SYMPOSIUM DD

Mineralization in Natural and Synthetic Biomaterials

November 29 – December 1, 1999

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* Invited paper

SESSION DD1: BONE AND BONDING OF SYNTHETIC MATERIALS TO BONE
Chair: Panjian Li
Monday Morning, November 29, 1999
Vineyard (M)

8:15 AM *DD1.1
TISSUE ENGINEERED BONE. Charles A. Vacanti, University of Massachusetts Medical School, Center for Tissue Engineering, Department of Anesthesiology, Worcester, MA.

This presentation reviews many important developments in the tissue engineering of new bone over the last 10 years. Tissue Engineering has been defined as "an interdisciplinary field in which the principles of engineering and the life sciences are applied toward the generation of biologic substitutes aimed at the creation, preservation or restoration of lost organ function." Early efforts combining cells with biocompatible materials are described and applications of this technology are presented with particular focus on uses in orthopaedics and maxillofacial surgery. Basic principles of Tissue Engineering focusing on cell biology and materials science as used currently in the field are presented. Finally, future challenges are outlined from the perspective of integrating technologies from medicine, biology, and engineering in hopes of translating Tissue Engineering to clinical applications.
In our laboratories, the goal is to isolate cells from a relatively small specimen of tissue, expand them in vitro, and deliver them, associated with a carrier material, in a configuration that will generate a new functional tissue. The ability to achieve this goal requires a keen understanding of extracellular matrix, structural framework and regulation of cell behavior. Consequently, input from biological scientists who give insight on the structure of tissue and behavior of cells, engineers who fabricate and process materials for guiding tissue development, is essential.
As early as 1908, Lexer described early attempts at tissue engineering of structural tissue in his report of the use of freshly amputated or cadaver allografts for joint reconstruction. Several decades later, the search for the elusive bone morphogenic proteins began. In 1965, Urist demonstrated the generation of bone by means of autoinduction. He described "wandering histiocytes, foreign body giant cells, and inflammatory corrective cells" being "stimulated by degradation products of dead matrix to grow in and repopulate the area of an implant of decalcified bone." The process as described was followed immediately by autoinduction in which both the inductor cells and the induced cells are derived from ingrowing cells of the host bed. Differentiation of the osteoprogenitor cell is elicited by local alterations in cell metabolic cycles that are as yet uncharacterized. The formation of bone that occurs secondary to bone grafts is of three origins; that is, transplantation of living cells, invasion by native osteoblasts, and transformation of host cells into osteoblasts.
Recent decades have given rise to a multitude of descriptions involving the replacement of bone lost to injury and disease. Autografts and allografts rely on creeping substitution. Bone substitutes and alloplastic materials have been used alone, and in conjunction with either demineralized bone or autogenous bone grafts. Mesenchymal tissue can be stimulated to differentiate into bone by polypeptides, demineralized bone powder or both. More recently, living cells have been delivered on alloplastic implants to produce bone. During the last decade, the principles of Tissue Engineering have been applied to virtually every organ system in the body, with a great deal of attention being focused on orthopaedic and maxillofacial applications.

8:45 AM *DD1.2
STRUCTURE AND ULTRASTRUCTURAL SPATIAL DISTRIBUTION OF APATITE CRYSTALS IN BONE; MECHANISM OF MINERALIZATION. Melvin J. Glimcher, Laboratory of Skeletal Disorders, Harvard Medical School, Children's Hospital, Boston, MA.

The nanocrystals of Ca-apatite play vital roles in functions of bone as a substance: (1) the mechanical properties of bone substance; (2) as an ion reservoir; (3) interactions with extracellular matrix proteins and cells. In turn these functions are dependent on chemical composition, size, shape and spatial distribution of the crystals and their specific chemical and ultrastructural interactions with matrix and cellular components. Bone crystals are extremely small (nanocrystals) thin platelets with an enormous surface area. They have long range order of apatite, but are not hydroxyapatite. The crystals are Ca deficient creating vacancies and contain both stable and labile HPO_4, CO_3, and PO_4 components, the labile groups probably on the crystal surfaces. Recent FTIR, proton NMR and inelastic neutron scattering have demonstrated that they do not contain OH groups in the apatite lattice. CO_3, HPO_4, particularly the labile components, vary with the age of the crystals (time spent in the tissue). Ultrastructurally the crystals are located within the collagen fibrils, initially within the hole zone channels and later between individual collagen molecules of a fibril (pore zone), their long, crystallographic c-axes roughly parallel to the long axes of the collagen fibrils. The mechanism of crystal formation is by heterogeneous nucleation within the collagen fibril, perhaps aided by organic phosphate groups of extracellular matrix phosphoproteins. Despite many studies the structure of the "initial" solid Ca-P phase deposited, critical for an understanding of the role of the 3-D structure of the crystals with the organic matrix and cellular proteins has been difficult to ascertain. We present new evidence of the initial structure of the crystals *insitu* in native bone.

9:15 AM DD1.3
ULTRASTRUCTURAL ARCHITECTURE OF BONE MINERAL REVEALED BY HIGH-RESOLUTION TEM AND FIELD-EMISSION LOW-VOLTAGE SEM. Valarie Benezra, underlineLinn W. Hobbs, MIT, Department of Materials Science & Engineering, Ca mbridge, MA; Myron Spector, Brigham & Women's Hospital and Harvard Medical School, Department of Orthopaedic Surgery, Boston, MA.

The apatitic mineral phase of bone imparts to that tissue its compressive, shear and inflectual strengths and moduli and serves as an important repository for calcium and macromolecular soluble regulators. The ultrastructure of this mineral phase has been studied by high-resolution 200-kV transmission electron microscopy (HRTEM) and 1-kV low-voltage high-resolution field-emission scanning electron microscopy (FESEM) in whole trabecular bone (canine distal femur) and in anorganic bone (Bio-Oss, Geistlich Biomaterials, Wollhausen, Switzerland) in which the organic material had been removed chemically. The FESEM specimens were observed uncoated, while the unstained HRTEM specimens were embedded in Spurr's resin and microtomed. The <2-nm resolution of the FESEM allowed substantial correlation between FESEM and HRTEM images of surface morphology and internal structure respectively. Several levels of ultrastructural architecture were observed to be present. Individual apatite crystallites of high perfection were organized along parallel fibrils 65-80 nm in diameter, comparable to the diameter of collagen fibers, which were in turn organized into columnar networks surrounding canaliculi. The platelet crystallites were planar in the a-c plane, ranging from 30-120 nm in length and width and 6-9 nm in thickness, with a long axis along [001]. Within the fibrils, crystallites were roughly parallel and distributed with an average periodicity of 65±4 nm, suggesting that mineralization takes place within the hole zones of a collagen precursor which exhibit similar periodicity. Their morphology was very different from that of crystallites in the synthetic-apatite bone-graft materials Osteograf/LD-300 (Ceramed, Lakewood, CO) or OsteoGen (Impladent, Holliswood, NY) and confirms the role of a collagen precursor in directing mineralization.

9:30 AM *DD1.4
BONE BONDING OF BIOMATERIALS AND APATITE FORMATION IN VIVO. Takashi Nakamura, Masashi Neo, Kyoto Univ, Dept of Orthopedic Surgery, Graduate School of Medicine, Kyoto, JAPAN, Tadashi Kokubo, Kyoto Univ, Dept of Material Chemistry, Graduate School of Engineering, Kyoto, JAPAN.

Mineralization of bone matrix occurs physiologically. Main inorganic component of bone is hydroxyapatite, which contains carbonate apatite and low Ca/P ratio. Ca and P ion concentrations in body fluid are too low for spontaneous apatite nucleation but it is sufficient for the growth of apatite crystal. In bone tissue the apatite-nucleation is thought to be induced by non-collagenous proteins like bone sialoprotein in bone matrix binding to calcium ions. This apatite formation plays an important role for bone bonding of bioactive ceramics or polymers. Bioactive ceramics are known, which can bind bone tissue chemically. The present authors tested bone-bonding strength of biomaterials using detaching test and observed the interface between bone and bioactive ceramics with transmission electron microscopy. An intervening apatite layer was observed at the interface of bone and bioactive ceramics. This layer was distinguished from bone apatite or ceramic. This apatite layer was formed within several days after implantation before bone was observed on the materials. Bisphosphonate is well known to inhibit apatite formation. The injection of bisphosphonate to rabbits concentration-dependently decreased bone-bonding strength of ceramics. The apatite layer was formed on bioactive ceramics in vitro by immersing them in simulated body fluid that contained similar concentrations of inorganic ions as plasma did. Using this apatite layer formed in vitro, it is possible to characterize the apatite layer. This apatite layer enhanced the differentiation of rat bone marrow cells to bone cells in vitro. When osteoclasts were cultured on this layer, they absorbed the apatite layer. These results suggested that this apatite layer not only played a key role for bone bonding but also behaved as bone-like tissues.

10:00 AM DD1.5
EARLY STAGES OF BONE MINERALIZATION ADJACENT TO HYDROXYAPATITE COATINGS ON IN VIVO IMPLANTS. Alexandra Porter, Oxford University, Department of Materials; Valarie Benezra, Joyce W. Lee, Linn W. Hobbs, MIT, Department of

Materials Science & Engineering, Cambridge, MA; Myron Spector, Brigham & Women's Hospital and Harvard Medical School, Department of Orthopaedic Surgery, Boston, MA.

A critical factor in successful orthopaedic and dental implantation is the attachment of the implant to surrounding bone. Hydroxyapatite (HA) coatings are currently being used as the attachment vehicle in several applications to encourage bone bonding, but it is unclear what roles the chemistry, surface morphology and crystallinity of the coatings play in the subsequent mineralization of apposing tissue. The sequence of mineralization has been studied in tissue developing adjacent to HA-coated Ti-6Al-4V 6.4-mm diameter rods implanted into femora and tibiae of dog models. The rods were coated by plasma spraying (PS) to a thickness of 40 micrometers, or by using ion-beam assisted deposition (IBAD) to 1 micrometer thickness, and were implanted–in both as-deposited and annealed states–for times between 3 hours and 10 days. Both coatings were substantially (60%) apposed by new bone after 10 days. Cross sections through the coating/bone tissue interface were examined by scanning electron microscopy (SEM) and by high-resolution transmission electron microscopy of unstained and stained thin sections. In the first 3 hours, isolated crystalline platelets of HA were observed to precipitate in tissue adjacent to as-deposited PSHA (significant amorphous fraction), appearing only after 3 days for annealed (substantially crystalline) PSHA coatings. By 3 days for as-deposited PSHA, HA crystallites had clustered into fibrous globular aggregates, which by 10 days for all coatings had begun to orient both parallel and normal to the coating interface. A poorly organized HA precipitation was also seen immediately adjacent the coating, particularly for IBAD coatings. HA platelet crystallites were distributed along the fibrous assemblies with a 65±nm spacing which, in stained specimens, was observed to be associated also with the presence of collagen fibers. The results suggest that initial stages of HA precipitation can be influenced by HA-coating solubility, but that later stages of bone tissue mineralization and bone apposition are mediated by influx of collagen, and probably of other fibrous and/or adhesion proteins observed in earlier immunohistochemical studies.

SESSION DD2: CALCIUM PHOSPHATES AS BONE SUBSTITUTES

Chair: Takashi Nakamura
Monday Morning, November 29, 1999
Vineyard (M)

10:30 AM *DD2.1

OSTEOINTEGRATION OF CALCIUM PHOSPHATE CERAMICS IN HUMANS AND ANIMALS. Patrick Frayssinet, Nicole Rouquet, DePuy-Bioland, Toulouse, FRANCE.

The cascade of the integration of porous calcium phosphate ceramics with healthy animal bone begins with surface modification of the ceramic implants. The ceramic surfaces start to change to carbonated apatite within a few minutes of implantation. The loose connective tissue then invades the pores. The connective tissue contains fibroblast-like cells, macrophages, mastocytes, pericytes and cells forming the microvessel network. The fibroblast-like cells differentiate preferentially at the ceramic surfaces into osteoblasts. Osteoblasts synthesise an osteoid matrix that will form immature bone. As this immature bone is remodelled and replaced by layered bone, the ceramics are gradually absorbed and finally replaced by mature bone. Biopsies of calcium phosphate ceramics implanted in humans for varying time lengths revealed that the response of human bone to these ceramic implants is similar to that observed in animals. However in humans, the invasion of connective tissue into the pores of the ceramic implants was preceded by the presence of macrophages and giant cells on the surface of the pore wall. The connective tissues in the pores prior to the formation of bone were found in several forms:
- A loose connective tissue in which most of the cells are TRAP+ with very little bone on the ceramic surfaces. No alkaline phosphatase positive cells could be identified.
- A dense connective tissue showing stellate shaped cells dispersed within the collagen matrix. These cells are strongly positive to alkaline phosphatase staining.
- A dense connective tissue containing thin spindle shaped cells showing weak alkaline phosphatase activity in the collagen matrix.
The findings raise the following questions:
- What role do the macrophages and multinuclear cells play on the ceramic surfaces before the invasion by connective tissue? *
- What are the respective roles of the different connective tissues found around the calcium phosphate ceramics?
The answers to these questions are important to determine the mechanism of the ossification process taking place around the calcium phosphate ceramics.

11:00 AM *DD2.2

BIOACTIVE GLASS STIMULATES BIOLOGICAL FUNCTION. Paul Ducheyne, Center for Bioactive Materials and Tissue Engineering, University of Pennsylvania, Philadelphia, PA.

In the U.S. alone, there are 1.23 million fractures that require a bone plate. Of that total, approximately 1 million require between 10 and 100 cc of graft material to stimulate bone repair. At this time, autogenous bone graft represents the gold standard: this graft is typically bone tissue taken from the patient's own iliac crest. Given the morbidity associated with this procedure and the frequently insufficient quantities available, extensive efforts for suitable alternatives are currently underway. Calcium phosphate ceramics and glasses, either by themselves or as carriers for bone (or "osteogenic") cells or various bone growth factors, are prime candidate materials for these applications. In this paper we will discuss key properties of these reactive materials, with the emphasis on the properties of the glasses. We will also illustrate the unique opportunities in orthopaedic care, as well as the applications in tissue engineering which have been brought about by progress in understanding the mechanisms by which these materials affect cells and biological function.
Since the effect of the glass is mediated by a reactive surface, it would appear that a granular material is most useful, as the surface area affecting bone growth is much greater than with blocks of the glass. We found that granules with a size range of about 300-355 mm reacted fully in vivo. Within these reacted particles a unique phenomenon took place: precursor cells for the bone tissue forming osteoblasts were triggered along their pathway to express the osteoblast function. This then led to the formation of bone tissue and a quick, extensive repair of the defect. Never before had this phenomenon been observed for a synthetic material in such an extensive, reproducible and expeditious way. These bioactive glass (BG) granules of narrow size range of 300 - 355 mm (Biogran*) are now used in a variety of treatments.
It is well known in engineering that new technologies can find their way to the market place even when there is not yet a full insight into what produces the useful effect. However, it is equally well established that continued progress depends upon a fundamental understanding of mechanisms. Given the major, unique effect of reacted BG on cell function, we addressed mechanisms by focussing on reproducing some of the in vivo findings by well-conceived in vitro experiments with and without cells. Our data show that various events take place in an integrated and overlapping manner at the BG implant - tissue interface: serum protein adsorption prior to and in parallel with solution-mediated reactions which lead to the formation of an amorphous Si-containing surface layer with accumulated Ca-P phases; selective adsorption of attachment molecules such as fibronectin; humoral and cell-mediated carbonated hydroxyapatite formation; cell proliferation and differentiation, and extracellular matrix formation. The effect of glass on biological molecules and osteoprogenitor cells opens up boundless perspectives for their use in tissue engineering procedures. Whereas tissue engineering has found its way into the clinical practice of restoring skin tissue in severely burnt patents and patients with chronic decubitus wounds, methods to regenerate tissues are only in their infancy in orthopaedic procedures. Since BG is a pre-eminent material to stimulate cells and molecules, it facilitates inventive treatments for better care of the orthopaedic patient population.

11:30 AM DD2.3

ENGINEERING CELL RESPONSE: THE EFFECT OF NANO-STRUCTURED HYDROXYAPATITE ON OSTEOBLAST ADHESION AND METABOLISM. Edward S. Ahn and Jackie Y. Ying, Massachusetts Institute of Technology, Dept of Chemical Engineering, Cambridge, MA.

