S25 because it has the largest pore size.

KK5.24

Coating Electrospun Poly(ε-caprolactone) Fibers with Gelatin and Calcium Phosphate for Bone Tissue Engineering Xiaoran Li[1,2], Jingwei Xie[1], Xiaoyan Yuan[2] and Younan Xia[1]; [1]Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, Missouri; [2]School of Materials Science and Engineering, Tianjin University, Tianjin, China.

Electrospinning was employed to fabricate fibrous poly(ε-caprolactone) scaffolds. The surfaces of the fibers were then coated with gelatin through layer-by-layer self-assembly, followed by functionalization with a uniform coating of bone-like calcium phosphate by mineralization in the 10 times concentrated simulated body fluid for 2 h. Transmission electron microscopy, water contact angle, and scanning electron microscopy measurements confirmed the presence of gelatin and calcium phosphate coating layers, and X-ray diffraction result suggested that the deposited mineral phase was a mixture of dicalcium phosphate dehydrate (a precursor to apatite) and apatite. It was found that the presence of gelatin facilitated a homogenous calcium phosphate coating. The scaffolds were then evaluated for the culture of pre-osteoblastic MC3T3-E1 cells. The cells attached, spread, and proliferated well with a flat morphology on the mineralized scaffolds. The proliferation rate of the cells on the mineralized scaffolds was significantly higher (by 1.9 fold) than that on the pristine fibrous scaffolds after culture for 7 days. These results indicated that the hybrid fiber scaffold containing poly(ε-caprolactone), gelatin, and calcium phosphate, which could mimic the structure, composition, and biological function of bone extracellular matrix, could serve as a new class of biomimetic scaffolds for bone tissue engineering.

KK5.25
Transferred to KK3.4

KK5.26
Self-healable Biopolymers for Drug Delivery and Tissue Engineering Xuanhe Zhao, Nathaniel D Huebsch, David J Mooney and Zhigang Suo; School of Engineering and Applied Science, Harvard University, Cambridge, Massachusetts.

Over the recent decades, there have been many advances in the development of biocompatible polymers as extracellular matrices for tissue engineering. The polymer matrices have dual functions both as mechanical supports for tissue growth and carriers of drugs such as growth factors. The release of large molecules of growth factors generally depends on the degradation of the polymer matrix, which sacrifices its function of mechanical support. Various efforts have been made on controlling the degradation rate of the polymer, but few on decoupling polymer degradation and drug release. In this work, a biocompatible polymer that can be crosslinked by divalent ions in vivo (e.g. $Ca2+$) has been selected as an extracellular matrix carrying growth factors (e.g. VGEF). The matrix is exposed to low frequency ultrasound, which causes cavitations in the matrix. As a consequence, the release of growth factors is greatly enhanced in a controlled manner. Meanwhile, physiological fluid containing divalent ions flows into the cavities, recrosslinks the polymers, and re-heals the cavities. In this way, we control the release of drugs from the polymer matrix, while maintaining its physical integrity and mechanical stiffness. Low frequency ultrasound has been frequently used to enhance the permeability of skins to large-molecule drugs by causing cavitations in skins. After the ultrasound is withdrawn, the cavities in skins re-heal. In this sense, our biopolymer matrix mimics the self-healing function of skin, without replicating skin's microstructures.

KK5.27
Thermal and Melt Property Characterization of Biodegradable Polyesters for Batch-Foaming. Qi Liao and Curt W Frank; Department of Chemical Engineering, Stanford University, Stanford, California.

Porous foams based on biodegradable polyesters are quite attractive for biomaterial applications such as medical implant and tissue engineering, and also as environment-friendly and energy-efficient building materials. The cellular structures not only affect the mechanical properties, biocompatibility, and degradation rates, but also provide the foundation for incorporating other phases and making hybrid biocomposites. In this work, we studied poly(3-hydroxyalkanoate)s (PHAs), a bacterial polyester family known for complete biodegradation. The focus was on small-batch foaming of these materials, as well as establishing the relationships between the foam structures and the thermal and melt viscoelastic properties of the bulk materials. Six different PHAs were compared, including poly(3-hydroxybutyrate)(PHB), poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(PHB-HV) and four poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)s (PHB-HHx) with different comonomer contents; their molecular compositions, melt properties and thermoprocessabilities were investigated. Methods used for polymer characterization included the gel permeation chromatography, differential scanning calorimetry, melt rheometry and scanning electron microscopy. Our results showed that among the six PHAs studied, PHB-HHx's with 4.6 mol.% or 6.9 mol.% HHx content has the best viscoelastic properties in the molten state and behaves the closest to conventional thermoplastics. This is in-line with the foaming results: Foams made of these two PHB-HHx's are the best in terms of cell size, wall intactness and gas-solid interface.

KK5.28
Amino- and Carboxy-functionalized Nano- and Microstructured Surfaces for Evaluating the Impact of Non-biological Stimuli on Adhesion, Proliferation and Differentiation of Primary Skin-cells. Petra Kluger[2], Kirsten Borchers[1], Achim Weber[1], Guenter E Tovar[1,3] and Heike Mertsching[2]; [1]Biomimetic Interfaces, Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Stuttgart, Germany; [2]Cell Systems, Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Stuttgart, Germany; [3]Institute for Interfacial Engineering, University of Stuttgart, Stuttgart, Germany.

Tissue Engineering is an interdisciplinary research field with the goal to manufacture in vitro tissues and organs. A crucial factor is the functional long-term cultivation of primary cells. Therefore, scaffolds have to be created, featuring structurally and functionally tailored substrates for the isolation and cultivation of primary cells in early differentiation stages. Insight into the cells' preferences concerning structure and the impact of chemical functionality of the substrate helps to built up improved cell culture systems as well as optimized biomaterials and tailored surfaces for implants without the use of cost-intensive biological components. To gain basic insight into the impact of non-biological features on cells' behaviour, primary keratinocytes and fibroblasts were cultured on functionalized planar, nano- and microstructured surfaces. Sintered layers of silica nano- or microparticles were used to fabricate structures in the range of naturally occurring structure-sizes. These nano- or micro-structured surfaces were functionalized with amino and carboxy-functions. Subsequently primary cells isolated from human foreskin were cultivated on these interfaces. Strong reactions on the differentiated interfacial properties were observed: Keratinocytes showed significantly better adhesion and proliferation on amino-functionalized surfaces than on carboxy- functionalized surfaces. On amino-functional surfaces an increasing proliferation-rate from microstructured to planar surfaces was detected. Cytokeratin 14, an early differentiation marker was detected. Markers for later stages of differentiation (cytokeratin 10 or filaggrin) could not be detected. Thus, structure and organic function can be employed to study and to conduct cell-behaviour at interfaces. We will present data on surface-characterization by ellipsometry, SEM, XPS and AFM. Adhesion, proliferation and differentiation of primary cells on various surfaces were evaluated by cell proliferation tests and immunohistochemical staining of cytoskeleton and differentiation markers.

KK5.29
Synthesis and Characterization of Novel Biodegradable and Injectable Hydrogels for Tissue Engineering based on PLA-PEG-PLA Block Copolymers. Kevin W. Worrell and Karl I Jacob; Polymer, Textile & Fiber Engineering, Georgia Institute of Technology, Atlanta, Georgia.

Biodegradable, injectable hydrogels have become very promising materials for a number of biomedical applications, particularly for non-intrusive tissue engineering strategies in which scaffolds with controlled release capabilities are also favorable. However, one of the greatest limitations of hydrogel scaffolds has been their poor mechanical properties, especially at high water contents. While a number of studies have been successful in the development of non-degradable hydrogels with mechanical properties that begin to match those of tissues such as articular cartilage, the mechanical properties of most biodegradable hydrogels are still inferior. Yet an increasing number of studies show that both the strength and modulus of biodegradable hydrogel scaffolds are important for the appropriate transduction of cues to cells seeded in the scaffolds. In this study, a recently developed interpenetrating polymer network (IPN) formation strategy utilizing a biomimetic pre-stress condition has been applied to the synthesis of partially and fully biodegradable hydrogels based on combinations of poly(lactic acid)-poly(ethylene glycol)-poly(lactic acid) (PLA-PEG-PLA) block copolymers and high-swelling polyelectrolytes. The equilibrium water content and the mechanical properties of the hydrogels have been found to be very dependent on the chemical interactions between the given components, as well as on the structural and morphological attributes of the system. In addition, the degradation rate and overall degradation profile of the hydrogels have been studied and found to be dependent on the initial equilibrium water content and the nature of the degradation products of the hydrogel components.

KK5.30

P(L,L-lactide) / pseudowollastonite-based Composites : New Biomimetic Materials for Bone Regeneration. Deborah Thérèse-Joséphine Barone[1], Pascal Viville[2], Jean-Marie Raquez[1], Alexandra Belayew[3], Roberto Lazzaroni[2] and Philippe Dubois[1]; [1]Laboratory of Polymeric and Composite Materials, University of Mons-Hainaut, Mons, Belgium; [2]Laboratory for Chemistry of Novel Materials, University of Mons-Hainaut, Mons, Belgium; [3]Laboratory of Molecular Biology, University of Mons-Hainaut, Mons, Belgium.

This study deals with novel bioactive and bioresorbable composites based on semi-crystalline poly(L,L-lactide) (P(L,L-LA)) and pseudowollastonite (psW) particles prepared by melt-blending and hot-pressing for bone-guided regeneration applications. The bioactivity of the composites was evaluated in simulated body fluid (SBF) at 37 °C and pH: 7.25 and evidenced by the ability to form a hydroxyapatite (Ca10(PO4)6OH2) (HA) layer at the surface. The polymer constituent was characterized, before and after immersion in SBF, in terms of molecular and thermal parameters by Gel Permeation Chromatography (GPC) and Differential Scanning Calorimetry (DSC). Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) were used to study the degradation of the composites and the changes in the surface morphology after the immersion tests. SEM combined with Energy Dispersive X-Ray Spectroscopy (EDS) analysis carried out on polished and carbon-coated cross sections provide information about the degradation profile of the composites and the formation of superficial HA over time, as evidenced by X-Ray Elemental Maps of Ca, Si and P. For instance, for the PLA/psW (20% in weight) composite the formation of an HA layer occurs in 16 weeks. Interestingly, the use of an amphiphilic Polyethylene oxide-b-Poly(L,L-lactide) (PEO-b-PLA) copolymer reduces the required time to form the HA layer down to 1 to 3 weeks of immersion. The toxicity of the materials was studied in vitro with osteoblastics SaOS -2 cells. The results showed a non-toxic behaviour for all those materials. Finally, the adhesion of these cells onto their surface was studied by Fluorescent Microscopy. The images revealed the presence of the SaOS-2 cells on all the biocomposites.

KK5.31

Enhancement of Osteoclastic Differentiation of Mouse Bone Marrow Cells Cultured on Hydroxyapatite/collagen Bone-like Nanocomposite. Masanori Kikuchi[1] and Atsushi Irie[2]; [1]Biomaterials Center, National Institute for Materials Science, Tsukuba, Japan; [2]Biomembrane Signaling Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.

