SYMPOSIUM F

F: Biomaterials for Tissue Engineering

December 2 - 4, 2003

Chairs

Joyce Y. Wong
Dept. of Biomedical Engineering
Boston University
44 Cummington St
Boston, MA 02215
617-353-2374

Christine E. Schmidt
Dept. of Biomedical Engineering
University of Texas-Austin
CPE 4.418, MC C0400
Austin, TX 78712
512-471-1690

Lonnie Shea
Dept. of Chemical Engineering
Northwestern University
2145 Sheridan Rd.
Evanston, IL 60208-3120
847-491-7398

Anne L. Plant
Biomolecular Materials Group
National Institute of Standards & Technology
MS 8313
100 Bureau Dr.
Gaithersburg, MD 20899-8313
301-975-3124

Christopher S. Chen
Dept. of Biomedical Engineering
Johns Hopkins University
Traylor Rm. 718
720 Rutland Ave.
Baltimore, MD 21205
410-614-8624

Arthur J. Coury
Genzyme Corporation
One Kendall Sq.
Cambridge, MA 02139-1562
617-768-8002

Symposium Support
†Asylum Research Corporation
Boston Scientific Corporation
Ethicon Inc./Johnson & Johnson
Genzyme Corporation
Guidant Corporation
The Whitaker Foundation
†2003 Fall Exhibitor

A Joint Proceedings with Symposium F/H/I to be published in extended summary format both in book form and online (see *ONLINE PUBLICATIONS* at www. mrs.org) as Volume EXS-1 of the Materials Research Society Proceedings Series

* Invited paper

SESSION F1: Overview and Challenges in Biomaterials I
Chair: Joyce Wong
Tuesday Morning, December 2, 2003
Back Bay A (Sheraton)

8:30 AM *F1.1
Novel Biomaterials. Robert S. Langer, Chemical Engineering, MIT, Cambridge, Massachusetts.

Approaches involving the synthesis and application of bioerodible polymers to serve as implantable scaffolds for mammalian cells to create new tissues and organs are being studied. This talk will discuss the design of new materials, in particular, synthetic polymers with specific ligands attached to them, photopolymerized materials, shape memory degradable polymers and materials with reversibly switching surfaces - that may have applications in these areas. We will also examine the use of materials coupled with human embryonic stem cells or other cells and the application of these approaches to the creation of new tissues such as spinal cord or blood vessels.

9:00 AM *F1.2
The Problem of Engineering a Whole Vital Organ.
Joseph Vacanti, [1]Harvard Medical School and the Massachusetts General Hospital, Boston, Massachusetts; [2]Pedi Surg, Massachusetts General Hospital, Boston, Massachusetts.

The field of Tissue Engineering is now established and is improving patient care in a number of areas. A most significant challenge is developing a strategy to generate vital organs to meet the terrible need for transplantation caused by the worsening organ donor shortage. New knowledge in stem cell biology is now offering the promise of contributing in this arena. Our laboratories have studied methods for solving the problem over the past 18 years. Over the past five years, we have put together teams of scientists and engineers at Massachusetts General Hospital, MIT, and Draper Laboratories to adapt the tools of microfabrication to aid in the effort. Our strategy lies in the realization that large masses of tissue are difficult to achieve relying on angiogenesis. The three to five days needed for capillary ingrowth limit the ultimate thickness of the resulting tissue. We decided to engineer a complete vascular circulation for the construct as we engineered the tissue of interest. For this, computational models of vascular network topology, fluid dynamics and microfluidics, and mass transport through the system have been generated. Based on these models, vascular circuits are etched in silicon and transferred to polymer devices. With a strategy of stacking of layers, circulations of many layers have been created, seeded with vascular endothelial cells and studied for flow characteristics. Recently, a second compartment of hepatocytes has been added and survival of the cells by flow through the vascular side has been demonstrated. Further work in optimization in vitro and then testing in animal models is underway.

9:30 AM *F1.3
The Tortuous Route From Scaffolds And Cells To Heart Muscle. Buddy Ratner, Bioengineering, University of Washington, Seattle, Washington.

The challenging goal of this tissue engineering program is grow heart muscle that might be useful clinically for cardiac reparative surgery. The principal muscle cells populating the heart, cardiomyocytes, have lost the ability to replicate. The heart muscle itself is highly vascularized. Muscle tissue is also aligned, organized with a mechanically appropriate extracellular matrix and innervated. Surgical considerations must be addressed. An interdisciplinary team funded through the NIH Bioengineering Research Partnership (BRP) program is exploring the feasibility of engineering heart muscle by addressing many of these challenges in a coordinated research effort. Individual investigator projects, interfaced through a strategic plan, are studying: (1) unique porous gels to stimulate angiogenesis, (2) novel polymers for tissue engineering, (3) three-dimensional muscle structures generated on 2-D and 3-D scaffold arrays (4) the controlled release of drugs, growth factors, angiogenic factors and genes (5) the plasticity of muscle cells, endothelial cells and purkinje cells (6) the development of a bioengineered microvessel system capable of sustaining heart muscle during development.

10:30 AM *F1.4
Strategies For The Engineering Of Organs. Anthony Atala, Surgery, Children, Boston, Massachusetts.

New advances in the field of Regenerative Medicine have led to the engineering of tissues and organs that may be used to restore and maintain normal function. Several ingredients are essential for the successful engineering of organs, including selective cell growth and expansion, biomaterials with organ-specific characteristics, favorable cell-biomaterial interactions, the appropriate design of three-dimensional constructs, and the use of coordinated systems for the enhancement of neo-vascularization and innervation. Cells for tissue reconstitution can be derived from the native organ to be replaced, thus avoiding rejection. In situations where normal native tissues are not available, different stem cell sources may be explored. Tissue engineering has been applied experimentally for the reconstitution of specific organs, such as the bladder. Recent progress suggests that other engineered organs created experimentally will also have clinical applicability.

11:00 AM *F1.5
Biologically Active Scaffolds used in Tissue and Organ Regeneration in Adults: Facts and Theories. Ioannis V Yannas, Biological Engineering, Mass. Institute of Technology, Cambridge, Massachusetts.

Scaffolds are highly porous, typically polymeric, materials that are used, either seeded with cells or unseeded, in protocols designed to synthesize tissue or organs in vitro or in vivo. A few among these (regeneration templates) have induced partial regeneration of three organs in animals and humans: skin, peripheral nerves along unprecedented distances, and the conjunctiva. The biological activity of regeneration templates depends critically on the presence of four structural features: selective melting of the quaternary structure of collagen to prevent platelet degranulation at the injured site (inhibits the inflammatory response following injury); incorporation of chemical composition that includes ligands specific for contractile fibroblasts (myofibroblasts are bound by these ligands); specific surface associated with the scaffold pore structure, that is sufficiently high (to bind almost all of the myofibroblasts at the injured site); and a degradation rate of intermediate value (to preserve the insoluble surface during, but not beyond, the entire period of active contraction at the injured site). Five sets of independent data are presented that support a basic conclusion: Contraction and regeneration are antagonistic processes during healing of wounds in most adult organs. The data are explained by the theory that regeneration of an adult organ in an injured site requires blocking of contraction; although necessary, blocking of contraction does not suffice to induce regeneration. Alternative interpretations of these observations are discussed. The mammalian fetus spontaneously closes wounds by regeneration while the adult closes wounds by contraction and scar synthesis. In the presence of a biologically active scaffold, wounds close instead by regeneration. It is suggested that an active scaffold blocks the adult healing response to severe injury, thereby uncovering the underlying fetal response.

11:30 AM *F1.6
Bioscaffolds Derived from Extracellular Matrix: Design Considerations and Factors that Influence In Vivo Remodeling. Stephen F Badylak, McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.