We have successfully synthesized nanostructured hydroxyapatite (HAP) with superior chemical homogeneity and sinterability than conventional HAP; nanostructured HAP has further demonstrated enhanced mechanical properties. Fully dense, transparent, nanostructured HAP has a three-point bending strength of 200 MPa and a compressive strength of 900 MPa whereas conventionally-prepared HAP has a bending strength between 38 to 113 MPa and a compressive strength between 120 to 800 MPa. The mechanical properties of nanostructured HAP can be further enhanced by dispersing either zirconia or silver within the HAP matrix. Because of the greater mechanical strength of nanostructured HAP, it has potential applications as a load-bearing implant. Nanostructured HAP has the additional advantage of enhanced bioactivity. By engineering the microstructure and surface chemistry of nanostructured HAP, osteoblast response can be controlled. The microstructure-dependence of bioactivity was examined by comparing nanostructured HAP (~125 nanometer grains) to coarse-grained HAP (~1 micron grains). The effect of surface chemistry on bioactivity was evaluated by comparing nanostructured HAP before and after carbonating the surface. Bioactivity was determined by the degree of spreading of osteoblasts on the substrate surface, by the rate of proliferation of osteoblasts, and by the concentration of alkaline phosphatase in the cell medium.

The effects on the production of extracellular matrix, collagen and bone mineral, on the different substrates will also be discussed.

11:45 AM DD2.4
NANOCRYSTALLINE APATITES FOR BONE RECONSTRUCTION. Sophie Cazalbou, Veronique Midy, Christian Rey, INPT-ENSCT, UPRESA CNRS 5071, Laboratoire Interface et Materiaux, Toulouse, FRANCE; Ali Tofighi, Dosuk D. Lee, Etex Corporation, Cambridge, MA; Michel Dard, Merck Biomaterial Gmbh, Darmstadt, GERMANY.

Poorly crystalline apatites (PCA) exhibit exceptional chemical and biological properties and have adapted to many living systems and physiologies. They are characterized by a non-stoichiometric composition, a high specific surface area and the existence of labile non-apatitic environments of mineral ions detected by spectroscopic techniques. The labile non-apatitic environments have been shown to be associated with water and they form probably a hydrated layer on the surface of the minuscule apatite crystals. This might explain the lability of the ions and the observed surface reactivity. Thus PCA can adsorb many active proteins or drugs. Growth factors (FGF-2 and VEGF) for example were strongly bound and preserved from degradation. They were not released in an uncontrolled fashion and remained mostly attached to the surface until the alteration of PCA crystals. Due to their instability, PCA cannot be shaped easily into materials, however, they have the ability to agglomerate irreversibly into solid body at low temperature. These low temperature ceramics show a controlled, variable pore size (5 to 25 nm) in which organic components can be trapped. They are only released as the ceramic is dissolved or degraded. several mineral ions which have been shown to have a biological activity can also be trapped at the surface or into the lattice of PCA. They are liberated by ionic exchange reactions or by dissolution with the possibility of immediate or delayed biological activity. This activity can also be modulated by using apatites at different maturation stage. PCA can be the base of smart biomaterials able to react differently depending on local conditions (bone remodeling rate, inflammation level, cell type)

SESSION DD3: BIOMIMETIC APATITE COATINGS
Chair: Tadashi Kokubo
Monday Afternoon, November 29, 1999
Vineyard (M)

1:30 PM *DD3.1
BIOMIMETIC CALCIUM PHOSPHATE FILMS AND THEIR BIOLOGICAL PERFORMANCES. Klaas de Groot, Biomaterials Research Group of Leiden University and Isotis bv, Bilthoven, THE NETHERLANDS.

More than 15 years ago, our research group started evaluating orthopedic implants coated with a thin 50 micron layer of hydroxylapatite (HA), in order to combine the mechanical strength of the metallic device, usually composed of Ti alloy, with the superior biological behavior of the bone like HA surface. Subsequent clinical studies by several groups, the most important one being lead by dr Geesink from the University Hospital of Maastricht University, showed that hipjoints coated with HA had a superior survival time: a group of 100 patients of average age 55 years had only three failures, not related to the coating, after a period of 13 years.
At that time, we produced the coatings by means of plasma spraying, a technique that because of its high temperature (the plasma reaches a temperature of up to 20,000 degrees C) has some drawbacks, despite its clinical success. These drawbacks are as follows: (1) due to the high temperature the coating composition is not always well defined and certainly not the same as the carbonate containing bony apatite, (2) plasmaspraying is aline of sight process and therefore not suited to coat complicated surfaces or porous insides, (3) one cannot include biological molecules like growthfactors (BMP) or antibiotics into the coating.
Therefore we, and other groups, have tried to develop coatings by a room, or body, temperature environment. Basically, a pretreated surface of preferably titanium or its alloys, is immersed in a supersaturated Ca phosphate solution. The supersaturation is such that spontaneous precipitation does not take place, but only a precipitation onto the pretreated surface. In doing so, one not only obtains a coating with a chemical and crystallographic compostion more similar to bone mineral than plasmasprayed coatings due to inclusion of carbonate, but one can include for example BMP in the supersaturated solution, thus obtaining coatings with true bone induction properties.
In our group we have succesfully tested such coatings, with and without BMP, in animals. First clinical trials of such socalled biomimetic coatings (without BMP) are expected in the fourth quarter of this year (1999).

2:00 PM *DD3.2
A NOVEL METHOD FOR SOLUTION DEPOSITION OF HYDROXYAPATITE ON TO THREE DIMENSIONALLY POROUS METALLIC SURFACES: PERI-APATITE HA. Joseph P. Zitelli, Paul Higham, Stryker Howmedica Osteonics, Advanced Technology Group, Rutherford, NJ.

Hydroxyapatite coatings on orthopedic implants for use in joint replacement surgery are currently, predominately based on high temperature deposition (thermal spraying) techniques. These involve the softening and partial melting of HA particles to place and adhere them to a metal substrate. Constrained within the high velocity thermal spray, the HA particles travel in a straight line.
On orthopedic implants with three dimensional porous ingrowth surfaces (which are used to fix the implant in the bone), a line-of-sight process will not uniformly coat the metal substrate. Portions of the ingrowth surface will be block by more prominent features and backside and undercuts of the metal will be blinded from the thermal spray process. These areas will not have the HA coating deposited on them.
To overcome this limitation a solution mediated deposition process for HA was developed, Peri-Apatite HA. The process evenly coats with HA the entire metal surface of a porous ingrowth zone. The HA is precipitated directly on the metal, as an extremely uniform coating, with an optimized thickness. These are ideal characteristics for a coating, so that it will not block off the desired porosity. The precipitation process permits the deposition of a bone-like HA coating. The physical (thickness, uniformity, morphology) and chemical (composition, phase purity, ionic groups) characterization of the HA coating will be reviewed. The unique aspects of the analytical techniques (XRD, FT-IR, SEM, TEM) used to develop the technical information will also be covered.

2:30 PM DD3.3
FROM SEA SHELLS TO BONE IMPLANTS. Guofeng Xu[1], Ilhan A. Aksay [2] and Jay T. Groves [1], Princeton University, Princeton, NJ, [1]Department of Chemistry, [2]Department of Chemical Engineering, Princeton, NJ.

Biological mineralization features an orchestrated balance among various controlling factors such as template promotion, crystal growth modification and cessation. Highly ordered calcium carbonate lamellae forming in the nacre (aragonite) of mollusk shells or in the semi-nacre (calcite) of brachiopod shells are excellent examples of such synergistic process. Mimicking the concerted interplay of template promotion and growth inhibition as often utilized in biomineralization, we have synthesized and characterized macroscopic-scale, continuous calcium carbonate thin films with a thickness which is comparable to that of an individual calcium carbonate lamella in sea shells. Detailed studies show that these thin films form through a multistep process, in which an amorphous phase is deposited and subsequently transformed into crystalline phase. This synthetic strategy is further applied to the formation of thin films of carbonated calcium phosphates. Upon heat treatment, the films transform into well crystallized continuous films with apatite structure. We are investigating the potential application of these phosphate films in biomedical research and bone implants.
Financial support from NSF MRSEC grant and Princeton Materials Institute is gratefully acknowledged.

2:45 PM DD3.4
FORMATION OF CALCIUM PHOSPHATE ON PHOSPHORUS-CONTAINING GROUPS INTRODUCED SUBSTRATE. Yoshiyuki Yokogawa, Kaori Nishizawa, Fukue Nagata, Tetsuya Kameyama, National Industrial Research Institute of Nagoya, Bioceramics Laboratory, Nagoya, JAPAN.

Recent studies have found a new surface functionalization method for creating favorable local conditions that lead to nucleation and growth of calcium phosphate over substrates. The introduction of phosphorus-containing groups into biological substrate (chitin, chitosan etc) was found to be effective for the induction of calcium phosphate growth over substrate in simulated body fluid (SBF) solution. The studies of the stimulation of calcium phosphate growth on surface functionalized substrate are of potential value in the design of biomaterials which induce calcification in human body, and of scaffold materials. In this work, a calcium phosphate coating over surface modified substrate was produced by a process based on introduction of phosphorus-containing groups on the surface of substrate, and SBF immersion. Phosphorus-containing groups were introduced onto substrate by phosphorylation using urea/H3PO4 in DMF solution. Phosphorylated samples were washed with water and dried. After immersion in Ca(OH)2 solution at ambient temperature, the Ca(OH)2-treated phosphorylated substrate were soaked into SBF solution. Nucleation of an initial calcium phosphate layer was stared to occur all over the substrate surface within 1-3 days. The growth of calcium phosphate layer after soaking for 1-6 days appears to proceed by nucleation, and a thicker coating was observed after soaking for

over 9 days. Phosphorus introduction was found to stimulate the growth of a calcium phosphate coating on their surfaces after soaking in SBF solution. The calcium hydroxide treatment facilitates the formation of a calcium phosphate precursor over the phosphorus ion introduced substrate, which in turn encourages the rapid growth of a calcium phosphate layer over the entire surface.

SESSION DD4: APATITE FORMATION ON INORGANIC SURFACES I
Chair: Klaas de Groot
Monday Afternoon, November 29, 1999
Vineyard (M)

3:30 PM *DD4.1
THE CONTROL OF MINERALIZATION ON NATURAL AND IMPLANT SURFACES. George H. Nancollas, Ruikang Tang and Wenju Wu, Dept of Chemistry, SUNY at Buffalo, NY.

The generation of minerals such as the calcium phosphates on the surfaces of dental and joint replacement implants is beneficial since the facilitation of bone formation permits their fixation. In contrast, the prevention of crystallization is desired on other surfaces such as kidney and cardiac valve prostheses. A key to the development of successful biomaterials is therefore an understanding of crystal growth and dissolution mechanisms in aqueous solution. The Constant Composition method was used to investigate the influence of factors such as solution composition, ionic strength, pH and temperature on the crystallization and dissolution of the calcium phosphates, brushite (DCPD), octacalcium phosphate (OCP), hydroxyapatite (HAP) and fluorapatite (FAP). In parallel with these studies, a contact angle method along with surface tension component theory was employed to investigate the roles of interfacial free energy in mineralization and demineralization. The interfacial tension values, -4.2, 4.3, 10.4 and 18.5 mJ/m2 obtained from contact angle measurements for DCPD, OCP, HAP and FAP, respectively, compare well with those calculated from dissolution kinetics experiments and provide information concerning the growth and dissolution mechanisms. The successful exploitation of these factors is illustrated in studies aimed at coating specific calcium phosphate phases on titanium metal and alloy surfaces and the nucleation and growth of OCP on polymer surfaces modified by silanization to produce amine- and carboxy-terminated end groups. In all these reactions of the calcium phosphates, concomitant dissolution processes are often involved. Constant Composition kinetic studies have shown that the rates of dissolution decrease markedly with time despite the sustained driving force, eventually approaching zero even though crystals remain in the undersaturated solutions. Dissolution can be reinitiated by exposing the crystals to the solutions of different undersaturations. These results suggest that the dislocation sizes play a significant role in the dissolution kinetic processes.

4:00 PM DD4.2
MOLECULAR ORBITAL STUDY OF APATITE NUCLEATION AT SILICA BIOCERAMIC SURFACES N. Sahai and J.A. Tossell, Department of Chemistry and Biochemistry, University of Maryland, College Park, MD.

Silica bioceramics, used as prosthetic bone and dental implants, promote apatite precipitation at their surfaces when immersed in simulated body fluid of composition similar to human blood plasma. Apatite formation occurs in stages but earlier studies disagree on the reaction path. According to one proposed mechanism, the calcium ion sorbs at silanol surface-sites followed by biphosphate (Scheme 1). A recent NMR study of apatite precipitation on bioceramics, however, suggests the reverse sequence resulting in direct Si-O-P bonds (Scheme 2). We have used molecular orbital calculations to determine the reaction sequence for apatite precipitation at bioceramic surfaces. Energies and Si-29, P-31 NMR shifts were calculated for sequential calcium and biphosphate sorption at model bioceramic surfaces. Optimized geometries and energies were obtained at the Hartree-Fock Level using effective core potentials and NMR shifts were calculated using the 3-21G* basis, as implemented in the programs GAMESS and GAUSSIAN 94. Defect and average sites were represented, respectively, by Si three-rings (Si3O6H6) and by Si seven-rings (Si7O12H10). Solvation effects were considered. Comparison of theoretical reaction energies and NMR shielding trends with experimental NMR trends suggests the formation of clusters consistent with Scheme 1 where biphosphate sorbs after calcium. Direct Si-O-P bonds, as in Scheme 2, are unlikely. At physiological pHs, a bidentate geometry is obtained for the calcium phosphate surface-cluster at Si3O6H6 sites. This geometry is similar to the local structure of brushite (CaHPO4. 2H2O). These results suggest that apatite is nucleated at defect sites by a brushite-like surface-cluster. We propose that molecular orbital calculations have been a previously under-exploited tool, and provide an effective method for exploring mechanisms of biomineralization reactions.

4:15 PM DD4.3
FABRICATION AND CHARACTERIZATION OF CALCIUM PHOSPHATE/POROUS SILICON/SILICON STRUCTURES DOPED WITH PLATINUM ANTI-TUMOR COMPOUNDS. Jeffery L. Coffer, Xin Li and John St. John, Texas Christian University, Ft. Worth, TX; Russell F. Pinizzotto and Yandong Chen, University of North Texas, Denton, TX; Jon Newey and Leigh T. Canham, DRA Malvern, Malvern Worcestershire, UNITED KINGDOM.

We have successfully developed a process which permits the encapsulation of the established class of platinum anticancer drugs such as cis-Platin (cis-diammine- dichloroplatinum(II)) within synthetic biocompatible calcium phosphate films that are electrochemically-grown on porous Si/Si substrates. Upon immersion of these structures into aqueous media, the desired platinum species can be released into the surrounding environment. These platinum complex- doped hydroxyapatite / porous Si / Si materials have been characterized by scanning electron microscopy (SEM), energy dispersive x-ray spectroscopy (XEDS), and secondary ion mass spectrometry (SIMS). In this presentation, we focus on the influence of initial platinum concentration in the deposition process on the rate and resultant amount of platinum complex that can be delivered to the surroundings in vitro. Both inductively- coupled plasma (ICP) spectroscopy and uv-visible absorption spectrometry have been employed to monitor the release of the encapsulated drug from the calcium phosphate layers. The impact of subsequent thermal annealing of the calcium phosphate / porous Si / Si structure on the rate of complex delivery to the surroundings will also be discussed.

4:30 PM DD4.4
HYDROXYAPATITE COATING ON THERMAL TITANIUM SUBSTRATE IN AQUEOUS SOLUTION. Masazumi Okido, Ryoichi Ichino, Kensuke Kuroda, Ryoji Ohsawa and Osamu Takai, Nagoya Univ, CIRSE & Dept of Engineering, Nagoya, JAPAN.

A hydroxyapatite, HAP, film was deposited on a titanium substrate in an aqueous solution, at an ambient temperature. The solution included 3 mmol/dm^3 $Ca(H_2PO_4)_2$ and 7 mmol/dm^3 $CaC1_2$ at pH 5.5. The temperature of the substrate surface was controlled in both methods of AC current through the Ti foil and high frequency induction heating using Ti ingot. In these method, only the substrate was heated up and the temperature graduation was formed between the substrate and the solution. The effects of surface temperatures, fluoride ions, additive inhibitor and heating time on the morphology of HAP crystals formed on Ti substrate were investigated in various conditions. The morphology changed from compact layer to dendrite layer with the HAP growing time in AC current method and the HAP film with the thickness of 200 μm can be obtained on Ti foil with cross section of 30 μm x 2 mm by heating for 20 min at 20 A-AC. The adhesion property is better and the film growing rate is faster in this method than that in cathodic electrode method which is one of the aqueous solution processes. On the other hand, the deposits consisted of algae-like whisker in the induction heating method. The XRD analysis and Ca / P ratio for the deposits were also examined.

4:45 PM DD4.5
EFFECT OF POST-TREATMENT ON DISSOLUTION AND BIOMINERALIZATION ON SURFACE OF HA COATINGS IN SIMULATED BODY FLUID (SBF). Jiyong Chen, Jie Weng, Qiyi Zhang, Jiaming Feng, Yang Cao, Xingdong Zhang, Sichuan University, Institute of Materials Science and Technology, Chengdu, CHINA.