Recently, Kikuchi *et al*. developed novel hydroxyapatite/collagen (HAp/Col) nanocomposite with bone-like nanostructure and chemical composition. The HAp/Col is incorporated into bone remodeling process and new bone is regenerated when it is implanted into bone defect. Kikuchi *et al.* also confirmed its enhancement effect of osteogenic activity of MG63, human osteoblastic cell line, even without addition of osteogenic supplements. In this study, we investigated influence of the HAp/Col on osteoclastic differentiation of primary cultured mouse bone marrow cells. The HAp/Col nanocomposite (HAp:Col=4:1 in mass ratio) was prepared by simultaneous titlation method. After filtration, 10 g of wet HAp/Col was press-dehydrated at 10 MPa for overnight. The plate obtained was punched out to 4 mmφ disk and crosslinked by a vacuum heating at 140 °C for 12 h or soaking in glutaraldehyde (GA) solution for 90 min. The GA crosslinked disks were washed with dH_2O and 2 mass% glycine solution to remove unreacted GA molecules. The disks were polished and sterilized. The HAp/Col membrane was also prepared by filtrating the HAp/Col followed by uniaxial pressing. Bone marrow cells and osteoblasts isolated from C57BL/6 mice were cocultured on the HAp/Col for 6 days in α-minimum essential medium containing 10% fetal bovine serum with or without osteoclast differentiation inducers, 10 nM 1,25-Dihydroxyvitamin D_3 and 1 μM prostaglandin E_2. The cells on the HAp/Col were then fixed, and stained for tartrate-resntant acid phosphatase, a marker enzyme of osteoclastic differentiation. Dentin slices HAp ceramics and tissue culture polystyrene (TCPS) plates were used as controls. Bone marrow cells cultured with the osteoclast differentiation inducers were very well differntiated into osteoclasts for all samples. Bone marrow cells cultured on the HAp/Col materials differentiated into osteoclasts even without the inbducers. Non-crosslinked and vacuum-heating HAp/Col showed weak differentiation and GA crosslinked HAp/Col showed strong differntiation. Contrarily, the non-crosslinked and GA crosslinked dentin slices demonstrated very weak differntiation and no differntiation without the inducers, respectively. HAp ceramics and TCPS showed no differntiation without the inducers. The differences between the HAp/Col and dentin could be nano-scale direction of hydroxyapatite and collagen on their surfaces. The HAp and collagen on the surface of dentin slice exposed *c*-face of HAp and terminal functional groups of collagen molecules. Thus, the cells on the dentin slice interacted with different mannar to that generally occurred in bone. Contrarily, the cells were interacted with the HAp/Col in the similar mannar to that observed in bone, *i.e.*, *a*-face of HAp and side functional group of collagen. In conclusion, the HAp/Col nanocomposite enhanced osteoclastic differentiation of mouse bone marrow cells *in vitro*.

KK5.32

Digital Image Correlation shows Localized Deformation Bands in Inelastic Tensile Loading of Fibrolamellar Bone. Michael Kerschnitzki[1], Gunthard Benecke[1], Peter Fratzl[1] and Himadri S Gupta[2]; [1]Biomaterials, Max Planck Institute of Colloids and Interfaces, Potsdam, Brandenburg, Germany; [2]School of Engineering and Materials Science, Queen Mary, University of London,

London, United Kingdom.

Bone is a hierarchically structured biocomposite, and as a consequence, its deformation as a response to externally applied load is expected to depend on the architecture at length scales from the supra-molecular to tissue level. Aim: Irreversible or plastic deformation in bone is associated with localized microdamage. Mechanisms at the molecular and mesoscopic level have been proposed to explain the nature of the irreversible deformation. Nonetheless, a quantitative correlation of mechanical yielding, microstructural deformation and macroscopic plastic strain still does not exist. To address this issue, we developed and applied a two-dimensional image correlation technique to the tensile deformation of bone, in order to determine the spatial distribution of strain fields at the length scale of 10 µm - 1 mm in bone during irreversible tensile deformation. Methods: Bovine fibrolamellar bone from the periosteum was shaped into long and thin (0.2 x 0.4 x 12 mm3 long) tensile specimens. To increase the precision of our image correlation measurements, the samples were speckled with ink in order to enhance natural sample-contrasts. Mechanical tests to determine local strain concentrations in the elastic as well as in the inelastic regime were carried out in a tensile tester, with the sample immersed in physiological saline. Results & Conclusions: We find that tensile deformation is relatively homogeneous in the elastic regime, and starts, at the yield point, showing regions of locally higher strain. Multiple regions of high deformation can exist at the same time over a length scale of 1 - 10 mm. Macroscopic fracture always occurs at one of the locally highly deformed regions, but the selection of which region cannot be predicted. Locally, strain rates can be enhanced by a factor of 3 - 10 over global strain rates in the highly deformed zones, and are lower but always positive in all other regions. Light microscopic imaging shows the onset of structural “banding” in the regions of high deformation, which is most likely correlated to microstructural damage at the inter - and intra - fibrillar level.

KK5.33

Abstract Withdrawn

KK5.34

In vitro Studies of DSS-8 Peptide on Nano-mechanical Behaviors for Remineralized Human Dentin Chia-Chan Hsu[1], Hsiu-Ying Chung[1], Elizabeth Marie Hagerman[2], Jenn - Ming Yang[1] and Benjamin M Wu[1,2]; [1]Materials Science and Engineering, UCLA, Los Angeles, California; [2]Department of Bioengineering, UCLA, Los Angeles, California.

Dental remineralization may be achieved by mediating the interactions between tooth surfaces with free ions and biomimetic peptides. We recently developed octuplet repeats of aspartate-serine-serine (DSS-8) peptide, which occurs in high abundance in naturally occurring proteins that are critical for tooth remineralization. In this paper, we evaluated the possible role of DSS-8 in dentin remineralization. Human dentin specimens were demineralized, exposed briefly to DSS-8 solution, and then exposed to concentrated ionic solutions that favor remineralization. Dentin nano-mechanical behaviors, hardness and elastic modulus, at various stages of treatment were determined by nanoindentation. The phase, microstructure and morphology of the resultant surfaces were characterized using the grazing incidence X-ray diffraction, variable pressure scanning electron microscopy, and atomic force microscopy, respectively. Nanoindentation results show that the DSS-8 remineralization effectively improves the mechanical and elastic properties for demineralized dentin. Moreover, the hardness and elastic modulus for the DSS-8 treated dentin were significantly higher that surfaces remineralized without DSS-8. The result also shows the elastic modului for the DSS-8 treated native dentin are decreased with increasing the concentration of SBF solution, but the hardness and elastic modulus for the DSS-8 treated demineralized dentin are enhanced with increasing the concentration of SBF solution due to changes of surface roughness.

KK5.35

Microstructural Investigation of Creep and Fatigue Properties of Cortical Bone. Claudia Fleck, Materials Engineering, Technical University of Berlin, Berlin, Germany.

Cortical bone is a hierarchically structured natural composite. The damage process during cyclic loading is characterised by non-linear deformation as well as the formation and growth of (micro)cracks. These lead to increasing non-elastic strain amplitudes, and to cyclic creep, which is especially pronounced for tensile mean loads. It has been stated, that the mentioned effects are due to static and cyclic deformation processes. In the present study, the (static) creep and (cyclic) fatigue deformation and damage behaviour have been investigated by loading cortical bone specimens from the horse tibia in static creep tests, cylic load increase tests, and cyclic single step tests as well as in tests combining static and cyclic loading. The tests were performed with axial or 3-point bending loading, under stress-control with the specimens kept wet in Hanks' solution at all times. A defined adaption of loading velocity was used to identify time and cycle dependent influences on the deformation. For defined loading times and/or cycle numbers, tests were interrupted for microstructural investigation of the specimens by the replica method, or by light and scanning electron microscopy. Distinct differences concerning the deformation as well as the damage behaviour could be shown between tensile and compressive loading. Deformation values proved to contain elastic, viscoelastic, and plastic components besides microcrack formation and growth, depending on loading function and rate. The measured deformation parameters were correlated to microstructural damage on the nano- and micro-scale.

KK5.36

The Substrate Effect on the Cell Properties from Indentation Measurement. Guoxin Cao and Names Chandra; university of Nebraska-Lincoln, Lincoln, Nebraska.

Atomic force microscopy (AFM) provides a convenient way to measure the local mechanical properties of cell. By indenting the cell attached on the substrate, the mechanical properties of cell can be identified from the recorded indentation force-depth relationship. However, since the substrate is usually much stiffer than cell, the substrate will affect the intrinsic indentation force-depth relationship especially for the thin region of cell with deep indentation. Therefore, it is very important to understand the substrate effect to the measurement of cell properties by using AFM. In current work, the computational simulations based on finite element modeling (FEM) are employed to investigate the mechanical properties of cell with substrate under nanoindentation, and the effects of indenter tip radius, contact modes and indentation loading modes are also considered. The cell can be simplified as standard linear viscoelastic material, which includes two elastic springs and one dashpot. The Poisson's ratio of cell is assumed as a constant. The rigid spherical indenter tip is selected. Three types connections are considered between the cell and substrate: fully adhered, partially adhered and nonadhered. In FEM simulation, the indenter is modeled as an axisymmetric 2D rigid surface, and the cell is modeled as 4-node axisymmetric element with reduced integration. Two different indentation loading profiles are used: (1)Quasi-static loading; (2)Dynamic loading: applying a small oscillatory displacement to the direct indentation, with amplitude and angular frequency. In quasi-static indentation, the relaxation modulus can be measured from the relaxation process, which can be also described in the first term of Prony series for the standard linear solid model. If there is the substrate effect, it can be incorporated by a factor, which is a function of the ratio of the indentation depth to the cell thickness. The dimensionless equation is established to solve elastic modulus using numerical simulations, which can fitted from FEM simulations. In dynamic indentation, a small oscillated displacement or force (e.g. sinusoidal with different frequency) is superimposed onto the direct indention component. The complex dynamic stiffness can be determined from the phase lag between the applied displacement/force and the resulted force/displacement. If there is the substrate effect, the dimensionless equation can be built to solve the dynamic indentation modulus, which can be fitted from FEM simulations. Based on the dimensionless equations, the effect of substrate is decoupled and the intrinsic viscoelastic properties of cells can be identified using the quasi-static and dynamic indentations. This result provides a very useful guideline to successfully characterize the viscoelastic properties of cells with substrate using the AFM nanoindentation.

KK5.37
Abstract Withdrawn

KK5.38
Indentation and Uniaxial Compression Study of Enamel's Elastic/plastic Behavior from Nanometer to Millimeter Length Scale. Siang Fung Ang[1], Stefan Habelitz[2], Arndt Klocke[3,4], Mike Swain[5,6] and Gerold A Schneider[1]; [1]Institute of Advanced Ceramics, Hamburg University of Technology, Hamburg, Germany; [2]Division of Biomaterials and Bioengineering, Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, San Francisco, California; [3]Division of Orthodontics, Department of Orofacial Sciences, University of California, San Francisco, San Francisco, California; [4]Department of Orthodontics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [5]Faculty of Dentistry, The University of Sydney, Sydney, New South Wales, Australia; [6]University of Otago, Dunedin, Otago, New Zealand.

