Thursday Morning, October 28, 1999

Biomaterial Interfaces Group
Room 613/614 - Session BI-ThM

Biomineralization

Moderator: J.J. Hickman, The George Washington University

8:20am **BI-ThM1 Nucleation and Growth of Calcium Phosphate on Self-Assembled Monolayers**, ***C.C. Chusuei,*** Texas A&M University; *B.J. Tarasevich,* Pacific Northwest National Laboratory; *D.L. Allara,* Pennsylvania State University; *M.J. Van Stipdonk, E.A. Schweikert, D.W. Goodman,* Texas A&M University

Calcium phosphate (CP) was adsorbed onto 16-carbon chain length self-assembled monolayers (SAMs) with various terminated functional groups from solution simulating ionic conditions found in blood plasma at the bone growth region at various exposure times to observe the onset of nucleation growth. X-ray photoelectron spectroscopy (XPS) was used to quantitate nucleation and growth of CP on the surface and compared with ellipsometric measurements. Secondary ion mass spectrometry (SIMS) was used for speciation, monitoring transformation from amorphous phases to hydroxyapatite and comparing them to standardized CP powders.@footnote 1@ Accelerated nucleation and growth of CP on anionically charged SAMs terminal functional groups, -COOH and -SO@sub 4@H, was readily observed at 310 K (consistent with physiological conditions) relative to room temperature (298 K). No growth was observed on the -CH@sub 3@ terminated SAMs. @FootnoteText@ @footnote 1@ Chusuei, C. C.; Van Stipdonk, M. J.; Justes, D. R.; Schweikert, E. A.; Goodman, D. W. Anal. Chem. 1999, 71, 149-153.

8:40am **BI-ThM2 Surface Modification of a Model Biomaterial by UV-Laser and/or Electron Beam Irradiation@footnote 1@**, ***M.L. Dawes,*** Washington State University; *Y. Kawaguchi,* Chugoku National Industrial Research Institute, Japan; *S.C. Langford, J.T. Dickinson,* Washington State University

Single crystal brushite (CaHPO@sub 4@@super .@2H@sub 2@O) is a model hydrated phosphate for studies of surface modification, etching, and biocompatible film growth by laser ablation. In this study we show that significant chemical and morphological changes are produced on such crystal surfaces by irradiation with electron and UV-laser beams. These changes are due to both photoelectronic and thermal effects, principally involving the anion, and are associated with high densities of point defects. We compare the spectroscopic and morphological changes generated by laser and electron beam irradiation as well as by thermal treatment in vacuum. All three treatments dehydrate the surface material; the resulting material forms subsurface, micron-sized platelets which can be exposed by spontaneous fracture of the surface layer. Spectroscopic evidence for reduced forms of phosphorus (primarily pyrophosphate but also elemental phosphorous) are observed on the treated material. Mass spectroscopy of laser-induced emissions from treated material show significant O@sub 2@ and PO@sub x@ emissions, consistent with this reduction. @FootnoteText@ @footnote 1@ This work is supported by the Department of Energy (DE-FG03-98ER14864) and the National Science Foundation (CMS-98-00230).

9:00am **BI-ThM3 Synthesis and Surface Characterization of Peptide-Modified Interpenetrating Polymer Networks that Control Biomineralization**, ***K.E. Healy,*** *T. Barber,* Northwestern University; *D.G. Castner, S.L. Golledge,* University of Washington **INVITED**

A major limitation in the performance of materials used in the medical device and pharmaceutical industries is that they lack the ability to integrate with biological systems through either a molecular or cellular pathway. We have designed and synthesized interfacial interpenetrating polymer networks (IPNs) that resist non-specific protein adsorption, and can be modified to tether bioactive groups such as peptides that mimic cell binding domains found on ECM molecules. An IPN was created by sequential photoinitiated synthesis of a thin layer of poly(acrylamide) [P(AAm)] followed by a secondary photoinitiation step using poly(ethylene glycol) [PEG] based monomers to create the network. Tethering of peptides was achieved by photoinitiated synthesis of PEG-monomethyl ether monomethacrylate, acrylic acid (AAc) and N,N-methylene-bis-acrylamide into the P(AAm) layer. A spacer of bisamino PEG (3400 MW) was then bonded to the AAc through a carbodiimide reaction. As a specific example of coupling bioactive molecules to the surface, peptides from the cell binding domain [CGGNGEPRGDTYRAY] and heparin binding domain [FHRRIKA] of bone sialoprotein were tethered to the remaining free PEG amine moiety via a sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate cross linker. Surfaces were characterized by contact angle measurements, spectroscopic ellipsometry, and X-ray photoelectron spectroscopy. The surface characterization confirmed the formation of the IPN and subsequent immobilization of the peptide. These surfaces resisted protein deposition and neither supported cell attachment nor growth without immobilization of the RGD-based biomimetic peptide from bone sialoprotein. Molecular modification of the non-adhesive IPN using a RGD-containing peptide led to rapid bone cell attachment independent of the presence of serum proteins, and subsequently to normal cell proliferation and normal phenotypic expression (e.g., synthesis of mineralized matrix).