Post-heat treatment is important for the long-term stability of plasma sprayed hydroxyapatite(HA) coatings in biological environment. The improvement on the stability of heated HA coatings is related to the phase transformation during heat treatment. The HA coatings were heated separately in vacuum,air and water vapour. The dissolution of the HA coatings was investigated by immersion in SBF. The changes in Ca,P ions in SBF were measured by ICP (inductively coupled plasma emission spectrometry). The starting HA powder for coatings was well-crystallized HA.The plasma spraying process resulted in amorphous phase and additional Ca-P phases (TCP,TTCP and CaO) because of the melt of HA particles in plasma flame and following solidification of melted HA particles striking against cool substrate. The as-received HA coating usually consisted of amorphous phase, additional phases and crystalline phase of unmelted inner cores of HA particles and recrystallized HA. The amorphous and additional phases were much easier to dissolve than crystalline HA. The as-received HA coatings with amorphous and additional phases were most dissoluble. Heat treatment in vacuum transformed the amorphous phase into crystalline phase. Besides this transform, the additional phases were also converted to crystalline HA after heating in humid environment. These phase transformations during heat treatment well explained the result that the dissolubility of HA coatings in SBF decreased in this order: as-received, heated in vacuum, in air and in water vapour. The

nucleation of bone-like apatite on the surfaces of HA coatings after a certain period of immersion in SBF was observed by SEM. The microenvironment with a sufficiently high degree of supersaturation of Ca, P ions is crucial for apatite to nucleate and grow in SBF. The dissolution of amorphous phase in coatings plays an important part in establishing the supersaturation of Ca, P ions.

SESSION DD5: POSTER SESSION
Chair: Paul Calvert
Monday Evening, November 29, 1999
8:00 P.M.
Exhibition Hall D (H)

DD5.1
AN IN-VITRO SYSTEM FOR THE SIMULATION OF ENAMEL GROWTH. Daniel Heidel[1], Hanson Fong[1], Blaire Burman[2], Michael L. Paine[3], Wen Luo[3], Malcolm Snead[3], Mehmet Sarikaya[1]; [1]Materials Science and Engineering, University of Washington, Seattle WA, [2]Shorewood High School, Shoreline, WA, [3]Craniofacial Molecular Biology, University of Soutern California, Los Angeles, CA.

The enamel of human teeth is composed of parallel rods (bundles) of closely-packed, long hydroxyapatite (HAP) Ca10(PO4)6(OH)2 (HAP) crystallites oriented normal to the tooth surface. Both the unique structure of the crystallites and the higher order structure of the rods is believed to be a consequence of interactions between proteins (such as amelogenins) secreted by the enamel forming tissues and the growing crystallites. A better understanding of the precise molecular mechanism that controls enamel crystallite formation could potentially lead to both novel biomimetic enamel repair strategies and crystal growth control systems. We have constructed a model biomaterialization system which utilizes the unidirectional flow of calcium ions using a dialysis membrane that separates two precursor solutions (kept at different pH values) used for HAP formation. The system generates ribbon-like crystallites similar to, but not exactly the same, the HAP particles found in maturing enamel. The morphology and structure of the resulting HAP crystallites are studied by x-ray diffraction, atomic force microscopy, and scanning and transmission electron microscopy. Based on these results, we are currently constructing an advanced fluid cell capable of reproducing the physiological environment of the maturing enamel in human tooth. Ultimately, we will incorporate the enamel protein amelogenin and genetically modified amelogenin derivatives into the crystallization chamber. This will allow the direct observation of the effect of amelogenin upon HAP crystallization in parallel with nanostructural studies of mouse enamel containing the same genetically modified amelogenin constructs.

DD5.2
BIOMIMETIC SYNTHESIS OF HA PRECURSORS IN UREA- AND ENZYME UREASE-CONTAINING SYNTHETIC BODY FLUIDS. Defne Bayraktar, Dept. of Metallurgical and Materials Eng., METU, Ankara, TURKEY; A. Cuneyt Tas, Max-Planck-Institute fur Metallforschung, Stuttgart, GERMANY.

An important inorganic phase of synthetic bone applications, calcium hydroxyapatite (HA, Ca10(PO4)6(OH)2), was prepared as a single-phase and sub-micron bioceramic precursor powder. Carbonated HA precursors were synthesized from calcium nitrate tetrahydrate and di-ammonium hydrogen phosphate salts dissolved in synthetic body fluid (SBF) solutions, containing urea (H2NCONH2) and enzyme urease, under the biomimetic conditions of 37°C and pH 7.4, by using a novel chemical precipitation technique. These powders were also found to contain trace amounts of Na and Mg ions in them, intentionally incorporated by using SBF solutions, instead of pure water, during their synthesis. The characterization and chemical analysis of the synthesized biomimetic HA precursors were performed by scanning electron microscopy (SEM), powder X-ray diffraction (XRD), Fourier-transformed infra-red spectroscopy (FT-IR), and inductively-coupled plasma atomic emission spectroscopy (ICP-AES).

DD5.3
DIP-COATING OF CALCIUM HYDROXYAPATITE ON TITANIUM ALLOY OR STAINLESS STEEL SUBSTRATES. Bora Mavis, Dept. of Metallurgical and Materials Engineering, METU, Ankara, TURKEY; A. Cuneyt Tas, Max-Planck-Institute fur Metallforschung, Stuttgart, GERMANY.

Titanium alloy (Ti-6Al-4V) and stainless steel (316L) are two of the most commonly used materials in the fabrication of orthopaedic implants. The main inorganic phase of human bones is calcium hydroxyapatite (HA: Ca10(PO4)6(OH)2). To achieve better biocompatibility with bone, metal implants made of 316L or Ti-6Al-4V are often coated with calcium hydroxyapatite ceramics. Dip-coating technique is scarcely used to apply HA onto metallic implants. This paper is to describe a chemical process of forming HA coating on metal surfaces via aqueous solutions containing sub-micron hydroxyapatite powders together with polyethylene glycol, glycerol and gelatine. The samples were characterized by means of SEM, EDXS and XRD.

DD5.4
Abstract Withdrawn.

DD5.5
Abstract Withdrawn.

DD5.6
PREPARATION OF APATITE/TITANIUM COMPOSITE COATINGS ON TITANIUM OR TITANIUM ALLOY SUBSTRATE USING RF PLASMA-SPRAYING PROCESS BY CONTROLLED FEEDING OF HA AND Ti POWDER. M. Inagaki, Y. Yokogawa and T. Kameyama, National Industrial Research Institute of Nagoya, Nagoya, JAPAN.

Plasma sprayed hydroxyapatite (HA) coatings on titanium substrates have been used practical for medical use. However, certainty with respect to the long-term stability such as 10-20 year's of HA coatings seems to be a worthwhile subject to investigate. To improve of adhesiveness of coatings, first, titanium powder was applied to titanium substrate to give the roughened surface to the substrate by RF plasma spraying process. Next, HA and Ti powder was applied with controlling the ratio of HA and Ti powder, aiming at reduction of the residual stress due to the large difference in the liner thermal expansion coefficient between the substrate and HA. The composition of HA/Ti composite coatings became HA-rich toward the outmost. Finally, HA powder was applied to the surface of HA/Ti composite coatings. To compare adhesiveness of coating layer to substrate, HA coatings without Ti powder spraying (direct HA coatings) or HA coatings on the prior titanium coatings were also prepared. Coating layer was evaluated by SEM-EDX, XRD, micro FT-IR and tensile testing. In case of direct HA coatings, ca. 50 μm thick coatings were obtained, however ca. 100 μm thick coatings were peeled off after plasma spraying. HA-rich layer near the surface of HA/Ti composite with thickness of over 100 μm were successfully obtained by present method. XRD analysis of coating surface shows that coatings have an apatite structure with a preferred <001> crystallographic orientation. The adhesiveness of HA-rich layer with 100 μm in thick on HA/Ti composite was ca. 50 MPa. The influence of RF inductive power on adhesive strength of HA/Ti composite coating was also studied.

DD5.7
HUMAN TOOTH ENAMEL HYDROXYAPATITE: AN ELECTRON DIFFRACTION STUDY. M. Reyes and J. Reyes-Gasga, Instituto de Fìsica, UNAM, Mèxico City, MEXICO.

Human tooth enamel is composed of hydroxyapatite mainly. Comparing this natural hydroxyapatite with the synthetic one, some important chemical differences are found. One of these is that the first presents many spurious elements induced in its unit cell such as Na, Mg, Cl, F, etc. Another difference is in their space group: synthetic hydroxyapatite obeys the P63/m (172) space group rules whereas the enamel hydroxyapatite does not. The study of these differences is quite important in the understanding of the properties that any biomaterials developed must contemplate it wants to be used as substitute of tooth enamel. In this work we have studied the human tooth enamel structure by electron diffraction, both in the conventional and convergent beam diffraction modes, in order to elucidate the origins of the space group differences with the reported one for the synthetic hydroxyapatite. We thank C. Angeles Chavez, P. Mexía, C. Flores and R. Hernãndez for technical help. This work was economically supported by CONACYT.

DD5.8
NANOMECHANICAL PROPERTIES PROFILES ACROSS DENTIN-ENAMEL JUNCTION OF HUMAN INCISOR TEETH. Hanson Fong[1], Mehmet Sarikaya[1], Shane White[2] and Malcolm Snead[2]; [1]Materials Science and Engineering, University of Washington, Seattle, WA, [2]Center of Craniofacial Molecular Biology, University of Southern California, CA.

Understanding how mechanical load is transferred from enamel to dentin and how the two structures function as a single mechanical unit during mastication requires studies of micro-mechanics in relation to microstructure of the dentin-enamel junction zone. In this investigation, nano-hardness and elastic modulus of human incisor teeth were studied across dentin-enaml-junctions (DEJ) by using a nanomechanical testing system attached to an atomic force microscope (AFM). It was found that, over a length scale of about 25 micrometers, there were decreasing trends in hardness (from about 4 to 1 GPa) and elastic modulus (from 85 GPa to 25 GPa) across the

DEJ zone profiling from enamel to dentin. Images obtained using AFM from polished surfaces of cross-sectioned samples showed that there is an interpenetration of structures of enamel and dentin at the DEJ zone; this result suggested that the nano-mechanical property profiles across DEJ were due to a continuous variation in the relative ratios of enamel and dentin structures. These characteristics of property and structure of the DEJ may imply significance in terms of coupling the two structures. By increasing the contact area across the interface between the two tissues the stresses are dissapated reducing interfacial stress concentrations at DEJ, thereby promoting load transfer from enamel to dentin while arresting the crack at the DEJ.

DD5.9
MICROHARNESS MEASUREMENTS IN HUMAN TOOTH ENAMEL. M.P. Gutierrez-Salazar and J. Reyes-Gasga, Instituto de Física, UNAM, México, MÉXICO.

In dental research, microhardness indentations measurements have found a wide number of the enamel properties, mainly focusing the mineralization phenomena. However, the microhardness values have showed an enormous variations in local measurements resulting in reported values with high standard deviation such as, for example, 344±49 [1] and 431±34 [2]. In this work we present a detailed set of microhardness values where a number of parameters which have an important influence have been controlled. These parameters are, for example, load and sample preparation among others. Vickers diamond was used with loads of 10, 25 and 50g which were applied during 10, 15, 20, 25 and 30s. The results indicate that enamel Vickers harness is around 350 and it remains constant all along the tooth sections.
We thank R. Trcjo, J. Cañetas, R. Hernández, P. Mexía and C. Flores for technical help. This work was economically supported by CONACYT.
References:
[1] Craig R.G. and Peyton F.A. The microhardness of enamel and dentine. J. Dent. Res. 1958 Vol. 37, No. 4: 661-668
[2] Collys K., Slop D., Cleymaet R., Coomans D. and Michotte. Load dependency and readability of microhardness measurements on acid-etched enamel surfaces.

DD5.10
CARBONATE APATITE-BEARING PURE TITANIUM IMPLANT. Kay Teraoka, Toru Nonami, Hiroshi Taoda, Katsuyoshi Naganuma, Yoshiyuki Yokogawa, Tetsuya Kameyama, National Industrial Research Institute of Nagoya, Ceramic Tech Dept, Aichi, JAPAN; Yutaka Doi, Asahi Univ, School of Dentistry, Gifu, JAPAN.

Titanium and some of its alloys are commonly accepted as orthopedic implants such as artificial tooth roots and artificial hip bones mainly due to its superior mechanical strength and low cytotoxicity. One of the keys for the successful implantation is fixation of the implants. Enhancement of bone formation around the implants can make a great contribution to long-term stable fixation of the implants. A practical way to enhance bone formation around the implants is coating of bioactive ceramics such as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAP) that is a main inorganic component of bone and teeth and is excellent in osteoconductivity. Therefore, the way to fix bioactive ceramics on the implants' surface has been a great interest. In this study, carbonate apatite (CHAP) ceramics were implanted in pits (ϕ 400 μm, depth 400 μm) on the surface of pure titanium substrates (ASTMB348-GR2) at 1023 K using a hot press with a vacuum furnace (JTT, Japan). The CHAP ceramics were well formed into the pits and were held firmly on the surface of the substrate by the implantation. The surface and the cross section of the CHAP-implanted substrate were observed by a scanning electron microscope. The CHAP-implanted substrates were implanted in mandible of beagle for animal tests. Tissue surrounding the CHAP-implanted substrates were observed by light microscope and investigated histologically at 2 weeks and 1 month postoperatively.

DD5.11
INVESTIGATION OF THE DYNAMICS OF BONE TISSUE REGENERATION BY ELECTRON-POSITRON ANNIHILATION METHOD. Adham A. Paiziev, Asad M. Karimov, Institute of Electronics, Dept. of Positron Diagnostics, Tashkent, UZBEKISTAN.

To study the dynamics of bone regenerator mineralization (Ca) experimental investigations on rats were undertaken. A sparing closed fracture of the shin bones of an extremity of a rat was performed under general anesthesia with subsequent immobilization of the extremity with a gypseous bandage. Histological studies were performed at 3, 7, 14 and 21 days with simultaneous measuring of the positron life time (P L T) spectra in the bone tissue samples. PLT spectra were measured on a spectrometer with the resolution of t = 400 ps. PLT spectra measured were calculated by the known "Positronfit" programme on an IBMcomputer. Data treatment show that the spectra of the samples studied fall under 2 time components, T1 and T2, with intensities I1 I2 respectively. The dependence of the parameters PLT on the regeneration time (Tr) war studied and compared with simultaneously run histological studies and an high data correlation was found. The most great change in PLT parameters is seen on the 7th day after the onset of the process of regeneration, the T1 and T2 parameters being the most sensitive to regeneration time (Tr). The maximum rate of these parameters in Tr region eguals 7 days and suggests on existence of signi-ficant bone tissue structural alterations. Indeed, histomorphological investigations demonstrated that formation of bone plates with osteocytes and osteofication of amorphous substance and fibres occurred on the 7th day. The I2 rate was substantially increased by the 7th day and after that it changed insignificantly. The fact seems to suggest that the formation of a crystal phase terminates by day 7.

DD5.12
CHEMICAL BONDING OF CEMENT ON A 3-D POLYMER SCAFFOLDING USING A BIOMIMETIC PROCESS. Carolyn Dry, University of Illinois, School of Architecture, Champaign, IL.

The overall goal of the project is to make a polymer/ceramic composite which mimics bone, especially it's toughness and strength, by imitating the process of bone fabrication. The composite is to have particular properties designed by varying the nature of the bond between the matrix ceramic and polymer components and by varying the amount of ceramic and polymer so that the relative strenths in compression, tension and toughness can be inlfuenced. In this part of the work the emphasis is on the intimate scaffolding connection between materials and a strong chemical bond. A liquid catalyst is released from hollow porous fibers into a powder matrix of a monomer and cement. First a polymer is formed. Then the cement reacts with the effluent of the polymer reaction, taking on the 3d pattern of the polymer and forming a chemical bond. SEMs reveal this templating. Also the composite is porous due to gas release during formation. It is therefore able to accept further chemical intrusion or ingrowth.

DD5.13
IMPROVEMENTS OF APATITE-FORMING ABILITIES ON PURE AND SODIUM-CONTAINING DIOPSIDE SUBSTRATES USING POROUS DIOPSIDE THIN FILMS AS NUCLEATING AGENT. Noriyuki Iwata, Katsuyuki Matsumoto, Tomomi Utsu, Makoto Tanaka and Sukenari Tsunakawa, Toin University of Yokohama, Faculty of Engineering, Department of Materials Science, Yokohama, JAPAN.