Enamel, the outermost layer of teeth, is a complex structure composed of mineral (mainly hydroxyapatite), organic and water phase. Mechanical properties of enamel are largely determined by its hierarchical structure. Previous studies have been aimed at the characterization of the elastic-plastic behavior of enamel. However, a systematic investigation at lengths scales of at least four hierarchical levels is lacking, namely macroscopic scale, several prisms, within one prism and a nanometer HAP crystallite. The objective of this study is to characterize enamel's yield behavior at these lengths scales with indentation and uniaxial compression. The determination of enamel's yield point is essential since loading beyond it may lead to fatigue and wear under cyclic loading. The yield point also gives insights into the deformability of the material before crack and fracture. The very first part of the nanoindentation loading curve was evaluated to obtain indentation stress-strain curve based on Tabor's theory. The yield point of enamel was found to vary from 0.9GPa to 17GPa depending on the probed length scale. Possible plastic processes of enamel are discussed in regard to their lengths scales. An attempt is made to use concepts of dislocation plasticity to describe the inelastic processes.

KK5.39
Modeling the Elastic and Creep Properties of Collagen Fibril. Fang Yuan[1], Anjali Singhal[1], L. C Brinson[2,1], David C Dunand[1], Jonathan D Almer[3] and Dean R Haeffner[3]; [1]Materials Science and Engineering, Northwestern University, Evanston, Illinois; [2]Mechanical Engineering, Northwestern University, Evanston, Illinois; [3]Advanced Photon Source, Argonne National Laboratory, Argonne, Illinois.

Bone is a family of materials with very complex hierarchical structure which imbues it with unique mechanical properties, such as high strength and high toughness at a low density. Because of these excellent mechanical properties, bone is intensely studied by the materials science community both to understand its native function and to create biomimetic analogs. A complete understanding of the hierarchical structure and properties of bone is dependent upon unraveling the structure-property relationships at the fundamental building block level - that of the collagen fibril (on the order of hundreds of nanometers or smaller). In this presentation, we focus on the collagen fibril level of the bone, and emphasize the relationship between the structure and mechanical properties of collagen fibril. In essence, the collagen fibril can be viewed as a nanocomposite: the weak, viscoelastic collagen phase as the matrix filled with hard, elastic plate-like mineral phase as the reinforcement. We create a two-phase cylinder-shaped model to represent the structure of the collagen fibril including the distribution of the discrete mineral plates axially and radially. By using finite element method (FEM) to simulate the response of this model, we discuss the dependence of the elastic properties of the collagen fibril on the dimension and spacing of the mineral plates, the volume fraction of the mineral phase, and inherent phase properties. We also compare our elastic modeling results with synchrotron x-ray diffraction experimental data which provide the deformation information of both the mineral plates and the connecting collagen matrix. The computational and experimental results match well, validating our approach. In addition to the elastic properties, we also simulate the creep behavior of the nanocomposite. To accomplish this, the viscoelasticity of the collagen matrix and interaction of the interface between two phases is incorporated into the model. The time-dependent deformation behavior in both phases are discussed and also qualitatively compared with the latest synchrotron x-ray diffraction experimental data. It is shown that the morphology and evolving interfacial properties under extended load are critical to capturing the material response.

KK5.40

Structure and Mechanical Properties of Horn Keratin. Ekaterina Evdokimenko[1], Luca Tombolato[1], Jerry Curiel[1], Po-Yu Chen[2] and Joanna McKittrick[2,1]; [1]Mechanical and Aerospace Engineering, University of California, San Diego, La Jolla, California; [2]Materials Science and Engineering, University of California, San Diego, La Jolla, California.

We report on the structure and mechanical properties of horns from a desert big horn sheep, Ovis canadensis. Horns must be strong and durable as they are subjected to extreme loading impacts, making them superior, impact resistant structural material. Horns are composed mainly of α-keratin, a fibrous, structural protein found in hair, nails, claws and hooves. The horn is a lamellar structure of keratin sheets (20-40 micrometers thick) enclosing tubes (about 90 micrometers in diameter) running in the longitudinal (growth) direction. The purpose of this work was to correlate the structure and the impact resistance properties, such as energy absorption and work of fracture, and to determine failure mechanisms. Hardness tests were conducted in longitudinal, transverse, and radial directions and samples were tested in bending and compression to failure in both wet and dry states. The fracture surfaces were examined by optical and scanning electron microscopy (SEM). Correlation of mechanical properties with the orientation will be discussed along with fracture mechanism. This research is supported by the National Science Foundation grant DMR 0510138.

KK5.41

Exploring Nucleation in Biomimetic Systems Through In Situ, Fluid Cell TEM. Michael H Nielsen[1,2], Jonathan R Lee[2] and James J De Yoreo[1]; [1]Lawrence Berkeley Lab, Berkeley, California; [2]Lawrence Livermore National Lab, Livermore, California.

One of the challenges in understanding templated growth of biominerals is probing the early events that determine the nucleation pathway and final mineral structure. Herein we report the development of an in situ transmission electron microscopy (TEM) technique suitable for imaging dynamic processes in liquid environments at high temporal resolution. The capability for in situ measurements is enabled by the combination of a custom designed TEM stage and cell. Significantly, the design of the cell and holder ensures temperature and electrochemical control over the reaction environment, which can be used to initiate processes of interest, such as the onset of crystal nucleation and nanoparticle growth. Moreover, because the gold electrode sits in the path of the electron beam, the systems allows for direct investigation of templated nucleation. Time-resolved imaging permits the observation of changes in the structural morphology of growing crystals. In conjunction with the solution parameters, these observations allow for determination of kinetic and thermodynamic factors that drive crystal nucleation and growth. Furthermore, dynamic diffraction allows for direct investigations into the pathways of crystal growth, enabling the determination of the underlying mechanisms that take a species from its solvated state to final crystalline form. Herein we report the observation of electrochemically driven calcium carbonate nucleation. We present data on the dependence of nucleation rates on driving force and temperature, from which we estimate interfacial energies and kinetic barriers, and on the morphological and structural evolution of the incipient nuclei. We show how this approach can be extended to observation of mineralization on biological structures such as protein cages and fibers.

SESSION KK6: Reversible Deformation and Fracture Mechanics of Biological Composites I

Chairs: Himadri Gupta and William Landis
Thursday Morning, April 16, 2009
Room 3024 (Moscone West)

8:30 AM *KK6.1

Phosphorylated Proteins May Play a Significant Role in the Fracture Resistance of Bone. Paul Hansma, University of California, Santa Barbara, California.

Phosphorylated proteins such as osteopontin [1] and dentin matrix protein [2} can form networks with the ability to dissipate large amounts of energy through the sacrificial bond and hidden length system. The system dissipates large amounts of energy with entropic and enthalpic forces while stretching out the hidden length of polymers in the “glue” that is exposed when sacrificial bonds break[3]. Dissipating the energy from impacts in this way protects strong bonds from irreversibly breaking. This mechanism works better in the presence of multivalent positive ions such as Ca2+ ions. Multivalent positive ions may be involved in forming bonds between negatively charged groups such as phosphorylated serine residues on the backbones of noncollagenous proteins such as osteopontin and bone sialoprotein, at physiological pH[2-5]. Evidence from Atomic Force Microscope indentation, pulling and imaging together with evidence from macroscopic testing suggests that collagen fibrils and mineral plates are not the only components of bone with a mechanical role. Bone also contains a protein-based “glue” that uses the sacrificial bonds and hidden length system. The “glue” binds mineralized collagen fibrils to other mineralized collagen fibrils and thus may also play a substantial mechanical role. Acknowledgements I thank Georg E. Fantner, Johannes H. Kindt, Philipp J. Thurner, Georg Schitter, Patricia J. Turner, Simcha F. Udwin, Marquesa M. Finch, Larry Fisher, Herb Waite, Galen Stucky, Dan Morse, Peter Fratzl, Ravi Nalla, John Kinney and Stephanie Lam for valuable contributions. This work is supported by the NIH grant RO1 GMGM65354. 1. Fantner, G.E. et al. Nanoscale ion mediated networks in bone: Osteopontin can repeatedly dissipate large amounts of energy. Nano Letters 7, 2491-2498 (2007). 2. Adams,J.et al.Molecular energy dissipation in nanoscale networks of dentin matrix protein 1 is strongly dependent on ion valence, Nanotechnology 19, 384008 (2008). 3. Smith BL, Schaffer TE, Viani M, Thompson JB, Frederick NA, Kindt J, Belcher A, Stucky GD, Morse DE, Hansma PK: Molecular mechanistic origin of the toughness of natural adhesives, fibres and composites. Nature 399, 761-763 (1999).

9:00 AM KK6.2

Statistical Model of the Dynamic Mechanical Response of Nacre. Mark H. Jhon[1,2] and Daryl C Chrzan[1,2]; [1]Department of Materials Science and Engineering, University of California, Berkeley, Berkeley, California; [2]Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California.

Structural biological materials such as nacre and bone have desirable mechanical properties, in particular a very high resistance to cracking relative to the toughness of the constituent materials. Such biological materials are, however, inherently difficult to model due to their hierarchical structure; features at many different length scales play a role in the macroscopic mechanical behavior. Existing models tend to focus on a particular feature of the microstructure, and do not address how features interact with with each other. In this study the mechanical properties of nacre are investigated using a multi-scale statistical mechanics model. At the smallest length scale, the organic adhesive is studied using a kinetic Monte Carlo model. The spring constant of an unfolding polymer array is found to soften during plastic deformation, while the force required to deform the polymer increases. Increasing the loading rate tends to increase the microscopic strength. This polymer model is coupled to a spring-block model of the macroscopic mechanical response. The spatial distribution of damage in the macroscopic model is found to depend strongly on the nature of the microscopic plasticity law. In particular, microscopic hardening, as displayed by the organic, tends to spread the spatial extent of damage. This is contrasted to the more brittle failure of a system with only mineral bridges. These break-down processes are accompanied by a power-law distribution of precursors to failure, which may be observed by acoustic emission measurements. This work was supported by the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

9:15 AM KK6.3

The Structural and Mechanical Design of Interfaces in Palms and Reeds. Markus Rueggeberg[1,2], Thomas Speck[2] and Ingo Burgert[1]; [1]Biomaterials, Max-Planck-Institute of Colloids and Interfaces, Potsdam, Germany; [2]Botanic Garden, Faculty of Biology, University of Freiburg, Freiburg, Germany.

In many plants, numerous stiffening elements consisting of elongated lignified fibre cells are embedded in relatively soft parenchymatous tissue. Differences in stiffness of more than one order of magnitude exist between the mechanically supporting tissues and the surrounding parenchyma. This puts some constraints on the design of the interfaces, as in case of mechanical loading, high stresses are generated in the stiff fibres, whereas the stresses in the relatively soft parenchyma cells stay rather low. Stress discontinuities are likely to occur at the interfaces under these conditions making such structures prone to failure. Hence, the structural and mechanical design of the interfaces in plants exhibiting such an inhomogeneous stiffness distribution is of particular interest from a biomechanics as well as biomimetics perspective. In the present study, we have investigated the structural and mechanical design of interfaces between stiff supporting tissue and relatively soft parenchymatous tissue at different levels of hierarchy taking the Mexican Fanpalm and the Giant Reed as model organisms. Micromechanical properties, cell parameters, cellulose microfibril orientation and lignification were studied using micromechanical testing, image analysis, synchrotron X-ray diffraction and UV-microspectrophotometry. The investigations revealed gradients in stiffness between the different tissues which were the result of alterations of cell and cell wall parameters. The principle of creating gradual transitions in stiffness was interpreted as a possible concept of lowering stress discontinuities at interfaces of tissues with considerable differences in stiffness and can be well transferred to the design of interfaces in technical fibre-reinforced composites.

