SYMPOSIUM LL

Orthopaedic/Dental Biomaterials

November 27 – 29, 2000

Chairs

C. Mauli Agrawal
Dept of Orthopaedics
Univ of Texas-Health Science Ctr
San Antonio, TX 78229
210-567-6495

John Brunski
Dept of Biomedical Engr
Rensselaer Polytechnic Inst
Jonsson Engineering Ctr Rm 7040
Troy, NY 12180-3590
518-276-6963

Warren O. Haggard
Wright Medical Technology
Arlington, TN 38002
901-867-4659

Joachim Kohn
Chemistry Dept
Rutgers Univ
Wright and Rieman Labs
Piscataway, NJ 08854
732-445-0488

Orhun K. Muratoglu
Orthopaedic Biomech & Biomatls Lab
Massachusetts General Hospital
Jackson 1206
Boston, MA 02114
617-726-3869

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SESSION LL1: ORTHOPAEDIC/DENTAL TISSUE ENGINEERING
Chair: C. Mauli Agrawal
Monday Morning, November 27, 2000
Berkeley (Sheraton)

8:30 AM *LL1.1
STRUCTURAL TEMPLATES AND BIOREACTORS FOR CARTILAGE TISSUE ENGINEERING. Gordana Vunjak-Novakovic and Lisa E. Freed, Massachusetts Institute of Technology, Division of Health Sciences and Technology, Cambridge, MA.

Functional substitutes of native cartilage can be created in vitro using chondrogenic cells, biodegradable polymeric scaffolds and bioreactors. Scaffolds provide a structural template for tissue formation, while bioreactors provide a controlled environment that embodies the chemical and physical regulatory signals for the development of a functional tissue. The structure (composition, morphology) and function (biosynthetic activity, integrative potential, mechanical function) of engineered constructs can be modulated by the conditions and duration of in vitro cultivation. The paper reviews the main requirements for scaffold and bioreactor design and recent progress in quantitative and modeling studies of engineered cartilage.

9:00 AM LL1.2
FORMATION OF THREE DIMENSIONAL CELL-POLYMER CONSTRUCTS IN BIOREACTORS FOR BONE TISSUE ENGINEERING. Vassilios Sikavitsas, Gregory Bancroft, Antonios Mikos, Rice University, Dept of Bioengineering, Houston, TX.

The aim of this study is to investigate the ability of bone marrow stromal cells seeded in three dimensional PLGA biodegradable scaffolds to be cultured in a spinner-flask or a rotating vessel. The studied bioreactors generate good mixing and thus better nutrient transport to the seeded cells. Poly(DL-lactic-co-glycolic acid) (PLGA) copolymer scaffolds have been selected for this study because they are osteoconductive and biocompatible, degrading into products that can be either metabolized or excreted. Rat bone marrow stromal cells were harvested from femurs and tibias of six week old male Sprague-Dawley rats and placed in T-75 culture flasks using DMEM with 10% fetal bovine serum from selected lots. On the third day non-adherent cells were removed and on the seventh day the cells were seeded on porous 75:25 PLGA cylindrical foams prepared by the method of solvent casting and particulate leaching and cultured in the spinner-flask, the rotary vessel and under static conditions in the presence of dexamethasone, β-glycerophosphate and ascorbic acid-2-phosphate. On the 14th, 21st and 28th day, foams were removed and the cell number, radio-labelled thymidine incorporation, alkaline phosphatase activity and osteocalcin, were measured. The foams were also stained for mineralization. The cell number demonstrated a higher number of cells on day 21 in the spinner-flask culture. The static culture had the lowest cell number but on the 28th day the difference in cell number in all three cultures was statistically insignificant. Histological sections of the foams on the 28th day showed higher cell densities at the exterior of the foam providing strong evidence for the existence of nutrient concentration gradients at the interior of the scaffolds. Although the good mixing provided in the spinner-flask allowed the cells to proliferate faster, high proliferation was limited to the cells located at the exterior of the foams.

9:15 AM LL1.3
DEVELOPMENT OF A NOVEL PHOTOCROSSLINKABLE HYALURONAN MATRIX FOR CARTILAGE REPAIR. Kimberly A. Smeds, Duke Univ, Dept of Chemistry, Durham, NC; Jean Y. Wang, Anthony E. Baer, Lori A. Setton, Duke Univ, Dept of Biomedical Engineering, Durham, NC; Mark W. Grinstaff, Duke Univ, Depts of Chemistry and Ophthalmology, Durham, NC.

Repair of degenerated articular cartilage with exogeneous scaffolds and cellular supplementation is an area of major interest in musculoskeletal research. In this study, we describe a novel scaffold for tissue-engineered cartilage repair which may be crosslinked in situ. This feature is extremely attractive for the ability to fill irregular defects and provide for optimal tissue integration. We have embedded chondrocyte cells in a photocrosslinakable hyaluronan based matrix. The photocrosslinkable methacrylated hyaluronan, HA-MA, was prepared by reacting a 2% w/v solution of the polysaccharide with a 20-fold excess of methacrylic anhydride for 24 hours at 5°C. The photocrosslinkable polysaccharide was next precipitated and washed with ethanol to remove remaining methacrylic acid and methacrylic anhydride. ^{1}H-NMR spectroscopy confirmed that 14% of the free hydroxyls on hyaluronan were modified. Upon exposure of the HA-MA to an argon ion laser (514 nm) in the presence of a photoinitiating system (eosin Y, 1-vinyl-pyrrilidinone, and triethanol amine) a stable hydrogel is formed. Next, primary chondrocytes were isolated from porcine knee joints and were mixed with a 2% solution of the HA-MA biopolymer at a concentration of 10^6 cells/ 1 mL. The suspension was divided into four equal portions and poured into cylindrical molds, photocrosslinked with an argon ion laser, and cultured out to two weeks. In control studies, the photoinitiating system was found not to be cytotoxic to chondrocytes at the concentrations required for facile photocrosslinking. Viability studies showed that by 6 hours 91% of the cells were still viable and by 13 days 77% were viable. Additional studies will focus on quantitative measures of DNA content, cell proliferation, S-GAG content, and collagen accumulation.

9:30 AM LL1.4
CHITOSAN/TRICALCIUM PHOSPHATE SCAFFOLDS FOR BONE TISSUE ENGINEERING. Yong Zhang, Miqin Zhang, University of Washington, Dept of Materials and Engineering, Seattle, WA.

This research involves synthesis and characterization of a biodegradable 3-dimensional polymer/calcium phosphate hybrid matrix as scaffolds for bone tissue regeneration. The scaffolds are comprised of chitosan and β-tricalcium phosphate (β-TCP) and have combined optimum mechanical and biological properties of the two materials. Chitosan is a biocompatible and biodegradable polymer, and has rich hydroxyl groups for surface modification. β-TCP with chemical composition close to the inorganic components of bone is used as a powder filler to reinforce the scaffolds and increase bioactivity of the scaffolds. The scaffolds are fabricated using a thermally induced phase separation technique. The principal advantages of this technique are its low cost, low shrinkage levels, low sintering temperatures, and its ability to produce a variety of microstructures of various shapes and sizes. The microstructure, mechanical properties, and degree of biodegradation of the scaffolds are controlled by varying the ceramic-polymer ratio and material processing conditions. The osteoblast-like MG63 cells are seeded on the scaffolds to study the attachment and proliferation of the cells. The hydroxyl groups on the scaffolds are used to graft polyethylene glycol (PEG) linked arginine-glycine-aspartate (RGD) peptides for promoting bone cell attachment and new tissue formation. The PEG chains are designed to suppress the nonspecific protein adsorption for the prevention of scar tissue formation.

10:15 AM LL1.5
CONTROLLING DIFFUSION OF SOLUTES THROUGH IONICALLY CROSSLINKED ALGINATE HYDROGELS DESIGNED FOR TISSUE ENGINEERING. Catherine K. Kuo, Peter X. Ma, Univ of Michigan, Dept of Biologic and Materials Sciences, Macromolecular Science and Engineering Center, Dept of Biomedical Engineering, Ann Arbor, MI.

Tissue engineering aims at creating new tissues as alternatives to organ transplants. Our approach is to incorporate cells into biodegradable polymer scaffolds designed to temporarily support new tissue formation. An important requirement of scaffolds is homogeneity. Homogeneity ensures structural integrity, uniform distribution of cells, and uniform porosity throughout the scaffold. Controlling pore sizes is necessary to regulate exchange of nutrients and waste products for cells. Pores too large can provide entryway to immune cells that can harm allogenic cells and developing tissue. We have fabricated three-dimensionally defined, homogeneous, ionically crosslinked alginate gels with a controlled slow-gelation system involving $CaCO_3$ and D-glucono-delta-lactone. We varied the structural parameters and alginate types of these gels to control the diffusion of glucose, vitamin B_{12} and FITC-dextran (molecular weights of 180, 1355 and 9500, respectively) through the gels. Experiments were performed with gel discs placed between side-by-side donor and receptor chambers in a humidified incubator at 37°C. Samples were taken periodically and measured on a UV-Vis spectrophotometer. Generally, diffusion coefficient (D) increased with decreasing solute size. Varying structural parameters of the gels did not have a significant effect on diffusivity of vitamin B_{12}. In contrast, for gels made with a Ca^2 to carboxyl molar ratio of 0.36, D of FITC-dextran increased from $2.88 \times 10^{-7} \pm 0.52 \times 10^{-7}$ to $4.66 \times 10^{-7} \pm 0.48 \times 10^{-7}$ cm^2/sec as alginate concentration decreased from 3.18% to 1.50%, respectively. D of FITC-dextran also increased from $3.17 \times 10^{-7} \pm 0.30 \times 10^{-7}$ to $4.66 \times 10^{-7} \pm 0.48 \times 10^{-7}$ cm^2/sec as crosslinking density decreased for 1.50% alginate gels from a Ca^2 to carboxyl molar ratio of 0.72 to 0.36, respectively. D was highest for alginate gels with the highest guluronic acid content. Controlling diffusivity allows alginate gels with specific properties to be fabricated for tissue engineering scaffolding and other biomedical applications

10:30 AM LL1.6
DEVELOPMENT OF BIODEGRADABLE POLYMER SCAFFOLDS FOR TISSUE ENGINEERING USING CO-EXTRUSION

TECHNIQUES. Newell R. Washburn, Alamgir Karim, Eric J. Amis, National Institute of Standards and Technology, Gaithersburg, MD; Kimberlee Potter, National Institutes of Health, Bethesda, MD.

The development of biodegradable polymeric scaffolds using co-extrusion techniques is presented. Poly(ϵ-caprolactone) (PCL) and poly(ethylene oxide) (PEO) were blended in a twin-screw extruder at 100°C. After subsequent annealing, the PEO was dissolved with water resulting in either open or closed porous materials with characteristic length scales in excess of 100 μm, depending on composition and processing conditions. Mechanical testing indicates the PCL scaffolds have compressive moduli on the order of 1 MPa at low loads. Scaffolds were seeded with osteoblasts isolated from embryonic chick calvariae. Non-invasive assessment of the mineralization process was performed using proton nuclear magnetic resonance microscopy and complementary information was obtained by histologic analyses.

10:45 AM LL1.7
TEMPORARY ENCAPSULATION OF RAT MARROW OSTEOBLASTS IN GELATIN MICROSPHERES FOR BONE TISSUE ENGINEERING. R.G. Payne[1], A.W. Yasko[2], M.J. Yaszemski[3] and A.G. Mikos[1]. [1]Inst. of Biosciences and Bioengineering, Rice University, Houston, TX. [2]Dept. of Orthopaedics, M.D. Anderson Cancer Center, Houston, TX. [3]Dept. of Orthopaedics, Mayo Clinic, Rochester, MN.