It was already reported that diopside ($CaMgSi_2O_6$), one of the pyroxene minerals, was an advantageous bioactive material because of its surpassing biological affinity and high mechanical strength. The present authors previously reported that the partial replacements of Ca with Na or Fe in the diopside crystals were achieved by sol gel or coprecipitation methods from solution.
In this paper, the synthetic process of diopside substrates with various Na/Ca ratios and the mechanical properties of them were presented. It was discovered that the crystallization temperatures, measured by DTA, were remarkably decreased with the increments of Na/Ca ratios. From the XRD pattern and density measurements, it was suggested that these sodium-containing diopsides showed better crystallinity than the pure ones, so these diopsides impregnated with sodium could be expected to be an artificial bone with higher mechanical strength. In order to improve the apatite-forming abilities of the substrates, they were dipped into the sol composed from $Ca(NO_3)_2$, $MgCl_2$ and TEOS, then heated at 850°C for 30 min. Their surfaces were successfully coated with porous diopside thin films.
T. Kokubo et al. proposed the simulated body fluid (SBF) methods as the simulation of the apatite forming. We used this SBF method as the preliminary evaluation of bioactivity. The apatite crystal growth onto these surfaces was carefully studied with XRD and SEM. The apatite formation was clearly predominant on the porous diopside surfaces, but on the non-coated surfaces, no apparent apatite growth was observed regardless whether pure or modified diopsides. The morphological studies of these surface structures in detail were in progress. We intend to develop this method for various bioactive applications.

DD5.14
ENHANCED MINERALIZATION ON ELECTRICALLY POLED BIOCERAMICS IN SBF AND MEM SUPPLEMENTED WITH 10% SERUM. Masataka Ohgaki, Hiroaki Takeda, Ayako Ishide, Ken-ichi Yamada and Kimihiro Yamashita, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental Univ, Chiyodaku, Tokyo, JAPAN.

Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$), a main component of bone and tooth minerals, has potential for use in a variety of oral, maxillofacial, and orthopedic applications. In hard tissue implantation, continuing study on bone-like crystal growth and osteoblastic cell activity on HAp ceramics is therefore of great importance for an advance of implantable biomaterial. we have recently reported that the accelerations and decelerations of bone like

crystal growth take place on the surfaces of electrically poled HAp in simulated body fluid by authors. In the present paper, we investigated the effects of bone-like crystal growth and biological behavior on electrically poled bioceramics. Electrically poled HAp ceramics were studied. For investigation of bone-like crystal growths on poled HAp surfaces, the poled specimens were immersed in a SBF (simulated body fluid), having the inorganic ion concentrations equal to those of human blood plasma, with pH 7.25 at 36.5OC, and in Eagle's minimum essential medium (MEM) supplemented with 10% foetal bovine serum (FBS). For Several days immersion, grown crystal layers were observed by scanning electron microscopy (SEM), and analyzed by X-ray diffraction and IR spectroscopy. As a result, the bone-like hydroxyapatite crystals grew rapidly on the negatively poled surface, while the growth was restricted on the positively poled surface in SBF and MEM. The biological activities on HAp ceramic surface were also enhanced by electrical poling. It was considered that the aligned dipoles of HAp ceramics accelerated the crystal growth and cell activation on the negatively poled surface, and decelerated on the positively poled surface.

DD5.15
INVESTIGATION OF POROUS GRADIENT TITANIUM COATING IMPLANTED BY NH2+ GROUP. Yunzhi Yang, Jiemo Tian, Jintao Tian, Tsinghua Univ, Beijing Fine Ceramics Lab, Beijing, P.R. CHINA; Zhiqing Chen, College of Stomatology, West China Univ of Medical Sciences, Chendu, P.R. CHINA.

The present work is part of a broad research in order to improve the surface biocompatibility and bioactivity of Ti and understanding of osteoblast cell response to biomaterials. In the paper bioactive porous gradient titanium biomaterials are investigated by hybrid plasma methods. Firstly, the porous gradient porous titanium coating on titanium substrate is prepared by low-pressure plasma spraying with the parameters of the spraying pressure 10 KPa and the spraying power 30-50 KW, in which the porous coating provides the ingrowth space of osteoblast tissue. Secondly, the porous gradient coating is implanted NH2+ group with the implantation parameters of 1017 atom/cm2 and 100 KeV, in which the surface having. NH2+ group will form the scaffolds to create new biological substances and promote the mineralization on surface of biomatreials. And the prepared bioactive porous gradient titanium is characterized with scanning electron microscopy (SEM), X-ray photoelectron spectroscope (XPS) and scanning Auger electron spectroscopy (AES). SEM shows that the coat thickness is about 250 um and the interface between coat and substrate is rather dense and the adhesion is strong. These pores within the coat are vertically permeated and their sizes gradually become bigger from near substrate to coat surface. And the average width size of outermost surface pore within coat reaches ~100um and the average depth size reaches 100um. XPS and AES show that implanted surface contains amino-group and it appears Gauss distribution in the surface layer. And the implanted specimens mainly consist of TiO2 surface oxide, which is covered by a carbon-dominated contamination.

DD5.16
SELF-ASSEMBLED β-SHEET ARCHITECTURES FOR BONE TISSUE ENGINEERING. Glen Spreitzer, John Doctor, David Wright, Duquesne University, Dept of Biology and Dept of Chemistry and Biochemistry, Pittsburgh, PA.

Advances in the understanding of biomineralization processes in a variety of organisms have revealed the critical role of three-dimensional scaffolding architectures to create a highly functionalized surface. These complex matrices function on a variety of length scales ranging from the macromolecular (10-100 nm) to the cellular (1-10mm) and larger. One dominant structural motif found in many of these architectures is macromolecules containing antiparallel β-pleated sheets. These hints from Nature have lead to the iterative design and development of a novel multipurpose platform technology based on self-assembled periodic peptide architectures for use in bone-tissue engineering is detailed. Combining molecular modeling, structural biochemistry, genetic engineering and synthetic techniques, we have produced two peptide beta-sheet architectures: a lamellar sheet and a hallow tube. These templates are capable of nucleating a variety of calcium biominerals. Characterization of these templates and analysis of their in vitro in vitro osteoconductive and osteogenic properties is reported.

DD5.17
A STUDY OF THE LINK BETWEEN THE CRYSTALLOGRAPHIC FORM AND CHEMICO-PHYSICAL FUNCTIONALITY OF CALCIUM CARBONATE. Alison Braybrook, Brigid Heywood, Kathryn Pitt, School of Chemistry and Physics, Keele University, UNITED KINGDOM.

It is known that the specific crystallographic form of an inorganic crystalline material will determine its physico-chemical properties, eg. solubility, catalytic activity, optical properties. Thus, there is potential for functionally optimising crystalline solids through the use of additives and dopants, which can selectively change the habit to a specific morphology. The design and activity of tailor-made additives is now well established and yet a predictive link between habit and functionality has yet to be systematically investigated. The objective of the present study was to use a targeted cohort of additives to provide a well-defined directory of crystallographic forms and then to correlate this information with measurements of selected physical parameters such as surface charge (zeta potential), dissolution profiles and reflectivity measurements. In this presentation we will review the first stages of the project; the production of calcium carbonate solids in a range of selected morphologies. One of the systems of interest involves the morphological modification of calcite through the addition of divalent cations. These studies have been paralleled by computational modelling of the solid state where the interaction of the dopant cation with the ionic solid has been examined. The prediction of calcite morphology is a crucial step towards understanding the controlled formation of this material and generating predictive models for future studies of structure-property-function relationships in both organic and inorganic materials.

DD5.18
MORPHOLOGICAL CHANGES IN CALCITE INDUCED BY AMINO-ACIDS: EMERGENCE OF CHIRALITY. Aleksandr Noy, Christine Orme, Mary McBride, Jim De Yoreo, Lawrence Livermore National Laboratory, Livermore, CA.

The presence of additives in the solution during crystal growth can have a profound effect on the equilibrium shape and the resulting crystal habit. The changes in crystal morphology are ultimately determined by the degree that the additive affects surface free energy of a particular set of crystal faces. However, these arguments cannot explain the presence of chiral features often encountered in biomineralization phenomena. We demonstrate that addition of chiral amino acids to the supersaturated solutions of calcium carbonate leads to the drastic changes in the morphology of the nucleating calcite crystals and introduces chirality into the system. Calcite crystals were nucleated and grown in presence and absence of aspartic acid. Patterned self-assembled monolayer substrates were used for nucleation to enhance the uniformity and to control spatial location of the crystals. SEM images of the grown crystals show that binding of aspartic acid molecules to the growth steps leads to the emergence of a new set of facets, in addition to the equilibrium set of (104) facets of pure calcite. Moreover, we found that D and L aspartic acid molecules stabilize opposite subsets of crystal faces and produce crystals that are related by mirror symmetry. In-situ AFM imaging of the growing calcite crystal steps confirmed that aspartic acid enantiomers were binding to the opposite set of steps and thus established the continuity between the microscopic and macroscopic events. We also present a simple steric model that shows how the chirality is induced.

DD5.19
MUCIN COATING ON HYDROPHOBIC POLYMER MATERIALS. Lei Shi, Dept of Materials Science and Engineering, Univ of Utah, Salt Lake City, UT; Karin D. Caldwell, Center for Surface Biotechnology, BMC, Uppsala University, Uppsala, SWEDEN.

Mucin is a class of glycoproteins, which are characterized mainly by their high molecular weight and high level of O-linked oligosaccharides. Mucin is the major constituent of mucus, which covers the lumenal surfaces of epithelial organs and serves as a physical barrier between the extracellular milieu and the plasma membrane. It has been found that mucin coating on hydrophobic material surfaces could greatly reduce protein adsorption, bacterial adhesion and cell adhesion. Mucin also shows surfactancy properties, which lower the surface tension of the aqueous solutions. Thus, it could be used to protect biomaterial surfaces for batter biocompatibility in a way of mimicking the epithelial surfaces in biological systems. In this work, the adsorption isotherm of bovine submaxillary gland mucin (BSM) onto a hydrophobic polystyrene surface (PS282-latex particles) was determined by using the solution depletion method, in which mucin concentrations were analyzed by amino acid analysis. Adsorption and desorption kinetics of BSM onto hydrophobic polystyrene surfaces (PS3090-latex particles) were also studied by the solution depletion method, in which mucin solution concentrations were determined by measuring UV adsorbance at a wavelength of 280 nm, together with a BCA colorimetric assay for calibration. From the adsorption isotherm, we found that the saturated surface concentration (Γ_{max})was 2.2 mg/m^2, and the adsorption constant (K) was calculated as 0.099 (ml/mg). By using a Langmuir adsorption model and non-linear fitting, kinetics parameters, k_{on} and k_{off}, were found to be 8.13x10^{-3} cm^3 mg^{-1} s^{-1} and 5.67x10^{-4} s^{-1}, respectively. The coating was found to be very stable with very limited desorption (less than 2%) from a long term observation (28 hours). The mucin coating layer thickness was investigated by several analytical techniques: flow Field-Flow-

Fractionation (FFF), Photon Correlation Spectroscopy (PCS), Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AEM). The thickness was measured as 3-5 nm, from which a monolayer coating was concluded. Finally, the weight average molecular weight of purified BSM was determined as $1.6x10^6$ Da by using static light scattering. After coating, the hydrophobic polystyrene surface lost its hydrophobicity to become hydrophilic, indicating that the mucin coating is able to increase the wettability of a hydrophobic polymer surface. Thus, mucin can be used to improve the biocompatibility of polymeric biomaterials by incorporating protein repelling properties to artificial surfaces.

DD5.20
CRYSTAL ENAMEL FORMATION. I.A. Belio, Facultad de Odontologia, UNAM, MEXICO; L.F. Jimenez, Facultad de Ciencias, UNAM, MEXICO; J. Reyes, Instituto de Fisica, UNAM, MEXICO.

Dental enamel comes from ectoderm tissue formed by hydroxyapatite, HAp (97%), organic material (1%) and water (2%). Secreted by ameloblast, in the middle of crystals, there is a central dark line formed by octacalcium phosphate (OCP) acting as HAp substrate. Chemical analysis with EDS was carried out in dental mouse enamel from foetus of 19 days. 16 inferior incisives were prepared using standard methods for TEM. Morphological characterization was obtained with micrographies of adult mouse dental enamel, as well as from foetus. Exactly in secretion area, in addition of HAp, Na and Cl were found. These elements focused our attention because their presence at the beginning of enamel formation, led us to think that they play an important role in the physical and chemical properties of mature enamel, according to the up-to date results reported in literature.

DD5.21
INFLUENCE OF THE MICROSTRUCTURE AND CRYSTALLOGRAPHIC TEXTURE ON THE MECHANICAL PROPERTIES OF EGGSHELLS LAID BY HENS OF DIFFERENT AGE. Alejandro Rodriguez-Navarro[1], Otto Kalin[1], Yves Nys[2] and Juan M. Garcia-Ruiz[1]; [1]Instituto Andaluz de Ciencias de la Tierra-CSIC, Granada, SPAIN, [2]Institut National de la Recherche Agronomique, Nouzilly, FRANCE.

The eggshell is a ceramic biomaterial made of calcite aggregates pervaded with an organic matrix, having excellent mechanical properties. As a polycrystalline material, its properties are expected to depend strongly on microstructure (grain morphology and size) and also on grain orientation (crystallographic texture), due to anisotropic nature of crystals. However, there is little or no information on the relationship between crystal orientation and the mechanical properties of the eggshell. We discuss, in detail, the relationship between crystallographic texture and mechanical properties, which could be of interest for this material, as well as any other polycrystalline ceramic. Also, as hens approach the end of the laying year, there is a remarkable decrease in eggshell strength. The fracture strength of eggshells laid by aged hens was found to be, on average, about half of that laid by young hens. In order to understand the origin of this decrease, the microstructure, crystallographic texture and other properties of eggshells laid by hens of different age were compared. In the case of eggshells laid by aged hens, the texture analysis revealed two preferred crystal orientations, after (001) and (104), compared to mainly one, after (001), in those laid by young hens. Our results suggest that crystallographic texture strongly affects eggshell strength. In particular, the fracture strength of eggshells decreases with increasing orientation of the constituting calcite crystals. Also, we observed that eggshells laid by aged hens showed a higher variability in properties such as eggshell breaking strength, thickness, grain morphology and crystallographic texture.

SESSION DD6: MINERAL FORMATION ON ORGANIC SELF-ASSEMBLED SURFACES I
Chair: Cheryl R. Scheid
Tuesday Morning, November 30, 1999
Vineyard (M)

8:30 AM *DD6.1
HIGHER-ORDER SYNTHESIS AND SELF-ASSEMBLY OF BIOMIMETIC MATERIALS. Stephen Mann, School of Chemistry, University of Bristol, UNITED KINGDOM.

Organized-matter chemistry is concerned with the synthesis, characterization and emergence of complex materials that exhibit order on length scales from the molecular to macroscopic. The study of biomineralization continues to be a source of inspiration for this endeavour, and many biomimetic strategies have been recently developed for the direct synthesis of organized inorganic-based structures with complex pattern and form. A key aspect of this approach is the integration of organic self-assembly and inorganic reaction chemistry to produce hybrid materials via direct or synergistic (coupled) methods of spatial and chemical templating. This lecture illustrates how organic mesophases and complex fluids for example, lyotropic liquid crystals, phospholipid helices, viroid tubes, polymer micelles, microemulsions, foams - can be used for the one-step synthesis of inorganic (silica, iron oxide, $BaSO_4$, Ca phosphate, $CaCO_3$ etc.) architectures with unusual morphological form ("morphosynthesis"). In particular, the lecture focuses on the emergence of organized colloidal superstructures, such as nested calcium phosphate-block copolymer micelles (collaborative work with Prof. M. Antonietti, Berlin) and linear chains of barium chromate nanoparticles assembled within compartmentalized fluids with a low instability threshold. The transformation of spatially confined systems into adaptive inorganic-organic structures, as well as the potential relevance for biomineralization and materials chemistry, will be discussed.

9:00 AM *DD6.2
INVOLVEMENT OF CELLULAR MEMBRANES AND THEIR LIPIDS IN NUCLEATION OF STONE FORMING CRYSTALS. Saeed R. Khan, Julie M. Fasano, Department of Pathology; Renal Backov, Daniel R. Talham, Department of Chemistry, Univ of Florida, Gainesville, FL.

More than 80% of human kidney stones consist of calcium oxalate and/or calcium phosphate. Human urine is generally metastable with respect to these salts and their nucleation is heterogeneous. Based on: 1. ultrastructural and immunohistochemical studies of stones in which membranous cellular degradation products and lipids were commonly seen in association with calcific crystals and 2. in vivo studies of nephrolithiasis in rat models where calcium oxalate (CaOx) and calcium phosphate crystals almost always formed and seen in association with cell membranes, we proposed that membranes and their lipids are involved in crystallization of these salts. To test our hypothesis we isolated organic matrix of kidney stones, its lipid contents and membrane vesicles from epithelial cells of rat kidney and incubated them in metastable solution of CaOx. Both membrane vesicles and matrix from the stones supported crystallization of CaOx and crystals formed in association with the membranes. Lipids of the stone matrix appeared better nucleators than whole matrix. Urine spends only minutes within the kidneys thus any nucleation which can lead to stone formation has to occur rapidly. In a separate study we showed that under specific circumstances relevant to conditions in the kidney, membrane vesicle-supported CaOx crystallization can occur within seconds, demonstrating the possibility of such events happening in the kidneys. We also studied CaOx monohydrate (COM) precipitation at Langmuir monolayers of dipalmitoylphosphatidylglecerol (DPPG), dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphati- dylserine (DPPS) showed precipitation to be heterogeneous and selective with a majority of crystals orienting with the 101 face of COM facing the monolayer. Apparently stone formation is similar to many other pathological calcification processes.