9:30 AM KK6.4

Nano-/micro-structural Response of the Collagen/matrix Composite in Human Arterial Adventitia Links to Mechanical Properties. Amenitsch Heinz[1], Fernando Cacho-Nerin[1], Fabian Schmid[1], Barbara Sartori[1], Michael Rappolt[1], Gerhard A Holzapfel[2] and Peter Laggner[1]; [1]Institute of Biophysics and Nanosystems Research, Austrian Academy of Sciences, Graz, Austria; [2]Institute for Biomechanics, Graz University of Technology, Graz, Austria.

The arterial adventitia, like many other soft biological tissues as composite materials, displays a highly anisotropic and nonlinear elastic mechanical behavior - a well-known J-shaped stress-stretch curve. The anisotropy of the tissue is due to the particular hierarchical arrangement of the collagen fibers around two preferential orientations embedded in a matrix material. A profound understanding of the correlation between structure of the collagen fibre arrangement and mechanical property is still missing. Therefore we have conducted an extensive uni- and bi-axial tensile testing of the adventitial layers combined with time-resolved fiber diffraction to reveal the reorientation as well as the load uptake of the collagen fibers under the various load protocols. By using this technique the mean orientation, the distribution and the d-spacing of the collagen fibers using the reflections of the gap-overlay repeat have been measured in situ under physiological conditions, together with the macroscopic force and sample deformation [1] This allows reconstruction of true strains and partly true stresses, which both can be compared to the predictions of nonlinear mechanical constitutive models valid on the macroscopic scale and established well in the literature, e.g. [2]. Uni-axial data are attributed first to a straightening, second to a reorientation of the collagen fibers, and third to an up-take of the increasing loads by the collagen fibers [3]. Remarkable only one fiber family is measured, which is oriented in load direction. The width of the distribution in the locking regime (high load) has been found to be independent of the anisotropy directions (longitudinal or circumferential) as well as the source of the adventitia. Under bi-axial deformations, the distribution of the fiber orientations depends on the ratio of the circular and axial stretches. Changing the ratio of stretches alters the distribution of the orientations, so that values close to 1 show two different fiber families, while for values far from 1 only one peak is seen as in the uni-axial experiment. This behavior is unexpected from the predictions of the constitutive models in the literature. These experimental findings will be discussed in detail; the peculiar response of the collagen orientation will be explained on bases of the network architecture as well as consequences for constitutive models based on multi level description will be given. This new insight and its consequences for new models will make therapies such as balloon angioplasty safer, will improve biocompatible implants such as stents and will allow for designing better artificial tissues. [1] F. Schmid, et al., NIMB, (2006), 246, 262. [2] T. C. Gasser, R.W. Ogden and G.A. Holzapfel, J. R. Soc. Interface, 2005, doi:10.1098/rsif.2005.0073 [3] F. Schmid, et al., J. Synchrotron Rad., 2005, 12, 727. This research has been funded by the FWF Austrian Science Fund under Project No. FWF P17922-N02.

9:45 AM KK6.5

AFM and PFM measurements of Enamel in order to Determine the Crack Tip Toughness and Cohesive Zone of Enamel Gerold A Schneider, Siang Fung Ang and Rodrigo Pacher Fernandes; Institute of Advanced Ceramics, Hamburg University of Technology, Hamburg, Germany.

Enamel, the outermost layer of teeth is a well-optimized hierarchical structure which has rendered its superior mechanical performance compared to those of its constituents. Studies have focused on the toughening mechanisms of enamel. This investigation reports the measurement of the crack opening displacement (COD) at the tip of a Vickers indentation crack. The COD is imaged under high-resolution atomic force microscopy (AFM). The crack tip fracture toughness is found to be slightly lower than the generally reported values in the literature. In addition a Barenblatt cohesion zone model is fitted to the measured COD profile in order to determine the cohesive stress and cohesive zone length. The structural mechanisms leading to the cohesive zone are discussed in regard to the organic (protein) sheets in between the hydroxyapatite crystallites. Piezoresponse force microscopy (PFM) is used to image the organic sheets as a function of their stress state in the vicinity of the crack tip.

10:30 AM *KK6.6
Probing Nanomechanical Behavior of Biological Fibrous Materials using Combination AFM-SEM. Asa H Barber, Fei Hang and Dun Lu; Department of Materials, Queen Mary, University of London, London, United Kingdom.

Many biological materials use fibers for a structural function. These fibers are often associated with a mineral phase which provides mechanical reinforcement. Bone is a prevalent example which uses collagen fibrils as building blocks for various hierarchical architectures. The mechanical properties of collagen fibrils and their associated mineral are still the subject of many studies. In this work we use a novel technique of atomic force microscopy (AFM) combined with scanning electron microscopy (SEM) to examine mechanical response of individual collagen fibrils. Manipulation of collagen fibrils is achieved using AFM while SEM allows in-situ monitoring of the process. Exposed individual collagen fibrils from a bone fracture surface are mechanically tested using the AFM-SEM with results producing a detailed tensile stress-strain curve. Thus, the complete deformation behavior to failure of individual fibrils and the effectiveness of the mineral reinforcement is examined.

11:00 AM KK6.7
Transferred to *KK9.1

11:15 AM KK6.8
Unique Structural Designs Leading to the Inelastic Deformation of Haversian Bone. Vincent Ebacher and Rizhi Wang; Materials Engineering, University of British Columbia, Vancouver, British Columbia, Canada.

Bone is known for its unique hierarchical structure from the nanometer scale to the macroscopic level. It has been generally hypothesized that these various hierarchical levels in bone complement each other to achieve the macroscopic mechanical functions. The most well-known hierarchical structure in human cortical bone is the Haversian system or secondary osteon, consisting of a central canal surrounded by concentric bone lamellae. Since the discovery of the Haversian system in human bone over three hundred years ago, researchers have been wondering about its mechanical advantages. Extensive comparative studies found Haversian bones to be less strong than plexiform bone, which commonly exists in large mammals such as cows. Despite some experimental evidences on the positive intervention of Haversian systems in the fracture process, the contributions of Haversian systems to bone fracture have been obscure. In this study, we compared the deformation-structure relation of human bone specimens with that of cow bone, by combining microscopy and digital image correlation technique with mechanical testing. We discovered a unique inelastic deformation mechanism in Haversian bone that may shine light on its structural advantages over other bones. It was shown that the inelastic deformation happened through the multiple crack nucleation and propagation processes, which were obviously governed by the unique structure of the osteonal lamellae and the distribution of the Haversian systems within the cortical bone. When compressed transversely, the concentric bone lamellae surrounding each Haversian canal allowed multiple arc-shaped cracks to develop intralamellarly. Groups of microcracks developed in high shear zones and radiated out in oblique directions from each Haversian canal. At the cortical bone level where the Haversian systems are randomly distributed within the interstitial bone matrix, multiple nucleation and stable development of such arc-shaped cracks happened to most osteons progressively. As a result, osteonal bone was not sensitive to the presence of Haversian canals and demonstrated a high inelastic strain at macroscopic level. Such remarkable hierarchical structure makes Haversian bones highly resistant to catastrophic failure.

11:30 AM KK6.9
Quasi-static and Dynamic Fracture Behavior of Elk Antler and Bovine Femur Bone. Po-Yu Chen[1], Robb M Kulin[1], Fengchun Jiang[2], Jerry Curiel[2], Fred A Sheppard[2], Kenneth S Vecchio[1,3] and Joanna McKittrick[1,2]; [1]Materials Science and Engineering, University of California, San Diego, La Jolla, California; [2]Mechanical and Aerospace Engineering, University of California, San Diego, La Jolla, California; [3]NanoEngineering, University of California, San Diego, San Diego, California.

Deer antlers have a primary function in intraspecific combat and have been designed for sustaining high impact loading and bending moment without fracture. Antlers have a similar microstructure as mammalian long bones, composed primarily of type-I collagen fibrils and carbonated apatite crystals, arranged in osteons in the compact bone and a lamellar structure in the cancellous bone. Antlers have much higher work to fracture and fracture toughness compare to other mammalian bones. It is important to understand the fracture behavior and toughening mechanisms of antler at high strain rate. In this study, quasi-static and dynamic (split-Hopkinson bar) bending tests (ASTM C1421) were performed on single-notched North American elk (Cervus canadensis) antler and bovine femur samples to measure the fracture toughness. Tests were conducted in the transverse (breaking) and the longitudinal (splitting) directions in both dry and re-hydrated conditions to study the effects of fiber orientation and hydration. Fracture toughness results in the transverse direction were much higher than that in the longitudinal direction and increased with degree of hydration for both antler and bovine femur. The fracture toughness of elk antler is ~ 50% higher than that of bovine femur. The double-notched samples were prepared and tested in quasi-static and dynamic modes. Fracture paths were then examined using scanning electron microscopy. Toughening mechanisms, including crack deflection by osteons, uncracked ligament bridging, and microcracks formation, were observed and discussed. Comparisons between antler and bone were made. This research is supported by the National Science Foundation grant DMR 0510138.

11:45 AM KK6.10

The Effect of Organic and Inorganic Modifiers on Hydroxyapatite Dissolution Studied by Atomic Force Microscopy Ki-Young Kwon[1,2], Eddie Wang[1,2] and Seung-Wuk Lee[1,2]; [1]Physical Biosciences Devision, Lawrence Berkeley National Laboratory, Berkeley, California; [2]Bioengineering, University of California, Berkeley, California.

The complexity of bone tissue and the lack of techniques for directly probing bone surfaces in vivo have hindered studies on the fundamental mechanisms of bone mineral remodeling. We are addressing these issues by using single crystal hydroxyapatite (HAP) as a well-defined bone surface model, and directly observing its surface under various aqueous solution environments using in situ atomic force microscopy. Specifically, we investigated the effects of 1) inorganic ions (NaCl and NaF) and 2) organic molecules (amino acids, and short peptide motifs) on the dissolution of HAP (100) surfaces. We have found that both NaCl and NaF strongly suppress HAP dissolution kinetics, but their inhibition mechanisms are different. We proposed that inhibition by NaCl is the result of competition for surface protonation sites between Na+ and H+ ions rather than the interaction of chloride ions with specific molecular steps. However, inhibition of HAP by NaF can be attributed to the interaction between fluoride ions and specific molecular steps, which results in the shape change of etch pits. Second, we found that HAP binding short peptides selected by phage display dramatically retards HAP dissolution and this inhibition is sensitive to pH. Our molecular level, real-time observations of HAP dissolution are significant for understanding bone resorption and dental carries and provide useful insights for the design of novel therapies for treating bone and teeth related diseases.

SESSION KK7: Reversible Deformation and Fracture Mechanics of Biological Composites II

Chair: Rizhi Wang
Thursday Afternoon, April 16, 2009
Room 3024 (Moscone West)

1:30 PM *KK7.1

The Role of the Organic Component in the Mechanical Behavior of Biomineralized Composites. George Mayer, Materials Science & Engineering, U. of Washington, Seattle, Washington.