We have developed a procedure which may have utility in applications involving cell/polymer constructs. One such application is the delivery of cells as part of an injectable material, such as a poly(propylene fumarate) based composite, for the purpose of bone regeneration. The temporary encapsulation is designed to isolate the cells from environmental effects for a short time period, such as composite curing. After approximately an hour, the gelatin would dissolve into the body fluid surrounding the hardened composite, leaving behind a porous network. The cells would attach to the walls of the pores, allowing them to receive nutrients via diffusion through these pores. Following sufficient bone formation, the composite would degrade and be removed from the body. The purpose of this study was twofold: 1) to determine if rat marrow derived osteoblasts could be encapsulated in gelatin microspheres, and 2) to determine if, after gelatin dissolution, the encapsulated cells retain their phenotypic expression. Briefly, primary rat marrow osteoblasts were suspended in a 10% gelatin solution in tissue culture medium at 37°C. That solution was added dropwise to stirred mineral oil at 10°C. The hydrophobic effect and temperature change caused microspheres to form. The microspheres were then rinsed, filtered, placed into tissue culture wells containing media, and incubated. Preliminary results indicate that the cells were successfully encapsulated, and that mineralization and osteocalcin expression do take place at later time points (i.e. 28 days). Additional studies are being conducted to help determine evolution of viability, proliferation, and phenotypic expression over time. These qualities will be elucidated by analysis of DNA content, ^{3}H-thymidine incorporation, alkaline phosphatase activity, osteocalcin levels, and mineralization of the encapsulated cells with respect to unencapsulated controls. We view temporary cell encapsulation as a promising technique with potential for use in a variety of orthopaedic applications.

11:00 AM LL1.8
DESIGNING BIOMATERIALS FOR USE IN BONE. Kenneth James, Howard Levene, Joachim Kohn, Department of Chemistry, Rutgers University, Piscataway, NJ; J. Russell Parsons, Department of Orthopaedics, UMD-New Jersey Medical School, Newark, NJ.

In a series of homologous, tyrosine-based polycarbonates, small changes in the chemical structure of the polymer pendent chain were found to affect the bone response in a long-term (1280 day) implantation study. Identically sized pins, prepared from poly(DTE carbonate), poly(DTB carbonate), poly(DTH carbonate), and poly(DTO carbonate) were implanted transcortically in the proximal tibia and the distal femur of skeletally mature New Zealand White Rabbits. The tissue response at the bone-implant interface was characterized in terms of the absence of a fibrous capsule (direct bone apposition) or the presence of a fibrous capsule (referred to as the encapsulation response). The relative frequency of direct bone apposition versus encapsulation was recorded for each polymer throughout the entire period of the study and compared with that of identical pins of poly(lactic acid). While all four polymers were tissue compatible, there was a correlation between the chemical structure of the pendent chain and the type of bone response observed, with poly(DTE carbonate) having the highest tendency to elicit direct bone apposition. Based on in vivo degradation data and the ability of model polymers with carboxylate groups at their surface to chelate calcium ions, it is proposed that the ability of poly(DTE carbonate) to elicit "bone apposition" is caused by the facile hydrolysis of the pendent ethyl ester groups which creates calcium ion chelation sites on the polymer surface. The incorporation of calcium chelation sites into the chemical structure of an implant material appears to be a key requirement if direct bone apposition/bone bonding is desired. This study demonstrates that very subtle changes in the chemical composition of an implant material can have significant effects on the long-term tissue response in a clinically relevant model.

11:15 AM LL1.9
A COLLAGEN/HYALURONATE BILAYER MATRIX FOR TISSUE REPAIR. Lin-Shu Liu, Andrea Y. Thompson, Robert C. Spiro, Orquest, Inc. Mountain View, CA.

The composition and structure of the extracellular matrix plays a key role in determining the cellular response that results in the formation of new or reparative tissue. In defects or wounds that involve more than one type of tissue, it would be ideal to have a matrix grafting that can simultaneously support distinct tissue repair requirements. For example, in most joint injuries, matrices with the potential to stimulate both bone and cartilage repair would have a significant clinical impact. We have developed a novel bilayer matrix composed of collagen (COL) and hyaluronate (HA) layers covalently cross-linked by divinyl sulfone. Fetal rat calvarial cells seeded on the matrix showed a distinct cellular morphology in each layer after 4 weeks of in vitro culture. Cells in the HA layer had a round, aggregated, and chondrocyte-like morphology, while those grown in the COL layer were flattened and spread. Biochemical analysis demonstrated that cells in the COL layer expressed high levels of alkaline phosphatase activity (ALP) and low levels of sulfated glycosaminoglycans (GAGs) compared to those in the HA layer. This phenomenon was enhanced by the addition of recombinant human growth and differentiation factor-5 (rhGDF-5). In response to rhGDF-5, cells within the HA layer formed extensive regions of cartilaginous nodules while only small patches of nodules were observed in the COL layer. The rhGDF-5 also increased the level of ALP activity in the COL layer compared to that found in the absence of growth factor or in the in HA layer. Intramuscular implants of the bilayer matrix with rhGDF-5 retrieved at 28 days revealed the presence of bone and cartilage tissue in the COL and HA layers, respectively. These results demonstrate that the differentiation of cells within distinct regions of the bilayer matrix can be influenced by specific compositional changes.

11:30 AM *LL1.10
RECONSTITUTING THE STRUCTURE AND INFORMATION CONTENT OF ACTUAL TISSUE SCAFFOLDS. Eugene Bell, Tissue Engineering, Inc.

ABSTRACT NOT AVAILABLE

SESSION LL2: DENTAL/MAXILLOFACIAL BIOMATERIALS
Chair: John Brunski
Monday Afternoon, November 27, 2000
Berkeley (Sheraton)

1:30 PM *LL2.1
OVERVIEW OF DEVICE RETRIEVAL AND ANALYSIS. Jack Lemons, University of Alabama at Birmingham, Department of Dental Biomaterials, Birmingham, AL.

Surgical implant devices constructed from synthetic origin biomaterials (metallics, polymerics, ceramics and composites of these) continue to be utilized in large numbers for musculoskeletal surgical reconstructions. A number of research laboratories conduct investigations to analyze characteristics of devices explanted at revision or removal surgery. The device is initially classified in terms of type, the role of the clinical history (from MD or DMD collaboration), and any observed surface or bulk characteristics that indicate device alterations associated with in vivo function. If unanticipated alterations are noted, more detailed in vitro studies are conducted after confirming adequate sample, source confidentiality and available resources. Approximately 5000 explanted devices have been evaluated in our laboratory since 1970 and current receipts are several hundred per year with the primary sources being orthopaedic and dental. Study outcomes will be reviewed in general, followed by selected examples of anticipated outcomes (Tier I), where device changes are found that have not influenced clinical outcome (Tier II) and unanticipated outcomes where in vitro studies have been conducted (Tier III).

2:00 PM LL2.2
DESIGN AND CHARACTERIZATION OF NEW Ti-Ag AND Ti-Ag-Sn ALLOYS FOR CRANIO-MAXILLO-FACIAL PROSTHESES MADE BY THREE-DIMENSIONAL PRINTING. Sang-Bum Hong, Harvard School of Dental Medicine, Boston, MA; Noam Eliaz, Gary G. Leisk, Ronald M. Latanision, Massachusetts Institute of Technology, H.H. Uhlig Corrosion Laboratory, Cambridge,

MA; Emanuel M. Sachs, Massachusetts Institute of Technology, Dept of Mechanical Engineering, Cambridge, MA; Samuel M. Allen, Massachusetts Institute of Technology, Dept of Materials Science and Engineering, Cambridge, MA.

New Ti-5Ag and Ti-5Ag-35Sn (wt.%) alloys were designed, synthesized by three-dimensional printing ($3DP^{TM}$), and characterized by means of microscopic observations, microhardness and electrochemical tests, and surface analysis. Silver nitrate was found to be an appropriate inorganic reactive binder for atomized titanium powder. The optimum parameters to allow densification of the printed material through sintering and liquid-Sn infiltration were determined for the Ti-Ag and Ti-Ag-Sn alloys, respectively. While the Ti-Ag alloy exhibited superior corrosion and mechanical behavior to the Ti-Ag-Sn alloy, the latter showed better dimensional stability. The applicability of $3DP^{TM}$ for fabricating complex cranio-maxillo-facial (and other) prostheses is discussed in detail.

2:15 PM LL2.3
STRUCTURE-NANOMECHANICAL PROPERTY STUDY IN ENAMEL USING MOLECULAR APPROACHES VIA BIOMIMETICS. Hanson Fong and Mehmet Sarikaya, Materials Science and Engineering, University of Washington, WA; Michael L Paine, Wen Luo and Malcolm L. Snead, The Center for Craniofacial Molecular Biology, School of Dentistry, The University of Southern California, Los Angeles, CA; Shane N. White, School of Dentistry, University of California, Los Angeles, CA.

Enamel is the unique composite material forming the outer covering of vertebrate teeth. It is the hardest tissue in the vertebrate body, containing long-, thin-crystallites of substituted hydroxyapatite (HAP). Enamel functions under immense loads, in a wet, bacterial-laden environment generally without catastrophic failure. Unlike most other bio-formed minerals, which are mesoderm in origin, enamel is elaborated by ectoderm derived cells called ameloblasts. We have focused our investigations on the formation of enamel using cell and molecular approaches and by coupling findings from these techniques to biomechanical investigations at the mesoscale. We have employed Koch's postulates to ascertain the role of amelogenin protein by creating "gain of function" and "loss of function" transgenic animal models. For amelogenin, the principle protein of forming enamel, we have identified two domains. We engineered amelogenin containing deletions to either of these two domains, and investigated enamel formation in transgenic mice. Morphological, structural and crystallographic variations were correlated to nano-mechanical properties of mouse teeth by electron and atomic force microscopy (nano-indentation) techniques. We found that there are significant changes in the mineralization behavior of the teeth generated using these domain deletions that correspond to decrease in mechanical performance. For example, nanohardness decreases by as much as 20% while elastic modulus up to 15% in comparison with nontransgenic teeth. The formation of enamel is an intricate biomineralization process requiring proper formation and assembly of amelogenin. Failure to do so results in altered processes leading to incomplete biomineralization of the hard tissue with inferior mechanical properties.
Supported by grants from the NIH, The National Institute of Dental and Craniofacial Research.

2:30 PM LL2.4
IN VIVO STUDY OF BIOACTIVE GLASS PASTE IN MOLARS OF MINI-PIGS. Sarah E. Efflandt, Lorraine F. Francis, Univ of Minnesota, Dept of Chemical Engineering and Materials Science, Minneapolis, MN; Manuela Lopes, Jorge Perdigao, William H. Douglas, Univ of Minnesota, Minnesota Dental Research Ctr for Biomaterials & Biomechanics, Minneapolis, MN; Ching-Chang Ko, Univ of Minnesota, Dept of Oral Science, Minneapolis, MN.

Bioactive materials are commonly used as implants in the fields of orthopaedics and dentistry because of their reaction with the biological environment leading to a contiguous bond with bone. The aim of this research is to explore these bioactive materials for use in dental restorations. Our previous results show an interaction between dentin from extracted human teeth and bioactive glasses leading to adherence. To study this phenomena further, $MgO\text{-}CaO\text{-}P_2O_5\text{-}SiO_2$ glass powder was placed in the molars of mini-pigs to determine its interaction with dentin in vivo. Powder mixed with water was placed into a cavity which was then sealed using a light-cured composite filling (Scotchbond, 3M company). After 30 days, the molars were extracted, fixed and dried using hexamethlydisilazane. The filled cavities were then cut down the center to reveal the cross-section and studied using SEM, TEM, chemical and x-ray analyses. Microscopy confirms the bioactive paste was still in place after sample preparation and was intimately associated with the dentin; however, electron microprobe and microdiffraction data reveal no certain apatite formation on the bioactive powder in vivo. Lack of accessability to supersaturated biological fluids (e.g. saliva) is the probable cause.

3:15 PM LL2.5
APATITE GROWTH ON BIOACTIVE GLASSES IN ARTIFICIAL SALIVA. Sarah E. Efflandt, Robert F. Cook, Lorraine F. Francis, University of Minnesota, Dept of Chemical Engineering and Materials Science, Minneapolis, MN.