9:30 AM DD6.3
LEARNING FROM BIOLOGICAL SYSTEMS: NOVEL ROUTES TO BIOMIMETIC SYNTHESIS OF ORDERED SILICA STRUCTURES. Jennifer N. Cha, Katsuhiko Shimizu, Yan Zhou, Sean C. Christiansen, Bradley F. Chmelka, Timothy J. Deming, Daniel E. Morse, Galen D. Stucky, Univ of CA, Santa Barbara, Dept of Chemistry, Materials, Molecular, Cellular and Developmental Biology and Chemical Engineering, Santa Barbara, CA.

In nature, biominerals are formed under the control of organic components such as proteins and polysaccharides at ambient pHs and temperatures. Yet up until now, there has been little success in utilizing such constituents as templates for biomimetic silica synthesis. Most prior work in this area has been limited to the use of self-assemblying amphiphiles. While some silica syntheses have been reported with polysaccharides and silicon catecholates, the formation of organized silica structures utilizing self assembly of biopolymers at neutral pH and low temperature has not been accomplished. Recent studies of the proteins occluded within the silica needles of the sponge, Tethya aurantia, demonstrated that these proteins, called silicateins, have the capability of acting as catalysts for the hydrolysis of silicon alkoxides at physiological pH and temperature, while also serving as scaffolds to organize the resulting silica or silsequioxane products. Further mutagenesis studies revealed that the key functional groups necessary for catalytic activity were those of the active site residues serine and histidine, which are organized to enhance the nucleophilicity of the serine residue. Based on these findings, we present here the design and utilization of synthetic lysine-cysteine block copolypeptides that catalyze hydrolysis and condensation of the silicon precursor, tetraethoxysilane (TEOS) at ambient temperature and pH. We have designed our polymer so that it not only is able to act as a catalyst for the SiO_2 formation, but also has self assemblying properties that direct formation of silica

structures with ordered morphologies. Since the degree of aggregation of this block copolypeptide can also be varied by oxidation of the cysteine sulfhydryl side chains, different structures can be produced from a single polymer ranging from transparent, hard silica spheres to organized bundles of silica fibers.

9:45 AM DD6.4
MORPHOLOGICAL CONTROL IN BIOMINERALIZATION- IS IT SIMPLER THAN WE THOUGHT?. Charley Malpass, Damian Odom, Carol Walker, Sung-hwan Yoon, Laurie Gower, Dept. of Materials Science & Engineering, University of Florida, Gainesville, FL.

The hallmark of biomineralization is the ability of organisms to form highly unusual crystal morphologies in their mineral deposits. Classic examples include the following: single-crystals with shapes that appear to have been molded by the vesicular compartment within which they are formed (echinoderm spines, plant raphides); the filling of collagen fibrils with iso-oriented nanocrystalline hydroxyapatite to form intrafibrillar composites (bone and dentin); the deposition of thin mineral tablets, films and coatings (mollusk nacre, dinoflagellate cysts); elongated crystals that have the appearance of extruded fibers (enamel prisms); mineralized cements (agglutinating forams, maturing dental enamel, kidney stones); high Mg-bearing calcites (echinoderms, coral spicules). We suggest that a simple and non-specific process, called the Polymer-Induced Liquid-Precursor (PILP) process, which has been demonstrated in vitro for calcium-based crystals grown in the presence of acidic macromolecules, could conceivably provide a viable explanation for the above-mentioned puzzling features of biominerals, and thus may lie at the foundation of morphological control in biomineralization. We have shown that under certain conditions, polyaspartic acid can transform the solution crystallization of calcite to a solidification process. The polyelectrolyte causes liquid-liquid phase separation of droplets of a metastable precursor phase, generating an aqueous biphasic solution. Because the precursor phase is in the form of a liquid, it can be molded and shaped by its container, which upon solidification, leads to crystals with non-equilibrium morphologies. For example, calcitic films and coatings have been generated, as well as spatially-delineated single crystals of calcite. We are currently investigating the calcium oxalates and calcium phosphates to determine if this PILP process can also be elicited in other calcium-based systems relevant to biomineralization.

SESSION DD7: APATITE FORMATION ON INORGANIC SURFACES II
Chair: George H. Nancollas
Tuesday Morning, November 30, 1999
Vineyard (M)

10:30 AM *DD7.1
CALCIUM PHOSPHATE CEMENTS: CHEMISTRY, PROPERTIES AND APPLICATIONS. Laurence C. Chow, American Dental Association Health Foundation, Paffenbarger Research Center, National Institute of Standards and Technology, Gaithersburg, MD.

Since the development in 1987 of the first self-hardening calcium phosphate cement (CPC) consisting of tetracalcium phosphate and dicalcium phosphate, a large number of different combinations of calcium and phosphate-containing compounds have been investigated as potential cement materials. Most of these cements form hydroxyapatite (HA), calcium deficient apatite, or carbonated apatite as the end product. Cement hardening, which can occur within several minutes in some systems, is believed to be caused by mechanical interlocking of the crystalline products formed in the setting reaction. Some of the cements have fairly high compressive (60 - 80 MPa) and diametral tensile (> 12 MPa) strengths, but CPC generally are brittle and have low fracture resistance. CPCs are microporous, with pore volume in the range of 30 to 40%. Most cements are pH neutral or slightly alkaline during and after setting, and they are highly compatible to soft and hard tissues. CPCs that form apatitic products are not soluble in physiological fluids (serum, saliva, etc.) but can dissolve under acidic physiologic conditions such as those in the osteoclast mediated process. As a result, CPC is slowly resorbable when placed in hard or soft tissues, and is replaced by new bone without a loss in volume when used to fill bone defects. The combination of cement properties, good mechanical strengths, biocompatibility, and osteoconductivity makes CPC useful in a number of clinical applications in which other existing materials do not work as satisfactorily. Recent research has been focusing on further improving physical properties and biological behavior of CPC. These include Incorporation of biocompatible or absorbable fibers into CPC to increases tensile and flexural strengths, formation of macropores sufficiently large to allow ingrowth of fully vascularized bone tissue, inclusion of bone growth factors in CPC to promote osteoinductivity, modifications of CPC composition to alter in vivo resorption rate. It is anticipated that CPC will have different compositions and properties engineered for specific clinical applications.

11:00 AM DD7.2
MECHANISM OF APATITE FORMATION ON BIOACTIVE TITANIUM METAL. Tadashi Kokubo, Hyun-Min Kim, Hiroaki Takadama, Masaki Uchida, Kyoto University, Department of Material Chemistry, Graduate School of Engineering, Kyoto, JAPAN; Shigeru Nishiguchi, Takashi Nakamura, Kyoto University, Department of Orthopaedic Surgery, Graduate School of Medicine, Kyoto, JAPAN.

The present authors previously showed that titanium metal, which was exposed to 5.0M-NaOH solution at 60°C for 24 h and heat-treated at 600°C for 1 h, spontaneously forms a bonelike apatite layer on its surface in the living body and bond to the bone through the apatite layer. In the present study, mechanism of the apatite formation on the bioactive titanium metal was investigated in an acellular simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma. A thin sodium titanate layer is formed on the surface of titanium metal by the NaOH and heat treatments. It has been assumed that Na^+ ion in the sodium titanate layer exchanges with H_3O^+ ion in the fluid in the living body. As a results, a lot of Ti-OH groups might be formed on the surface of titanium metal, and ionic activity product of the apatite in the surrounding fluid might be increased. The Ti-OH groups might induce the apatite nucleation and the increase ionic activity product accelerates the apatite nucleation. In the present study, X-ray photoelectron spectroscopy (XPS) of the surface of the titanium metal soaked in SBF showed that Na^+ ion in the sodium titanate layer actually released via exchange with H_3O^+ ion in SBF within 1 d, and Ti-OH groups are formed on the surface of the titanium metal. Analysis of apatite formation on pure titania gels with different structures, however, showed that the Ti-OH groups on amorphous structure is not effective for the apatite nucleation, but those on anatase structure is effective. XPS of the titanium metal soaked in SBF showed that the Ti-OH groups do not induce the apatite nucleation directly, but through formation of calcium titanate.

11:15 AM DD7.3
FAST DEPOSITION OF APATITE COATINGS ON IMPLANTS. F. Barrère[1,2], P. Layrolle[1], C.A. van Blitterswijk[1,2,3], K. de Groot[1,3] [1]IsoTis BV, Bilthoven, THE NETHERLANDS, [2]iBME University of Twente, THE NETHERLANDS, [3]BRG, Leiden University, THE NETHERLANDS.

Calcium Phosphate (Ca-P) layers can be deposited on orthopedic implants by using a biomimetic method. The metal implants are immersed into simulated body fluids (SBFs) allowing the precipitation of bone mineral-like apatite films on their surface. Several authors have reported the deposition of Ca-P layers on chemically treated metal implants [1,2] by immersion over long periods (e.g. 1 week). In this paper, we describe an effective method for coating implants within 24 hours. Our method is based on the higher solubility of Ca-P salts in acidic media. At pH=6, it is then possible to concentrate more than 8 times SBF solution without any precipitation. As the pH in the solution gradually increases, supersaturation is reached and a Ca-P film precipitates on the Ti6Al4V surface. This so-produced coating was characterised by SEM, FTIR, XRD, XPS and the adhesion of the Ca-P film on the metal was determined by measuring the tensile bonding strength. The coating covers the whole surface of implants independently of their shape. This coating is dense and uniform with a thickness of 1-2microns. Amorphous carbonated Ca-P globules strongly attached to the substrate (tensile bonding strength: 56.1±10.6MPa) composed this layer. A subsequent crystalline coating composed of octacalcium phosphate, carbonated apatite or hydroxyapatite can be deposited on top of the amorphous film under crystal growth conditions. Furthermore, this biomimetic coating method can be applied to porous implants and allows the co-precipitation of growth factors. References: 1: F. Miyaji, H.M. Kim, T. Kokubo, T. Kitsugi and T. Nakamura, 'Bioactive titanium alloys prepared by chemical surface modification', Bioceramics 8, 323-329 (1995) 2: P. Layrolle, C.A. van Blitterswijk and K. de Groot, 'Biomimetic Hydroxyapatite coating on Ti6Al4v induced by pre-calcification', Bioceramics 11, 465-468 (1998)

11:30 AM DD7.4
APATITE FORMATION ON ELECTROCHEMICALLY TREATED TITANIUM. Kanji Tsuru, Shinji Takemoto, Satoshi Hayakawa, Akiyoshi Osaka, Okayama Univ., Faculty of Engineering, Biomaterials Lab, Okayama, JAPAN.

Titanium oxide gel was electrochemically prepared on Ti with a cell consisting of Ti as the working electrode, Pt as the counter one, AgCl as the reference one, and an aqueous solution of 0.1 mol/L $Ca(NO_3)_2$ as the electrolyte solution. The Ti electrode was kept at 9.5V for 1 hr for oxidation and subsequently kept at -3.0V for 10 min (Ca9.5-3.0) by changing polarity: calcium ions were expected to be adsorbed at

the latter treatment. An other Ti specimen was kept at -3.0V for 10 min (Ca-3.0). Both specimens were soaked in a simulated body fluid (Kokubo solution) and deposited apatite to prove bioactive.

11:45 AM DD7.5
ATOMIC DISPLACEMENT PARAMETERS OF CARBONATE APATITES FROM POWDER NEUTRON DIFFRACTION DATA.
Theodora Leventouri, Florida Atlantic Univ, Dept of Physics, Boca Raton, FL; Bryan C. Chakoumakos, Oak Ridge National Laboratory, Oak Ridge, TN; Hadi Y. Moghaddam, Nearchos Papanearchou, Camelia E. Bunaciu, Florida Atlantic Univ, Dept of Physics, Boca Raton, FL.

Carbonated apatite most closely resembles biological apatite, and it has been extensively studied. However, the enigma of the precise determination of the crystalline configuration remains while several structural models of the carbonate substitution have been proposed. Here we present structural details from Rietveld refinements of powder neutron diffraction data of a natural carbonate fluorapatite (francolite) and of low temperature synthetic carbonate hydroxyapatites with various carbonate concentrations. We focus on the temperature dependence of the anisotropic displacement parameters to identify the contributions of static versus dynamic disorder for each of the atomic sites. A series of samples of low temperature carbonate hydroxyapatite was prepared via hydrolysis of calcium phosphate and sodium bicarbonate of 0-0.35M. X-ray diffraction characterization reveals a strong dependency of the product of the hydrolysis on the processing parameters. Single-phase specimens were prepared for solutions of 0.075-0.275M. Our experiments provide strong evidence that the planar carbonate ion substitutes on the mirror plane of the tetrahedral phosphate ion. The anisotropic displacement parameters of the tetrahedral site are mostly affected by the carbonate presence. Analysis of the refinements of the carbonate fluorapatite after the carbonate is driven off from the structure through heat treatments, show that lattice constants, bond lengths, angles and atomic displacement parameters simulate the ones of a fluorapatite with no carbonate in its structure. Results on the atomic positional disorder caused by the carbonate substitution in both carbonate fluorapatite and hydroxyapatite will be presented.

SESSION DD8: MINERAL FORMATION ON ORGANIC SELF-ASSEMBLED SURFACES II
Chairs: Paul Calvert and Mehmet Sarikaya
Tuesday Afternoon, November 30, 1999
Vineyard (M)

1:30 PM *DD8.1
CONTROLLED NUCLEATION AND GROWTH OF MINERALS ON ORGANIC TEMPLATES.
Ilhan A. Aksay, Princeton University, Department of Chemical Engineering and Princeton Materials Institute, Princeton, NJ.

In biogenic hard materials, organic templates organized on length scales of 1$\bar{1}$00 nm act as frameworks for the growth of specifically oriented and shaped inorganics (e.g., $CaCO_3$, SiO_2, Fe_3O_4, hydroxyapatite). A general assumption is that these structurally organized organic surfaces induce growth of specifically oriented, dissimilar constituents through a direct nucleation process from the liquid state. An alternative path is a two-step process where the inorganic phase first nucleates in an amorphous form and then crystallizes through a solid state phase transition. Our recent work on various systems including silica-based micellar structures, barium titanate, and calcite illustrate that solid state crystallization path may be a more common path.

2:00 PM DD8.2
29Si NMR CHEMICAL SHIFTS FOR SILICA-SERINE AND SILICA-POLYOL COMPLEXES AS INDICATORS OF SILICA BIOMINERALIZATION MECHANISMS IN DIATOMS AND PLANTS.
Nita Sahai, John A. Tossell, Dept of Chemistry and Biochemistry, University of Maryland, College Park, MD.

Serine- and polysaccharide-enriched organic matrix is associated with biogenic silica (diatom tests, phytoliths). The nature and role of the silica-organic bonding, however, has remained unresolved in the literature. We have used molecular orbital theory to determine the relative stability and 29Si NMR shifts of direct Si-O-C bonds versus hydrogen-bonds between silicon and the alcohol residue of serine and aliphatic polyalcohols (proxy for polysaccharides). To date, we have investigated Si-ligand complexes in 1:1 stoichiometric ratio with silicon being in both four-fold and five-fold coordination. Si:ligand stoichiometric ratios of 1:2 and 1:3 will also be investigated in the future. Structures and energies of the complexes were obtained at the Hartree-Fock level using effective core potentials as implemented in the program GAMESS. 29Si shifts were calculated relative to tetramethylsilane using the 6-31G* basis set in the program GAUSSIAN 94. Preliminary results for 1:1 Si:ligand complexes suggest that H-bonded complexes are more stable than direct Si-O-C bonded complexes, where Si is four-fold coordinated. 29Si shifts of H-bonded complexes and of four-fold coordinated Si-O-C bonded complexes range from -55 to -73 ppm, far above -92, -102 and -110 ppm observed experimentally for solid biogenic silica. Formation of five-fold coordinated Si-O-C bonded complexes is endothermic relative to the four-fold coordinated complexes, but they yield shifts from -96 to -107 ppm. These preliminary results suggest that formation of five-coordinated Si-O-C bonds requires catalysis. If such bonds exist in solid biogenic silica, they may have escaped detection because of their low concentration, and overlapping NMR peak positions with inorganic four-fold coordinated Si. Based on sol-gel literature, however, a more likely explanation is that the four- and five-fold coordinated Si-O-C bonded complexes are short-lived dissolved precursors to silica oligomers. The challenge lies in isolating such dissolved precursor complexes from diatoms and silica-concentrating plants.

2:15 PM DD8.3
STRUCTURAL CHARACTERIZATION OF PEPTIDE-AMPHIPHILE TEMPLATES FOR HYDROXYAPATITE NUCLEATION.
Nathan Lockwood, Matthew Tirrell, Dept of Chemical Engineering and Materials Science; Kevin Mayo, Dept of Biochemistry, Univ of Minnesota, Minneapolis, MN.