The roles of minor organic layers in controlling the mechanical response of such biomineralized composites such as mollusk shells and sponge spicules have been investigated. The mechanisms whereby such minor constituents govern energy dissipation in rigid biomineralized structures are described, and a rationale for new modes of toughening that may relate more generally to families of ceramic- or glass/organic composites is offered.

2:00 PM *KK7.2

3D High-resolution and High-sensitivity X-ray and Neutron Imaging of Wet Dentine in Teeth. Paul Zaslansky, Biomaterials, Max Planck Institute of Colloids and Interfaces, Potsdam, Brandenburg, Germany.

Dental tissues have been the subject of intense study for more than 150 years. Presumably, both materials properties and details of the intricate microstructure are important for the excellent performance of teeth in mammals, specifically when considering the harsh conditions of function - under varying humidity, temperature and mechanical loads. The hierarchical organizations of different tissues within teeth, dentine being an example, result in findings of markedly different structure-function relations when examined at the nanometer, micrometer or sub-millimeter length scales. To this end, measurements of responses to load determined across distances that differ by several orders of magnitude are important and difficult to obtain. Newer large-scale facilities of high-brilliance X-ray or neutron sources make it possible to image whole or relatively large samples, so as to detect structural or compositional characteristics that are of significance at micron or sum-micron length scales in 3D. Using such methods, we have studied differences that exist in dentine microstructure in various regions of teeth. Our results demonstrate the ability to detect tubules sized less than one micrometer in diameter, within large mm-sized dentine samples. We found a great variability in tubule orientation very near enamel yet very well defined orientations just 500 micrometers deeper in. Significant differences of the density of protein exist in the dentine microstructure within the crown, although all samples seem to have similar composition and organization. We have further used these methods to demonstrate differences in water content throughout the dentine bulk, and to identify discontinuities and voids at interfaces with dental restorations placed in teeth. These results are important to refine our understanding of the details of how different parts of the whole tooth structure respond to load, which we believe is important to understanding the longevity of sound, intact teeth. These and similar new non-destructive methods thus help us better understand both the fundamental mechanisms involved in the adaptation of biological materials such as dentine, and are important to understand what are important design strategies (not necessary identical microstructures) that may have use in man-made devices.

2:30 PM KK7.3

High Energy X-ray Diffraction Measurement of Load Transfer between Hydroxyapatite and Collagen in Bovine Dentin Alix Christine Deymier[1], Jonathan D Almer[2], Dean R Haeffner[2] and David C Dunand[1]; [1]Materials Science and Engineering, Northwestern University, Evanston, Illinois; [2]Advanced Photon Source, Argonne National Lab, Argonne, Illinois.

Dentin is a load-bearing composite consisting of a ceramic phase, hydroxyapatite (HAP), and a polymeric phase, collagen. Under load (e.g, during chewing or biting), load is transferred, as in all composites, from the compliant collagen to the stiff HaP phase. Wide-angle synchrotron x-ray diffraction measurements were performed on incrementally loaded bovine dentin samples. Elastic strains in the HAP phase increase linearly with the applied stress, resulting in a slope of 18±2 GPa. This value is much lower (i.e., HaP strains are much higher) than predicted by the Voigt model using HaP and protein volume fractions determined by thermo-gravimetric and chemical analysis, as well as the HaP handbook Young's moduli (EHAP=120 GPa). This discrepancy can only be explained by assuming that the Young's modulus of the nanometric HAP platelets is about 47GPa. This strong reduction from bulk HaP stiffness values is attributed to the very small thickness of the HaP platelets (< 5 unit cells), as also observed in inorganic thin films.

2:45 PM KK7.4
Micropillar Compression of Individual Osteoarthritic Bone Trabeculae. Andy Bushby, F. Ahmed and H. S Gupta; Centre for Materials Research, Queen Mary, University of London, London, United Kingdom.

In recent years micropillar compression tests have become commonplace for metallic materials. However, the elegant techniques developed can also provide insights into the failure of other micro- and nano-scale structures such as hierarchically built mineralized tissues, where structural units at scales from angstroms to metres can play a decisive mechanical role. In this study, individual trabeculae (of about 100 micrometre diameter) were excised from osteoarthritic human femoral heads. Uniaxial compression and flexure tests were performed using a UMIS 2000 nanoindentation instrument as a load frame. Under monotonic and repeated loading conditions the development of damage could be identified from the load displacement data. To correlate these mechanical events to structural damage, individual trabeculae were embedded in PMMA resin after indentation and examined in the FIB/SEM using a ‘slice and view’ process to build up 3D image data sets through the damage zones. This approach provides new information about the development of damage at the micro to nano-scale in bone under controlled loading conditions.

SESSION KK8: Structure-Property Relationships in Biomimetic Composites III
Chair: Pablo Zavattieri
Thursday Afternoon, April 16, 2009
Room 3024 (Moscone West)

3:30 PM KK8.1
Does the Incorporation of Calcium or Phosphate Control the Rate of Brushite Mineralization? Jennifer L Giocondi[1], George H Nancollas[2], Alex A Chernov[1] and Christine Orme[1]; [1]LLNL, Livermore, California; [2]SUNY, Buffalo, New York.

Traditionally mineral growth rate is considered to be a function of the solution supersaturation. In the case of a two-component mineral such as brushite ($CaHPO_42H_2O$), this implies that the growth rate depends on the cation and anion activity product. However, if the activation barriers associated with incorporating into the crystal differ for the two ions, the ion with the slower incorporation rate will control the crystal growth rate. In this case, the cation to anion ratio (as well as the product) will influence rate. In this study we show that under conditions of constant supersaturation the atomic step kinetics vary by a factor of two depending on the Ca^{2+} to $HPO4^{2-}$ ratio. We find that the growth rate is limited by HPO_4^{2-} incorporation. These experiments provide estimates of the relative activation barriers for Ca^{2+} and HPO_4^{2-}. Surprisingly, the step kinetics are limited by the incorporation of phosphate anions rather than calcium, as is typically assumed. This may be due to the oxygen coordination within the crystal. In the solution phase, calcium has a hydration shell that typically contains 8 water molecules. Within the brushite crystal, the calcium is also coordinated with 8 oxygens, two of them in the form of water. Thus, less re-arrangement may be needed to incorporate within the $CaHPO_42H_2O$ crystal as compared to other biominerals such as calcite. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. Portions of this work were supported by the National Institutes of Health (NIDCR DE03223).

3:45 PM *KK8.2
Nanoscale Phase Ordering in Polymer-Derived Ceramic Composites. Julin Wan[1], Patrick Malenfant[1], Seth Taylor[2] and Mohan Manoharan[1]; [1]Ceramic and Metallurgy Technologies, GE Global Research Center, Niskayuna, New York; [2]Northrop Grumman, Redondo Beach, California.

Materials scientists have long endeavored to reproduce the hierarchical structure of naturally-occurring materials such as nacre in the microstructural design of engineered ceramics. Unique properties including high damage tolerance are generally attributed to the distinct, ordered architecture of many biological ceramics. But efforts to mimic biologically-inspired microstructures in engineered ceramics have traditionally been limited in their ability to control microstructure at the nanometer length scale, this is particularly true with high temperature, nonoxide ceramic materials. Currently, a major effort in nanotechnology research is to develop novel processing schemes for the nanoscale assembly of materials with long-range order. These so-called bottom-up approaches to materials processing make use of various structure-directing agents to engineer microstructure in a controlled fashion. We seek to exploit these novel processing techniques to fabricate hierarchical, bio-inspired structures that feature chemical or phase ordering at nanometer length scales. Our approach utilizes the self-assembly of block copolymers to dictate structure while phase chemistry is controlled via the incorporation of one or more ceramic precursors. Various microstructures ranging from lamellar to cylindrical to other ordered architectures are described, and their evolution is shown to vary sensitively with silicon carbide (SiC) and silicon carbonitride (SiCN) precursor chemistry and loading, solvent type, and temperature. We also demonstrate, for the first time, the use of a hybrid organic-inorganic block copolymer in which the ceramic constituent is an instrinsic component of the BCP and thus ceramic phases can be achieved without the addition of precursors. Using this approach, we synthesize boron carbonitride (BCN) and boron nitride (BN) ceramics, as well as a multitude of metal-doped variations of these ceramics. In these novel materials, phase ordering is maintained following pyrolysis at temperatures as high as 1400 degree C, promising a new approach to the facile synthesis of high temperature ceramic composites with nanoscale order.

SESSION KK9: Biomaterials in Tissue Engineering
Chair: Lara Estroff
Friday Morning, April 17, 2009
Room 3024 (Moscone West)

8:30 AM *KK9.1

Nature-inspired Design of Highly Toughened Materials. Maximilien E Launey[1], Etienne Munch[1], Daan H Alsem[1,2], Eduardo Saiz[1], Antoni P Tomsia[1] and Robert O Ritchie[1,3]; [1]Materials Sciences Division, Lawrence Berkeley National Laboratoy, Berkeley, California; [2]National Center for Electron Microscopy, Lawrence Berkeley National Laboratoy, Berkeley, California; [3]Department of Materials Science and Engineering, University of California, Berkeley, California.

One of the major scientific challenges for new, more efficient, energy-related technologies is the development of lightweight structural materials with improved combinations of strength and toughness. Natural materials such as bone, nacre or wood achieve readily this through the creation of sophisticated hierarchical composite structures with characteristic features at nano- to macroscopic dimensions that generate toughening mechanisms acting at multiple length-scales. So far, the biggest obstacle to replicate these mechanisms in synthetic materials has been the lack of processing techniques able to achieve, in practical dimensions, such complex hierarchy. In this work we use a new technique, freeze casting, to build bulk hybrid materials with unique hierarchical structures. Specifically the directional freezing of a ceramic suspension is used to create lamellar ceramic scaffolds whose structure can be manipulated by controlling the freezing conditions. We have fabricated porous scaffolds, with ~5 to 100 µm thick lamellae oriented over macroscopic dimensions, which are then infiltrated with a second metallic or organic phase to generate hybrid structures with lamellar and “brick-and-mortar" architectures. We apply this technology to the fabrication of model materials that combine a hard ceramic, Al_2O_3, with a relatively soft polymeric (polymethylmethacrylate) or metallic phase (Al-Si). The strength of the hard/soft interface is manipulated at the microscopic level by controlling the roughness of the ceramic lamellae and at the molecular level through chemical grafting or the addition of active elements, e.g. Ti, that are known to segregate to oxide/metal interfaces. The final hybrid (up to 80% ceramic) materials exhibit rising R-curve behavior with fracture toughnesses up to 400 times higher (in terms of J) than their main constituent, Al_2O_3. Akin to natural materials, this spectacular fracture resistance derives from a confluence of mechanisms acting at multiple length scales with the microstructural damage and resulting toughening distributed over very large (millimeter) dimensions. The architecture (lamellar vs. brick-and-mortar) and interface strength have a decisive influence. In polymer/ceramic materials brick-and-mortar structures with strong interfaces exhibit larger toughness that far surpass what can be expected from the simple “law of mixtures” of their constituents and are comparable to that of metallic engineering alloys, with K_{Jc} values as large as 30 MPa√m. Preliminary investigations on lamellar metal/ceramic materials also show a remarkable combination of strength and toughness (~300 MPa and 30 MPa√m respectively). This new approach for the design and fabrication of nature-inspired hierarchical composites can be translated to many different material combinations including other ceramic/polymer and ceramic/metal composites. This work was supported by the U.S. Department of Energy under Contract No. DE-AC02-05CH11231

9:00 AM KK9.2

A New Multilayered Composite Bioceramic for Bone Graft. Jose I Arias[1], Andronico Neira-Carrillo[2], Mehrad Yazdani-Pedram[2], Maria S Fernandez[2] and Jose Luis Arias[2]; [1]Instituto Ciencias Clinicas Veterinarias, Universidad Austral de Chile, Valdivia, Chile; [2]Animal Biology, Universidad de Chile and CIMAT, Santiago, Chile.