Bioactive materials have been widely researched for orthopaedics and dental implants because of their ability to bond to bone. When bioactive materials are placed against bone in vivo, they form a layer of apatite allowing adjacent bone growth. The formation of an apatite layer on soaking in simulated body fluid correlates with these in vivo results. Like bones, teeth are primarily composed of hydroxyapatite. Our results show dentin from extracted human teeth adheres to bioactive glasses when soaked in saliva at 37°C. To understand this result further, bioactive glasses in the $Na_2O\text{-}CaO\text{-}P_2O_5\text{-}SiO_2$ and $MgO\text{-}CaO\text{-}P_2O_5\text{-}SiO_2$ systems were soaked in artificial saliva and the growth of crystalline apatite on their surfaces monitored. Apatite growth over soaking times varying from 1 to 42 days was assessed as well as the effects of three different glass surface finishes. Samples were analyzed using x-ray diffraction, ion beam scattering, infrared spectroscopy and electron and optical microscopy. X-ray data reveals apatite peaks on the Na_2O-containing glass as early as 10 d of exposure with continuous evolution of more crystalline material through 42 d; in contrast, apatite peaks are not apparent on the MgO-containing glass until 42 d. The most interesting observation is that apatite development was greatest on the surfaces of intermediate roughness, implying competition between nucleation and growth on the glass surface. In addition to the difference in the rate of apatite development, the morphology of the apatite layer was different for the two glasses: micro-crystalline and plate-like for the Na_2O and MgO-containing glasses, respectively.

3:30 PM LL2.6
SYNTHESIS AND CHARACTERISATION OF HYDROXYAPATITE FROM MELLITE EDUARDO BARROSOI SP. NOV. AND MONETITE. V. Rodriguez-Lugo, G.A. Camacho-Bragado, J.A. Ascencio, C. Angeles-Chavez, V.M. Castao.

In the present work we present the theoretical and experimental results from the synthesis of hydroxilapatite by using natural precursors. Mellite Eduardo Barrosoi sp. Nov, was used as raw material in the synthesis of the precursors: Calcium Oxide (CaO), Calcium Hydroxide (COH) and Calcite ($CaCO_3$). The reaction among the precursors was carried out under hydrothermal conditions. Two distinct pressures were selected, 1.4 MPa and 6.5 MPa. The reaction time was varied from 2, 4, 6, 8 10 to 20 hours. In order to compare the products of the stoichometric reaction and the effect of adding an excess of CaO, both sets of products were characterised by XRD, LV-SEM, EDS, FTIR and TEM. One observed several crystalline habits such as fibre-like shapes with diameters from 1 to 3mm and lengths of 36 mm, needle-like structures of 0.9 mm of diameter and 8 mm of length, those habits correspond to hydroxyapatite and portlandite. Calcite and monetite were identified by XRD demonstrating the yield dependence on the reaction conditions. The gradual formation of hydroxyapatite is studied by comparison of the experimental results with the theoretical calculations obtained by molecular simulation.

3:45 PM LL2.7
ATOMIC SCALE INTERFACIAL STRUCTURE OF HYDROXY-APATITE: HIGH-RESOLUTION TRANSMISSION ELECTRON MICROSCOPY. Kimiyasu Sato[1], Toshihiro Kogure[1,2], Toshiyuki Ikoma[1,3], Yuri Kumagai[1] and Junzo Tanaka[1,3]. [1]CREST, Japan Science and Technology, Saitama, JAPAN. [2]Department of Earth and Planetary Science, Graduate School of Science, University of Tokyo, Tokyo, JAPAN. [3]National Institute for Research in Inorganic Materials, Ibaraki, JAPAN.

Hydroxyapatite (HAp) is a main mineral component of bone and tooth, and is widely used in orthopaedic, dental and industrial fields. To achieve the design of novel bioactive materials by combining HAp with organic substances, a thorough understanding of the surface structure of HAp is fundamentally important. HAp crystals are often facetted with six equivalent faces, expressed as {100} planes in hexagonal symmetry. We tried to elucidate the atomic configuration of the {100} faces in stoichiometric HAp crystals by high-resolution transmission electron microscopy (HRTEM). We prepared carbonate-free and almost stoichiometric HAp sintered body. HAp studied has been proved to have monoclinic symmetry, because ordered hydroxyl ions lower the symmetry of the crystal structure. As crystallographic difference between hexagonal and monoclinic symmetries is, however, quite insignificant, the crystal structure is formally described by the hexagonal symmetry in the present paper. The sintered HAp was polished mechanically and thinned by argon ion-milling; HRTEM observations were performed at 200 kV using a Hitachi HF-2000. To determine the atomic configuration, HRTEM

images were compared with calculated images obtained by multislice image simulations. Grain boundaries with {100} planes were observed from the [001] direction. The HAp grains had interfaces crossing hydroxyl columns, where existed PO_4 tetrahedra and Ca(2) ions. When specimens were subjected to electron beam radiation for a long duration, HAp crystals partially vitrified. According to TEM observations from the [001] direction, the vitrified regions took roughly hexagonal shapes surrounded with {100} planes. The interfacial structures between crystalline and amorphous regions were identical to that observed at the grain boundaries. As both grain boundaries and crystalline/amorphous interfaces were terminated with the {100} faces of the same atomic configuration, such structure was conjectured to be essential for inter-atomic bonds at the HAp crystal interfaces, and gives us a fundamental design concept for organic/HAp composites.

4:00 PM LL2.8
THE NANOSCALE MECHANICAL PROPERTIES OF HUMAN MOLAR DENTAL ENAMEL. Adrian B. Mann, Suhn Kim, Janet Cuy, Ken Livi, Mark Teaford, Timothy P. Weihs, The Johns Hopkins University, Dept of Materials Science and Engineering, Baltimore, MD.

The mechanical behavior of dental enamel has been the subject of many investigations. These initially assumed the tooth enamel was a more or less homogeneous material with uniform mechanical properties. Now it is generally recognized that the mechanical response of the enamel depends upon location, prism orientation and chemical composition. This study used nanoindentation testing to map out the properties of dental enamel over the axial cross-section of an upper 2^{nd} molar. The local variations in mechanical characteristics are correlated with changes in microstructure and chemical content across the cross-sectioned enamel sample. SEM is used to examine the microstructure and microprobe techniques are used to examine changes in chemical composition. The ranges of hardness (H) and elastic modulus (E) observed over an individual tooth have been found to be far greater than previously reported. At the enamel surface H > 6 GPa and E >115 GPa, while at the amelodentinal junction H < 3GPa and E < 70 GPa. These variations correspond closely to variations in the prism orientation. At the enamel surface the prisms axial positions are generally perpendicular to the occlusal surface, while at the amelodentinal junction they are more scattered in orientation with many lying parallel to the occlusal surface. The chemical composition of the enamel was also found to vary in a similar manner to the mechanical properties with the constituents of hydroxyapatite (P_2O_5 and CaO) and chlorine highest at the surface. There was no discernible trend in fluorine content. By the amelodentinal junction the amount of Na_2O and MgO were found to increase. Intriguingly, on the buccal side of the tooth, at the occlusal surface, the enamel was harder and more elastic than on the lingual side. The interior enamel, however, was softer and more compliant on the buccal side than the lingual side.

4:15 PM LL2.9
MAXILLOFACIAL IMPLANTS OF POLARIZED HYDROXY-APATITE PLASMA-SPRAY-COATING TITANIUM. Hideki Sagawa, Takayuki Kobayashi, Masato Ueshima, Satoshi Nakamura, Masataka Ohgaki, Kimihiro Yamashita, Tokyo Medical and Dental Univ, Inst of Biomaterials and Bioengineering, Tokyo, JAPAN.

We have proved that sintered hydroxyapatite (HAp) ceramics are electrically polarizable and that the polarized HAp provokes characteristic interactions with bone tissues both on the positively and the negatively charged surfaces. In this research, electrical polarizability of plasma-sprayed HAp coatings on titanium was demonstrated by thermally stimulated current (TSC) measurement. Moreover, the biological reactions of the polarized HAp coatings were investigated using bones of beagle dogs. The HAp layer with a thickness of 30 μm was coated on titanium by plasma-spray techniques. The HAp coated titanium was electrically polarized in a dc field of 1 $kVcm^{-1}$ at 300°C. The samples were implanted into the defects formed on bones by dental burs. The bones were extracted at 1, 2 and 4 weeks after the administration and fixed with 20% neutral buffered formalin solution. The bone tissue sections were observed by light microscopy and scanning electron microscopy. The electrical storage of the polarized HAp coatings was clearly detected by TSC measurement. The new bone formation in contact with the N-surface was observed at 1 week after the operation. The bone formation in the vicinity of the negatively charged surface (N-surface) was significantly promoted compared to that of the non-polarized HAp coating surface. The histological results suggest that the electrical polarization treatment is effective for osteoconductivity improvement of the HAp coating layers and confidently predicted that the polarization technique is applicable to maxillofacial implantation systems of HAp plasma-spray coated titanium.

4:30 PM LL2.10
DESIGNING MONOLAYER DENTAL CERAMIC CROWN STRUCTURES. Yan Deng, Hae-Won Kim, Young-Woo Rhee and Brian R. Lawn, National Institute of Standards and Technology, Materials Science and Engineering Laboratory, Gaithersburg, MD.

Simple relations are derived for the onset of competing fracture modes in ceramic coatings on compliant substrates from Hertzian-like contacts. These structures are intended to simulate the basic features of ceramic crown systems in oral function. Special attention is given to a deleterious mode of radial cracking that initiates at the lower coating surface beneath the contact, believed responsible for the bulk of all-ceramic crown failures. Critical load relations for the onset of cracking are expressed in terms of well-documented material parameterselastic modulus, toughness and hardness; and geometrical parameterscoating thickness and sphere radius. Data from selected ceramic coating materials on polycarbonate substrates are used to demonstrate the validity of the relations. The formulation provides a basis for designing ceramic crowns with optimum damage resistance.

4:45 PM LL2.11
CONTACT DAMAGE MODES IN MULTILAYERED STRUCTURES FOR DENTAL APPLICATIONS. Pedro Miranda, Fernando Guiberteau, Universidad de Extremadura, Dept Electrónica e Ingeniería Electromecánica, Badajoz, SPAIN; Antonia Pajares, Francisco L. Cumbrera, Universidad de Extremadura, Dept Física, Badajoz, SPAIN; Brian R. Lawn, Materials Science and Engineering Laboratory, National Institute of Standards and Technology, Gaithersburg, MD.

We have characterized contact damage modes induced in model brittle bilayer coatings on soft substrates that emulate the essential trilayer structure and material properties of dental crowns. To simulate occlusal conditions we have conducted indentation tests using hard spheres as indenters, over clinically relevant loads. Experiments have been performed on model transparent trilayer structures, to enable in situ observation during the contact. Individual layer surfaces are preferentially abraded to introduce uniform flaw states and so allow each crack type to be studied separately and controllably. Fracture occurs by cone cracking at the top surface or by radial cracking at the bottom surface of the brittle coating layers. Critical loads for crack initiation are measured as a function of layer thickness and elastic modulus mismatch. Finite element analysis (FEA) has been used to calculate contact tensile and shear stress fields in the layer structure using ABAQUS codes, with material parameter input (modulus, yield stress and work hardening, strength) obtained from independent indentation tests on monolithic materials. Fracture criteria are imposed to calculate critical loads for each crack mode for comparison with experimental values. The results yield functional fracture mechanics relations for the critical loads, providing the basis for design of trilayer systems with optimal damage thresholds.

SESSION LL3: ORTHOPAEDIC BIOMATERIALS
Chair: Orhun K. Muratoglu
Tuesday Morning, November 28, 2000
Berkeley (Sheraton)

8:30 AM *LL3.1
Harry Rubash, Massachusetts General Hospital, Boston, MA.

ABSTRACT NOT AVAILABLE

9:00 AM LL3.2
STRUCTURE AND PROPERTIES OF A NEW NANOCOMPOSITE POLY(METHYL METHACRYLATE) BONE CEMENT. Anuj Bellare, Andreas Gomoll, Wolfgang Fitz, Richard D. Scott, Thomas S. Thornhill, Dept of Orthopaedic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; David A. Baker, Lisa A. Pruitt, Dept of Mechanical Engineering, University of California at Berkeley, Berkeley, CA.