The nucleation and growth of hydroxyapatite in bones and teeth is regulated by a number of proteins, polysaccharides, and glycoproteins. In particular, nucleation in dentin tissue appears to be mediated by certain β-sheet domains in the protein phosphophoryn. In an effort to understand the stereochemical relationships between these domains and hydroxyapatite crystal nuclei, we are incorporating phosphophoryn sequences into peptide-amphiphiles by linking a dialkyl lipid tail to the N-terminus of the peptide. Our initial work is with the hexapeptide sequence $(\text{phosphoserine-aspartic acid})_3$, a highly acidic motif believed to be present in the β-sheet domains of phosphophoryn. Previous work in our group has demonstrated the ability of peptide-amphiphiles to stabilize native secondary structures in triple-helical and α-helical sequences; we believe this stabilization will also apply to the β-sheet sequences of interest in this work. Circular dichroism and nuclear magnetic resonance experiments are underway to determine the conformation of the peptide portion of the amphiphilic molecules in solution. Future investigations include examining the influence of these molecules on hydroxyapatite nucleation via Langmuir-Blodgett monolayers of the $(\text{Pse-Asp})_3$ amphiphiles to construct nucleation templates. The combination of conformational and nucleation properties of the amphiphiles will eventually be used to develop a model of the stereochemical relationships between the $(\text{Pse-Asp})_3$ template and hydroxyapatite crystals.

2:30 PM DD8.4
LABILE IONS OF CALCIUM-PHOSPHATE APATITE CRYSTALS IN THIN LAYER FORMED WITH FLUORIDE OR GALLIUM.
Hyun-Man Kim, Kil-Hee Lee, Yoonji Kim, Jea Seung Ko, Dept of Oral Anatomy and Institute of Dental Research, College of Dentistry, Seoul National University, Seoul, KOREA; Christian Rey, Laboratoire de Physico-Chimie des Solides, Institut Polytechnique de Toulouse, C.N.R.S., Toulouse, FRANCE.

Trace elements incorporated into calcium-phosphate (Ca-P) apatite crystals can change the physicochemical properties of crystals, especially the surface of the Ca-P crystals. The surface modification, then, can affect the biological response of the Ca-P which is the event at the interface between the surface inorganic ions of the biomaterials and organic molecules in tissue. Fluoride and gallium are known to alter behaviors of bone cells, osteoblasts or osteoclasts. Their effects on the labile ions, which are believed to be on the surface of crystals, of low crystalline crystals in the thin layer of Ca-P apatite crystals were studied. Thin layer of low crystalline Ca-P apatite crystals was formed on the solid surface by inducing heterogenous nucleation of Ca-P apatite crystals. Highly metastable calcium and phosphate ion solution ($[Ca^+]$ 4.52 mM, $[PO_4{}^{3-}]$ 3.73 mM) was applied over the surface of culture dishes to form thin film of apatite crystals at 8° C for 24 hours. Then the temperature was increased to 20° C with adding of sodium fluoride or gallium nitrate at various concentrations. The bone-like low crystalline nano-apatite crystals of the thin layer were confirmed with transmission electron microscopy and x-ray diffraction. Analysis with Fourier transformed infrared spectroscopy showed that apatite crystals formed with fluoride decreased labile non-apatitic $HPO_4{}^{2-}$ and $PO_4{}^{3-}$ in a dose dependent way from 10 to 1000 mM. And there was also a decrease in the relative amount of labile non-apatitic $HPO_4{}^{2-}$ and $PO_4{}^{3-}$ in the Ca-P crystals formed in the presence of gallium nitrate from 10 nM to 10 mM. These results indicate that incorporation of fluoride or gallium may be a way to

control the amount of labile ions in the thin layer of low crystalline Ca-P crystals which are known to affect the inorganic-organic interactions.

2:45 PM DD8.5
A TWO-COMPONENT TEMPLATING STRATEGY FOR THE FABRICATION OF INORGANIC MATERIALS. Brigid Heywood, Susan Hill, School of Chemistry and Physics, Keele University, UK.

In biological systems, it is reported that specific molecular interactions between an assembled organic substrate and the nascent biocrystal play a fundamental role in promoting and controlling the deposition of a particular mineral phase. This process can be translated to synthetic, in vitro systems, where organic matrices of precise molecular design can be used to control nucleation and growth of the inorganic phase. These macromolecular templates can be used to present a highly ordered array of chemical functionality, and thus a specific molecular motif which is capable of inducing controlled mineralisation. In this work, we have taken this concept one step further by using functionalised organic films which are further modified by the selective adsorption of a secondary unit. These secondary recognition molecules are selected for their ability to interact with the primary organic film via electrostatic or hydrogen bonding. The assembly of these novel two-component films produces a molecular template capable of initiating and controlling crystal growth. By varying the chemical identity of the secondary recognition component, the polymorphic form and crystallographic orientation of the mineral particles can be specifically and selectively directed.

3:30 PM *DD8.6
CERAMIC THIN FILMS ON ORGANIC SELF-ASSEMBLED MONOLAYERS: SYNTHESIS AND THE MECHANISM OF FORMATION. Mark R. De Guire, Case Western Reserve University, Dept. of Materials Science and Engineering, Cleveland, OH.

In recent years, biomimetic or bio-inspired techniques for the synthesis of ceramic thin films from aqueous solutions at low temperatures have been pursued by several research groups. Most of these routes use thermal or chemical hydrolysis to drive the precursor salt solutions towards metastable or unstable conditions with respect to precipitation of the desired phase for film formation. The substrates range from inorganic materials (metals, glass, single-crystalline silicon) without special surface preparation, to functionalized organic surfaces such as self-assembled organic monolayers (SAMs). Our results on the deposition of ceramic thin films on SAMs will be reviewed. Crystalline films of TiO_2, ZrO_2, FeOOH, AlOOH, SnO_2, and In_2O_3 have been formed directly from solutions below 100°C. Films of ZnO, Y_2O_3, $ZrTiO_4$, Y_2O_3-ZrO_2, Fe_3O_4, and γ-Fe_2O_3 have been formed using heat treatments at temperatures lower than typical sol-gel or vapor phase deposition processes. Our current understanding of the role of these ordered organic surfaces on the deposition process will be presented. In particular, the following topics will be discussed: In what respects is the present approach 'biomimetic'; and what is the mechanism of formation of these films – heterogeneous nucleation, or assembly of colloidal particles on the substrate?

4:00 PM DD8.7
COMBINATORIAL APPROACHES TO PEPTIDE-ENCAPSULATED CdS NANOCLUSTERS. Glen Spreitzer, Jacqueline M. Whitling, David W. Wright, Duquesne University, Dept. of Chemistry and Biochemistry, Pittsburgh, PA.

As many properties of group II-VI semiconductor nanocrystallites arise from their quantum confined nature, the synthesis of these clusters is of great interest. The challenge of synthesizing functional nanoclusters is, however, complicated by the limited stability of metal-chalcogen surfaces under highly oxidizing conditions and Ostwald ripening. Ironically, plants and yeast, using short cysteine-rich matrix peptides (phytochelatins), are capable of biosynthesizing the target particles. Our objectives are to develop methods for the synthesis of biogenic CdS semiconductor nanocrystallites that address the following issues: (1) What properties of the ligand peptide are important in stabilizing and producing CdS particles of any desired size? (2) Will hybrid peptides using non-natural amino acids stabilize the nanoclusters while incorporating new surface functionality? (3) Is it possible to use novel peptides to modify the particle surface, thereby, controlling the photophysical properties of the CdS cluster? Our approach consists of parallel combinatorial techniques and computational methods for the discovery and optimization of peptide and peptidomimetic ligands to stabilize size-defined clusters of CdS. The effects of cysteine separation on CdS particle size by varying the number of bonds with rotational freedom within the spacer amino acids is examined. Furthermore, the properties of the resulting CdS nanoclusters will be reported.

4:15 PM DD8.8
MORPHOLOGY OF MESOPOROUS SILICA GROWN ON ORGANIC SURFACES: EFFECTS OF SURFACE FUNCTIONAL GROUPS AND MICROSTURCTURES. Atsushi Hozumi, Yoshiyuki Yokogawa and Tetsuya Kameyama, Bioceramics Laboratory, Ceramics Technology Department, National Industrial Research Institute of Nagoya, Nagoya, JAPAN; Katsumasa Hiraku, Hiroyuki Sugimura and Osamu Takai, Department of Materials Processing Engineering, Graduate School of Engineering, Nagoya University, Nagoya, JAPAN; Masazumi Okido, Center for Integrated Research in Science and Engineering, Nagoya University, Nagoya, JAPAN.

Mesoporous silica (MPS), synthesized based on an organized surfactant templating technique has attracted attention as materials for high separation and catalytic carrier etc.. In this study, we have focused on morphology control of MPS, which depends on substrate surface properties, such as wettability, charge, and microstructures. First, each Si substrate was coated with an organosilane self-assembled monolayer (SAM) of (heptadecafluoro-1,1,2,2-tetrahydrodecyl) trimethoxysilane (FAS), octadecyltrimethoxysilane (ODS) or N-(6-aminohexyl) aminopropyltrimethoxysilane (AHAPS) by chemical vapor surface modification (CVSM). These SAM-Si surfaces were so hydrophobic that their water-contact angles came up to 110 degrees or more. Next, some of the SAM-Si substrates were photoirradiated using an Xe excimer lamp. Then micrometer-scale patterns composed of hydrophobic/hydrophilic regions were formed on the SAMs due to photodegradation of the SAMs. Micropatterned SAM-Si substrates were immersed in a solution consisting of tetraethoxysilane, cetyltrimethylammonium chroride, hydrochloric acid and water, MPSs were formed on the substrates. MPSs deposited on the FAS- and ODS-Si formed into films, while one deposited on the AHAPS-Si with amino gropus as end radicals showed rope-like shapes. Morphology of MPS was found to depend not only on hydrophobic/hydrophilic properties of the substrates but also on acidic/basic properties of the substrates. In addition, when MPS grew on the micropatterned samples, MPS was preferentially deposited on the hydrophobic regions due to hydrophobic interaction between the SAM and surfactant molecules. The spatial control of surfactant assembling can be achieved, in micrometer scale by changing chemical properties of substrates.

4:30 PM DD8.9
BIOMIMETIC APPROACH TO NANO-STRUCTURED SILICA-TYPE MATERIALS. Ulrich Wiesner, Cornell Univ, Dept of Materials Science and Engineering, Ithaca, NY.

Currently, a great deal of attention is being paid to the synthesis of nano-structured ceramic type materials with long range order. A biomimetic approach typically employed is to use organic molecules with the ability to self-assemble as structure directing agents. The final morphology is then determined by the cooperative organization of organic and inorganic species into three dimensionally structured arrays, a central concept discussed in the field of biomineralization. Here the effect of block copolymers as structure directing agents will be discussed. We show that changing from conventional silicon precursors to organically modified silicon precursors in the block copolymer directed synthesis, unprecedented morphology control is obtained for the resulting nano-structured organically modified ceramic materials (ORMOCERS) (1). Indeed, most of the mesophase morphologies observed in block copolymers or mixtures with their parent homopolymers are obtained for such organic-inorganic hybrid materials. The basis for this morphology control is a unique polymer-ceramic interface which is characterized by solid state NMR techniques (2). Mesophases with low volume fraction of inorganic can be employed to prepare 'hairy' nano-objects of controlled shape, size and composition (3). This may open access to, e.g., nano-engineering of ceramic materials. Mesophases with high volume fraction of inorganic can be used to prepare mesoporous materials with large pore sizes through calcination with possible applications in the field of, e.g., separation technology and catalysis. (1) M. Templin, A. Franck, A. Du Chesne, H. Leist, Y. Zhang, R. Ulrich, V. Schädler, U. Wiesner, Science 278 (1997), 1795 (2) S.M. De Paul, J.W. Zwanziger, R. Ulrich, U. Wiesner, H.W. Spiess, Interface Structure of Inorganic-Block Copolymer Composites, J. Am. Chem. Soc. (1999), in press (3) R. Ulrich, A. Du Chesne, M. Templin, U. Wiesner, Adv. Mater. 2 (1999), 141

4:45 PM DD8.10
REMOVAL PATHWAY OF BIOACTIVE GLASS RESORPTION PRODUCTS FROM THE BODY. William Lai, Paul Ducheyne, Jonathan Garino, Dept of Bioengineering and Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA; Catherine Flaitz, Division of Oral & Maxillofacial Pathology, Dental Branch, University of Texas, Houston, TX.

Bioactive glass (45S5) granules (BGG) with a narrow size range (300-355 μm) fully react in vivo to form internal silica gel cores which are subsequently degraded, leaving external, biologically formed calcium phosphate shells. To date, no study has determined the

destination and path of removal of the silicon-containing products from implanted BGG. In this study we traced and quantified the silicon released from BGG in vivo.
1500 mg of BGG was implanted in the paraspinal muscle of 7 rabbits weighing approximately 4 Kg. Blood samples and 24-hour urine samples were obtained over a 24 week period. Local muscle tissue as well as the following organs were harvested for chemical and histological analyses: brain, heart, kidney, liver, lung, spleen, and thymus. Flame atomic absorption spectrophotometry was used to measure the concentration of elemental silicon in all the samples after digestion. Tissues and fluids from a control group of 7 rabbits without BGG were obtained in a similar manner.
The urinary silicon of the implanted group was significantly higher than in the control group. From the data, the calculated average excretion rate was approximately 3 mg/day, and as such, 100 percent of the implanted silicon was excreted in 15 weeks. No elevated concentrations of silicon were found at the implant site or in the other organs after 24 weeks. Histological appearance of all organs was normal for all animals in the study.
The concentrations of silicon measured in the urine were well below saturation and since no significant increase in silicon was found in the kidney or in the other organs, the increased silicon excretion rate was within the physiological capacity of rabbits. Therefore, it can be concluded that the resorbed silica gel is harmlessly excreted in soluble form through the urine.

SESSION DD9: PATHOLOGICAL MINERALIZATION AND PREVENTION
Chair: Robert J. Levy
Wednesday Morning, December 1, 1999
Vineyard (M)

8:30 AM *DD9.1
TISSUE HEART VALVE MINERALIZATION: CLINICAL IMPORTANCE, MECHANISMS, PREVENTION AND FUTURE DIRECTIONS. Frederick J. Schoen, Brigham and Women's Hospital, Department of Pathology, Boston, MA.

Substitute heart valves composed of human or animal tissues (tissue valves) are used in 40% or more of valve replacements worldwide, predominantly as stented porcine aortic valves and bovine pericardial valves preserved by glutaraldehyde (GLUT) (collectively termed bioprostheses). The principal disadvantage of tissue valves is limitation of durability, owing to progressive calcific and non-calcific deterioration which causes high rate of progressive and age-dependent structural valve deterioration (failure rate nearly 100% in 5 years in those <35 years old but <10% in 10 years in those > 65). The natural aortic valve is cellular and layered; its specialized extracellular matrix (ECM) accommodates marked cyclical changes in shape and dimension, transfers stresses to the adjacent aortic wall, and repairs ongoing injury. Although GLUT bioprostheses mimic natural aortic valve structure, they have 1) non viable cells which cannot remodel ECM proteins, 2) a mechanically locked cuspal microstructure, and 3) markedly different mechanical properties from natural aortic valve cusps.
Calcification is a direct consequence of the inability of the non-viable cells of the GLUT preserved tissue to maintain normally low intracellular calcium. Consequently, nucleation of calcium-phosphate crystals occurs at the phospholipid-rich membranes and their remnants. Valvular collagen and experimental leaflet heart valves composed of synthetic polymers also calcify. Tissue valve mineralization has complex host, implant and mechanical determinants. Moreover, non-calcific degradation in the absence of physiological repair mechanisms of the valvular ECM is increasingly being appreciated as a critical yet independent mechanism of valve deterioration.
Active basic research, industrial development and clinical investigation seek to improve tissue valves. Particularly exciting is *tissue engineering*, in which an anatomically appropriate construct containing cells seeded on a resorbable scaffold is fabricated in vitro, then implanted. Remodeling in-vivo, stimulated and guided by appropriate biological signals incorporated into the construct, is intended to recapitulate normal functional architecture.

9:00 AM DD9.2
EFFECT OF CALCIFICATION ON THE FATIGUE BEHAVIOR OF FLUORINATED POLYURETHANES. Roberto S. Benson, Univ. of Tennessee, Materials Science and Engineering, Knoxville, TN; Hyung-Joong Kim, Kongju Univ, Materials Science and Engineering, Kongju, KOREA.