The extracellular matrix (ECM) can be considered as Nature's bioscaffold and is an important modulator of tissue and organ development during embryogenesis and of host inflammatory and reparative responses following tissue injury in adult mammals. The ECM consists of a mixture of structural and functional proteins arranged in a unique three-dimensional ultrastructure and is a determinant of both cell phenotype and the organization and spatial arrangement of various cell populations. Autologous, allogeneic, and xenogeneic ECM have been used as a scaffold for tissue reconstruction in numerous preclinical animal studies and in human patients. These scaffolds have been engineered to take many physical forms and to have tissue-specific mechanical properties; however, the effect of ECM processing methods upon biologic properties of the scaffolds and the host response to these scaffolds has received relatively little attention. The material properties of ECM scaffolds derived from different tissues such as dermis, tendon, pericardium, small intestine, urinary bladder, and liver vary significantly. The uniaxial and biaxial tensile strength, suture retention strength, isotropic properties, and the ability to support tissue-specific cell differentiation not only depend upon tissue source but also upon the methods used to prepare these ECMs as bioscaffolds for therapeutic use. The rate of scaffold degradation and the fate of scaffold degradation products depends upon the in vivo application and factors such as chemical cross-linking that may be used to prepare such materials. Factors that influence the rate and mode of in vivo tissue remodeling include: 1) the formulation/configuration of the scaffold substrate; 2) the environmental stressors such as mechanical loading that are placed upon the scaffold; 3) the rate of scaffold degradation, and; 4) the blood supply to the developing tissue. In summary, the ECM represents an excellent scaffold for tissue reconstruction but its ultimate utility will depend upon the ability to properly engineer this material for biomedical applications.

SESSION F2: Overview and Challenges in Biomaterials II
Chair: Christine Schmidt
Tuesday Afternoon, December 2, 2003
Back Bay A (Sheraton)

1:30 PM *F2.1
The Engineering Challenges For Biomaterials In Tissue Engineering. Gail K. Naughton, College of Business, San Diego State University, San Diego, California.

A crucial mainstay of tissue engineering is the biomaterial from which scaffolds used for cell-seeding are fashioned. Products initially commercialized in this field utilized simple mesh structures composed of degradable synthetic polymers which predominantly provided a structural function. Methods have been utilized to optimize cell attachment and secretion of natural extracellular matrix components in vitro. Multiple studies have shown that even within the class of cells of stromal origin significant differences exist in porosity, thickness, and degradation rate of the biomaterial scaffold required for optimal cell and tissue growth. Traditionally utilized animal derived biomaterials have been shown to induce an allergic reaction and lack of persistence. The development of human bioengineered collagen and other extracellular matrix materials offers a reproducible material which will prevent such reactions and may improve persistence of the scaffold material. Production and characteristics of recently developed human materials will be described. As we progress to more sophisticated structures where multiple cell types are required along with specific mechanical characteristics (i.e. blood vessels, urinary repair products) new biomaterials and scaffold designs must be employed. Most scaffolds used to date have been biocompatible but not biointeractive. Scaffolds have been designed to release growth factors that induce cellular differentiation and tissue growth in vitro, or cell migration into the implant in vivo. Biomaterials can also be designed to deliver anti-rejection or antimicrobial agents at the site of implantation. Results on the characteristics of scaffolds optimized for a variety of stromal and parenchymal cell seeding and growth will be described, along with new methods utilizing solid free-form fabrication techniques along with MRI and computerized tomography.

2:00 PM *F2.2
Designing Biologically Relevant Biomimetic Materials. Barbara D Boyan[1] and Zvi Schwartz[1,2]; [1]Georgia Tech/Emory Center for the Engineering of Living Tissues, Georgia Institute of Technology, Atlanta, Georgia; [2]Department of Periodontics, Hebrew University, Hadassah, Jerusalem, Israel.

In vivo, osteoblasts initiate bone formation on a bone surface that has been preconditioned by osteoclasts during bone resorption, resulting in a textured substrate with micro and nanotopographical features. Features of this natural surface can be fabricated on material surfaces, leading to altered osteoblast responses to a number of regulatory factors including steroid hormones, fluid induced shear force, growth factors and cytokines. The effects of surface microtopography are also dependent on the state of maturation of the responding cell population. Studies examining the mechanisms involved show that part of the response to surface rugosity is mediated by Cox-1 and part by Cox-2 based on the relative decrease due to resveratrol and NS-398. The responses to surface microtopography are mediated by complex signaling pathways that act in concert to promote osteogenic differentiation, including PKA, PKC and ERK MAP kinase. Use of model surfaces with well-defined microarchitecture indicates that specific structural features differentially modulate physiological responses of the cell. Controlling cell attachment can mimic some of these effects. Behavior of the cells due to microarchitecture can be restored to behavior on tissue culture plastic by promoting integrin binding, suggesting that current approaches to tissue engineering may be optimized for cell culture and not for in vivo requirements. This work was supported by a grant from the ITI Foundation. Institut Straumann, Waldenburg, Switzerland, supplied Ti disks. The authors acknowledge the contributions of their collaborators, students and staff to this work.

2:30 PM *F2.3
In Vitro-In Vivo Correlations of Inflammatory Cell Interactions with Surface-Modified Biomaterials. James Morley Anderson, Pathology, Case Western Reserve Univ., Cleveland, OH, Ohio.

Implantation of biomedical polymers and drug delivery systems results in the normal sequence of inflammatory and wound healing events, resulting in the foreign body reaction at the tissue/material interface. Even with biocompatible and biodegradable materials, the normal foreign body reaction consists of macrophages and foreign body giant cells at the tissue/material interface. Macrophages and foreign body giant cells are capable of not only facilitating degradation of biomedical polymers and drug delivery systems, but also are capable of modulating bioactive agent release patterns and adversely influencing bioavailability of agents from drug delivery systems. Our studies have focused on developing a mechanistic understanding of the influence of biomaterial surface chemistry on the formation and activity of macrophages and foreign body giant cells in the foreign body reaction. Studies utilizing materials with a broad range of surface chemistries have been used to investigate the capability of surfaces to induce macrophage and foreign body giant cell apoptosis, i.e., programmed cell death. Correlative in vitro and in vivo studies will be presented, which identify certain surface chemistries that facilitate programmed cell death and a reduction in the foreign body reaction.

3:30 PM *F2.4
Antibody delivery from implanted biomaterials: a model for preventing infection. David W. Grainger, [1]Chemistry, Colorado State University, Fort Collins, Colorado; [2]Microbiology, Colorado State University, Fort Collins, Colorado; [3]Chemistry, Colorado State University, Fort Collins, Connecticut.

Protein delivery for tissue neo- or re-genesis is a popular for implant and tissue engineering work. Protein-based agents for tissue engineering and implant-based delivery include growth factors and other cytokines, antimicrobial and apoptotic agents, immuno-stimulants or vaccines, bio-active enzymes and antibodies. We have recently reported several different antibody-releasing systems in the context of implant-centered infection. Current concerns about widespread anti-biotic resistance to many clinical antibiotics combined with the resurgence of interest in custom, humanized antibodies as drugs (as opposed to targeting agents) makes the use of antibody-based antimicrobials attractive. Over 200 humanized monoclonal antibodies are now in Phase I and Phase II trials as drugs for treating many pathologies. In vitro characterization of release and antimicrobial properties, and in vivo animal efficacy data against infection in implant and trauma models have recently been published (see below). In general, pooled polyclonal antibodies exhibit several interesting general therapeutic properties in vivo, and due to their abundance and low cost, offer a practical experimental alternative to expensive monoclonal agents in developing baseline models, delivery modes and efficacy studies. Our approach uses polyclonal human antibodies as suitable protein drug models in pioneering several implant-based releasing systems that should prove useful for extension to other models for many therapies. Specifically, human antibodies have been delivered to mice implant and trauma models of infection. Antibodies are formulated into hydrophilic polymer coatings or delivery devices in proximity to a surgical or trauma injury site, or biomedical implant. Local and systemic bio-distribution of the antibodies in a murine host are assayed using ELISA assays, and the correlation of these kinetics with various infection markers are tracked. While therapeutic value in infection models using clinically relevant gram positive pathogens (e.g., S. aureus) is limited, human antibodies are shown consistently in our studies to produce dramatic improvements in infection incidence and complications in murine models using relevant gram negative pathogens (e.g., P. aeruginosa, E. coli). Murine burn wounds, surgical wounds and implant-associated infection models have all been fully characterized. This antibody-derived benefit is due to local delivery in smaller doses than required systemically. Additionally, the therapeutic benefit is witnessed in the context of implanted biomaterials and, significantly, in treating antibiotic-resistant strains of bacteria as well. Finally, antibody delivery is shown to be synergistic with systemic conventional antibiotic therapy, meaning that both local protein and systemic drug treatments are compatible and clinically efficacious in this context.