Orthopaedic bone cement is widely used for fixation of total joint replacement prostheses. These cements usually comprise a pre-polymerized poly(methyl methacrylate) or a poly (methyl methacrylate-co-styrene) random copolymer powder and a methyl methacrylate liquid component. The powder component also contains a reaction initiator to initiate setting when the two components are mixed. In addition, micrometer size filler particles of either barium sulfate or zirconium oxide are present in the powder to radiopacify the cement, thereby enabling the orthopedic surgeon to monitor fractures in the implanted cement using x-ray radiographs. Incomplete dispersion of the radiopacifier particles during mixing of the two components is known to result in the formation of particle agglomerates of 50-200 micrometer diameter. These large defects can reduce the fracture toughness of PMMA cements, leading to early

fracture of the cement and loosening of the implant, ultimately necessitating early revision surgery to replace the implant. In this study, the micrometer size radiopacifier barium sulfate particles were replaced by commercially available nano-sized (100 nm diameter) barium sulfate radiopacifying particles. The resulting nanocomposite PMMA bone cements were thereafter characterized using low voltage high resolution scanning electron microscopy, and by ultra-small angle x-ray scattering at the UNICAT beamline of the Advanced Photon Source, Argonne National Laboratory. The nanocomposite and microcomposite PMMA bone cements were thereafter subjected to uniaxial tensile and fatigue tests. These tests revealed a higher work-of-fracture and number of cycles to failure, respectively, of the nanocomposite cement. These results, the morphological observations and the structure-property relationships governing fracture toughness of PMMA cements will be presented.

9:15 AM LL3.3
COMPARATIVE EARLY STAGE MINERALIZATION ADJACENT TO PROSTHETIC IMPLANTS IN CANINE- AND HUMAN-MODEL BONE EXPLANTS. Alexandra Porter, Cambridge University, Dept of Materials Science & Metallurgy, Cambridge, UNITED KINGDOM; Melanie J. Coathup and Gordon W. Blunn, Royal Orthopaedic Hospital, Biomedical Engineering Centre, Stanmore, Middx, UNITED KINGDOM; Myron Spector, Brigham & Womens Hospital and Harvard Medical School, Department of Orthopaedic Research, Boston, MA; Linn W. Hobbs, MIT, Dept of Materials Science & Engineering, Cambridge, MA.

Explants of femoral bone containing hydroxyapatite(HA)-coated implants from canine and human models have been studied to compare the course of early-stage mineralization of new bone forming adjacent to the coated implants. The canine explants were from distal femur sites implanted with 6-mm HA-coated Ti-6Al-4V rods for periods between 2 hours and 14 days. The human explants come from an inventory of 90 hip replacements involving HA-coated femoral stems which were retrieved at autopsy; the shortest residence time was 14 days. Explants were fixed in formalin and embedded in PMMA, mechanically cross-sectioned, then embedded in epoxy and microtomed for transmission electron microscopy (TEM) in the vicinity of the bone/implant interface. Diffraction contrast and high-resolution TEM were used to study bioapatitic mineral nucleation and subsequent mineralization of the extra-cellular matrix, while heavy metal stains were used to locate fibrous proteins in the interface region. The canine model studies revealed that HA platelet precipitation occurs as early as 2 hours without apparent foci and cluster nucleation of HA ribbons within 3 days; collagen invades by 10 days and directs the subsequent mineralization of HA platelets which appear to nucleate in the hole zones of the collagen molecular fibril assemblies. Results from the ongoing human explant studies will be compared.

9:30 AM LL3.4
BIOLOGICAL REACTION OF POLARIZED HYDROXYAPATITE PROSTHESIS IN BONE MARROW. Tomoko Sakai[1,2], Takayuki Kobayashi[1], Masato Ueshima[1], Satoshi Nakamura[1], Sadao Morita[2], Ken-ichi Shinomiya[2], Kimihiro Yamashita[1], Tokyo Medical and Dental Univ. [1]Institute of Biomaterials and Bioengineering. [2]Dept of Orthopedics, Tokyo, JAPAN.

We have already reported that hydroxyapatite (HAp) is electrically polarizable and that the higher rate of bioactivity is attained on the polarized HAp. The objective of this study was to evaluate the electrically polarized HAp surface by histological examination in vivo. HAp disks with a size of 10 mm diameter x 1 mm thickness were prepared. These specimens were cramped with Pt plates, heated up to 300°C, then subjected to the polarization treatment in a dc field of 1 $kVcm^{-1}$. As a control, HAp disks without the polarization treatment were used. The femora of six Japanese white rabbits were fenestrated and the samples were implanted. The rabbits were sacrificed at 1, 2 and 4 weeks after the operation and the samples were harvested with the surrounding bones. The specimens of femora were fixed and examined by optical microscopy and scanning electron microscopy. The positively charged surface (P-surface) demonstrated the obvious new bone formation at 2 weeks after the operation. On the other hand, the new bone formation appeared on the surface of the non-polarized hydroxyapatite at 4 weeks after the operation. The negatively charged surface also demonstrated the new bone formation at 2 weeks after the operation, though the thick and direct bone attachment was extensively recognized on the P-surface. These facts showed that the surfaces of the electrically polarized HAp have the different osteoconductivities from the conventional HAp. The earlier new bone formation appeared on the both surfaces of the polarized HAp. These findings suggest the electrical polarization can accelerate the new bone formation on HAp surfaces and will be of great benefit to the bone regeneration in orthopedic surgery.

10:15 AM LL3.5
STRENGTH AND TOUGHNESS OF AN APATITE CEMENT. Victoria C. Jew, Reinhold H. Dauskardt, Stanford Univ, Dept of Materials Science and Engineering, Stanford, CA.

Apatitic materials resembling bone mineral have received considerable attention due to their biocompatible and osteoconductive properties. Particularly when formed cementitiously at physiological temperature, they present significant potential for orthopaedic applications such as rapid bone repair, fracture fixation, and augmentation of load bearing hardware. To date, the strength and resistance to fracture of such apatites have been extremely low, limiting their use in load-bearing applications. To better characterize the mechanical properties of a hydroxyapatite bone cement, this study investigates its fracture toughness and flexure strength, as well as the mechanisms of failure. Strategies to enhance the mechanical properties of this bone cement are also examined, utilizing the addition of organic phases such as collagen. Second phase additions are found to increase cement strength and reliability. Micromechanical models have been developed to account for these strengthening and toughening effects. Implications for the integrity and reliability behavior of such synthetic bone mineral substitutes in load bearing applications are considered.

10:30 AM LL3.6
INFLUENCE OF HIGH SPEED MIXING ON AGGLOMERATION OF NANOMETER BARIUM SULFATE IN BONE CEMENT. Wolfgang Fitz, Andreas Gomoll, Anuj Bellare, Richard D. Scott, Thomas S. Thornhill Orthopaedic Research Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Bone cements revolutionized implant fixation of the low friction arthroplasty introduced by Sir Charnley in the mid 1950s. The bone cement attaches the implant to the bone and transfers mainly cyclic loading during most normal daily activities between the artificial implant and the bone. However, there is the current consensus that mechanical failure of the bone cement is the leading cause of aseptic loosening. A comparison of radiolucent bone cement with cements with different percentages of barium sulphate resulted in an almost two-fold reduction of impact strength of different bone cements with different amounts of radiopacifiers added. It is our opinion, that the presence of radiopaque fillers weakens the interbead matrix. Either a nonuniform particle distribution, agglomeration of the radiopacifiers or the presence itself may be the cause for the inferior mechanical properties. Our hypothesis is that a uniform dispersion of barium sulphate within the interbead matrix will not weaken the mechanical performance compared to radiolucent cement. Furthermore using principals of the emerging nanotechnology we hope to strengthen the fracture toughness with the use of nanometer sized barium sulphate particles compared to radiolucent bone cement and even surpass its mechanical properties without changing the composition, setting time and handling characteristics of this new nanocomposite acrylic bone cement. We claim that agglomeration of radiopacifiers can be prevented with high speed mixing of small uncoated barium sulfate particles with an average particle size of 100 nm, without significantly changing viscosity and setting times. Fractographic analysis will be used to demonstrate the beneficial effect of high speed mixing of nanometer sized radiopacifier compared to commercial formulas and radiolucent cement. . In this study an experimental high-speed mixed nano-composite cement is compared to vacuum mixed Simplex P, CMW1 (radio-opaque and radiolucent) and high-speed mixed Simplex P. JEOL 6320FV low voltage scanning electron microscope (LVHRSEM) examined the surface fracture of all cured cements. Agglomeration of barium sulfate fillers were not seen in the experimental nanometer-cement as compared to the commercial formulas. However we observed a uniform dispersion of the 100 nm large barium sulfate particles. High speed mixing of nanometer sized radiopacifier prevails a uniform dispersion with no agglomeration and no significant changes in viscosity and setting time. Further mechanical testing will provide the benefit of this new nanocomposite bone cement.

10:45 AM LL3.7
CONDITIONS LEADING TO INCREASED INTERFACIAL POROSITY IN CEMENTED FEMORAL STEMS. Stephen Spiegelberg, Cambridge Polymer Group, Inc., Somerville, MA.

This paper examines the dependence of the rheological properties of bone cement on the femoral stem-bone cement interfacial porosity in total hip and knee replacements. A study by James et al. (1993) showed that interfacial porosity from in vivo and in vitro components did not vary with surface finish, stem material, or centrifugation. They concluded that the rheological, or flow, behavior of the bone cement could explain the presence of the pores. We present a study that combines bone cement rheometry and stem insertion studies that fully characterizes the cure behavior of bone cement, and provides a quantitative analytical approach to explain the conditions that will lead to femoral stem-cement interfacial porosity. It was proposed that

the level of porosity at the cement-femoral stem interface is a function of the advancing contact angle at the bone cement wetting line, which in turn is a function of the stem insertion rate and the response time of the bone cement. This latter relationship is known by the dimensionless parameter the Deborah number. The response time of the cement, which varies with cure time, was measured with shear rheometry. Model stem insertion studies conducted at different cure times and insertion velocities provided a range of Deborah numbers. The stem-cavity samples were sectioned and examined with light microscopy to quantify the interfacial porosity. The micrographs show that the interfacial porosity increases as the Deborah number increases. This increase in Deborah number is accomplished by either increasing the stem insertion rate, or by waiting longer during the cure process before stem insertion, which yields a higher relaxation time for the bone cement. These results indicate the operating window, based on time during cure and stem insertion rate, by which a surgeon can minimize interfacial porosity.

11:00 AM LL3.8
BIOACTIVITY RESPONSES OF DIFFERENT UHMWPE PARTICLES FROM IN-VIVO AND IN-VITRO TESTS.
YoungSoo Park, Stephen Hsu, National Institute of Standards and Technology, Gaithersburg, MD; Sang Y. Yang, P. Wooley, Wayne State University, Detroit, MI; K. Merritt, U.S. Food and Drug Administration, Rockville, MD; Michael J. McNallan, University of Illinois at Chicago, Chicago, IL.

The ultra-high molecular weight polyethylene(UHMWPE) wear debris is generated from articulating surfaces in total joint replacements. The etiology of aseptic loosening remains unclear, but the generation of small wear particles from the prosthetic components is known to contribute to this pathology. These particles stimulate local cellular responses, and provoke inflammation, the release of soluble factors including cytokines, and subsequent bone resorption. The hypothesis is that different wear particle size and shape may have different activity in provoking cellular and cytokine responses. Different UHMWPE wear particles can be generated using metal counter surfaces having textures. Two textures, one with cross-hatched and the other with uni-hatched grooves, were employed for particle generation by the pin-on-flat wear tester using UHMWPE pins (GUR415, extruded, gamma irradiated with 25 kGy in air). These two textured surfaces produced two distinct populations of wear particles. One is larger and more elongated (fibril shapes) than the other. The mean sizes and aspect ratios of the particles are at the range of 5 μm to 25 μm and about 1.5 to 3 respectively. These particles were produced in a sterilized environment and no further sterilization steps were taken to preserve the as produced wear particle characteristics. In this study, generation of UHMWPE particles as well as the nature of the cellular and cytokine responses to wear particles was examined through in-vitro and in-vivo tests. Macrophages RAW 264.7 and the murine air-pouch model of inflammation were employed to characterize the effect on the particle size and shape. Once a preliminary understanding of the relationship between particle (size and shape) and bioactivity is developed, new particle generation processes that minimize the production of specific sizes and shapes may be developed for more extensive tests.