Fatigue crack propagation behavior of fluorinated and non-fluorinated polyurethane-calcium blends were studied. The polyurethanes were based on MDI, ethylene diamine and soft segment of polytetramethylene glycol and perfluoropolyoxyalkyl. The fatigue crack propagation (FCP) behavior of the polyurethanes exhibited a dependence on the chemical composition of the polymer and calcium salt. The non-fluorinated PTMG2000 did not undergo crack propagation. While the fluorinated PTMG2003F exhibited a propagation rate of 7.8×10^{-6} m/cycle at constant strain amplitude and tearing energy range. The incorporation of calcium chloride into PTMG2000 did not promote any changes in the FCP behavior, no crack propagation. The addition of hydroxyapatite (HAP) to PTMG2000 allowed cracks to grow at a rate of 3.33×10^{-6} m/cycle. In the case of PTMG2003F, the addition of calcium chloride did not lead to crack propagation; while the addition of HAP produced a material in which cracks propagated at a rate of 10×10^{-6} m/cycle. The difference in response between the non-fluorinated and fluorinated polyurethane-calcium chloride blends to cyclic loading is attributed to morphological changes manifested in the form of domain disruption and strain (chain orientation) at the crack tip. The cohesive force index and crack tip orientation were determined using FTIR. The non-fatigued samples of calcium blends of PTMG2000 and PTMG2003F have lower cohesive force indices than corresponding fatigued samples, thus indicating a higher degree of domain disruption. It was found that higher strain at the crack tip tends to arrest growth. The strain at the crack tip for PTMG2000 is originally large and decreases with the addition of calcium chloride, but not enough to induce crack propagation. For PTMG2003F, the addition of calcium chloride increased the strain at the crack tip leading to an arresting effect on the crack growth. The changes in the fracture behavior of the polyurethane-HAP blends were attributed to filler effect.

9:15 AM DD9.3
MINERALIZATION RESISTANT GLUCOSE SENSOR MEMBRANES FABRICATED FROM Fe(III)-ASSISTED SELF-ASSEMBLIES OF NAFION. I. Galeska, K. Ray, T. Valdes[1], F. Moussy[1], and F. Papadimitrakopoulos; Department of Chemistry, Polymer Science Program, Nanomaterials Optoelectronics Laboratory, Institute of Materials Science, University of Connecticut, Storrs, CT; [1]Center for Biomaterials & Surgical Research Center, University of Connecticut Health Center, Farmington, CT.

Nafion, the perfluorinated ion exchange copolymer of tetrafluoroethylene and perfluoro-[2-(fluorosulfonylhexoxy)propylvinyl ether], has been investigated as a transport membrane for miniaturized, implantable glucose sensors. The in vitro study in DMEM nutrient mixture revealed however, a series of limitation associated with calcification of the dip-coated and thermally annealed Nafion films. These are associated with calcium phosphate mineralization, nucleated from the sulfonate groups of Nafion. In an effort to address this issue, ferric cations were incorporated into the Nafion membrane films via electrostatic layer-by-layer self-assembly. Characterizations of these films revealed significantly lower levels of calcification. Because of glucose oxidase enzyme damage at low pH, pH and salt effects on the self-assembly were studied to arrive at an optimum deposition conditions. UV and FTIR studied hydrolytic stability of the self-assembled films might eliminate the need of the thermal annealing step, required by nafion, which might contribute to partial damage of glucose oxidase. Kinetic studies are presently underway to further optimize the deposition conditions and glucose transport of these self-assembled metallorganic membranes.

9:30 AM DD9.4
MECHANISMS OF ELASTIN IMPLANT CALCIFICATION. Naren Vyavahare, Robert J. Levy, Children's Hospital of Philadelphia, PA.

Elastin, an abundant structural protein present in the arterial wall, is prone to calcification in a number of disease processes including porcine bioprosthetic heart valve calcification and atherosclerosis. The mechanisms of elastin calcification are not completely elucidated. In the present work, we demonstrated calcification of purified elastin in rat subdermal implants (Ca^{2+} = 89.73±9.84 μg/mg after 21 days vs. control, unimplanted Ca^{2+}= 0.16±0.04 μg/mg). X-ray diffraction analysis along with resolution enhanced FT-IR spectroscopy demonstrated the mineral phase to be a poorly crystalline hydroxyapatite. Our results further demonstrated that $AlCl_3$ pretreatment of elastin led to complete inhibition of elastin calcification using 21 day rat subdermal implants (Ca^{2+}= 1.57±0.18 μg/mg for $AlCl_3$ pretreated elastin vs. 89.73±9.84 for untreated elastin). The $AlCl_3$ pretreatment caused irreversible binding of aluminum ions to elastin as assessed by atomic emission spectroscopy. Moreover, aluminum ion binding altered the spatial configuration of elastin as shown by circular dichroism (CD), fourier transform infrared (FTIR), and ^{13}C nuclear magnetic resonance (NMR) spectroscopy studies, suggesting a net structural change including a reduction in the extent of beta sheet structures and an increase in coil-turn conformations. In addition we have localized high expression of matrix metalloproteinase (MMP-2 & MMP-9), tenascin-C and alkaline phosphatase proximal to the initial calcific deposits. Inhibition of MMP activity in rats by daily injection of an MMP inhibitor

(hydroxamate-based) significantly reduced early (7 day) elastin implant calcification (control injection Ca^{2+}= 21.71±1.19 μg/mg, MMP inhibitor injection Ca^{2+}= 5.43±1.03 μg/mg elastin) suggesting an important role of MMPs in the elastin calcification mechanism. Thus, it is concluded that purified elastin calcifies in rat subdermal implants, and MMPs, tenascin-C, and alkaline phosphatase are actively involved in the initiation of elastin calcification. The $AlCl_3$ pretreated elastin completely resists calcification due to irreversible aluminum ion binding and subsequent protein structural alterations.

9:45 AM DD9.5
CALCIFICATION RESISTANT POLYURETHANES MODIFIED WITH GEMINAL BISPHOSPHONATE GROUPS. Ivan Alferiev, Narendra Vyavahare, Robert J. Levy, Children's Hospital of Philadelphia, Philadelphia, PA.

Due to their excellent material properties and biocompatibility, polyurethanes are used in biomedical applications. However, thrombosis, calcification and oxidative degeneration are still major problems for their long term use. We have modified commercial polyurethanes by covalently attached bisphosphonate groups. Thus, 3 types of medical grade segmented polyurethanes: polyurethane-urea BioSpan, polycarbonate-urethane Bionate (Polymer Technology Group Inc., Berkely, CA), and a polyether-urethane (Sulzer Carbomedics Inc., Austin, TX) were modified with 0.06 - 0.12 mmol/g of geminal bisphosphonate groups via base-induced N-alkylation of the urethane NH sites with 1,6-dibromohexane and the following attachment of thiol-containing bisphosphonates to bromoalkyl groups created on the polymer backbone. Another approach to the bisphosphonate modification employed attachment of carboxy-groups to polyurethanes, either by direct base-induced carboxyalkylation with bromocarboxylic acids lithium salts, or by interaction of bromoalkylated polymers with mercaptocarboxylic acids. The carboxy-groups attached to the polymer backbone were activated via N-hydroxysuccinimide esterification and reacted with 3-amino-1-hydroxypropylidene-1,1-bisphosphonic (pamidronic) acid. The reactions were monitored by ^{1}H and/or ^{31}P NMR spectroscopy. It was shown that the urea NH sites of polyurethane-urea BioSpan are not involved into the alkylation. As follows from GPC-analysis and measurements of intrinsic viscosities, the molecular weights of polymers remain practically unaffected in the conditions of modifications. The mechanical properties of bisphosphonate modified polyurethanes were close to those of the starting non-modified polymers. Water absorption increased up to 20% depending on the extent of modification. Polyurethane-urea (BioSpan) modified with 0.09 mmol/g of 2,2-diphosphonoethylthio groups showed a significantly lower calcification (Ca = 72.33 ± 26.8 ng/mg for bisphosphonate-modified PU compared to 387.44 ± 154.7 ng/mg for control PU) in the rat subdermal calciphylaxis model.

SESSION DD10: CALCIUM CARBONATE FORMATION
Chair: Angela M. Belcher
Wednesday Morning, December 1, 1999
Vineyard (M)

10:30 AM *DD10.1
PROTEINS FROM OYSTER SHELL: BIOMINERALIZATION REGULATORS AND COMMERCIAL POLYMER ANALOGS. A.P. Wheeler, Department of Biological Sciences, Clemson University, Clemson, SC; C.S. Sikes, The Mineralization Center, Department of Biological Sciences, University of South Alabama, Mobile, AL.

Molluscan shell is a composite made up of um-sized CaCO3 crystals and an organic phase (matrix). This report outlines our studies on the structure and activities of matrix proteins isolated from the inner calcite layer of shell of the Eastern oyster, including their cellular origin, molecular genetics and their relationship to the crystalline mineral phase. In addition, we present results of the synthesis and commercialization of polypeptide polymers which are based on the structure and activities of the oyster proteins. Extracted shell proteins are polyanionic and range in size from relatively small soluble forms to those which are crosslinked and insoluble. The soluble forms are capable of adsorbing to calcite in vitro and in the process changing its growth habit and acting as threshold growth inhibitors. Their function in vivo is not understood, but they may serve to control shell crystal morphology. The insoluble protein forms gels readily and may serve to provide resiliency to the shell and, from in vitro and in situ observations, appears to serve as a site for nucleation of crystals. However, from studies in vitro, these gels do not lower the energy of activation for nucleation, as previously expected. Matrix protein aggregates are identifiable by AFM on the surface of crystals, but as such do not serve as nucleation sites for new crystal growth. If the aggregates are removed, then ectopic crystal growth proceeds readily revealing orientation of the underlying crystals. All the matrix proteins contain domains rich in aspartic acid, are heavily phosphorylated, cross-react in antibody studies and may belong to a limited number of gene families with individuals modified post-synthesis. The proteins are made by a specialized group of cells located primarily some distance from the growing edge of the shell and appear to be assembled into sheets soon after secretion. Based on the structure and activity of the matrix proteins soluble anti-scalants and crosslinked insoluble water absorbents have been developed. These are primarily poly(aspartates) which can be made in large scale via thermal polycondensation of aspartic acid. The soluble forms are commercially used as biodegradable water treatment chemicals among other applications.

11:00 AM DD10.2
REGENERATION OF HARD TISSUE IN THE SHELL OF STROMBUS GIGAS, THE GIANT QUEEN CONCH. X. Su and A.H. Heuer, Department of Materials Science and Engineering, Case Western Reserve University, Cleveland, OH.

Shell (hard tissue) regeneration is an important biomineralization process in molluscs. Rapid regeneration is very important in avoiding loss of fluids and attacks by predatory animals. We have studied the regeneration processes of Queen conch shell by two different methods: insertion of an abiotic glass cover slide between the mantle tissue and the shell; and by removal of a piece of hard tissue from the last whorl of the shell. The regenerated materials were removed after mineralization periods extending from 6 hours to 18 days and were analyzed by X-ray diffraction, and scanning and transmission electron microscopy. Although the CaCO3 in native Queen conch shell is exclusively aragonite, calcite was detected in the regenerated materials grown on glass substrates. Calcite formation occurred only during the very early stage of mineralization and the initial minerals formed were soon overgrown by aragonite. The initial aragonite minerals were spindle-like clusters of fine-grained crystals, and were followed by poorly oriented elongated crystals; after this transition period, the microstructure was recognizable as the crossed-lamellar structure of the natural shell. The regenerated material induced by removing a piece of shell consisted entirely of aragonite. Thick white-colored organic materials were secreted before the deposition of the crossed-lamellar microstructure, and longer periods were required for the mature shell structure to develop. In both types of regeneration experiments, once the crossed-lamellar microstructure was formed, further tissue development was identical to natural shell growth.

11:15 AM DD10.3
TEMPLATE MEDIATED BIOMINERALIZATION OF HEMOZOIN. David W. Wright, James Ziegler, Duquesne University, Dept. of Chemistry and Biochemistry, Pittsburgh, PA.

During the intraerythrocytic phase of its life cycle, the malaria parasite can degrade up to 80% of an infected erythrocyte's hemoglobin to obtain requisite amino acids for its maturation. The catabolism of hemoglobin occurs in specialized digestive vacuoles (pH 4.5-5.2) via a defined metabolic pathway. While hemoglobin proteolysis yields needed amino acids, it also releases toxic free heme (Fe(III)PPIX). To balance the metabolic requirements for amino acids against the toxic effects of heme, malaria parasites have evolved a detoxification mechanism which involves the formation of an epitaxial crystalline heme aggregate known as hemozoin (malaria pigment, β-hematin). Recently, Sullivan et al. have shown that a family of histidine-rich proteins (HRP) mediates the formation of hemozoin and is inhibited by traditional antimalarial compounds. To study this unique biomineralization process, we have designed a family of peptide dendrimeric bionucleating templates (BNT I and BNT II) capable of nucleating the formation of hemozoin. Each template binds significant amounts of the natural substrate, Fe(III)PPIX. Binding of the metal-free base protoporphyrin IX to the templates suggests that substrate recognition is based on the porphyrin moiety rather than specific metal recognition. Such a supposition is further supported by the fact that Zn(II)PPIX, as well as the structurally related metal-free and metallo- phthalocyanines, can bind the templates. Further, it was shown that the dendrimeric BNT I and BNT II were capable of supporting the polymerization of hemozoin and are inhibited by antimalarial drugs. A comparison of the template activity with that of HRP II strongly suggests that this protein serves as a critical nucleating scaffold for the biomineralization of heme within the parasite.

11:30 AM DD10.4
AN IN SITU AFM INVESTIGATION OF THE EFFECT OF ASPARTIC ACID ON THE ENERGETIC AND KINETIC FACTORS CONTROLING THE GROWTH OF CALCITE. H.H. Teng, P.M. Dove, School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA; J.J. DeYoreo, Department of Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, CA.

Because calcite occurs ubiquitously in biomineralizing systems and is easily crystallized, it has emerged as a model system for developing an understanding the process of biomineralization, particularly when combined with aspartic acid (ASP) rich protein mixtures. However little is known about the physical mechanism by which these proteins alter the growth process. In an attempt to quantify the energetic and kinetic effects of ASP on calcite crystallization, we have used in situ atomic force microscopy to investigate growth on the {104} faces of calcite in both pure and ASP bearing solutions. Results were obtained in solutions with well-characterized chemistry and saturation state (s = $\ln[(a\mathrm{Ca}^{2+})(a\mathrm{CO_3}^{2-})/\mathrm{Ksp})$ in the range of s = 0.04 - 1.42. Measurements of the dependence of step speed on concentration and step length, as well as the dependence of critical step length on supersaturation were used to determine the step edge energies and kinetic coefficients for calcite growth. The results show that the step edge energy is significantly reduced upon addition of moderate amounts of ASP and that, while in ASP-free solutions it is independent of supersaturation, in ASP-bearing solutions it exhibits a strong supersaturation dependence. In contrast, the addition of ASP has little or no effect on the kinetics of growth. Addition of solutions containing high levels of ASP alters the growth hillock morphology, leading to expression of a new step direction with reduced step edge energy. These results support a model of ASP-calcite interaction in which the primary effect of the amino acid is to alter the equilibrium thermodynamic state of the growth surface perhaps by formation of an adsorption compound. The results are consistent with conclusions based on qualitative observations reported in the literature of the effects of more complex, naturally occurring protein mixtures on $CaCO_3$ crystallization.

11:45 AM DD10.5
THREE DIMENSIONAL FINITE ELEMENT MODELING OF MICROSTRUCTURAL DEVELOPMENT OF NACRE IN SEA SHELLS AND IMPLICATION ON MINERALIZATION OF $CACO_3$.
Dinesh R. Katti, Department of Civil Engineering, North Dakota State University, Fargo, ND; Kalpana S. Katti, Department of Polymers and Coatings, North Dakota State University, Fargo, ND.

Three dimensional finite element models of nacre are constructed based on reported microstructural studies on the 'brick and mortar' microarchitecture of nacre. 3D eight noded isoparametric brick elements are used to design the microarchitecture of nacre. Simulated mechanical tests are performed on the constructed models and properties such are strength and elastic modulus are calculated and compared to micromechanical studies on abalone nacre and geological aragonite reported in literature. A parametric study of mineralization and evolution of microstructure of nacre in terms of size is performed on the models. Stress deformation response at organic-inorganic interface, inside aragonitic bricks and organic mortar are found quantitatively. The consistent mismatch between modeled 'perfect nacre' structure and experiments with identical microstructures sheds light on mineralization of $CaCO_3$ in nacre at early crystallization stage with significant involvement of defects and development of such microstructural features such as porosities. Our work quantitatively shows that these defects are instrumental in defining the complexities of mechanical behavior of nacre.

SESSION DD11: BIOMIMETIC HYDROXYAPATITE-POLYMER COMPOSITE
Chair: Tadashi Kokubo
Wednesday Afternoon, December 1, 1999
Vineyard (M)

1:30 PM *DD11.1
BIOMOLECULAR RECOGNITION AND CONTROL OF BIOLOGICAL AND NON-BIOLOGICAL INORGANIC MATERIALS FROM SEA SHELLS TO SEMICONDUCTORS.
Angela M. Belcher, Sandra R. Whaley, Erin E. Gooch and Christine E. Flynn, Dept. of Chemistry and Biochemistry and The Texas Materials Institute, The University of Texas at Austin, Austin TX.