4:00 PM *F2.5
Understanding The Mechanism Of Action Of Biomaterials And Disease Modifying Agents In Models Of Osteoarthritis In Vitro. Ross Tubo and Peter DiBenedetto; Genzyme Corporation, Framingham, Massachusetts.

Human articular chondrocytes cultured at high density form a three-dimensional articular cartilage graft (ACG) within three weeks. ACGs express articular cartilage specific genes, including type II collagen and aggrecan, and possess many of the histological hallmarks of articular cartilage, including metachromatic matrix staining and chondrocytes in lacunae with some columnar organization. Treatment of ACG with agents known to negatively impact cartilage health in vivo resulted in decreased expression of type II collagen and aggrecan and increased expression of matrix metalloproteinases, known to be active in cartilage matrix degradation. We have used this model to determine the impact of biomaterials and other agents on cartilage matrix degradation and synthesis.

4:30 PM *F2.6
New Developments in Spinal Cord Repair: Novel Nanostructured Biopolymer-Microglial Cell Implants.

Eugene P Goldberg[1,2], JB Stopek[1,2], WJ Streit[3,2] and JP Mickle[4,2]; [1]Biomaterials Center, Department of Materials Science & Engr, University of Florida, Gainesville, Florida; [2]McKnight Brain Research Institute, University of Florida, Gainesville, Florida; [3]Department of Neuroscience, University of Florida, Gainesville, Florida; [4]Department of Neurosurgery, University of Florida, Gainesville, Florida.

This paper will review recent studies in our laboratory devoted to a novel tissue engineering approach to Central Nervous System (CNS) cell regeneration using Biopolymer-Microglial Cell compositions. This research is especially aimed at Spinal Cord Repair. Results to date suggest that further development of this approach may prove practical to restore neuromuscular function for patients paralyzed by spinal cord injuries. There are no clinically effective therapies for repair of injured CNS tissues and spinal cord damage is especially debilitating. More than 250,000 Americans are affected and more than 50% are paraplegic or quadriplegic. Annual health care costs exceed $10 billion. Tissue engineering approaches with various polymer scaffolds and tissue growth factors have not been clinically successful. Studies reported here result from a unique multi-disciplinary program at the University of Florida involving the College of Engineering Biomaterials Center and the College of Medicine Brain Research Institute as well as Neuroscience and Neurosurgery departments. Research has involved the synthesis, characterization, and in vivo evaluation (including noninvasive high field MRI) of biodegradable implants comprising microglial cells contained in porous alginate or vegetable DNA implants, some with phospholipid nanosurface modification. Since microglia are the natural CNS repair cells, the strategy employed has been to develop implants containing viable microglia in porous biocompatible biopolymer matrices; composite structures designed to control the complex sequencing of synthesis and regulation of natural neurotrophic factors which repair the CNS and stimulate the growth of neurons. In a rat spinal cord injury model, effective wound healing and neural regeneration has been demonstrated with elimination of cystic cavity complications. The remaining challenge is to optimize the compositions, implant design, and surgical procedure for such implants to show that neuromuscular recovery can be routinely achieved in preclinical animal studies and thereby encourage human clinical trials.

SESSION F3: 3D Constructs
Chair: Lonnie Shea
Wednesday Morning, December 3, 2003
Back Bay A (Sheraton)

8:30 AM F3.1
Abstract Withdrawn

8:45 AM F3.2
On the Optimal Rate of Freezing Native and Artificial Tissues. Sreedhar Thirumala and Ram V Devireddy; Mechanical Engineering, Louisiana State University, Baton Rouge, Louisiana.

The effect of various parameters on the predicted optimal cooling rate (Bopt) of an arbitrary native or artificial biological system has been studied using a well-defined water transport model. The various parameters investigated are apparent activation energy (ELp), reference permeability of the membrane to water (Lpg), osmotically inactive cell volume (Vb), and the diameter of a spherical cell (D). Bopt is determined assuming a damaging criterion for initial percentage of water trapped (%WT), along with the end temperature at which water transport is assumed to cease (Tend). A significant observation of this study is that there is an exact inverse relationship between the ratio of initial volume of intracellular fluid (water) to the surface area available for water transport (denoted as, WV/SA) and the predicted optimal cooling rate (Bopt). This relation is then used to develop a Generic Optimal Cooling Rate Chart (GOCRC) with the activation energy (ELp) as the abscissa and a cooling rate (Bgraph) as the ordinate. By using this GOCRC we can calculate Bopt values for any combination of other given parameters assuming a predetermined value for %WT and Tend, by using a simple mathematical equation. Significantly, the GOCRC can be used irrespective of the cell geometry (spherical, cylindrical or a Krogh cylinder) or type (native or artificial) as long as the physiologically relevant data is provided. ACKNOWLEDGMENTS: Funding is provided by Louisiana Board of Regents {LEQSF (2002-05)-RD-A-03}. Thanks are also due to Prof. Mehmet Toner for the original idea.

9:00 AM F3.3
Design of DNA releasing scaffolds for efficient delivery. Lonnie D. Shea[1,2], Brian Anderson[1] and Jae-Hyung Jang[1]; [1]Chemical Engineering, Northwestern University, Evanston, Illinois; [2]Biomedical Engineering, Northwestern University, Evanston, Illinois.

Polymer scaffolds capable of controlled DNA delivery have shown the potential for directing progenitor cell development into functional tissues. However, the design parameters for these scaffolds that optimize and control transfection are not well understood. Tissue engineering scaffolds have been fabricated by the assembly and fusion of microspheres, with which the DNA is either associated or encapsulated. Naked DNA can be incorporated with high efficiency and the release rates can be controlled though the method of incorporation, the microsphere size, and the polymer molecular weight. The quantity and duration of protein production by polymeric release of DNA has been determined by noninvasive imaging of luciferase activity in vivo. Expression was dependent upon the DNA loading, release rate, and geometry of the scaffold. For porous tissue engineering scaffolds, luciferase activity was maintained within a factor of 2 for at least 28 days following implantation. Scaffolds have also been developed for the incorporation of DNA polyplexes, which can extend this approach to the in vitro engineering of tissues. The polymer can function to regulate the distribution whereas complexation provides for more efficient internalization and trafficking. DNA polyplexes incorporated into the polymer transfect cells throughout the scaffold in vitro. For release of DNA polyplexes, electrostatic interactions between the complex and the scaffold can be designed to retain the DNA at the surface. Complex association with the scaffold must be balanced with the need for cellular internalization. The interaction between polyethylenimine (PEI) complexed DNA and tissue culture polystyrene (TCP) was examined as a model. Unmodified TCP was incubated with PEI/DNA complexes, and was subsequently seeded with NIH/3T3 cells. PEI/DNA complexes readily associated with the surface, but the complexes were unable to associate with the cell and transfection was limited. Modification of the TCP to alter the electrostatic interactions allowed for the association of similar quantities of DNA as unmodified TCP; however, the altered electrostatic interactions allows for cellular association of the complexes and substantial levels of transfection. Confocal microscopy demonstrated that the complexes on the treated surface were either internalized by the cell or associated with the upper surface of the cell membrane. For substrate-mediated delivery, the mechanism of DNA internalization appears to be a combination of complex internalization from beneath the footprint of the cell and from aggregation of complexes on the cell membrane. In summary, polymers for DNA delivery can be designed to either release or retain the complex, with both approaches having opportunities to regulate gene transfer within the scaffold.