9:40am **BI-ThM5 Osteoblast Behavior on Surfaces with Varied RGD Peptide Surface Concentrations Prepared Using Gold-Thiol Self-Assembly**, ***G.D. Moodie,*** *D.M. Ferris, R.F. Henn, N.J. Wimmer,* Brown University; *R.F. Valentini,* Brown University / Rhode Island Hospital

In this work we evaluate the response of osteoblasts to changing concentrations of the integrin-binding RGD peptide immobilized on gold-coated surfaces. Surfaces were prepared by evaporating 100 Å of titanium onto glass cover slips followed by 800 Å of gold. The peptide Arg-Gly-Asp-Cys (RGDC) and the diluent Cys were bound to the gold through the Cys thiol. 1:0, 1:1, 1:10, 1:100, 1:1000, 1:10000, and 0:1 RGDC:Cys solutions were tested. XPS and SIMS verified peptide immobilization. Osteoblasts isolated from 6-day old rat calvaria were plated at a density of 10,000 cells/cm@super 2@ for one hour and then fixed. Co-localization of actin and vinculin indicates the presence of integrin-based focal adhesions. Vinculin was stained with mouse anti-human vinculin IgG and a rhodamine conjugated secondary antibody. Actin staining was done with FITC / phalloidin. Peptide stability was first assessed by aging uncoated, RGDC coated, and fibronectin coated substrates for 3, 9, 14, and 28 days in serum-free media. Co-localizations were observed on 85-90% of cells on RGDC substrates that had been aged for 3 to 28 days. In contrast, co-localization on fibronectin coated surfaces showed a steady decline with aged specimens and was at the level of plain gold by day 28 (about 30%).The percent of cells showing co-localizations, the number of co-localizations per cell, and cell area all decreased as peptide concentration decreased and were statistically different from 100% RGDC at and below the 1:100 dilution. This study shows that RGDC binds to gold surfaces and influences osteoblast response in a dose-dependent fashion.

10:00am **BI-ThM6 Molecular Recognition at the Protein-Biomineral Interface**, ***J.R. Long,*** *W.J. Shaw, G.P. Drobny, P.S. Stayton, P. Bower,* University of Washington

Biological organisms exhibit sophisticated crystal engineering capabilities that underlie the remarkable material properties of mineralized tissues such as bone and nacre. While nature's biomineralization processes are a complex blend of finely controlled nucleation and growth events that are not currently well understood, it is known that organisms produce acidic proteins which play a key directoral role in controlling biological crystal growth. We have taken a systematic approach with model proteins and biological proteins and peptides to elucidate how small, acidic proteins interact with biological crystals and control their growth rates. Solid-state NMR results investigating protein conformation and orientation on HAP surfaces will be reported.

10:20am **BI-ThM7 Kinetics and Interfacial Energy Studies of Biomineralization**, ***G.H. Nancollas,*** *W. Wu,* State University of New York, Buffalo **INVITED**

The ability of surfaces to nucleate minerals such as the calcium phosphates is important in a wide range of biological events. The kinetics of crystallization and dissolution of the mineral surfaces has received considerable attention from the point of view of parameters such as solution composition, ionic strength, pH, temperature, and solid surface characteristics. However, a factor which is usually ignored in discussions of such induced crystallization reactions is the surface free energy of the nucleus/substratum interface. The Constant Composition method is especially useful for investigating the mechanisms of these reactions and surface free energies, measured using thin layer wicking methods can be used to corroborate crystal growth and dissolution mechanisms determined from kinetics experiments. Kinetic studies have been made using calcium phosphate phases such as dicalcium phosphate dihydrate (DCPD), octacalcium phosphate (OCP), hydroxyapatite (HAP), and fluorapatite (FAP). The much smaller interfacial tensions of OCP and DCPD in contact with water as compared with those of HAP and FAP support the widely held suggestion that the former phases are precursors in HAP and FAP biomineralization. On substrata consisting of minerals, polymers or typical implant materials such as the titanium oxides, the ability of the

surfaces to nucleate calcium phosphate minerals is closely related to the magnitude of the interfacial energies.