Biological systems have a unique ability to control crystal structure, phase, orientation and nanostructural regularity of inorganic materials. An example is seen with the control of crystallographic phase and orientation of calcium carbonate with the polyanionic proteins isolated from shells of the marine gastropod, the abalone. We are currently investigating the principles of natural biological molecular recognition in materials and developing new methods to pattern useful non-biological electronic and magnetic materials on new length scales. A peptide combinatorial approach has been employed to identify proteins that select for and specifically bind to inorganic structures such as semiconductor wafers, nanoparticles and quantum confined structures. This approach utilizes the inherent self-organizing, highly selective properties of biologically derived molecules. We are currently investigating peptide recognition and interaction with III-V and II-VI semiconductor materials, magnetic materials and calcium carbonates and phosphates. We have selected peptides that can specifically bind to and discriminate zinc-blende III-V semiconductor surfaces. These peptides show crystal face specificity and are being used to organize nanoparticles heterostructures. Long term potential application of these materials would include optoelectronic devices such as light emitting displays, optical detectors and lasers, and nano-meter scale computer components.

2:00 PM DD11.2
PREPARATION OF HYDROXYAPATITE/COLLAGEN COMPOSITES USING BIOMIMETIC PROCESS AND THEIR BIOCOMPATIBILITY.
Masanori Kikuchi, Junzo Tanaka, Natl Inst for Res in Inorg Mater, Ibaraki, JAPAN; Soichiro Itoh, Shizuko Ichinose, Yoshihisa Koyama, Kazuo Takakuda, Tokyo Med. and Dent. Univ., Tokyo, JAPAN; Katsuyoshi Nagaoka, Shigeo Tanaka, Nihon Univ., Kanagawa, JAPAN.

Nanocomposites of hydroxyapatite (HAp) and collagen (Col) were prepared by a biomimetic coprecipitation method under controlled pH and temperature. Transmission electron micrographs showed that the c-axis of HAp nanocrystals aligned along collagen molecules by a self-organization mechanism; a very similar alignment to bone structure was found in the sample prepared at near- biological conditions, $i.e.$ pH8-9 and 40°C, which consisted of fibers with length of 20μm. Such self-organized nanostructure did not appear at pH7.4 of biological condition. The condition where the self- organization appeared was explained in terms of the charged state and dehydration of collagen, and the steady growth of HAp crystals. The three-point bending strength was dependent of the degree of self-organized alignment; the maximum value was 40MPa, corresponding to a half strength of bone. From the material scientific point of view, therefore, osteoblasts may control the chemical condition in a small area around the cells to pH 8-9 as well as supply raw materials of bone, hydroxyapaite and collagen, at the initial state of osteogenesis. The biocompatibility of the composites was examined by animal tests using beagles. At 2 weeks after implantation, the composites gradually degraded from the surface where osteoclasts and osteoblasts were induced; then, the composites were absorbed and new bone was formed as rapidly as a bone remodeling process. The HAp/Col composites prepared by biomimetic process were expected

2:15 PM DD11.3
GROWTH OF BONE-LIKE MINERAL ON POLY(LACTIC-CO-GLYCOLIC ACID) FILMS AND SCAFFOLDS IN VITRO.
William L. Murphy, Katherine A. Gilhool, David H. Kohn, David J. Mooney, University of Michigan, Depts of Chemical and Biomedical Engineering and Biologic and Materials Sciences, Ann Arbor, MI.

Novel strategies to engineer bone have focused on the use of natural or synthetic degradable materials as scaffolds for cell transplantation or as substrates to guide bone tissue development. The basic requirements of the scaffold material are biocompatibility, degradability, mechanical integrity, and osteoconductivity. A major design problem is satisfying each of these requirements with a single scaffold material. This study addresses the problem by describing an approach to combine the biocompatibility and degradability of a polymer scaffold with the osteoconductivity and mechanical reinforcement of a bone-like mineral film. We report the nucleation and growth of a continuous carbonated apatite mineral on the interior pore surfaces of a porous, degradable polymer scaffold via a one step, room temperature incubation process. A three dimensional, porous scaffold of the copolymer 85:15 poly(lactic-co-glycolic acid) (PLGA) was processed by a solvent casting, particulate leaching process. FTIR spectroscopy and SEM analysis after different incubation times in a simulated body fluid (SBF) demonstrate the growth of a continuous bone-like apatite layer on the polymer surface without an appreciable decrease in scaffold porosity. Quantification of phosphate on the scaffold displays the growth and development of the mineral film over time, with an incorporation of 0.43mg of phosphate after 16 days in SBF. The compressive moduli of polymer scaffolds increase 2.5-fold after a 16 day incubation time as compared to control scaffolds. Analysis of mineral growth on two-dimensional 85:15 PLGA films reveals changes in growth rate, constitution, and morphology of the mineral formed as a function of surface hydrolysis treatment. In summary, this biomimetic treatment provides a simple, one step, room temperature method for surface functionalization and subsequent mineral nucleation and growth on biodegradable polymer scaffolds for tissue engineering.

2:30 PM DD11.4
EXTRUSION FREEFORM FABRICATION OF BIOMIMETIC MINERALIZED GELS.
Paul Calvert and Chad Souvignier, Arizona Materials Labs., Tucson AZ.

Bone growth occurs by the deposition of layers of swollen collagenous matrix and its subsequent mineralization. These processes occur continuously with mineralization proceeding deep within the matrix, away from the layer of osteocytes. This raises questions about how nucleation can be induced to occur preferentially at a site remote from the cells and how a dense structure can be formed when the source of material is ahead of the growth front. Freeform fabrication is also a layerwise process for forming materials which we have been using to make bone-like composites. Hydrogels, such as agarose, can be freeformed containing large concentrations of partly-soluble salts. Subsequent treatment with aqueous solutions of the appropriate counter ions can mineralize the gel with calcite or hydroxyapatite. During drying, these materials retain their shape, while undergoing a 50% linear shrinkage, to form dense, strong composite materials. The resulting materials show surprisingly good toughness and strength. Comparison with the natural process sheds some light on gaps in our understanding of bone growth. The potential for using this freeforming method for bone prostheses and for strong synthetic composites will be discussed.

2:45 PM DD11.5
BIOACTIVE HYDROXYAPATITE-POLYSULFONE COMPOSITE FOR TISSUE REPLACEMENT. Benedict Chua, Min Wang, Chee Yoon Yue, School of Mechanical and Production Engineering, Nanyang Technological University, SINGAPORE.

Based on the theory that the best material for replacing a body tissue should be identical or at least similar to that tissue, a variety of bioactive composites have been developed for tissue substitution since the early 1980s. In this investigation, a new material consisting of hydroxyapatite (HA) and polysulfone (PSU) was produced and evaluated for potential medical applications. Hydroxyapatite is a synthetic bioceramic that resembles bone apatite while polysulfone is a high strength, biocompatible polymer. The HA/PSU composite containing up to 20vol% of HA was studied at the initial stage. Composite with higher HA contents will be produced and assessed. The procedure for manufacturing HA/PSU composite included drying, blending, compounding and injection/compression moulding. Defect-free composite samples (rectangular bars, discs and dumbbell specimens) could be obtained by injection moulding. Thick composite plates could be made by compression moulding. Raw materials were fully characterised before composite processing. Both compounded materials and moulded parts were assessed using various techniques. It was found through scanning electron microscopy (SEM) that HA particles (mean particle size: 7.32μm) were well dispersed in the PSU matrix. Thermogravimetric analysis (TGA) verified the amount of HA in the composite. Differential scanning calorimetry (DSC) results indicated that the glass transition temperature (T_g) of the polymer matrix was not affected by the incorporation of HA. Rheological analysis revealed that PSU and the composite exhibited pseudoplastic flow behaviour at the processing temperatures. For unfilled PSU, its viscosity decreased with an increase in temperature. The viscosity of HA/PSU composite increased with an increase in the HA volume fraction. It was shown through dynamic mechanical analysis (DMA) that the storage modulus of the composite was increased with an increase in HA volume percentage below T_g of the polymer, while tan δ was maintained at nearly the same level. It was established that water uptake reached an equilibrium after 7 days' immersion in distilled water for PSU and HA/PSU composite. It was found that after 7 days' immersion in distilled water, the storage modulus of PSU and HA/PSU composite was decreased, with the reduction being less for HA/PSU composite than for PSU. Other tests, including biaxial fatigue testing, are being conducted in Ringer's solution and simulated body fluid.

SESSION DD12: BIOMOLECULE-MINERAL INTERACTIONS
Chair: Paul Calvert
Wednesday Afternoon, December 1, 1999
Vineyard (M)

3:30 PM DD12.1
SELECTIVE BINDING OF CHIRAL AMINO ACIDS TO ATOMIC STEPS OF CALCITE. Christine Orme, Aleksandr Noy, Mary McBride, Jim De Yoreo, Lawrence Livermore National Laboratory, Livermore, CA.

Proteins regulate the polymorph and macroscopic morphology of inorganic crystals found in biological systems. Molluscan proteins which regulate calcium carbonate growth are rich in acidic amino acids such as aspartic acid and glutamic acid. In this study we investigate the interaction of calcite atomic steps with aspartic acid. We find that the D and L forms of aspartic acid break the crystal symmetry by selectively binding to different faces that are related by a glide plane. This suggests that the binding involves the backbone of the amino acids not simply the residue. The evolving surface of the (104) cleavage plane of calcite was imaged using an AFM under conditions of both growth and dissolution. During calcite dissolution the addition of aspartic acid changes the etch pit shape from the rhombohedral symmetry associated with pure calcite to a trapezoidal shape. None of the new facets coincide with any of the original stable calcite step directions. Three of the new facets are the same for both D and L aspartic acid but one of the facets is related by a mirror symmetry and forms according to the enantiomer. During growth this same pattern emerges making growth hillocks grown in the presence of D and L aspartic acid related by a mirror symmetry. SEM images of nucleated crystals grown in the presence of these amino acids also confirm these changes and show the macroscopic effect of the modification of the atomic step directions.

3:45 PM DD12.2
IMPORTANCE OF ELECTROSTATIC INTERACTIONS BETWEEN CALCITE SURFACES AND PROTEINS. Alejandro Rodriguez-Navarro, Russell Messier, The Penn State Univ, Materials Research Lab, PA; Concepcion Jimenez-Lopez, Juan Manuel Garcia-Ruiz; Univ of Granada, IACT-CSIC, Granada, SPAIN.

We have studied the electrostatic interactions of proteins with the calcite surfaces during its subsequent nucleation and growth on a substrate. In doing so, a model system of four globular proteins (lysozyme, ribonuclease, myoglobin and a-lactalbumin), having the same size and conformation, but differing in surface properties (i.e. surface charge) was used. Depending on the nature of the charge on the protein, its morphological effect on calcite growth (inhibition of specific crystal faces) varies, with this effect becoming more pronounced as the protein is more negatively charged. To study how the adsorption of proteins affects the growth of calcite along different crystal directions, calcite plates cut with different crystallographic orientations (i.e. (001), (104), (100) and (110)) were used as substrates. The nucleation density on the calcite substrates increased with protein concentration, further increasing as the protein charge become more negative. At high protein concentration (10 mg/mL), calcite crystals cover the substrate surface, forming a continuous film. The overgrowing calcite crystals show the same orientation as the substrate. The nucleation density also varies with the crystallographic orientation of the calcite substrates, increasing in accordance with the sequence: (110), (100), (104) and (001). The (001) orientation coincides with the disposition of the alternating carbonate and calcium layers of the calcite structure. Interestingly, on substrates with an orientation parallel to the c-axis, overgrowing crystals nucleate in lines perpendicular to the c-axis. To study how the protein itself controls the orientation of crystals, we used amorphous substrates (glass). After incubation on the glass substrates with negatively charged proteins, an oriented nucleation of the calcite crystals was induced. As the concentration of the protein and/or the time of incubation increases, the number of crystals preferentially oriented also increases. At high incubation times, all calcite crystals were oriented with their c-axis perpendicular to the substrate.

4:00 PM DD12.3
CONTROL AND CHARACTERIZATION OF PROTEIN ADSORPTION ON CERAMIC SURFACES. Michael Read, Massachusetts Inst of Technology, Dept of Materials Science and Engineering, Cambridge, MA; Sandra Burkett, Amherst College, Dept of Chemistry, Amherst, MA; Anne Mayes, Massachusetts Inst of Technology, Dept of Materials Science and Engineering, Cambridge, MA.

Protein adsorption to implant surfaces is an early step in the mechanism of biomineralization, and is influenced by surface charge and hydrophobicity. Adsorbed protein amounts and conformational changes may in turn mediate subsequent cell adhesion and inorganic deposition. Lysozyme, a model protein, is thought to bind to HAp through electrostatic interactions without large changes in conformation. Similar adsorbed amounts, reversibility, and H/D exchange properties are observed for aluminophosphate. When the surface composition, charge, and hydrophobicity are varied in a controlled manner through the addition of silica, interesting differences are observed.

4:15 PM DD12.4
MODIFICATION OF ASSEMBLED STRUCTURES OF STEROIDS ADSORBED ON APATITE CERAMIC SURFACE BY ELECTRICAL POLING. Satoshi Nakamura, Akiko Obata, Kimihiro Yamashita, Tokyo Medical and Dental Univ, Inst of Biomaterials and Bioengineering, Tokyo, JAPAN.

Steroids are biogenic materials forming biomembranes combined with phospholipids and endowing the membranes with various functions. The modification of the aggregate modes of steroids is important for control of the function of biomembrane containing steroids, a study on the mineralization of cholesterol gallstones, and development of the

delivery system of steroids as drugs. The aggregate structures of steroids adsorbed on electrically-poled hydroxyapatite (HAp) ceramics were investigated by X-ray diffractometry (XRD) and infrared spectroscopy (IR). Effectivity of the poled HAp on modification of the steroid assembly modes was discussed by estimation of the arrangements of the steroid molecules and the azimuthal relationship between the steroids and the poled HAp substrates. The dense HAp ceramics were obtained from the pelletized HAp powder precipitated from calcium hydroxide suspension and phosphoric acid using conventional sintering at 1250°C under the saturated water vapor pressure. The HAp ceramic blocks were poled in a DC electrical field with being heated at more than 300°C. The HAp ceramics were immersed in the ethanol solution of steroids at 20°C. The steroid crystals were overgrown onto the HAp substrates by recrystallization method. In the case of cholesterol, the tabular crystals of cholesterol monohydrate with well developed (001) faces were overgrown on all of the HAp ceramic surfaces. The XRD reflection intensity ratio of the overgrown crystals on the negatively (N) and positively (P) poled HAp surfaces was significantly different from the ratio of the crystals on the HAp surfaces without poling treatment (0-surfaces). The thickness of the cholesterol layers on the P-surfaces was considerably less than those of the N- and 0-surfaces. It was revealed that the electrically-poled HAp ceramic surfaces altered the aggregate structures and the crystal growth rates of steroids.

DD12.5
Abstract Withdrawn.

DD12.6
GENETICALLY-ENGINEERED BIOMIMETIC PROTEINS FOR FUNCTIONAL MATERIALS ASSEMBLY. Mehmet Sarikaya[1], Daniel Heidel[1], Hanson Fong[1], Richard Humbert[1], Malcolm L. Snead[2] and Stanley Brown[3]; [1]Materials Science and Engineering, University of Washington, Seattle, WA, [2]Craniofacial Molecular Biology, University of Southern California, Los Angeles, CA and [3]Molecular Cell Biology, University of Copenhagen, Copenhagen, DENMARK.

Structural control of inorganic materials at the nanometer-scale is a key to synthesis of materials with new and improved physical properties. Biological hard tissues may serve as models for novel engineered materials as biocomposites have excellent combination of physical properties that are related to their highly ordered hierarchical structures. The intricate nano- and micro-architecture of biocomposites are controlled at the molecular level by macromolecules through interactions with mineral phases. In this presentation, human and mice enamel, mollusc shells, and sponge spicules will be discussed as biocomposite examples that offer materials science and engineering lessons for functional materials assemblies (piezoelectric, mechanical, and optical). The central issue, mimicking of biological structures, requires the use of macromolecules, in particular proteins, that have affinity to inorganic surfaces. Although proteins can be isolated from biological tissues, a more practical strategy is to use genetic engineering techniques to develop novel none-natural proteins with high affinity to inorganic surfaces. The second part of the presentation will discuss combinatorial genetic techniques that permit isolation of specific recognition elements for inorganic surfaces, including those not realized by natural proteins, in the absence of apriori prediction of necessary structures. The results could have significant implications in tailoring surfaces, and formation and assembly of ordered structures of metals, functional ceramics, semiconductors, and ferroelectrics in applications of nanotechnology, smart materials, bioimplants, and biomimetics.