9:15 AM F3.4
Robotic Deposition of 3-D Periodic Hydroxyapatite Scaffolds for Bone Implants. Sarah M Michna[1], Jennifer A Lewis[1], Amy J Wagoner Johnson[2], Jennifer G Dellinger[1], Russell D Jamison[1], James E Smay[3], Joseph Cesarano[4] and Russ Parsons[5]; [1]Materials Science and Engineering, University of Illinois, Urbana, Illinois; [2]Mechanical Engineering, University of Illinois, Urbana, Illinois; [3]Chemcial Engineering, Oklahoma State University, Stillwater, Oklahoma; [4]Sandia National Laboratories, Albuquerque, New Mexico; [5]New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, New Jersey.

Robocasting, a robotic deposition method, was used to directly assemble 3-D periodic scaffolds with hydroxyapatite inks. Such inks were engineered to have the desired elastic moduli and yield stresses required to create self-supporting structures on several length scales. The ink was deposited through a cylindrical nozzle (diameter = 250 - 400 microns) and both the rod diameter and rod spacing was varied during the study. Here, we explore the processing parameters and ink characteristics needed to build these scaffolds. In vivo experiments are underway to characterize their biocompatability and the effect of scaffold geometry on bone ingrowth. We are also extending our fabrication process to incorporate controlled porosity within the HA phase through the addition of polymer microspheres. These HA scaffolds will be characterized by mercury intrusion porosimetry and SEM to evaluate their pore architecture.

9:30 AM F3.5
Microfabricated Biodegradable Scaffolds For Tissue Engineering Of Vital Organs. Jeffrey T Borenstein[1], Edward Barnard[1,2], Brian Orrick[1], Wing Cheung[2,3], Cathryn Sundback[2,3] and Joseph P Vacanti[3,2]; [1]Draper Laboratory, Cambridge, Massachusetts; [2]Massachusetts General Hospital, Boston, Massachusetts; [3]Harvard Medical School, Boston, Massachusetts.

Microfabrication has been demonstrated as a platform technology for the fabrication of scaffolds for tissue engineering of vital organs. The principal advantage of this technology over existing approaches is the ability to fabricate micron-scale features such as capillaries which are critical to the function of the engineered organ construct. Biocompatible polymers such as PDMS comprise useful templates for demonstration of the technology, since they leverage a substantial foundation of microfabrication process development, and they may ultimately provide tissue engineered constructs useful for a range of

wearable organ assist devices. However, a fully implantable replacement organ construct will require the use of biodegradable polymers and microfabrication techniques capable of fashioning these materials into organ scaffolds with high resolution. Earlier reports by this group described the development of microfabrication techniques capable of producing microfluidic structures for organ vasculature using the biodegradable polymer PLGA (poly(lactic-co-glycolic acid.)) Such structures were used to build a replica of organ vasculature through seeding and culturing of endothelial cells. In this paper, a process for the fabrication of high resolution three-dimensional scaffolds capable of co-culture of vascular and parenchymal cells is described. Processing of these constructs requires the bonding of multiple sheets of micromachined PLGA vessel networks together with layers which comprise the parenchymal compartments of the organ. In order to separate the vascular and parenchymal components, thin nanoporous membranes of PLGA are inserted and bonded in a sandwich structure. These three-dimensional constructs may then be seeded with the appropriate cell types and cultured to form organoid replicas. This technology will ultimately be scaled up to produce sufficient numbers of cells to replace the function of vital organs such as the kidney and liver.

9:45 AM F3.6
Computational modeling of cell-polymer interactions as part of the combinatorial design of biomaterials. Sascha D Abramson[2], Gabriela Alexe[3], Peter L Hammer[3], Doyle Knight[4,5], Agnes Seyda[1,2], Jackson Smith[2,5], Norbert Weber[1,2] and Joachim Kohn[1,2]; [1]Chemistry, Rutgers University, Piscataway, New Jersey; [2]New Jersey Center for Biomaterials, Rutgers University, Piscataway, New Jersey; [3]RUTCOR - Rutgers University Center for Operations Research, Rutgers University, Piscataway, New Jersey; [4]Department of Mechanical and Aerospace Engineering, Rutgers University, Piscataway, New Jersey; [5]Center for Computational Design, Rutgers University, Piscataway, New Jersey.

To be of practical utility, combinatorial approaches to biomaterials require (i) the availability of parallel synthesis techniques to generate large libraries of polymers, (ii) high through-put assays for the rapid characterization of bio-relevant material properties, and (iii) predictive computational models of the biological response of cells in contact with biomaterials. Here we report for the first time the integration of these 3 methodologies and illustrate the potential of this approach to accelerate the development of new degradable polymers for tissue engineering. The parallel synthesis of a library of 112 polyarylates had been reported previously. This library was used to develop high-throughput screening techniques to determine bio-relevant polymer properties such as the amount of protein adsorption on polymer surfaces using an immunofluorescent assay in a 384-well plate format, or the rapid measurement of gene expression in macrophages of inflammatory cytokines (IL-1beta, IL-6) by RT-PCR on large sets of polymers. The reliability of the data obtained by these high-throughput screens was validated. The data was then used, together with structural descriptors of polymer composition, as the inputs into two types of semi-empirical models - Artificial Neural Network (ANN) and Logical Analysis of Data (LAD). From the library of 112 tyrosine-derived degradable polyarylates, 62 polymers were randomly selected as the 'training set'. Rat lung fibroblasts (RFL) and non-transformed human fetal fibroblasts (NFF) were seeded on solvent cast polymer surfaces and cell growth was measured. These data were used to train the models which then predicted cell growth on the 50 remaining polymers in the library for which no cell growth data had been collected. Subsequent experimental validations indicated that the ANN accurately predicted cell growth for the high growth polymers, but did not provide accurate predictions for low cell growth polymers. LAD correctly predicted the high and low cell growth polymers and, additionally, found optimal ranges for polymer chemical composition, surface chemistry, and bulk properties. Both models correctly identified lead polymer compositions which were superior to tissue-culture polystyrene plates for the growth of NFF cells. The ease by which these lead compositions could be identified illustrates the potential utility of combinatorial/computational approaches in biomaterials design.

10:30 AM F3.7
Bioactive Scaffolds Based on Bioartificial Biodegradable Hollow Microfibers. Luigi Lazzeri, Maria Grazia Cascone, Lorenzo Pio Serino, Serena Danti and Paolo Giusti; Department of Chemical Engineering, University of Pisa, Pisa, Italy.

Both synthetic and natural polymers have been investigated for use as tissue engineering scaffolds. In particular degradable polymers are used that allow tissue growth into the matrix while eliminating the need for a second surgery to remove the implant. In order to promote tissue growth a scaffold must have a large surface area to allow cell attachment. This is usually obtained by producing highly porous matrices with a pore size large enough so that cells penetrate the pores and with interconnected pores to facilitate nutrient and waste exchange by cells deep within the scaffold. Porosity and pore size are properties often dependent on the production method. Several methods have been developed to create highly porous scaffolds that include fiber bonding. Biodegradable hollow microfibers containing particles loaded with active agents could be used to produce a special kind of substrate for tissue engineering. This substrate should be able to work as a scaffold and at the same time it should act as a system releasing drugs such as growth factors, able to stimulate cell growth inside the construct. Aim of the present work was the preparation of polymeric scaffolds based on hollow biodegradable microfibers. The fibers were produced by a dry-wet spinning procedure, using poly(L-lactic acid) (PLLA) and PLLA added with different natural macromolecules (bioartificial fibers) such as: dextran, chitosan etc. The spinning procedure enabled the fibers to be loaded with biodegradable microparticles containing suitable active agents or drugs. The morphology of both fibers and particles was investigated by scanning electron microscopy. The mechanical and thermal properties of the fibers were studied. In vitro release tests were performed to evaluate the release of the drugs from the fibers loaded with the particles. In vitro tests based on the cell culture method were performed to investigate cell adhesion and growth into the scaffolds.