11:00am **BI-ThM9 Incorporation of Dye Molecules into Calcium Oxalate Host Crystals**, ***L.A. Touryan***, *R.W. Gurney,* University of Washington*; M.J. Lochhead,* University of New Hampshire*; B. Kahr, V. Vogel,* University of Washington

Biological systems direct inorganic mineral synthesis and subsequent composite growth via molecular interactions between macromolecules and mineral phases. However, describing the relationship between the organic and inorganic molecules and their interactive functions at the molecular level remains difficult. Exactly how additives orient within host lattices is not known, as biomineral crystals accommodate their presence but they do not give rise to sufficient intensity in x-ray diffraction studies. Nevertheless, it is this integration of large biomolecules into much smaller unit cells that profoundly changes the materials properties of biominerals and make their synthetic recreation desirable for novel material design and the enhanced biocompatibility of biomedical implants. Here we discuss the use of sensitive optical techniques, in conjunction with modeling, to determine the spatial orientation of organic additives within the host lattice of a calcium biomineral. We have found that common aryl-carbonium dye molecules such as eosin and fluorescein incorporate into the lattice structure of calcium oxalate, the primary biomineral of kidney stones. These dyes tend to incorporate along the fastest growing crystal planes, and can be detected through fluorescence microspectroscopy. We have measured the intensity of polarized fluorescence on two well-developed crystal faces, calculated dichroic ratios, and used the data to model the direct orientation of the transition dipole moments of organic dyes that incorporate within the inorganic host lattice.

11:20am **BI-ThM10 Molecular Orientation in Artificial Joint Polymers: Characterizing the Precursors of Wear with Soft X-ray Absorption**, ***D.A. Fischer,*** National Institute of Standards and Technology*; S. Sambasivan,* Brookhaven National Laboratory*; M. Shen,* University of Maryland, College Park*; S. Hsu,* National Institute of Standards and Technology

Over half a million patients receive artificial joint replacements annually and practically all the replacements consist of a sliding pair represented by a polymer (ultra-high molecular weight polyethylene -UHMWPE) and a hard counterface (metal or ceramic). For the past 30 years UHMWPE has remained the dominant polymer in artificial joints due to its outstanding wear resistance properties. It has been recognized that wear of UHMWPE contributes to the loosening of the implants and is the main cause for the failure of long-term implants. Hence there is an urgent need to understand the mechanism and the surface morphology leading to wear and failure of the artificial joint. Molecular orientation in biomaterials is thought to be critical in characterizing the precursors of wear and the production of debris during the wear process. Current methods of inferring or deducing orientation are not accurate and often rely on staining and cutting specimens. In this study we use the electric field polarization dependence of soft x-ray absorption to directly determine molecular orientation in UHMWPE and evaluate the utility of this technique for evaluating artificial joint materials. We have measured the change in molecular orientation of ultra high molecular weight polyethylene (UHMWPE) samples subjected to various wear motions and duration. Two motions were used: a unidirectional and a cross-shear (motion to form figure-eight) motion. The observed orientations of the UHMWPE molecular chains using soft x-ray absorption are discussed and contrasted with the current understanding of the wear process in UHMWPE.

11:40am **BI-ThM11 Peptide Functionalized Titanium Alloy Surfaces for Orthopedic and Dental Materials**, ***F.A. Akin***, *L. Hanley,* University of Illinois, Chicago*; H. Zreiqat, C.R. Howlett,* University of New South Wales, Australia

Surface modification to a biomaterial may improve long term survival of prosthetic devices. The modulation of bone behavior was examined by surface chemical modification of titanium alloy (Ti-6Al-4V) using peptides. RGDSC (arginine-glycine-aspartate-serine-cysteine) was covalently bound to the Ti-6Al-4V surface by 3-aminopropyltriethoxysilane. Surface characterization of amine-, cysteine-, and RGDSC-terminated Ti-6Al-4V was determined using x-ray photoelectron spectroscopy, roughness assesment, and scanning electron microscopy. All elemental peaks as well as the valence band are employed in the x-ray photoelectron spectral analysis of RGDSC on Ti6Al4V. The S(2p) peak was used to determine the atomic percentage of S on the surface, providing information on the peptide surface density. The valence band of the XPS also showed significant differences between the three surfaces. The attachment and proliferation of human bone-derived cell (HBDC) to the amine-, cysteine-, and RGDSC-terminated Ti-6Al-4V were examined using colorimetric and immunohistochemical assays.

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