11:15 AM LL3.9
MECHANISM OF OSTEOGENESIS AROUND POLARIZED HYDROXYAPATITE IMPLANTS.
Takayuki Kobayashi, Satoshi Nakamura, Masato Ueshima, Kimihiro Yamashita, Tokyo Medical and Dental Univ, Inst of Biomaterials and Bioengineering, Tokyo, JAPAN.

It has been reported that the electrical charges induced on hydroxyapatite (HAp) ceramics by electrical polarization accomplish acceleration or deceleration of the bone-like crystal overgrowth in simulated body fluid. In the present study, We confirmed that acceleration effect of the polarized HAp on new bone growth by biological estimations. The mechanism of osteogenetic enhancement was also discussed histologically. Rectangular HAp ceramic blocks were cramped with a pair of electrode plates and polarized in a dc field of $1 kVcm^{-1}S$ for 1 hour. The negatively and the positively charged HAp surfaces were indicated as N-surface and P-surface, respectively. The surfaces of samples without polarization were named 0-surface. The electrically polarized HAp samples were implanted in femoral and tibial bones of beagle dogs. The bones containing the HAp samples were extracted on 7, 14 and 28 days after the implantation. The formation of new thick bones with osteoblast layer was observed on the N-surface 7 days after the implantation. On the contrary, the newly formed bones observed around the P-surface did not directly contact the HAp surface. The thickness of the new bone contacted the N-surface on 14 days increased 2 times more than that of 7 days after the implantation. In the vicinity of the P-surface, the new bone formation was developed and directly contacted parts of the matured bone surfaces on 14 days. A small volume of new bone directly contacted the 0-surface on 14 days. The N-surface of the polarized HAp promoted the new bone formation in the neighboring territories, compared with the P- and 0-surfaces of HAp. It was suggested that the negative charges on the polarized HAp activated osseous cells and induced them to contact on the N-surface.

11:30 AM *LL3.10
EVALUATION OF ARTIFICIAL KNEE JOINTS.
Peter Walker, Royal National Orthopaedic Hospital Trust.

ABSTRACT NOT AVAILABLE

SESSION LL4: MUSCULOSKELETAL BIOMATERIALS
Chair: Warren O. Haggard
Tuesday Afternoon, November 28, 2000
Berkeley (Sheraton)

1:30 PM *LL4.1
MUSCULOSKELETAL TISSUE ENGINEERING: CLINICAL AND BIOMATERIALS CONSIDERATIONS.
Mike Yaszemski, Mayo Clinic.

ABSTRACT NOT AVAILABLE

2:00 PM LL4.2
STEREOLITHOGRAPHIC PROCESSING OF CERAMIC/ ORGANIC COMPOSITES FOR ORTHOPAEDICS.
Jim H. Lee, Robert K. Prud'homme and Ilhan A. Aksay, Princeton University, Dept. of Chemical Engineering and Princeton Materials Institute, Princeton, NJ.

Ceramic stereolithography (CSL) is used to fabricate complex-shaped ceramic/organic composites by laser photocuring a concentrated ceramic dispersion in photocuring solutions layer-by-layer. The main processing parameters in CSL such as layer thickness, resolution, hatch spacing, and overcure depend on the knowledge of light propagation in concentrated multiple scattering dispersion. By incorporating biocompatible polymers as the matrix phase, biomaterials with controlled microstructure may be constructed. In studies dealing with the processing of these reinforced biocompsosites, we investigated the depth of curing for model resin systems as a function of photoinitiator concentration. Alumina (Al_2O_3) was used as the reinforcing phase in a matrix of *2,2-bis(4-(2-hydroxy-3-methacryloxypropoxy)phenyl) propane* (Bis-GMA), a commonly used monomer in dental fillings. Free radical polymerization was initiated with *2-benzyl-2-N,N-dimethylamino-1-(4-morpholinophenyl)-1-butanone* (DBMP). An optimal photoinitiator concentration that maximized the gel cure depth was observed. Two regimes were shown to exist in which the swell ratio was minimized or maximized. The study showed that photoinitiator plays a significant role in controlling the quality and performance of the formed gel network, with special regard to thickness of cured layers. This holds strong implications not only for stereolithographic production of macroscale biocomposites, but for dental restorations in which coatings are photocured, as well.

2:15 PM LL4.3
PROCESSING AND PROPERTIES OF NANOSTRUCTURED HYDROXYAPATITE-BASED BIOMATERIALS FOR ORTHOPEDIC APPLICATIONS.
Edward Ahn, Jackie Ying, Massachusetts Institute of Technology, Dept of Chemical Engineering, Cambridge, MA; Myron Spector, Harvard Medical School, Dept of Orthopedic Surgery, Boston, MA.

Hydroxyapatite (HAP) has generated great interest in the search for advanced orthopedic and dental implant materials as it elicits a favorable biological response. However, it is sensitive to non-stoichiometry and impurities in its synthesis and processing. Thus, conventionally processed HAP materials often lack phase purity and homogeneity, resulting in poor mechanical properties. Nanostructure processing is employed for microstructural control to achieve the desired mechanical characteristics for HAP. It also further enhances surface reactivity to promote tissue integration. Through nanostructure processing, superior chemical homogeneity, microstructural uniformity, and ultrafine grain sizes (125 nm) have been achieved so that flaw sizes can be significantly reduced in fully dense HAP monoliths. As a result, nanostructured HAP monoliths provide for superior compressive (900 MPa) and bending (200 MPa) strengths and fracture toughness (1.3 $MPa*m^{1/2}$). Nanocomposite processing is also utilized to further improve mechanical properties by dispersing zirconia nanoparticles in the HAP matrix. The effects of yttria-stabilized zirconia dispersions and unstabilized zirconia dispersions on the mechanical properties have been investigated. The incorporation of highly dispersed unstabilized zirconia (3 wt%) significantly increased the fracture toughness (2.0 $MPa*m^{1/2}$) and bending strength (280 MPa) of our HAP-based systems. Our high strength, nanostructured HAP monoliths also provide a model two-dimensional

surface to examine the effect of microstructure and surface chemistry on MC3T3 adhesion, proliferation, and mineralization. Surface properties were evaluated by X-ray photoelectron spectroscopy and by contact angle measurements prior to cell culture. Protein adsorption isotherms using fibronectin and vitronectin were measured, and an atomic force microscopy was used to determine whether proteins were localized along the grain boundaries. Our studies indicated that high volume fraction of grain boundaries increased cell adhesion, proliferation, and mineralization relative to coarse-grained HAP (~3 micron grains) and Ti6Al4V.

2:30 PM LL4.4
MICROPATTERNED SURFACES MODIFIED WITH SELECT PEPTIDES PROMOTE EXCLUSIVE INTERACTIONS WITH OSTEOBLASTS. M.E. Hasenbein, S.C. Deslandes, T.T. Andersen*, R. Bizios, Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY. *Department of Biochemistry and Molecular Biology, Albany Medical College, Albany, NY.

Osseointegration is an integral part of the clinical success for orthopaedic/dental implants and is dependent on the interaction of osteoblasts with material surfaces. For anchorage-dependent cells, such as osteoblasts, a crucial event in this process is adhesion, a prerequisite for subsequent cell functions. Micropatterning of bioactive, adhesive peptides represents a novel, design approach to enhance osteoblast/biomaterial interactions at the tissue/implant interface by promoting and spatially-directing cell attachment to implant surfaces. In the present study, standard microcontact printing techniques were used to pattern circles (diameters 10, 50, 100, and 200 micrometers) of N1[3-(trimethoxysilyl)propyl]diethylenetriamine (DETA), a hydrophilic compound, surrounded by octadecyltrichlorosilane (OTS), a hydrophobic compound, on borosilicate glass, a model substrate. The DETA regions were further modified (Dee et al., 1995) by immobilization of either the adhesive peptides Arginine-Glycine-Aspartic Acid-Serine (RGDS) and Lysine-Arginine-Serine-Arginine (KRSR) or the non-adhesive peptides Arginine-Aspartic Acid-Glycine-Serine (RDGS) and Lysine-Serine-Serine-Arginine (KSSR). After four hours under standard cell culture conditions, adhesion of either osteoblasts or fibroblasts in Dulbecco's Modified Eagle Medium in the absence of serum on surfaces modified with the non-adhesive peptides RDGS and KSSR was random and low. In contrast, both osteoblasts and fibroblasts adhered and formed clusters onto the DETA circles modified with the adhesive peptide RGDS; whereas only osteoblasts adhered and formed clusters onto DETA circles modified with KRSR, a peptide that selectively promotes adhesion of osteoblasts (Dee et al., 1998). These results provide evidence that patterning of select peptides can direct adhesion of specific cell lines exclusively to predetermined regions on material surfaces. Establishing such region-selective compatibility specifically for osteoblasts (but not for fibroblasts) on the surface of the next generation of orthopaedic/dental biomaterials will promote proactive interactions at the bone/implant interface leading to improved in vivo osseointegration of implants.
References:
Dee et al., Tissue Engineering, 1:135-145, 1995; Dee et al., J. Biomed. Mater. Res., 40:371-377, 1998.

3:15 PM LL4.5
MECHANICAL BEHAVIOUR OF POLY-HYDROXY-ALKANOATES/HYDROXYAPATITE COMPOSITES AS BONE IMPLANT BIOMATERIALS. Ruben Sanchez, Marcio Moriera, Norma Galego, Polymer Section, Advanced Materials Laboratory, North Fluminense State University, UENF; Campos dos Goytacazes, RJ, BRAZIL.

Various synthetic materials have been usually utilized as reinforcement in fracture fixation to the human bone. The poly(b-hydroxyalkanoates) (PHA) could replace traditional polyolefins based on their biocompatibility, adequate fracture thoughness and fatigue strenght. The composites were developed using previously characterized poly-hydroxyalkanoates (PHB and PHO) with 30, 40 and 50% of polymer composition; and natural hydroxyapatite (HA) with 0,1 mm particle diameter. A polymer solution was previously prepared and mixed with HA. The mixture was homogenizated and pressed by hot uniaxial pressing at 10 C above polymer melting point. The mechanical properties of the composites were studied by compression test, and the fracture area was explored by Scanning Electron Microscopy (SEM). HA was used due to its chemical and structural similarity with the human bone. On the other hand bacterial PHB was used as second composite component, besides being biocompatible and introduce an appropriate mechanical property, as tensile strenght values between dense bone (137,8 MPa) and cortical bone (41,4 MPa). The rate of chemical hydrolysis of PHAs is very slow in vitro, may be in off to biomedical implants where the bioactivity of HA and the biodegradability of PHAs can be combined to avoid particle migration from the implant area before growth of a new tissue and a favorable answer to induce a bone formation taking advantages of the slow polymer degradation.

3:30 PM LL4.6
A NOVEL COMPOSITE BIOMATERIAL FOR APPLICATIONS INVOLVING ELECTRICAL STIMULATION OF BONE.
Peter Supronowicz, Rensselaer Polytechnic Institute, Department of Biomedical Engineering, Troy, NY; Pulickel Ajayan, Rensselaer Polytechnic Institute, Department of Materials Science and Engineering, Troy, NY; Klaus Ullmann, Rensselaer Polytechnic Institute, Department of Biomedical Engineering, Troy, NY; Bernard Arulanandam, Dennis Metzger, Albany Medical College, Center for Immunology and Microbial Disease, Albany, NY; Rena Bizios, Rensselaer Polytechnic Institute, Department of Biomedical Engineering, Troy, NY.