Bone grafts need to comply with some criteria of biocompatibility, including favoring neovascularization, new bone formation, and discourage inflammatory response and graft rejection. It is also expected that these materials should have mechanical properties similar to those of natural bone, that is, having enough pores to permit osteoprogenitor cells and vascular endothelium penetration but maintaining strength and flexibility. Here, a new resistant and flexible tridimensional multilayered bioceramic composite was obtained by using chitosan and hydroxyapatite in combination with cells and their associated growth factors from the bone marrow tissue, allowing the development of a biocompatible bone graft. This multilayered graft made out of chitosan functionalized with phosphate groups and mineralized with calcium phosphate (hydroxyapatite) was analyzed with scanning electron microscopy (SEM), X ray diffraction (XRD), energy dispersive X-ray analysis (EDX) and Fourier transform infrared spectroscopy (FTIR) to assess the degree of phosphorylation and the amount of hydroxyapatite present in the graft. The composite was further characterized by mechanical testing (Vicker's microhardness test), in vitro osteoblasts culture citotoxicity tests, simulated body fluids (SBF) stability tests and in vivo histological analysis of mouse subcutaneous inflammatory response and biocompatibility in rabbit tibial bone defect The results showed that this multilayered graft has mechanical properties comparable to that of trabecular bone and it was capable of stimulating the generation of new bone by means of osteoconduction, osteointegration, osteoinduction discouraging inflammatory response, making it possible to regenerate bone 16 weeks after implantation.

9:15 AM KK9.3

Poly(lactic-co-glycolic acid) Nanoparticles Improve the Viability of Liver-derived Cells Encapsulated in a Poly(ethylene glycol) Hydrogel. Wonjae Lee[1], Nam-joon Cho[2,3], Menashe Elazar[3], Jeffrey S Glenn[3] and Curtis W Frank[2]; [1]Mechanical Engineering, Stanford University, Stanford, California; [2]Chemical Engineering, Stanford University, Stanford, California; [3]Medicine, Stanford University, Stanford, California.

Poly(ethylene glycol) (PEG) has been successfully utilized as a cell-encapsulating material due to its biocompatibility, hydrophilicity, and highly tunable structural properties. However, the viability of encapsulated liver-derived cells has remained poor compared with other cell types. We have demonstrated that the low viability of encapsulated liver-derived cells appears to be caused partially by the limited permeability within the PEG matrix to support the high metabolic activity of hepatocytes. In order to improve permeability within PEG matrices, we have attempted to increase the network mesh size by changing the molecular weight or solid content of the PEG, but the theoretically calculated mesh sizes do not correlate consistently with the viability of encapsulated liver-derived cells. These data imply that the theoretical mesh size is not the appropriate spatial feature to determine the matrix’s permeability. We showed that encapsulated cells in PEG hydrogels could be infected with hepatitis C and pseudotyped lentiviruses, and that progeny infectious virus could be recovered from the media supernatants. Because theoretically calculated mesh sizes of 3.4k and 8k PEG were around 4nm, while the size of the hepatitis C virus (HCV) and the lentivirus particles are 50 nm and 100 nm, respectively, this suggests that defects in the network are large enough to allow for virus particles to penetrate within the PEG network structure. We expect that the level of network defects may be a primary determinant of the PEG network structural characteristics, which will affect the hydrogel permeability and the consequent ability to support metabolic activities of encapsulated cells. We therefore sought a way to augment the network defects by integrating poly (lactic-co-glycolic acid) nanoparticles into the hydrogel, expecting that hydrophobic nanoparticles could induce loose crosslinking within the interface of the hydrophilic PEG network during the polymerization process. Gong et al have reported that hydrogels that were synthesized in contact with hydrophobic surfaces showed physical properties attributed to the lower crosslinking density and existence of graft-like dangling chains as defects 1. By analogy, for our system, the large interfacial zone between the hydrophobic particles and the surrounding aqueous PEG-DA solution leads to higher network defects in the vicinity of the particles. The addition of hydrophobic nanoparticles significantly enhanced the permeability and, as a result, the viability (up to 331 ± 58% increase, n=12, p-value =3.4×10-13, two-way ANOVA) and hepatic function, as assessed by albumin secretion (up to 411 ± 140% increase), improved. These results have implications for a potential novel technology platform for studying 3D scaffold designs, hepatic virology, drug development, and regenerative medicine. 1. Gong et al, J Am Chem Soc 2001, 123, (23), 5582-3.

9:30 AM KK9.4
Abstract Withdrawn

10:15 AM *KK9.5

Self-Assembling Peptide Nanofiber Hydrogels Targeted for Dental Tissue Regeneration. Kerstin M Galler[1,2,3], Lorenzo Aulisa[1], Adriana C Cavender[2], Schmalz Gottfried[3], Rena N D'Souza[2] and Jeffrey D Hartgerink[1]; [1]Bioengineering, Rice University, Houston, Texas; [2]Biomedical Sciences, Baylor College of Dentistry, Dallas, Texas; [3]Restorative Dentistry and Periodontology, University of Regensburg, Regensburg, Germany.

Recent isolation of human pulp-derived stem cells opens possibilities for novel treatment strategies in regenerative dentistry. These cells differentiate into various lineages, and they produce dentin after transplantation in vivo. Combining them with a suitable scaffold might enable us to engineer dental tissues in the near future. Peptide-based hydrogels are particularly interesting as short peptide monomers can self-assemble into nanofibrous networks and serve as a matrix for cell encapsulation. Their chemical versatility allows for incorporation of bioactive molecules to stimulate specific cell-matrix interactions and promote the formation of dental soft and mineralized tissues using tooth-derived stem cells. Objectives: The aim of this study was to optimize peptide-based hydrogels for adhesion, proliferation and differentiation of dental stem cells. Methods: Different peptide hydrogels were tested for their mechanical properties and compatibility with dental stem cells. Peptides were modified by incorporation of a cell adhesion motif, an enzyme-cleavable site and a heparin-binding domain for binding of growth factors. Degradation rates, growth factor release profiles and gel strength were determined. Cell proliferation was evaluated and collagen formation and mineral deposition were assessed histologically. Peptide-based systems were compared to other hydrogels including PuraMatrix®, collagen and fibrin in terms of gel strength and conductivity for cell proliferation. Results: Cells proliferate within the gels, degrade the matrix and produce collagen and mineral, but compatibility varies with peptide design. Proliferation rates are similar to commercially available systems, but modifications of peptide-based hydrogels prove to be advantageous to support cell differentiation. Conclusion: Peptide hydrogels are a versatile system allowing for incorporation of bioactive sequences and molecules, promoting dental stem cell growth and differentiation. They can be injected into small defects and provide a promising and potent tool to engineer soft or mineralized dental tissues. This research was supported by the Alliance for Nanohealth.

10:45 AM KK9.6

Histological and Mechanical Evaluation of the in vivo Bone-bonding Ability on the K2TinO2n+1/β-Ti Alloy as a Novel Bioactive Material. Chunxiang Cui[1], Yumin Qi[1], Shuangjin Liu[1], Mingfang Zhang[2], Xuelian Xue[1] and Nan Huang[2]; [1]School of Materials Science and Engineering, Hebei University of Technology, Tianjin, Tianjin, China; [2]Department of Pathology, Tianjin Medical University, Tianjin, Tianjin, China.

The purpose of this study was to histologically and mechanically appraise the in vivo bone-bonding abilities of K2TinO2n+1 coated and uncoated Ti-15Mo-3Nb (TMN) implants. According to GB/T16886/6-1997 biological evaluation of medical devices Part 6:Tests for local effects after implantation, the two types of implants were implanted into the proximal metaphyses of Chinese white rabbits' femurs for 12, 26 and 52 weeks and investigated by pushing out test, scanning electron microscopy (SEM) attached to an energy-dispersive X-ray micro-analyzer (EDX) and light microscopy. The bone-bonding abilities of the K2TinO2n+1 biocoating /Ti-15Mo-3Nb (KBT) gradient biomaterial implants were higher than those of T implants at different periods of implantation. The K2TinO2n+1 biocoating (KB) could stimulate new bone rapid formation at the early stages of implantation. And the implants with the biocoating eventually bonded to bone directly, with no intervening soft tissue layer, that was an osseocoalescence. However, the type of bone-bonding between TMN titanium alloy implants and bone was a simple osseocoaptation. The more excellent bone-bonding ability of the KBT implants should be attributed to the superficial characteristics, the bioactivity of low potassium titanate and biostability of high potassium titanate.

11:00 AM KK9.7

Enamel Matrix Guided Growth of Apatite Vuk Uskokovic[1], Li Zhu[2], Wu Li[2] and Stefan Habelitz[1]; [1]Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, San Francisco, California; [2]Department of Oral and Craniofacial Sciences, University of California, San Francisco, San Francisco, California.

Amelogenesis is one the most peculiar mineralization processes in the biological realm. Not only does it produce dental enamel, the hardest tissue among vertebrates, but it is also a process during which the extracellular matrix disintegrates as it gives rise to a 96 - 98 wt% mineralized tissue. Owing to the unusual, constructive degradation of the protein matrix, the proteolysis of the enamel matrix is supposed to play a crucial role in the formation of enamel. The aim of our research is exploring the effects of the proteolytic interaction between the recombinant version of human amelogenin, the major protein of the developing enamel matrix, and matrix-metalloprotease-20, one of the two main proteases involved in the hydrolysis of amelogenin, on the crystallization of hydroxyapatite. The experimental setting we employ to mimic amelogenesis in vitro is based on continuous titration of precursor ions with a preprogrammed control, typically under pH-stat or constant titration rate modes, with glass ceramic substrates comprising preferentially oriented fluoroapatite crystals used as seeds onto which the ions nucleate/absorb. We have evidenced that only protein concentrations higher than 0.8 mg/ml yield crystal growth rates higher than 20 - 40 nm/day. Despite its mainly hydrophobic nature, dispersed amelogenin nanospheres, the size and surface charge properties of which were analyzed using Dynamic Light Scattering (DLS) and electrophoretic fingerprinting, are shown to promote nucleation of apatite by decreasing the nucleation lag time proportional to their concentration. Electrophoretic analyses indicate a selective interaction between the full-length amelogenin and shorter peptides obtained by its proteolytic cleavage, with a particularly emphasized effect of the 146-residue fragment. Atomic force microscopy, spectrophotometric ionic content analyses, MALDI-TOF mass spectrometry, and Raman spectroscopy have presented additional characterization techniques used in this study. The project is supported by the NIH/NIDCR grants R01-DE017529 and R01-DE015821.