10:45 AM F3.8
Abstract Withdrawn

11:00 AM F3.9
It looks like bone, it acts like bone, but is it bone?- A Resorbable Bone Graft Substitute. Stephen A Doherty, David D Hile and Debra J Trantolo; Cambridge Scientific, Inc., Cambridge, Massachusetts.

Approximately one million bone grafting procedures are performed world-wide, with roughly half of them performed in the USA. Grafting is required in many bone repair scenarios, resulting in tissue demand far exceeding the supply. Autograft is complicated by the need for a secondary harvesting site and insufficiencies in the supply of donor stock; allograft raises safety concerns regarding the risks of disease transmission. These issues have encouraged the development of synthetic bone graft substitutes (BGS). Tricalcium phosphate and hydroxyapatite are among the most common synthetics, possessing compositions similar to that of native bone while providing osteoconductive surfaces which encourage new bone formation. In some applications, though, these materials do not yield full functional and cosmetic recovery. Owing to resorbablity, defect sites are not replaced by native bone and dimensional stability is sometimes compromised. Poly(propylene glycol-co-fumaric acid) (PPF) is a unsaturated biopolymer that can be used as the basis of a BGS that can provide an adjunct to the aforementioned grafting materials. A biopolymeric-based BGS with scaffolding properties can overcome many of the drawbacks associated with currently used grafting material, particularly those owing to contouring limitations. PPF can be crosslinked in the presence of effervescent agents, directly applied to defect site, and cured in situ. The curing process generates a porous osteoconductive scaffold with morphological and mechanical properties comparable to bone. This polymer-based BGS has an interconnected pore structure in the size range of 50-400 microns. The initial mechanical properties are similar to that of cancellous bone and the temporal mechanics suggest that the material is absorbed at a rate commensurate with new bone formation. The material has been shown in preclinical trails of intriguing use for a number of periodontal and orthopaedic applications. The parametric study of sensitive formulation variables will be presented to demonstrate material manipulation for endpoint properties aligned with specific clinical indications.

11:15 AM F3.10
Vascular Tissue Engineering: Effect of Scaffold Architecture on Smooth Muscle Cell Response. Sumona Sarkar, Patrick Rourke, Tejal Desai and Joyce Wong; Biomedical Engineering, Boston University, Boston, Massachusetts.

One of the most sought after goals in the field of vascular tissue engineering is the construction of functional small diameter (< 6 mm) blood vessels in vitro. While there have been significant advances in the tissue engineering of blood vessels, the mechanical properties are still orders of magnitude below that of vessels found in vivo. Smooth muscle cells (SMCs) and their surrounding matrix are thought to provide much of the structural support in blood vessels. Since the structure of native blood vessels is highly organized, we hypothesize that this spatial and hierarchical organization of SMCs and matrix has evolved to provide sufficient burst strength, compliance and stability. To test this hypothesis, we first examined the effects of scaffold topography on SMC response in vitro. While the use of topographical cues has been used extensively to control cell morphology in vitro, we are not aware of studies examining topographical effects on smooth muscle cell response. We used soft lithography to create PDMS (polydimethylsiloxane) scaffolds

patterned with grooved relief structures. We systematically varied groove width to investigate the role of relief structure dimensions on SMC morphology (aspect ratio), orientation (alignment), and attachment strength. SMCs seeded on substrates with grooved relief structures were more aligned (>90%) and elongated (aspect ratio > 11) than those seeded on smooth scaffolds (alignment: < 20%; aspect ratio: < 5). Furthermore, SMC attachment was higher on grooved (>70%) than on smooth (< 8%) scaffolds. We found that groove width is an important determinant of SMC morphology: decreasing groove width elicited elongation of smooth muscle cells and enhanced attachment of cells to the scaffold. Statistical analysis indicated that there is a high correlation between SMC orientation, aspect ratio, and attachment strength. These findings support our hypothesis that topographical cues in the form of grooves on the tissue-engineered scaffolds have a significant effect on SMC response. Techniques used in this study can be used to test the role of structural organization of smooth muscle cells in relation to scaffold architecture on SMC response. This approach may be useful for the development of novel scaffold architectures to control SMC response in order to achieve the required physical properties for blood vessels.

11:30 AM F3.11
Cell-controlled growth factor and gene delivery enhances wound healing in a novel model of tissue regeneration. David J. Geer[1], Pedro Lei[1], Daniel D. Swartz[2] and Stelios Andreadis[1]; [1]Chemical Engineering, SUNY - Buffalo, Amherst, New York; [2]Chemical Engineering, SUNY at Buffalo, Amherst, New York.

Natural biomaterials, such as fibrin, may be useful in promoting reepithelialization of skin wounds by providing a conduit for controlled migration and/or a matrix for the delivery of therapeutic proteins and genes to cells. Fibrin has been used in multiple medical applications including Achilles tendon repair, remodeling of bone grafts, skin grafting after burn or injury, repair of peripheral nerves and induction of angiogenesis. In addition, it has also been used for facilitating the delivery of growth factors (e.g. FGF-1 and neurotrophic factors (NTFs)) and genes (e.g. EGF-encoding DNA) to promote wound healing. Controlled delivery of growth factors using fibrin gels has been recently demonstrated with the development of techniques for incorporating peptides that effectively link heparin-binding proteins into the fibrin matrix during polymerization. We developed a novel method to covalently conjugate KGF and genes encoding for KGF into the fibrin matrix during polymerization. Controlled delivery of the bound KGF or plasmid DNA is then achieved via fibrinolysis by cells migrating into the wound space through activation of the plasminogen system. Fibrinolysis and KGF delivery can be controlled by addition of aprotinin to the gel. To demonstrate the effectiveness of the KGF delivery system we developed an in vivo model of wound healing using tissue engineered skin transplanted onto athymic mice. Human epidermal keratinocytes were seeded on acellular dermis, grown for 5 days at the air-liquid interface and then grafted to the dorsum of athymic mice. Four weeks later, the transplanted tissues were infiltrated by mouse mesenchymal cells and showed completed integration with the surrounding mouse skin. Next, the transplanted tissues were punch wounded and treated with fibrin supplemented with KGF. Tissues were excised at 7 and 14 days post-wounding and processed for histological analysis. Approximately 33% of KGF-treated tissues (N = 9) showed complete reepithelialization by 7 days post-wounding in contrast to untreated controls (N = 4) that did not heal at this time. Surprisingly, the blood vessel density in the granulation tissue at 7 and 14 days was also higher in tissues that received KGF treatment suggesting a stimulatory effect on the migration of mouse endothelial cells. Our results suggest that fibrin may be a suitable biomaterial for controlled release of growth factors to increase cellular migration and growth, ultimately promoting faster healing. Furthermore, development of biomaterials as scaffolds for cell migration and as matrices for drug delivery may be facilitated by development of in vitro and in vivo biomimetic models of wound regeneration.

11:45 AM F3.12
Characterizing Fibroblast Attachment, Proliferation, and Migration on Self-Assembled Collagen Threads. Kevin G. Cornwell[1,2] and George D. Pins[1]; [1]Biomedical Engineering, Worcester Polytechnic Institute, Worcester, Massachusetts; [2]Graduate School of Biomedical Sciences, University of Massachusetts Medical School, Worcester, Massachusetts.