Literature reports have shown that bone repair (for example, in animal models) occurs at an accelerated rate under electrical stimulation delivered to the injury site via implantable, metal electrodes. Novel biomaterials (for example, current-conducting composites) provide as yet unexplored alternatives for biomedical applications that require delivery of electrical stimulation for the purpose of bone-fracture repair. Materials with current-conducting properties could eventually lead to alternative therapeutic methodologies; in conjunction with cultured cells, they also provide models to examine and, thus, elucidate the cellular/molecular-level mechanisms responsible for osteogenesis under electrical stimulation. In the present in vitro study, the effects of electric current stimulation on select functions of rat calvarial osteoblasts (the bone-forming cells) on novel current-conducting polylactic acid/carbon nanotube (80:20 w/w %) composites were investigated. Compared to controls (that is, osteoblasts cultured on polylactic acid/carbon nanotube composites under standard cell culture conditions, but no electrical stimulation), exposure of osteoblasts (cultured on the novel composites) to 10 μA at 10 Hz for 6 hours daily for 2 consecutive days resulted in a 46% increase in cell proliferation. Moreover, exposure of osteoblasts to electrical stimulation of 10 μA at 10 Hz for 6 hours daily for 21 consecutive days resulted in a 300% increase in the concentration of extracellular matrix calcium. Osteoblasts exposed to 10 μA at 10 Hz for 6 hours resulted in upregulation of Collagen Type I mRNA expression; during the same time interval, there was no Collagen Type I mRNA expression by controls, that is osteoblasts cultured under similar conditions but no electrical stimulation. These results provide cellular/molecular evidence that electrical stimulation promotes osteoblast functions responsible for the chemical composition and structure of the organic and inorganic phases of bone.

3:45 PM LL4.7
FABRICATION AND CHARACTERISATION OF CALCIUM PHOSPHATE AND LIPOSOMES COMPOSITES AS AN IMPLANT COATING. Tarinee Pongsaanutin, Jan T. Czernuszka, Department of Materials, University of Oxford, Oxford, UNITED KINGDOM.

Calcium phosphate ceramics, especially hydroxyapatite have been effectively used for dental and orthopaedic applications due to their excellent osteoconduction and biocompatibility. It is also known that liposomes are widely used as targetable drug delivery devices. The purpose of this paper is to show how we synthesise composites of calcium phosphate and liposomes. This composite can be deposited electrophoretically as a coating. Thus, we combine the osteogenic potential of hydroxyapatite with the potential for targeting drugs and active ingredients to bone. This will lead to improved treatments for such conditions as osteoporosis, arthritis and tumours. Agents to prevent infection and enhance bone formation may also be added. Liposome vesicles were prepared by sonication of phosphotidylcholine and then introduced into an aqueous solution of calcium and phosphate ions supersaturated with respect to hydroxyapatite. Precipitation occurred on the outer surface of the liposome vesicles. These composite precipitates were deposited onto a cathode using an electrodeposition method at physiological temperature (37°C). Scanning electron microscopy (SEM), transmission electron microscopy (TEM), powder x-ray diffraction (PXRD) and Fourier transform infrared spectroscopy (FTIR) were used to analyse the morphology, structure and chemical composition of the composite coating. The results from PXRD and FTIR show a mixture of amorphous and poor crystalline hydroxyapatite. Enhanced crystallinity developed on ageing in the calcifying medium. This was verified by electron diffraction and TEM. Dark field images confirmed that the precipitated HAP deposited solely at the outer surface of the liposome. SEM micrographs demonstrated a thin uniform coating at the microstructural level. These results suggest that liposomes can be coated externally with calcium phosphate. The fraction of crystallinity can also be altered. It is expected that these liposome composite assemblies will have widespread use in aiding the treatments of bone disorders.

4:00 PM LL4.8
TRANSFORMATION BEHAVIOUR, MECHANICAL PROPERTIES AND BIOCOMPATIBILITY OF A BINARY NiTi AND A TERNARY NiTiCu ALLOY. Mohammed Es-Souni, University of Applied Sciences, Materials Testing and Joining, Kiel, GERMANY; Martha Es-Souni, Helge Fischer-Brandies, Clinic of Orthodontics, Christian-Albrechts University, Kiel, GERMANY.

In the present paper, the transformation behaviour, mechanical properties and biotoxicity of two commercial NiTi and NiTiCu shape memory arch wires are investigated. The differential scanning calorimetry results in the temperature range from -80 to +80 C and the bending properties in the temperature range from 6 to 60 C are reported. Furthermore, the results of biotoxicity experiments using the MTT (3,(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide)-test on epithelial cell cultures from human gingiva in the presence of both alloys are described. The morphology of the cells after incubation with the alloys is also investiagted using SEM. It is shown that the mechanical properties are strongly temperature dependent. Biocompatibility is shown to depend on the alloy composition with the binary alloy being characterised by a higher biocompatibility.

4:15 PM LL4.9
NANOCERAMICS AS THE FUTURE ORTHOPAEDIC/DENTAL BIOMATERIAL. Thomas J. Webster, Richard W. Siegel*, Rena Bizios, Departments of *Materials Science and Engineering and Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA.

The objective of the present *in vitro* study was to investigate, for the first time, cytocompatibility properties of nanophase (that is, novel material formulations with grain sizes less than 100 nm) ceramics pertinent to orthopaedic/dental applications. Nanophase and conventional (that is, grain sizes greater than 100 nm) alumina, titania, and hydroxyapatite compacts were prepared as previously described (Webster *etal.*, 1999). Neonatal rat calvaria osteoblasts (bone-forming cells) were seeded (at a cell density of 40,000 cells/cm^2) onto the ceramics of interest to the present study and cultured under standard cell culture conditions (that is, a 37°C, humidified, 5% CO_2/95% air environment) in Dulbeccos Modified Eagle Medium (DMEM) (supplemented with 10% fetal bovine serum (FBS), 50 μg/mL ascorbic acid, and 10 mM β-glycerophosphate) for 21 and 28 days. At that time, calcium content in the extracellular matrix was quantified using a commercially available kit (Sigma) and following manufacturer's instructions. Primary osteoclast-like cells (bone-resorbing cells) isolated from femurs of Wistar rats were seeded ($2x10^7$/cm^2) onto the ceramics of interest to the present study and cultured under standard cell culture conditions in DMEM (supplemented with 10% FBS, 10^-8 M 1-alpha, 25-(OH_2) vitamin D_3, and 1% antibiotic/antimycotic solution) for 10 and 13 days. At that time, resorption pits on the ceramic surfaces were visualized and counted using reflected light microscopy (Olympus Ix70) with image analysis software (Image Pro). Compared to conventional ceramics, deposition of calcium-containing mineral by osteoblasts and formation of resorption pits by osteoclast-like cells were significantly (p ¡ 0.01) greater on respective nanophase ceramics after all time periods tested in the present study. By demonstrating that bioceramics can be designed and fabricated through control of grain size to possess improved cytocompatibility properties for select osteoblast and osteoclast function, the results of the present study demonstrated for the first time, that nanophase ceramics have great potential to become the next generation, choice orthopaedic/dental biomaterial to enhance bonding to juxtaposed bone and, thus, increase implant efficacy. Webster *etal.*, *Biomater.* 20:1221-1227, 1999.

4:30 PM LL4.10
SYNTHESIS AND CHARACTERIZATION OF CORALLINE HYDROXYAPATITE GRAFTED WITH PMMA FUNCTIONAL MICROSPHERES FOR OSTEOGENIC AND BONE FILLING MATERIALS. M. Sivakumar and K. Panduranga Rao, Biomaterials Laboratory, Central Leather Research Institute, Adyar, Chennai, INDIA.

Many efforts have been made towards the development of new bone substitute materials. Among these, hydroxyapatite/polymer composites have attracted much attention since such composite have some unique advantages over their conventional constituting components. Poly(methyl methacrylate) [PMMA] as an adhesive in bone arthroplasty applications is well known. In the present paper, attempts were made to prepare functional microspheres of PMMA having epoxy and hydroxy functionality using glycidyl methacrylate and chain transfer agent, mercaptoethonal. PMMA functional microspheres were prepared by using dispersion polymerization techniques and these microspheres were subsequently grafted with coralline hydroxyapatite using coupling grafting technique via diisocyanate. These grafted ceramic-polymeric composites materials were characterized by various techniques such as XRD, FT-IR, TGA, DSC and optical microscope. These microspheres and grafted materials were analyzed for particle size distribution using particle size analyzer and found to be 260 microns and 310 microns respectively. The optical micrographs clearly indicated that the coralline hyroxyapatite was grafted onto the functional microspheres. The percentage grafting of CHA-PMMA composite material was determined and it was 120%. Gentamicin was incorporated in these grafted materials and in its in-vitro release was carried out in phosphate buffer. It is aimed to use these grafted microspheres in orthopaedics particularly in the repair and regeneration of bone and dental.

SESSION LL5: POSTER SESSION
ORTHOPAEDIC/DENTAL BIOMATERIALS
Tuesday Evening, November 28, 2000
8:00 PM
Exhibition Hall D (Hynes)

LL5.1
POLYURETHANE BIOMATERIALS WITH ANTI-INFECTION PROPERTIES. Erkesh O. Batyrbekov, Rinat M. Iskakov, Bulat A. Zhubanov, Institute for Chemical Sciences, Almaty, KAZAKSTAN.

Biomaterial-related infection are frequently observed with prosthetic implants and these may result in post-surgical failure of the device. In present study the strategy for the prevention of foreign body infection by incorporation of antimicrobial agents in polyurethane biomaterials is described. A procedure has been developed for making antibiotic-loaded polyurethane films. Rifampicin, oxacillin and ciproflaxacin were used as antibacterial agents. Antibiotics were incorporated in segmented polyuretanes based on various polyetherdiols, diisocyanates and butanediol. The antibiotics release kinetics and biological efficacy has been investigated. It was founded that the release rate depends on the concentration of incorporated drugs and show two significant periods consisting of "burst effect" and typical Fickian diffusion-controlled release. The high antibacterial effect of antibiotic-loaded biomaterials against five different strains of bacteria was shown. The preliminary results in rabbits show the successful application of obtained polyurethane implants for the prevention of foreign body infection. The continuos release of effective doses of antibiotics over a long period at the interface where the biomaterial is contacted with the body can prevent the bacterial colonization of the implants.

LL5.2
NOVEL POLYMER/CERAMIC NANOCOMPOSITES FOR ORHOPAEDIC/DENTAL APPLICATIONS: MECHANICAL AND CYTOCOMPATIBILITY PROPERTIES. Anastasia J. McManus, Richard W. Siegel*, Robert H. Doremus*, Rena Bizios, Rensselaer Polytechnic Institute, Departments of Biomedical Engineering and *MS&E, Troy, NY.

To date, homogeneous, large (over 200 nm)-grain size, traditional materials that either satisfy mechanical (e.g., metals) or cytocompatibility (e.g., ceramics) requirements have been used almost exclusively for orthopaedic/dental applications. For a variety of reasons, however, these materials have not produced ideal bone implants. In an attempt to more closely match the microstructure and mechanical properties of bone (a composite 30% organic/protein, 65% inorganic/ceramic nanophase material), novel polymer/ceramic nanocomposite formulations were designed, fabricated, and characterized (i.e., mechanical and cytocompatibility properties). Formulations of polylactic acid (PLA) composites with varying (30, 40, 50, and 60) weight percent of nanophase alumina, hydroxylapatite, and titania loadings were prepared. The mechanical properties, including bending modulus and fracture toughness, of all materials tested in the present study were determined. The nanocomposites exhibited mechanical properties that were very different than those of the constituent component materials. For example, the polylactic acid/nanophase alumina composites exhibited bending moduli one and three orders of magnitude larger than those of the homogeneous polymer and ceramic constituent components, respectively; most importantly, the bending moduli of these novel composites were in the range of values (1- 20 GPa) of human cortical bone. The cytocompatibility of the nanocomposites was determined by comparing osteoblast and fibroblast adhesion and proliferation; formation of mineral deposits in the extracellular matrix by osteoblasts was also examined. Osteoblast adhesion on the alumina composite formulations increased as a function of nanophase alumina weight percent; the results on the 50% nanophase alumina/PLA composite surfaces were similar to those obtained on the homogeneous alumina surfaces, on which osteoblast adhesion was a maximum. The results of the present study provide evidence that these novel polymer/ceramic nanocomposite formulations closely match the

mechanical properties of native bone, an advantage over traditional orthopaedic implant materials; most importantly, because of their enhanced cytocompatibility, these nanocomposites could promote osseointegration, which is a crucial requirement for the clinical success of orthopaedic implants.

LL5.3
PHYSICAL AND MECHANICAL PROPERTIES OF A PHOTOCROSSLINKED HYALURONAN FOR BIOMEDICAL APPLICATIONS. Kimberly A. Smeds and Mark W. Grinstaff, Duke University, Department of Chemistry, Durham, NC.