11:15 AM KK9.8

Early Stages of Collagen Mineralization Studied by Cryo-TEM: Starting at the Overlap Region? Fabio Nudelman[1], Paul Bomans[1], Koen Pieterse[2], Laura Brylka[1], Gijsbertus de With[1] and Nico Sommerdijk[1]; [1]Laboratory for Materials and Interface Chemistry and Soft Matter Cryo-TEM Research Unit, Dept. of Chemical Engineering and Chemistry, Eindhoven University of Technology, Eindhoven, Netherlands; [2]Biomodeling and Bioinformatics, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands.

Bone is a hierarchically structured biocomposite whose main building block is the mineralized collagen fibril. The collagen molecules in a fibril are organized in staggered parallel arrays that give rise to a periodicity of 67 nm and to the characteristic banding pattern. The mineral phase is composed of carbonated hydroxyapatite crystals that are located inside grooves or channels within the type I collagen fibril, with their c-axis oriented parallel to the long axis of the fibrils. The intimate ultrastructural relation between the collagen fibrils and the mineral is clear evidence that collagen mineralization is not random, but is a tightly regulated biological process, where the collagen fibril serves as a scaffold onto which the mineral is deposited. Thus, the precise mechanisms through which the collagen fibril - from its amino acid sequence to its supramolecular structure - controls mineral formation during osteogenesis are of great interest. Cryo-Electron Microscopy (cryo-EM) is a powerful tool to study collagen mineralization in vitro. Fast-freezing of the sample in liquid ethane ensures near native preservation of the molecular structure of the collagen, avoiding artifacts caused by dehydration and chemical fixation. By combining cryo-TEM, cryo-tomography and cryo-STEM, we are capable of studying the early stages of mineral formation in 3-dimensions, with unprecedented resolution. Collagen type I from equine tendon at pH 2.5 was adsorbed on TEM grids and allowed to assemble into fibrils at pH 7.4. The grids were then incubated either in simulated body fluid or in a CaCl2 and K2HPO4 solution, in the presence of polyaspartic acid (polyasp) as a substitute for the non-collagenous proteins and inducer of intrafibrilar mineralization. After different amounts of time the samples were fast-frozen in liquid ethane for cryo-EM. Cryo-TEM and tomography of unmineralized collagen fibrils demonstrated their proper assembly and revealed, for the first time, finer details of collagen structure with unprecedented resolution. Cryo-TEM and cryo-STEM showed that at the early stages of mineralization, calcium phosphate precipitates are detected associated mostly to the overlap regions of the collagen fibril. Cryo-tomography showed the presence of calcium phosphate particles inside the collagen fibrils, also located mainly at the overlap region. Preliminary elemental analysis using cryo-EELS confirmed that the precipitates are indeed composed of calcium phosphate, and preliminary electron diffraction experiments showed that the early mineral phase is amorphous. We are currently in the process of correlating the structural features of the collagen fibril observed using cryo-EM with the known amino acid sequence and crystal structure of the collagen fibril. Taken together, our results lead to an understanding on how the chemistry and supramolecular structure of the collagen fibril directs and controls intrafibrilar mineralization and hence osteogenesis.

11:30 AM KK9.9

Structural and Mechanical Properties of the Mineral and Protein Phases in Bone. Po-Yu Chen[1], Damon Toroian[2], Paul A Price[2], Fred A Sheppard[3] and Joanna McKittrick[1,3]; [1]Materials Science and Engineering, University of California, San Diego, La Jolla, California; [2]Biology, University of California, San Diego, La Jolla, California; [3]Mechanical and Aerospace Engineering, University of California, San Diego, La Jolla, California.

Bone is a hierarchically structured composite consisting of a protein phase (type-I collagen) and a mineral phase (carbonated hydroxyapatite). Mineralized collagen fibrils comprised of nano-sized collagen molecules and mineral platelets are arranged in osteons in compact bone and a lamellar structure in the cancellous bone. The mineral phase is thought to be aligned and clustered between the collagen fibrils. Our goal was to investigate the structural and mechanical properties of the mineral and protein phases in bone by demineralization and deproteination. Compact bone and cancellous bone from bovine femur and elk antler (Cervus elaphus canadensis) were examined in this study. Structural features of demineralized, deproteinated, and untreated samples at different hierarchical levels were characterized by micro-computed tomography (CT), optical microscopy, SEM, TEM and TEM tomography. Both the deminerlized and deproteinated bone samples appeared identical at maco-scale. The concentric ring structure in the osteons was undisturbed after demineralization yet the pure mineral phase showed no such concentric rings - rather the mineral was evenly distributed around the central blood vessels. Electron micrographs showed that the minerals were aligned in a coherent manner, forming a continuous network. Compression tests were performed in dry and re-hydrated conditions. Results showed that the sum of the stress-strain curve for demineralized and deproteinated bone was far lower than that of the untreated bone, indicating a strong synergetic effect between the two phases. This research is supported by the National Science Foundation grant DMR 0510138.

SESSION KK10: High Resolution Imaging Techniques for Characterizing Organic-Inorganic Composites

Chairs: David Kisailus and Ulrike Wegst
Friday Afternoon, April 17, 2009
Room 3024 (Moscone West)

1:30 PM *KK10.1

The use of CryoTEM to Study the Early Stages of Biomimetic Mineral Formation. Nico A.J.M. Sommerdijk, Laboratory of Materials and Interface Chemistry, Eindhoven University of Technology, Eindhoven, Netherlands; Soft Matter CryoTEM Research Unit, Eindhoven University of Technology, Eindhoven, Netherlands.

The in situ study of the development of minerals in the presence of structure directing organic molecules can make an important contribution to the understanding of the processes involved in biomimetic mineralization experiments. It is however not a trivial matter to obtain morphological and structural information of such systems in their native hydrated state. We recently have presented the possibility to use cryoTEM as a quasi time-resolved method to investigate the early stages of template controlled mineral formation without removing the developing particles from their aqueous environment.[1] As TEM essentially provides only 2D projections the visualization and accurate description of the interaction between the template and the developing mineral structures is not a trivial matter. Here we show how cryo electron tomography (cET) allows to study the interaction of synthetic as well as biological molecules with biominerals in their early stages of development. We further show that by combining cryoTEM and cET, techniques developed within the life sciences, with techniques derived from materials science such as cryoEDX, cryoEELS and low dose electron diffraction we can correlate the observed morphologies with both structural and chemical information. Using these techniques we will demonstrate the development of amorphous precursor phases and their development into the crystalline forms both for calcium carbonate and calcium phosphate. Moreover with unprecedented detail, high resolution cryoTEM provides information about the pre-nucleation stages that lead to the formation of these amorphous phases. [1] B.P. Pichon, P.H. H. Bomans, P.M. Frederik N. A. J. M. Sommerdijk, A quasi-time resolved cryo-TEM study of the nucleation of $CaCO_3$ under Langmuir monolayers, J. Am. Chem. Soc. 130, 4034 (2008).

2:00 PM *KK10.2

Biomineral Ultrastructure. Pupa Gilbert[1], Rebecca A Metzler[1], Christopher E Killian[1], Susan N Coppersmith[1], Yurong Ma[2], Yael Politi[2], Steve Weiner[2] and Lia Addadi[2]; [1]Physics, University of Wisconsin, Madison, Wisconsin; [2]Weizmann Insttitute of Science, Rehovot, Israel.

The ultrastructure of biominerals is one of the most fascinating aspects of these materials, attracting the attention of materials scientists as well as chemists, physicists and engineers. Typically, the ultrastructure is revealed and analyzed at multiple scales by electron microscopies. Another experimental method, only recently introduced into the study of biominerals, is synchrotron spectromicroscopy. As this method detects both the organic and mineral components, the bonds at their interface, and crystal orientation at the nano-scale, it revealed several biomineral formation and transformation mechanisms (1-3). Here we discuss recent results on the biomineral ultrastructure, with particular focus on the relative arrangement of crystal orientation in the biomineral tissue of two distinct systems (i) Columnar and sheet nacre from a variety of mollusk shells, which are all different from one another. In gastropod columnar nacre we observed gradually ordering tablet crystal orientation with distance from the prismatic-nacre boundary. This was not observed either in cephalopod columnar nacre or in bivalve sheet nacre. A theoretical model explains the kinetics of this ordering mechanism with distance in terms of competition for space (4, 5). (ii) The sea urchin tooth, contrary to conventional wisdom, is not composed of plates, needles and polycrystalline matrix all diffracting as a single crystal. Rather it comprises two distinct and alternating crystal layers A and B, stacked as ABABAB. The angle between A and B crystals is less than 6°. This ultrastructure may explain how the tooth self-sharpens as it grinds limestone, thus far a puzzle in biomineral materials science (6). 1. Labrenz M, Druschel G, Thomsen-Ebert T, Gilbert B, Welch S, Kemner K, Logan G, Summons R, De Stasio G, Bond P, et al. (2000) "Sphalerite (ZnS) deposits forming in natural biofilms of sulfate-reducing bacteria" SCience 290, 1744-1747. 2. Chan C, De Stasio G, Welch S, Girasole M, Frazer B, Nesterova M, Fakra S, & Banfield J (2004) "Microbial polysaccharides template assembly of nanocrystal fibers" Science 303, 1656-1658. 3. Politi Y, Metzler R, Abrecht M, Gilbert B, Wilt F, Sagi I, Addadi L, Weiner S, & Gilbert P (2008) "Mechanism of transformation of amorphous calcium carbonate into calcite in the sea urchin larval spicule" Procs Natl Acad Sci USA in press. 4. Metzler RA, Abrecht M, Olabisi RM, Ariosa D, Johnson CJ, Frazer BH, Coppersmith SN, & Gilbert P (2007) "Architecture of columnar nacre, and implications for its formation mechanism" Phys Rev Lett 98, 268102. 5. Gilbert P, Metzler R, Zhou D, Scholl A, Doran A, Young A, Kunz M, Tamura N, & SN C (2008) "Gradual Ordering in Red Abalone Nacre" J Am Chem Soc accepted for publication. 6. Ma Y, Aichmayer B, Paris O, Fratzl P, Meibom A, Metzler R, Politi Y, Weiner S, Addadi L, & Gilbert P "The grinding tip of the sea urchin tooth: high magnesium calcite crystal organization and characterization" submitted.

2:30 PM KK10.3

Quantitative X-ray Microtomography to Establish Structure-Property Linkages in Hybrid Materials Philipp Hunger[1], Amalie E Oroho[1], Adrian P Sheppard[2], Peter Cloetens[3] and Ulrike Gesa K. Wegst[1]; [1]Materials Science and Engineering, Drexel University, Philadelphia, Pennsylvania; [2]Applied Mathematics, The Australian National University, Canberra, Australian Capital Territory, Australia; [3]European Synchrotron Research Facility, Grenoble, France.