Aligned collagen scaffolds for tendon and ligament repair should possess mechanical strengths and hierarchical substructures that recapitulate the native tissue. These qualities are expected to enhance tissue regeneration by promoting rapid cell attachment, proliferation and migration while maintaining mechanical integrity. Previously, we developed a biomimetic approach to self-assembling solutions of collagen molecules into collagen threads that exhibited mechanical properties and aligned fibrillar substructure comparable to native tendon structures. In this study, we developed a series of experimental techniques to assess the capacity of individual collagen threads to facilitate tissue regeneration by measuring cell attachment, proliferation and migration in vitro. Self-assembled collagen threads were extruded from solutions of collagen molecules acid-extracted from rat tail tendon, and were subsequently cross-linked at 105°C under vacuum. Fibroblasts were isolated from human neonatal foreskin dermis or tendon tissue and cultured in DMEM with 10% FBS. Fibroblast attachment was assayed by affixing collagen threads to sterile tissue culture coverslips and seeding the scaffolds with aliquots of cells. After allowing the cells to adhere to the threads for 4 hours, the fibers were incubated with medium containing MTT stain, the cells were lysed and a colorimetric assay was used to correlate the amount of MTT stain with the number of attached cells. The rate of cell proliferation was determined by measuring changes in MTT staining of fibroblast-seeded collagen threads on days 1, 4, and 7. To evaluate cell migration, fibroblast-seeded collagen threads were stained with Neutral Red and visualized by phase contrast microscopy. For each thread, cell migration distances and relative cell confluence were measured as a function of time. The results of this study indicate that self-assembled collagen threads enhance the rates of fibroblast attachment, proliferation, and migration relative to those observed on native tendon fibers control materials such as Prolene threads. These findings suggest that self-assembled collagen threads may provide an aligned scaffolding material to enhance tissue regeneration and to facilitate the rapid repair of injured tendons and ligaments.

SESSION F4: Natural and Artificial Polymers
Chair: Christine Schmidt
Wednesday Afternoon, December 3, 2003
Back Bay A (Sheraton)

1:30 PM F4.1
Abstract Withdrawn

1:45 PM F4.2
In Vitro Biocompatibility Assessment of Anionic Amino Acid or Peptide Conjugated Polyhema Co-Polymers for Bone Tissue Engineering Applications. Jie Song[1], Catherine Klapperich[1] and Carolyn Bertozzi[2,1,3]; [1]Materials Sceinces Division, Lawrence Berkeley National Laboratory, Berkeley, California; [2]Departments of Chemistry and Molecular and Cell Biology, University of California, Berkeley, California; [3]Howard Hughes Medical Institute, University of California, Berkeley, California.

We have generated a library of polyHEMA-based hydrogel polymers conjugated with anionic amino acid and peptide ligands that induce the formation of calcium phosphates in vitro under various mineralization conditions. This results in a polymer/ceramic composite material. The microstructure and crystallinity of the nucleated mineral has been analyzed using SEM and energy dispersive x-ray analysis and vary as a function of mineralization conditions. These materials were designed as bone mimics. In order to determine how the anionic amino acid and peptide ligands are interacting with bone cells at the molecular level, we have performed a series of high throughput gene expression experiments using Affymetrix Human Genome Arrays (Affymetrix, Santa Clara, CA). In order to isolate gene expression changes due to the interactions of the peptides and amino acid ligands on bone cells, we made flat glass surfaces displaying the library of conjugates. Cells exposed to these test slides and to tissue culture polystyrene control surfaces were studied using the microarrays. The data from these experiments were analyzed using a robust mean analysis at the probeset level and analysis of variance at the chip level. A set of genes that were significantly differentially expressed ($p<0.001$) between the control cells and the cells grown on the model surfaces containing anionic mineral-nucleating ligands was identified. Relating this list of genes back to what is known about the biology of healthy bone, we can begin to determine how closely our material mimics bone matrix in its ability to guide mineralization and bone cell activity.

2:00 PM F4.3
Combinatorial Screening of Augmented Polysaccharides for Cartilage Repair. Newell Washburn[1], Michael Weir[1], Wan-Ju Li[2] and Rocky Tuan[2]; [1]NIST, Gaithersburg, Maryland; [2]NIH/NIAMS, Bethesda, Maryland.

We have prepared hyaluronic acid and alginate derivatives functionalized with peptides in order to enhance their capabilities in promoting cartilage repair. To polysaccharides were covalently linked peptides that are known to provide improved mechanical properties, specific interactions with chondrocyte alpha5beta1 and alpha6beta1 integrins, and insulin-like growth factor binding. Design-of-experiment methods were used to screen the response of fetal bovine chondrocytes to these hydrogels. The effects of hydrogel composition,

concentrations of TGF-beta, FGF, and IGF-1, and cell density were all probed. Measures of cellular response include proliferation, and production of collagen type II and aggrecan. We have found a stronger dependence of culture conditions on samples with lower cell density and will present characterization of the aggregate response surface.

2:15 PM F4.4
Controlling Mammalian Cell Activity on Micropatterned Polyelectrolyte Multilayers. Michael Berg[1], Sung Yun Yang[2], Paula T. Hammond[1] and Michael F. Rubner[2]; [1]Department of Chemical Engineering, MIT, Cambridge, Massachusetts; [2]Department of Materials Science and Engineering, MIT, Cambridge, Massachusetts.

Polyelectrolyte multilayers have many interesting properties and potential applications as biomaterials due to their ease of processing, conformal coating of any geometry, and wide range of applicable materials. This work focuses on patterning multilayer surfaces our group has found to be bio-inert (resistant to cell adhesion) to present specific areas for cell attachment to study the effects of varying ligand density on adhesion, spreading, cytoskeletal organization, and migration. The bio-inert surfaces consist of hydrogen-bonded multilayer films made from polyacrylic acid (PAA) and polyacrylamide (PAAm). We have been successful in using polymer-on-polymer stamping to pattern poly(allylamine hydrochloride) (PAH) onto the surface when PAA is the top layer of the film. Ligands containing the adhesion peptide, RGD, can be tethered onto the primary amine groups of PAH to promote cell adhesion. Varying the stamping conditions leads to control over the RGD density, which can be monitored by quartz crystal microbalance (QCM) or with radiolabled peptides. Wild type (WT) NR6 cells (mouse fibroblasts) were seeded on patterned surfaces presenting various RGD densities to study such activities as spreading, motility, and cytoskeletal protein organization. The observed cell interactions were indeed RGD specific since the fibroblasts detached in the presence of soluble RGD. The specificity of the cell interactions was also confirmed by substituting a ligand, which is not known to promote adhesion. We have also looked at patterns specific to tissue engineering applications such as gradients to control the direction of cell movement and study the haptotaxis process.

2:30 PM F4.5
Polymer Scaffolds for Multi-cellular Architectures. Padmavathy Rajagopalan, Francois Berthiaume, Arno W. Tilles, Mehmet Toner and Martin L Yarmush; Center for Engineering in Medicine, Massachusetts General Hospital, Harvard Medical School, Shriners Hospital for Children, Boston, MA, Massachusetts.

Chronic liver failure is a significant cause of fatality in the United States. Bioartificial livers consisting of functional hepatocytes can provide temporary support to patients with fulminant liver failure and save lives of patients awaiting orthotopic liver transplantation. The optimal function of a bioartificial liver depends on the ability to maintain hepatocytes in an environment that resembles conditions in vivo. In the liver, layers of hepatocytes and non-parenchymal cells form a continuous three-dimensional tissue. Although, such cell-cell communications between multiple cell types in vivo is essential in maintaining differentiated cell function, the design of such complex systems in vitro has proven to be difficult. To enhance hepatocyte function in liver-assist devices our goal is to build multi-cellular 3-D constructs that resemble the in vivo structure of liver sinusoids. These constructs consist of confluent layers of hepatocytes and nonparenchymal cells (e.g. fibroblasts, and endothelial cells) with ultra-thin polymer layers between adjacent cell layers. Polymer layer deposition is accomplished by taking advantage of the chemistry and electrostatic charge on cell surfaces. A dilute solution (0.001-0.003 %w/v) of a cationic polyelectrolyte solution such as polyethylenimine or chitosan is deposited on a confluent layer of primary hepatocytes or 3T3 fibroblasts. This polymer layer serves as a substrate for the subsequent deposition of multiple cell types. Using this technique, multi-cell architectures consisting of a hepatocyte layer on fibroblasts as well as double hepatocyte layers have been cultured. The presence of polyethylenimine was verified by depositing an anionic fluorescent dye. Cell viability is not adversely affected by the deposition of the polyelectrolyte solution. Preliminary results indicate that the functional capability of hepatocytes, specifically, albumin production is significantly enhanced when hepatocytes are maintained in multi-cellular layers. The use of polyelectrolyte solutions as ultra-thin polymer scaffolds is a promising technique for the design of complex multi-cellular architectures.