Novel biomaterials that can be polymerized in situ via an optical trigger are of current interest to a variety of disciplines including dentistry, angioplasty and orthopaedics. Hydrogels are one class of biomaterials currently under investigation for these applications. We have synthesized a novel hydrogel based on the natural polymer hyaluronan (HA). HA, an alternating co-polymer of β(1-4) linked 2-acetemide-2-deoxy-D-glucose and β(1-3) linked D-glucuronic acid, is known to be biocompatible, non-antigenic and generally non-tissue reactive. By modifying HA with methacrylate moieties (HA-MA), the polymer can be photocrosslinked in the presence of a radical initiating system of eosin Y and triethanolamine to form a solid, transparent, soft, flexible hydrogel. This rapid photo-induced hydrogel formation occurs on glass and Teflon surfaces as well as dry and moist tissues. The surface properties of this novel hydrogel were characterized by SEM and AFM and revealed a uniform and smooth surface. Swelling studies showed that the HA-MA hydrogel can absorb over ten times its weight in water. The mechanical properties of this photo-crosslinked polysaccharide were evaluated using compression and creep compliance experiments. The HA-MA hydrogel exhibits solid-like (elastic) behavior with a low phase angle and a G* value of 1.6 kPa. The physical and mechanical properties of this novel hyaluronan hydrogel and the ability to be rapidly photocrosslinked suggest that this polymer is useful for islet cell encapsulation and in situ ophthalmic wound repair.

LL5.4
EFFECTS OF DEMINERALISED BONE MATRIX IMPLANTS FOR FRACTURE HEALING IN CANINES. R.V. Suresh Kumar, Department of Surgery and Radiology, College of Veterinary Science, Tirupati, INDIA.

The aim of this study is to evaluate Demineralised bone matrix (DBM) with tricalcium phosphate (TCP) and hydroxyapatite (HA) implants for fracture healing in dogs. The same were compared to untreated and cancellous bone graft treated animals. Under general anesthesia transverse fractures segmental defects were created in all animals and stabilized with intramedullary pinning. The defects were filled with materials in three groups and left unfilled in control groups. Postoperatively various parameters like clinical, biochemical, radiological and histological changes were recorded and compared. Early functional limb usage was noticed in DBM TCP implanted group. Biochemical parameters did not show any significant changes post operatively in the study. Radiological studies showed visible segmental defect even at the end of the 12th week in untreated animals. The union of graft to host bone was observed at 9th week in cancellous bone grafted group whereas complete union was visible at 6th week in DBM implanted groups. Periosteal reaction was a prominent feature in untreated and cancellous bone grafted groups. Angiographic changes revealed slightly hypervascularity at the fracture site in DBM implanted groups which came to normal by 9 weeks. However these findings are not significant in other groups. Osteomedullograms showed medullary cavity continuity and no leakage of constrast medium at 12 weeks in all groups. Histological studies revealed fibrocellular reaction in early stages of control groups whereas osseous spicules were noticed in treated groups. 12th week radiographs showed excellent formation of osseous tissue and periosteum with incomplete harvesian system. Based on the above observations it is concluded that DBM implants are proved to be the best substitutes compared to autogenous cancellous bone grafts.

LL5.5
PHOTOCROSSLINKED POLY(PROPYLENE FUMARATE) SCAFFOLDS FOR ORTHOPEDIC APPLICATIONS. John P. Fisher, Theresa A. Holland, Antonios G. Mikos, Rice University, Department of Bioengineering, Houston, TX.

A tissue engineering scaffold based upon the biodegradable polymer poly(propylene fumarate) (PPF) has been produced using a UV crosslinking/porogen leaching strategy. PPF (Mn = 1400 to 2700) was crosslinked using 365 nm ultraviolet light and the photoinitiator bis(2,4,6-trimethylbenzoyl) phenylphosphine oxide (BAPO; 5 mg BAPO/g PPF) by forming covalent crosslinks across fumarate units of PPF chains. With the inclusion of a NaCl porogen (50, 70, 80, and 90 wt%) and its removal by water leaching after UV crosslinking, a porous PPF scaffold was formed. Porosity was characterized by weight loss, mercury porosimetry, and scanning electron microscopy. Results showed that porosity can be controlled by porogen content and that scaffolds of at least 70 wt% porogen possess an interconnected pore structure. Fourier transform infrared spectroscopy was used to evaluate the crosslinked PPF, showing that the exterior surface of the porous samples undergoes greater crosslinking than the interior of the sample. Scaffold compressive mechanical strength was found to increase with increasing PPF molecular weight and decrease with increasing porogen content. The strongest samples which possessed an interconnected pore structure (PPF Mn = 2200 and 70 wt% porogen) were found to have an elastic modulus of 41.8 /- 15.2 MPa and a compressive strength at 1% yield of 1.84 /- 0.79 MPa. This work has shown that the UV crosslinking/porogen leaching technique is a simple method to form porous solids from photoinitiated materials and therefore allows for the material's further evaluation for tissue engineering applications.

LL5.6
FABRICATION OF AN INJECTABLE POROUS BIODEGRADABLE HYDROGEL FOR DENTAL TISSUE ENGINEERING. Esfandiar Behravesh, Antonios G. Mikos, Rice Univ, Dept of Bioengineering, Houston, TX.

Three-dimensional porous scaffolds have been utilized as conduits for guided tissue regeneration. Existing methods for the manufacturing of porous scaffolds require prefabrication before implantation and are not suitable for injectable systems. A tri-block copolymer of poly(propylene fumarate) and poly(ethylene glycol)-monomethylether combined with poly(ethylene glycol)-diacrylate was crosslinked utilizing a water soluble persulfate-ascorbic acid initiation system. The composition of the initiation system was varied to assess its effect on the sol fraction and swelling characteristics for thin hydrogel films. The sol fraction was measured to be less than 5% while the equilibrium water content ranged from 40-60%. Porous three-dimensional hydrogels were further fabricated by carbon dioxide formation from the dissolution of sodium bicarbonate in water in the presence of ascorbic acid; the sodium bicarbonate was added to the initial formulation. The effect of the sodium bicarbonate and ascorbic acid reaction on the porosity of the resulting hydrogels was also determined for porous scaffolds of porosity up to 90%. Pore morphology was analyzed using scanning electron microscopy for dried hydrogels and cryosectioning combined with stereology for hydrated hydrogels. Interconnected porous scaffolds with a controllable porosity were synthesized which hold promise for dental applications.

SESSION LL6: NOVEL ORTHOPAEDIC/DENTAL BIOMATERIALS
Chair: Joachim Kohn
Wednesday Morning, November 29, 2000
Berkeley (Sheraton)

8:30 AM *LL6.1
POSTERIOR ALL-CERAMIC CROWNS - A DAMAGE/FATIGUE PROBLEM. Van Thompson, University of Medicine and Dentistry of New Jersey, NJ.

Clinical results with all-ceramics dental crowns on molar teeth indicate that high stress states result in significant failure rates for monolithic materials. Recent investigations have shown that initial strength of a ceramic material has little to do with its clinical performance. Instead, tolerance to damage, introduced a number of different ways, is a more important characteristic. Damage from repeated Hertzian contact (blunt, cyclic contact similar to occlusal contact) accumulates, leading to catastrophic failure. Both near field (cone cracking) and far field (radial cracking) can occur. Routine sandblasting can create damage equivalent to a million loading cycles for some ceramics. Esthetic monolithic materials are susceptible to significant reduction in strength/failure after damage equivalent to 4-5 years of clinical use. Layered ceramic structures, soft esthetic porcelains on stiff and strong substrates, offer an alternative with improved damage tolerance. Unfortunately, recent investigations suggest that fatigue life remains a significant problem as these ceramic systems must perform in thin cross section (1-2 mm) on a low elastic modulus dentin substrate(15-17 GPa) while cemented with an even lower modulus resin layer (2-8 GPa) of significant thickness (20-50 microns). The wet oral environment with pH transients and millions of complex load cycles presents a challenge for designing future esthetic crown systems This presentation will compare Hertzian contact laboratory fatigue studies of monolithic and layer ceramics results with long-term clinical performance data on molar crowns.

9:00 AM LL6.2
DESIGN, SYNTHESIS AND CHARACTERIZATION OF HYDROXYAPATITE PARTICULATES. Chun-Wei Chen, Kullaiah Byrappa, Charles Oakes, Wojciech Suchanek, Richard Riman, Rutgers

Univ, Dept of Ceramic and Materials Engineering, Piscataway, NJ; Mamoru Senna, Keio Univ, Faculty of Science and Technology, Yokohama, JAPAN; Kelly Brown, Kevor TenHuisen, Victor Janas, Johnson & Johnson Corp Biomaterials Center, Somerville, NJ.

Biological hydroxyapatite [HAp, $Ca_{10}(PO_4)_6(OH)_2$] is nonstoichiometric with a wide range of cationic and anionic lattice substitutions. Use of synthetic HAp-based materials depend upon the ability to design HAp particulates with well-controlled chemical and physical characteristics. A rational approach to this problem is to compute thermodynamic equilibria as a function of processing variables, generate equilibrium diagrams to map processing variable space for the phases of interest, design hydrothermal experiments to test and validate computed diagrams, and utilize processing variable space to explore opportunities for control of reaction and crystallization kinetics. This paper will detail our progress in making such designer HAp particulates. Predictive phase diagrams were constructed with a focus on HAp on HAp formation from 50 to 350°C. Yield diagrams were constructed illustrating regions in which 99% of the calcium should precipitate as HAp. Thermodynamic calculations were completed using temperature-dependent functions for the relevant solution phase and solid-liquid equilibria and solute species activity coefficients. Model accuracy was then evaluated through experiment at 50, 100 and 200°C and a pH range between 2.2 and 8.9. The thermodynamic calculations and experimental results are in good agreement. Stoichiometric, crystalline HAp has also been prepared by heterogeneous reaction of $Ca(OH)_2$ powder and aqueous $(NH_4)_2PO_4$ at room temperature using mechanochemical-hydrothermal methods. Using this approach we have accomplished cationic and anionic substitutions not previously achieved with a high level of reproducibility. HAp products prepared via the above methodologies have been characterized via powder X-ray diffraction, IR spectroscopy, thermal and chemical analyses. BET, SEM, TEM and dynamic light scattering were used to characterize surface and morphological features. HAp surface areas vary from 45 to 150 m^2/g depending upon synthesis conditions with average particle sizes varying from 240 nm to 400 nm.

9:15 AM LL6.3
EFFECTS OF DOPING SUBSTITUTIONS IN HYDROXYAPATITE ON OSTEOBLAST ADHESION. Thomas J. Webster, Celaletdin Ergun*, Robert H. Doremus*, Rena Bizios, Departments of Biomedical Engineering and *Materials Science and Engineering, Rensselaer Polytechnic Institute, Troy, NY.

The present *in vitro* study investigated the effect of dopants, specifically, Magnesium, Cadmium, Zinc, and Yitrium, present in hydroxyapatite (HA) on osteoblast adhesion. For this purpose, HA substrates were prepared by adding calcium nitrate (used either undoped or containing 2 mole% of either Magnesium, Cadmium, Zinc, or Yitrium) to ammonium phosphate:distilled water solutions. The resultant solutions were stirred for 24 hours, centrifuged, filtered, dried, and sintered at 1,100°C for 60 minutes. Neonatal rat calvaria osteoblasts were seeded (3,500 cells /cm^2) in Dulbecco's Modified Eagle Medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (Sigma) and were allowed to adhere under standard cell culture conditions (that is, in a 37°C, humidified, 5% CO2/95% air environment) for 4 hours. Adherent cells were fixed, stained, and counted *in situ* using fluorescence microscopy. Proteins adsorbed onto the substrates of interest to the present study were quantified following immersion of each sample separately either in DMEM supplemented with 10% fetal bovine serum (containing undetermined amounts of proteins such as albumin, laminin, denatured collagen, fibronectin, vitronectin, *etc.*) or in a solution of 5 μg each of albumin, laminin, denatured collagen, fibronectin and vitronectin (all chemicals from Sigma) per mL of phosphate buffered saline under standard cell culture conditions for 4 hours. Adherent proteins were then desorbed; protein concentration in each eluant was determined using a commercially available kit (BCA Assay; Pierce). Osteoblast adhesion was significantly ($p < 0.01$) greater on HA doped with Yitrium than on any other HA formulation tested in the present study. Moreover, compared to HA doped with either Zinc or Cadmium, significantly ($p < 0.01$) greater amounts of fetal bovine serum, denatured collagen, and vitronectin adsorbed on HA doped with Yitrium. For the first time, these results demonstrated that modification of HA with Yitrium enhances adsorption of select proteins (specifically, denatured collagen and vitronectin) that, subsequently, promote osteoblast adhesion.