The design requirements for a synthetic bone substitute material appear deceptively simple: to provide a porous matrix with interconnecting porosity that promotes rapid bone ingrowth and, at the same time, possesses sufficient strength and toughness to prevent crushing under physiological loads until full integration and healing is reached. Yet, the challenge persists to synthesize a material that mimics both the structure and the mechanical performance of bone. Despite extensive efforts and first encouraging results, current porous materials for bone regeneration tend to suffer a common limitation: the inherent lack of strength associated with porosity. Engineered high-performance composites with effective properties that, like natural bone and nacre, exceed by orders of magnitude the properties of their constituent are rare. The reason for this is that the multi-level hierarchical composite structure, which is thought to be the origin of the observed ‘mechanical property amplification’ is difficult to emulate in synthetic materials. One process with which it is possible to manufacture complex, nacre and bone-like hybrid materials is freeze-casting. It utilizes the intricate process of ice formation to create hybrid materials with complex lamellar or brick-and-mortar-like structures that can be controlled across several length-scales. First results show that both strength and toughness of freeze-cast ceramic-polymer and ceramic-metal hybrid materials increase in a non-additive manner that goes well beyond the simple composite 'rule of mixture'. To date, little is know, how to predict the mechanical performance of such lamellar composites based on volume fractions of the different constituents, on lamellar geometry and surface roughness, and on interfacial bonding. We use quantitative X-ray microtomography to establish first, simple structure-property linkages, to identify mechanisms that control mechanical behavior over multiple length-scales, and to propose new design concepts to guide the synthesis of custom-made, hierarchically structured hybrid materials.

2:45 PM KK10.4

Collagen Fibril Orientation Mapping of Mineralized Tissue by Polarized Raman Spectroscopy. Admir Masic[1], John W Dunlop[1], Markus A Hartmann[1], Jong Seto[1], Sonja Gamsjaeger[2], Paul Zaslansky[1] and Peter Fratzl[1]; [1]Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany; [2]4th Medical Department, Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, Vienna, Austria.

The collagen molecule is a fundamental structural building block for various types of natural tissues (1). Its characteristic hierarchical structure, from atomic to tissue levels allows for the fulfillment of a variety of mechanical functions, particularly in vertebrates. It is a major constituent of tendons and ligaments, as well as the organic matrix of bone and dentin—it is also present in skin and arteries. In all the aforementioned biological materials, the orientation of collagen fibers plays a fundamental role in the overall mechanical properties of the tissue (2, 3). In this study, Raman micro-spectroscopic and imaging analyses were used to elucidate collagen fibril orientation within different biological materials (tendon, bone, dentine etc.). A major experimental result reported here is the possibility to map collagen fibril orientation of a tissue directly in-situ at the micron scale based on evaluating a molecular response within the tissue. The results demonstrate the versatility of the analytical technique, and provide insights into the organization of collagen based mineralized tissues at the ultrastructural level. Additional Raman measurements were performed on bovine cortical bone samples to correlate tissue architecture and its changes due to mechanical loading in compression to specific spectroscopic band shifts. The symmetric vibration band position of the phosphate group was used to evaluate local stresses within the sample. Different stress values were found as function of collagen fiber orientation and compression directions. 1. Fratzl, P. 2008. Collagen: Structure and Mechanics, an Introduction. In Collagen. P. Fratzl, editor. Springer, New York. 1-13. 2. Fratzl, P., and R. Weinkamer. 2007. Nature's hierarchical materials. Progress in Materials Science. 52:1263-1334. 3. Seto, J., H. S. Gupta, P. Zaslansky, H. D. Wagner, and P. Fratzl. 2008. Tough lessons from bone: Extreme mechanical anisotropy at the mesoscale. Advanced Functional Materials. 18:1905-1911.

3:30 PM *KK10.5

Quantitative Mechanical/Chemical Imaging of Bone from Dmp1 Null Mice. Xiaomei Yao, Lynda Bonewald, J David Eick and Yong Wang; University of Missouri Kansas City School of Dentistry, Kansas City, Missouri.

Dentin matrix protein 1 (DMP1) is an acidic noncollagenous protein which plays an important role in mineralized tissue formation. Dmp1 null adult mice are ricketic and osteomalacic and are a model for hypophosphatemic rickets. Mutation in humans results is Autosomal Recessive Hypophosphatic Rickets (Feng et al, 2006). The degree of bone mineralization significantly contributes to bone tissue mechanical properties, but precise relationships and interactions between chemical and mechanical variables are unknown. Objective: To relate the changes of chemical properties to the mechanical properties in the Dmp1 wildtype (WT) and null (KO) mouse femoral cortical bone by using FTIR imaging and Scanning Acoustic Microscopy (SAM). Methods: Six femurs from 3 month old Dmp1 WT and KO mice (n=3) were fixed in 70% ethanol, and embedded in PMMA. The embedded blocks were cut longitudinally to 3-μm-thick sections which were then placed on BaF_2 discs for FTIR chemical imaging. The chemical images were collected by using the Spectrum Spotlight 300 system, with 8 cm-1spectral resolution and 6.25 μm spatial resolution. The acoustic images were collected from the remaining blocks by using the KSI SAM with a 110 MHz transducer. Results: Interactive mechanical (elastic modulus) and chemical images (i.e., mineral/matrix ratios) were generated from the same region of bone at a lateral resolution of ~10 um. Mechanical analysis showed that bone in Dmp1KO had ~4 times lower elastic modulus (4.3±1.4 GPa) than that in WT (17.5±1.9 GPa). The mineral-to-matrix ratios in Dmp1KO (4.81±0.51) were ~2 times lower than that in Dmp1 WT (8.55±0.62). The mineral crystallinity and collagen crosslink ratios were not significantly different between KO and WT. Conclusions: The results relate the bone elastic modulus changes in Dmp1WT and KO mice with chemical changes by specific sites. These measurements provide a new tool for describing the variability of bone chemical and mechanical properties.

4:00 PM *KK10.6

Probing the Molecular-level Structure of Biominerals. Melinda J Duer[1], David G Reid[1], Erica R Wise[1], Serena M Best[2], David Zou[2], Christian Jaeger[3], Catherine M Shanahan[4] and Michael Schoppet[5]; [1]Chemistry, University of Cambridge, Cambridge, United Kingdom; [2]Materials, University of Cambridge, Cambridge, United Kingdom; [3]Federal Institute for Materials Research and Testing, Berlin, Germany; [4]Cardiovascular Division, Kings College London, London, United Kingdom; [5]Internal Medicine and Cardiology, Philipps University, Marburg, Germany.

The material properties of biominerals, such as bone, arise at least in part from the interplay between the various components that make up the material at a molecular level. This talk will show how solid-state nuclear magnetic resonance (NMR) spectroscopy can be used to characterise the mineral phase in bone and its interaction with the surrounding organic matrix. The mineral phase is studied with 31P and 1H NMR and two-dimensional spectroscopic techniques which allow spatial correlations between neighbouring phosphate groups and between phosphate groups and hydrogen atoms to be determined. This allows us to distinguish two distinct regions for the mineral crystals: a relatively crystalline, hydroxyapatite-like phase and a more disordered phase containing hydrogen phosphate and water. Further NMR experiments show that individual crystals contain both phases and that the apparently disordered phase is locally ordered. We hypothesize that the core of the crystals contains the relatively ordered hydroxyapatite phase, whilst the disordered region containing hydrogen phosphate and water makes up the surface regions of the crystals. As such, at any given depth into the surface, this phase is expected to be largely ordered in two dimensions with the structure varying with depth. This conclusion is supported by first-principles electronic structure calculations. [1] The surface structure of the crystals is clearly very important as it is to this, not the bulk structure, that the protein matrix binds so these experiments provide essential information. The REDOR [2] experiment probes the interface between the mineral crystal surfaces and the surrounding protein matrix and has shown that chondroitin sulphate-like glycosaminoglycans (GAGs) are the predominant species bound to the mineral crystal surfaces.[3] Even more interesting is that we find the same species associated with the calcification deposits in atherosclerotic plaques, suggesting that there is a bone-like control mechanism for the formation of this pathological material.[4] Further work on synthetic samples allows us to conclude that chondroitin sulphates acting in a templating role result in nano-structures very similar to those in bone.[5]. [1] H.F. Chappell, M.J. Duer, N.S. Groom, C.J. Pickard, P.D. Bristowe. PCCP 10 (2008) 600-606. [2] T. Gullion and J. Schaefer, J. Magn. Reson. 81 (1989) 196. [3] E.R. Wise, S. Maltsev, M. E. Davies, M. J. Duer, C. Jaeger, N. Loveridge, R. C. Murray, and D. G. Reid. Chem. Mat. 19 (2007) 5055-5057. [4] M. J. Duer, T. Friščić, D. Proudfoot, D. G. Reid, M. Schoppet, C. M. Shanahan, J. N. Skepper, E. R. Wise, Arteriosclerosis, Thrombosis and Vascular Biology 28 (2008) 2030 - 2034. [5] S.M. Best, M.J. Duer, D.G. Reid, E.R. Wise, D. Zho. Magn. Reson. Chem. 45 (2007) 1-7.

4:30 PM KK10.7

Compressive Failure of Human Trabecular Bone Studied by X-ray Microtomography. Farhat Ahmed[1,2], Graham R Davis[2] and Andy J Bushby[1]; [1]Centre of Materials Research, Queen Mary University of London, London, United Kingdom; [2]Dental Biophysics, Queen Mary University of London, London, United Kingdom.

The understanding of deformation and fracture behaviour of human trabecular bone is instrumental in providing understanding of the relationship between mechanical and structural failure in bone and bone related diseases. X-ray Microcomputed Tomography (XMT) has been widely used in the understanding of structural geometry and analyzing qualitative data. In this study we present a quantitative approach to the evaluation of bone fracture using a high-definition XMT system (Davis and Elliott, J. Phys. 104:131 - 134, 2003). Human cancellous bone cylinders measuring 5mm in diameter and 10mm in length were taken from major, partial and non-loading bearing areas of osteoarthritic femoral heads obtained from patients undergoing total arthroplasty. They were scanned at 0% strain in situ and after the application of 2, 4, 6, 8 and 10% overall strain, using an in-house strain device. 3D data sets were obtained from a series of 601 2D projections and reconstructed to provide images relating to the linear attenuation coefficient. Using a 2D transfer function thresholding approach, it was possible to identify regions of collapsed trabeculae and relate them to them to the compressive strain of the bone cylinder. The failure of individual trabeculae can be followed through the variable strains and identified, giving new insights into the local strain conditions that lead to trabecular collapse or fracture. This study provides a novel and quantitative approach to the understanding of bone fracture using XMT.

4:45 PM KK10.8

Transmission X-ray Microscopy of the Nanostructure of Bone Trabeculae. Joy Andrews[1], Eduardo Almeida[2], Josh Alwood[3], Marjolein van der Meulen[4], Jie Chen[5] and Piero Pianetta[1,3]; [1]SSRL, SLAC, Menlo Park, California; [2]NASA Ames Res Ctr, Moffett Field, California; [3]Stanford University, Stanford, California; [4]Mechanical & Aerospace Eng, Cornell University, Ithaca, New York; [5]IHEP, Beijing, China.

In space travel, exposure to weightlessness and radiation can affect bone structure. We will present transmission x-ray microscopy (TXM) images, taken at 8 keV in Zernike phase contrast with 40 nm resolution, of trabeculae from mice exposed to radiation and to hind limb unloading (a NASA-developed method to simulate weightlessness). Details of osteocytic, lacunar and canalicular organization may yield insight into the effects of space flight on bone. The Xradia full-field TXM at the BL6-2 wiggler beam line at SSRL has been designed to operate in the photon energy range from 5-14 keV in absorption contrast, and at 8 keV and 5 keV in Zernike phase contrast. 2D full-field images and raster-scanned mosaics can provide details on the structure of biological and mineralized tissue, as well as nanomaterials and environmental samples. 3D tomography reveals further detail on three-dimensional arrangement.