2:45 PM F4.6
Angiogenic Potential of Synthetic, Degradable Hydrogels Fabricated From Tyrosine-Derived Polycarbonates. Kristen Labazzo[1,3], Durgadas Bolikal[1,3], Margaret Schwarz[2] and Joachim Kohn[1,3]; [1]Chemistry, Rutgers University, Piscataway, New Jersey; [2]Department of Surgery, Robert Wood Johnson Medical School, New Brunswick, New Jersey; [3]New Jersey Center for Biomaterials, Rutgers University, Piscataway, New Jersey.

When utilizing biomaterials for tissue engineering, vascularization of implanted devices is necessary for successful tissue integration and survival. Rarely does a device demonstrate inherently angiogenic properties without the use of exogenous biological substances. Our lab has synthesized hydrogels by crosslinking poly(DTE-co-10%DT carbonate), a degradable, tyrosine-derived polycarbonate, with PEG-dihydrazide. In previous studies, one of the degradation products, PEG-di-DT hydrazide, appeared to induce angiogenesis. To further examine this phenomenon, an in vitro endothelial cell migration assay, and an in vivo subcutaneous rat model were utilized. The endothelial cell migration assay, commonly used for angiogenic determination, was used to test PEG-di-DT hydrazide. Briefly, human aortic endothelial cells (HAEC's) were plated in transwells, with fibrogen precoated membranes of 0.8 μm. The bottom wells contained the test substances which consisted of media (baseline), the dilutions of PEG-di-DT hydrazide (1 μg/mL and 1000 μg/mL), or 0.01 μg/mL basic FGF-β, a naturally-occurring angiogenic substance. The cells were incubated at 37 C for six hours, were fixed and stained with Diff Quik (Sigma) and quantitated under an inverted microscope. The in vitro results consistently demonstrated that the PEG-di-DT hydrazide behaved as a chemoattractant to the HAEC's compared to FGF-β. Initial results show that there may be a dose-dependent effect, where increased concentrations of PEG-di-DT hydrazide encourage more cells to migrate. To assess the basic biocompatibility of the entire hydrogel, an in vivo rat model was used. Porous scaffolds of 8 mm diameter were implanted subcutaneously in rats for up to 15 weeks. The explanted tissue was fixed and stained with Hematoxylin and Eosin, and qualitatively observed under a light microscope. In vivo, the implanted hydrogels induced increased vasculature and tissue infiltration over time compared to control implants. Except for the 1-week time point, little inflammation was observed. Although the in vivo observations of increased vasculature and tissue infiltration could potentially be associated with the transient inflammatory response as opposed to angiogenesis, the in vitro endothelial cell migration results support the hypothesis that PEG-di-DT hydrazide may stimulate angiogenesis.

3:30 PM F4.7
Photocrosslinkable Hyaluronic Acid Hydrogels for Tissue Engineering. Jennie B. Leach[1] and Christine E. Schmidt[2,3]; [1]Chemical Engineering, University of Texas at Austin, Austin, Texas; [2]Biomedical Engineering, University of Texas at Austin, Austin, Texas; [3]Texas Materials Institute, University of Texas at Austin, Austin, Texas.

Comprehensive tissue engineering therapies should be bioactive, provide a degradable scaffold, promote natural wound healing, and facilitate regeneration. To meet these aims, our goal was to design a hydrogel biomaterial composed of hyaluronic acid (HA; also called hyaluronan). HA is a naturally occurring non-immunogenic polymer that plays key roles in several physiological processes, including angiogenesis and extracellular matrix homeostasis. As a biomaterial, HA provides a combination of unique advantages: an ease of production and modification, a hydrophilic and non-adhesive character, and an enzymatically-mediated degradation. With these advantages in mind, we synthesized glycidyl methacrylate-HA (GMHA) conjugates, which were subsequently photocrosslinked to form hydrogels. A range of hydrogel degradation rates was achieved as well as a corresponding, modest range of material properties (e.g., swelling, mesh size). Increased amounts of conjugated methacrylate groups were associated with increased crosslink densities and decreased degradation rates and had no significant effect on human aortic endothelial cell cytocompatibility and proliferation. Rat subcutaneous implants of the GMHA hydrogels indicated good biocompatibility and little inflammatory response. We also synthesized GMHA-peptide conjugates and characterized their material properties and ability to support in vitro cell adhesion. Finally, we investigated the feasibility of GMHA-based hydrogels to controllably release a model protein, bovine serum albumin. We conclude that these novel GMHA hydrogels are promising scaffolding biomaterials for the support of repair or regeneration in a variety of soft tissue engineering applications.

3:45 PM F4.8
Tissue Engineering Scaffold Materials from Self-Assembling Modular Artificial Proteins. Lixin Mi, Stephen Fischer and James L Harden; Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, Maryland.

We have utilized de novo protein design and recombinant DNA methods to develop a library of self-assembling proteins with specific biofunctional attributes for cell and tissue engineering scaffolds. These artificial proteins include engineered molecular recognition elements designed to direct the self-assembly of multi-component hydrogels

with tailored microstructure, topology, and biofunctional activity. The proteins are fibrilar, telechelic designs based on a modular, multi-block architecture that includes independent inter-chain binding and linker domains. The central linker domains of each protein are water soluble, disordered sequences that encode a unique biofunctional feature. The associating end blocks are amphiphilic helices designed to serve as smart cross linking agents of the hydrogel. In this talk, we present studies of a series of proteins with complimentary associating end blocks that form trimer aggregates. The center blocks of each these proteins include a cell binding ligand. The multi-functional hydrogels that self-assemble from these components have been characterized for their materials properties and for their interactions with human cell lines. In particular, confocal fluorescence microscopy studies and metabolic assays show that cell adhesion, cytoskeletal reorganization, and the formation of focal adhesion complexes are sensitive to the types and relative concentrations of biofunctional domains in the hydrogels. These studies highlight the potential of such modular, hydrogel-forming proteins for use in tissue engineering applications

4:00 PM F4.9
Chitonsan/alginate hybrid scaffolds for bone tissue engineering. Zhensheng Li, Hassna Rehman Ramay and Miqin Zhang; Materials Science and Engineering, University of Washington, Seattle, Washington.

For the past decade, a large number of natural and synthetic porous polymeric materials have been extensively investigated for use as scaffolding materials for bone tissue engineering. Although these efforts have resulted in notable progress in some aspects and to varying degrees, development of 3D bioresorbable scaffolds that have both desired mechanical strength and biological properties remains a formidable challenge. In the present study, we report the development of a novel hybrid biodegradable porous scaffold made of naturally derived chitosan and sodium alginate, with significantly-improved mechanical and biological properties. The scaffolds have a three-dimensional interconnected porous structure and are fabricated through a thermally induced phase separation process. Physical, mechanical, and biological properties of the scaffolds were examined with various characterization techniques. The results showed that the compressive yield strength of the chitosan-alginate scaffolds was as high as $\sim$ 0.46 Mpa, presumably attributed to the ionic bonding between the amino groups of chitosan and carboxyl groups of alginate. The studies of cell-material interaction indicated that osteoblast cells seeded on the alginate-chitosan scaffolds and cultured without osteogenic medium appeared to attach and proliferate well on the scaffolds, leading to the formation of bone nodules. The chitosan-alginate polymer scaffolds do not swell and are structurally stable in solution of neutral PH.

4:15 PM F4.10
Polyelectrolyte Multilayer Films As Cytophobic and Antibacterial Coatings. Sung Yun Yang[1], Michael C Berg[2] and Michael F Rubner[1]; [1]Materials Science and Engineering, M.I.T., Cambridge, Massachusetts; [2]Chemical Engineering, M.I.T., Cambridge, Massachusetts.