10:00 AM LL6.4
A BIOACTIVE GLASS FIBER REINFORCED COMPOSITE. Anthony B. Brennan, Univ of Florida, MS&E and Biomed Eng, Gainesville, FL; Rodrigo Orefice, Fed Univ of Minas Gerais, Dept of Metl and Mater Eng, Belo Horizonte-MG, BRAZIL; A.E. Clark, Univ of Florida, Prosth Dept, Gainesville, FL; Larry L. Hench, Imperial College, London, UNITED KINGDOM.

Bioactive fibers were produced using a sol-gel method. The rheological properties of two different sol compositions prepared from a mixture of TEOS, phosphorous alkoxide and calcium nitrate or calcium chloride in a water-ethanol solution are reported. The sols were extruded through a spinneret to produce continuous 10 m-diameter fibers. Discontinuous fibers and fibrous mats were prepared by air-spraying the multicomponent sols. The sol-gel fibers were converted to the bioactive fibers by three different thermal treatments at either 600, 700 or 900°C for three hours. SEM, BET, EDX and FTIR were used to characterize the morphology and structure of the fibers. The BET measured surface area of the fibers sintered at 900°C was zero m^2/gm compared to a value of 200 m^2/gm for a typical sol-gel derived particle of similar composition. Both the continuous and discontinuous fibers exhibited in vitro bioactivity in a simulated body fluid. For the first time, the bioactivity of the glass is demonstrated to be dependent upon composition. A hybrid composite prepared with a novel surface active polymer is described. The preliminary viscoelastic properties and in vitro bioactivity are presented for polymeric composites containing the discontinuous bioactive glass fibers.

10:15 AM LL6.5
SYNTHETIC BIODEGRADABLE POLYMER NETWORKS MODULATING MARROW STROMAL OSTEOBLAST FUNCTION. Heungsoo Shin, Antonios G. Mikos, Dept of Bioengineering, Houston, TX.

We are interested in the synthesis of new orthopaedic biomaterials for the treatment of bony defects by guided tissue regeneration. We have previously shown that composite scaffolds based on poly (propylene fumarate) (PPF) exhibit suitable mechanical properties for trabecular bone replacement. We have further demonstrated that PPF supports marrow stromal osteoblast adhesion in vitro. In this study, we have synthesized new unsaturated polyesters based on PPF with covalently bound peptide sequences in an attempt to modulate cellular function on the polymer. The polyester is a linear oligomer of poly(ethylene glycol) and fumaric acid (OPF) with the peptide covalently attached to the PEG end hydroxyl group. We have investigated the effects of the peptide content in the polymer network for a model GRGD peptide as well as the PEG spacer length on the function of attached marrow stromal osteoblasts. Contact angle analysis confirmed that the hydrophilic domain of OPF and immobilized peptide were homogeneously distributed throughout the polymer network of hydrophobic PPF. The functionalized polymer network may provide a new means of modulating cellular function and therefore would be useful as a scaffold for bone tissue engineering.

10:30 AM LL6.6
CRYSTAL MORPHOLOGY CONTROL BY MELT PHASE SEPARATION IN BIODEGRADABLE POLYMER BLENDS. Yvonne A. Akpalu, Rensselaer Polytechnic Institute, Department of Chemistry, Troy, NY; J. Carson Meredith, Georgia Institute of Technology, School of Chemical Engineering, Atlanta, GA; Eric J. Amis, Polymers Division, National Institute of Standards and Technology, Gaithersburg, MD.

The effect of lower critical solution temperature (LCST) phase separation on the crystal growth mechanism and fine structure of poly(ϵ-caprolactone) PCL in PCL/poly(D,L-lactide) (PDLA) blends is studied by simultaneous small-angle x-ray scattering (SAXS) and wide-angle x-ray scattering (WAXS). We have followed the structural changes (2 nm to 100 nm) during crystallization at 45°C of critical and off-critical blend compositions from both one-phase and two-phase melts. Phase separation is induced by controlled temperature jumps into the LCST (two-phase) region, which is above the melting temperature (60°C) of PCL. The large-scale morphology (1-100 μm) resulting from phase separation is observed with optical microscopy. The SAXS profiles arise from two scattering species: (1) amorphous domains outside the lamellar stacks that give rise to diffuse scattering and (2) lamellae in lamellar stacks that give rise to discrete scattering. We determine morphological changes occurring during crystallization for the two scattering contributions by fitting the observed scattering to a model that accounts for a significant amorphous phase between lamellar stacks. Some morphological parameters are independent of blending and melt phase separation. The fraction of crystals within lamellar stacks (60%) and the size of the amorphous regions surrounding stacks (29 nm) are the same for pure PCL and for blends, and are independent of blend composition and the extent of melt phase separation. The long period (17 nm) obtained for PCL is also independent of blend composition and melt phase separation, indicating that PDLA is excluded from the lamellar stack. Other morphological parameters are determined by blend composition and the extent of melt phase separation. For example, the periodicity in stacking (48 nm) obtained for PCL is reduced to 45 nm in the blends. In the off-critical blends, the fraction of crystals that develop outside stacks increases from 0% to 6% as melt phase separation increases. In the critical blend, however, crystals are restricted to lamellar stacks. Our results show that the melt morphology can determine the extent

to which new crystals are formed in the amorphous regions surrounding lamellar stacks, the ultimate crystallinity obtained, and the primary crystallization rate. The effective use of melt phase separation for controlling the crystal morphology is discussed.

10:45 AM LL6.7
NON-WOVEN SHEETS OF BIOACTIVE FIBERS PRODUCED BY A SOL-GEL PROCESS. Rosana Domingues, Universidade Federal de Minas Gerais, Dept of Chemistry, BRAZIL; Arthur Clark, University of Florida, College of Dentistry, FL; Anthony Brennan, Dept of MS&E, University of Florida, FL.

A high velocity spray process was used to prepare SiO_2 CaO_2 P_2O_5 fibers from sol-gel method. Both, dispersed fibers and non-woven sheets of bioactive fibers were produced. Temperature and pH of the solutions were evaluated during all synthesis and used to evaluate the progress of sol-gel reaction. Two compositions having different silica contents were produced. Sol viscosities were studied and the optimized range for produced non-woven sheets was determined. The formation of hydroxy carbonate apatite, HCA, on the surface of fibers was used to evaluate the kinetics of the bioactivity in a simulated body fluid. Diffuse Reflectance Infrared Fourier Transform Spectroscopic analysis confirmed the presence of HCA formed after 3 hours immersion in the test fluid for both compositions tested. Samples higher in silica exhibited faster kinetics. Scanning Electron Microscopy revealed a homogeneous layer of HCA on surface of fibers. Fiber morphology was maintained almost unchanged after 30 days of immersion. Potential applications are as scaffolds for both mineralized and non-mineralized structural tissues.

11:00 AM LL6.8
SYNTHESIS OF NOVEL POLYSACCHARIDE MIMICS. Matthew T. Sheehy, Elisabeth B. Walsh and Mark W. Grinstaff, Duke Univ, Dept of Chemistry, Durham, NC.

Polysaccharides are of wide-spread use in the biotechnology, food and pharmaceutical industries. We have synthesized two novel analogs of alginic acid, poly(5,6-dihydroxynorbornane carboxylic acid) and poly(5,6-dihydroxyoxanorbornane carboxylic acid). Ring opening metathesis polymerization (ROMP) of a cyclic olefin in the presence of Grubbs catalyst yielded these new polymers. Further modifications then afforded hydrophilic polymers possessing a repeating cyclic ring structure, two secondary alcohols, and a carboxylic acid. These polymers were characterized by 1H NMR, IR, and GPC. The synthetic procedure described involves altering the functional groups of the polymer such that its chemical, physical, and mechanical properties can be optimized for a specific tissue engineering application.

11:15 AM LL6.9
SURFACE TREATMENT OF BIOMEDICAL POLYMERS FOR ENHANCED ADHESION. Valerie Barron, Dept of Physics, Trinity College Dublin, IRELAND; Martin Buggy, Dept of Materials Science and Technology, University of Limerick, IRELAND.

As part of a project to develop a biomimetic artificial hip joint, it was necessary to bond carbon fibre reinforced polyetheretherketone to thermoplastic polyurethane. In order to produce a strong and durable adhesive joint, surface treatment was necessary prior to bonding. Surface treatments may involve one or more of the following; removal of a weak boundary layer, increase in surface roughness, change in chemical nature of the surface, or modification of the physical structure of the surface. Methods of surface treatment for thermoplastic polymers involve altering surface tension and surface chemistry using techniques such as plasma treatment, corona discharge, oxidising flame treatments and laser treatments. Prior to bonding, techniques such as surface tension and wettability were examined to optimise the method of surface treatment. Surface chemistry was examined using Fourier transform infrared spectroscopy (FTIR) and x-ray photoelectron spectroscopy (XPS). In addition surface topography was examined using a number of techniques including atomic force microscopy (AFM), scanning electron microscopy (SEM). Mechanical testing by peel testing according to British standards was carried out to establish the durability of these adhesive joints in a biomedical environment. The human body is one of the most aggressive environments that an adhesive bond has to endure. The durability of these APC2/polyurethane joints is investigated in various aqueous environments including Ringers solution and distilled water. While the bond was stable in air for over 1 year, storage in aqueous media leads to rapid deterioration of bond strength. This phenomenon was observed both in distilled water and in Ringers solution. Measured diffusion rates for both environments were very similar and it is suggested that the observed faster rate of loss of bond strength in Ringers solution is due to the interaction of buffered components from the Ringers or solubilisation of low molecular weight material by the Ringers.

11:30 AM LL6.10
THE SYNTHESIS OF HYDROXYAPATITE / POLYSACCHARIDES NANOCOMPOSITES THROUGH A SELF-ORGANIZATION MECHANISM. Toshiyuki Ikoma[1,2] and Junzo Tanaka[1,2]. [1]National Institute for Research in Inorganic Materials, Ibaraki, JAPAN. [2]CREST, Japan Science and Technology, Saitama, JAPAN.

The purpose of this study is to prepare novel inorganic/organic nanocomposites using a self-organization process. Both nanocomposites, hydroxyapatite (HAp)/hyaluronic acid (HyA) and HAp/chondroitin sulfate (ChS) composites, were prepared by a coprecipitation method using a calcium hydroxide suspension involving polysaccharides and a diluted phosphate solution. HyA and ChS are the ubiquitous glycosaminoglycan found in almost all tissues, which are the linear polysaccharides consisting of glucuronic acid and N-acetylglucosamine (HyA) or N-acetylgalacutosamine (ChS). Therefore, it is expected that HyA and ChS are bioactive and can interact with HAp crystal surfaces via carboxyl groups and/or sulfate groups. According to TEM observations, the HAp/HyA composites consisted of island-like assembled particles of 300nm in length and 30nm in width, while the HAp/ChS composites those of 150nm in length and 50nm in width. When electron diffraction was separately took for respective particles, 002 and 004 diffraction spots ascribed to HAp were observed along the longitudinal axes of the particles; therefore, the c-axes of HAp nanocrystals (30nm in size) were regularly aligned along polysaccharide chains in the respective assembled particles. This result means that the HAp nanocrystals can be self-organized through the nucleation and growth of HAp crystals on the carboxyl groups in ChS/HyA. The self-organization mechanism was elucidated by a molecular orbital method using a cluster of HAp surface and carboxyl group, in which calcium ions were assumed to be coordinated by seven oxygen ions on the HAp {100} surface. As a result, the configuration of a -CO_2 plane of the carboxyl group was stable when it was perpendicular to the c-axis of HAp, and the chemical bond formed between Ca and -CO_2 was considerably covalent with the bond order of 0.15. It was considered that such coordination bond play an essential role for the self-organization mechanism.