Polyacrylamide (PAAm) containing H-bonded multilayers exhibit superb long-term resistance to mammalian cell adhesion. In addition, their micropatterning capabilities make it possible to study surface-mediated cell behavior. The swelling behavior of these multilayer films was previously found to be the most important factor in determining surface cell-resistance. In an extended study involving a new cell type, poly(acrylic acid) (PAA)/PAAm multilayers were also found to be resistant to primary rat hepatocytes and non-cytotoxic to this cell type. In addition, using in-situ metal synthesis, silver nanoparticles can be loaded into the PAA/PAAm multilayer films to render them suitable for anti-bacterial coating applications. We have also explored the use of copolymers to deposit polyelectrolyte multilayers. PAAm containing ionic copolymers, i.e. PAAm with anionic or cationic comonomers were also investigated as candidates for bio-inert surfaces. Because the PAAm copolymer-multilayers are assembled mainly through electrostatic interactions, they are stable at high pH conditions without the need for any further treatment. These copolymer systems exhibit cytophobic and cytophilic behavior based on their assembly conditions. Suitable designed PAAm copolymer multilayer systems also exhibit antibacterial activity.

4:30 PM F4.11
In Vitro and In Vivo Biocompatibility Analysis of Poly (Glycerol Sebacate) as a Potential Nerve Guide Material. Cathryn Sundback[1], Jeffrey Y Shyu[1], Timothy P Sheahan[2], Yadong Wang[3], William C Faquin[2], Robert S Langer[3], Joseph P Vacanti[1] and Tessa A Hadlock[2]; [1]Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; [2]Department of Otolaryngology, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, Massachusetts; [3]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Intraluminal structures within nerve guidance conduits, such as fibers, sponges, and aligned gels, enhance peripheral nerve regeneration. These materials are biocompatible and bioresorbable, but tend to have poor mechanical properties and precise guidance patterns cannot be produced. A novel biodegradable tough elastomer, poly (glycerol sebacate) (PGS), has rubber-like elasticity and good mechanical properties obtained through covalent crosslinking and hydrogen bonding. PGS is tougher than most hydrogels and is capable of large reversible deformation. Using conventional processing techniques, PGS can be processed into precision patterns that may enhance guided axonal regeneration. The potential use of PGS in nerve guide repairs was assessed through in vitro biocompatibility tests and in vivo soft tissue response in comparison with poly (lactic-co-glycolic acid 50:50) (PLGA). In vitro, NIH3T3 fibroblast monolayers were exposed to diluted polymer extracts to assess cell viability (Neutral Red) and metabolic activity (MTT). In vitro biocompatibility tests were conducted with Schwann cells on PGS and PLGA to assess cell viability, metabolic activity, apoptosis, cell attachment (N-acetyl-β-D-hexosaminidase), and DNA synthesis activity (Proliferating Cell Nuclear Antigen); minimal cytotoxic and bioactive effects were observed with PGS. We implanted PGS and PLGA pieces in direct apposition to the sciatic nerve in Fisher rats, and evaluated fibrous capsule formation (Masson's Trichrome), inflammatory response (H&E), and newly recruited ED1+ macrophage presence. PGS gradually resorbed without swelling, decreasing to 40% of its original thickness at 35 days and resorbing fully at 60 days; increasing crosslinking density will increase resorption time. PLGA swelled to 190% of its original thickness at 21 days. PGS caused minimal tissue reaction with no chronic inflammation; macrophage density was comparable to that of sham samples. In contrast, PLGA caused thick fibrotic bands, significant chronic inflammation, and increased macrophage density. Based on these data, PGS is a potential nerve guidance conduit material.

4:45 PM F4.12
Biodendrimers For Repair Of Corneal Wounds: In Vitro And In Vivo Results. Mark Grinstaff[1,2], Michael Carnahan[1], Paul Kang[2], Stella Finkelstein[2], Andy Velazquez[2] and Terry Kim[2]; [1]Departments of Biomedical Engineering and Chemistry, Boston University, Boston, Massachusetts; [2]Department of Ophthalmology, Duke University, Durham, North Carolina.

Dendrimers are monodisperse macromolecules composed of distinct structural entities including a focal point, interior region, and numerous end groups. As a consequence of this structure, dendrimers possess unique properties which are of interest for medical applications. We are investigating dendrimers composed of biocompatible monomers (e.g., glycerol, lactic acid, and succinic acid), termed "biodendrimers." For tissue engineering applications, we are exploring the use of photocrosslinkable dendrimers for the repair of corneal lacerations. We have shown in vitro that we can seal 4 mm linear and stellate wounds more effectively than sutures. One dendritic macromolecule ([G1]-(PGLSA-MA)2-PEG) seals the wound and can withstand intraocular pressures up to 171 mmHg without leakage at the wound site. We will also report the initial results from our in vivo studies, which further support the use of this novel adhesive for repairing corneal wounds. This sutureless procedure is approximately five times faster than suturing the wound, potentially reducing surgical time and intervention. Moreover, the crosslinked gel is transparent, elastic, and adhesive. The tissue sealing mechanism is likely one of physical entrapment where an inter-penetrating network (IPN) is formed between the crosslinked copolymer and the tissue.

SESSION F5: Poster Session: Biomaterials for Tissue Engineering I
Chair: Lonnie Shea
Wednesday Evening, December 3, 2003
8:00 PM
Exhibition Hall D (Hynes)

F5.1
Investigation Of Effect Of Morphology And Hydration On Mechanical Properties Of Polymer Scaffolds For Tissue Engineering. Sascha Abramson[1,2], Adam Kaufman[1,2] and Joachim Kohn[1,2]; [1]Chemistry, Rutgers University, Piscataway, New Jersey; [2]New Jersey Center for Biomaterials, Rutgers University, Piscataway, New Jersey.

A wide variety of techniques have been used for the fabrication and evaluation of polymeric scaffolds for tissue engineering. Small changes in the fabrication method can lead to dramatic changes in scaffold properties, including architecture. We examined the effects of small variations of a solvent casting/poragen leaching technique on the

morphology and compressive strength of scaffolds made from poly(DTE carbonate), a tyrosine-derived degradable polycarbonate, which has been explored for tissue engineering. Scaffolds were mechanically tested under compression in ambient conditions (room temperature and dry) and in physiological conditions (37 C and hydrated). Scaffolds were fabricated by poring a 10% w/v solution of polymer in dioxane and water over a bead of salt of a known size distribution. After the solution had spread, immersing the scaffolds in liquid nitrogen induced phase-separation of the water and dioxane. Scaffolds were then freeze-dried and repeated water washings leached the salt out. To vary the scaffold morphology, the amount and the size distribution of the salt was varied systematically. Scaffolds exhibited a bimodal distribution of pores. Macropores were imprints left by the salt crystals and corresponded to the size distribution of the salt used to fabricate the scaffold. Micropores were 1-10 μm and had similar organization regardless of fabrication conditions. Pore size was found to have little effect on compressive strength of the scaffolds. Hydration had a dramatic effect on the compressive modulus. Scaffolds tested under physiological conditions consistently showed lower moduli than dry scaffolds. For example, the standard sponge made in our laboratory, using 200-400 μm sieved salt, had a $\sim$40% drop in compressive modulus when tested under physiological conditions. Over all, sponges tested under physiological conditions maintained 79% of the compressive modulus of samples tested dry. A second more detailed study on a selected group of scaffolds confirmed these results.

F5.2
Creation of Hepatocyte Spheroid Array for High Throughput Screening. Akihiro Hirano[1], Hidenori Otsuka[2], Yukio Nagasaki[3], Yasuhiro Horiike[2], Teruo Okano[4] and Kazunori Kataoka[1,2]; [1]Department of Materials Science, University of Tokyo, Bunkyo-ku, Tokyo, Japan; [2]Biomaterials Center, National Institute for Materials Science, Tsukuba, Ibaraki, Japan; [3]Department of Materials Science and Technology, Tokyo University of Science, Noda, Chiba, Japan; [4]Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan.

High-throuput screening(HTS) using high-density microplates is the primary method for the discovery of novel lead candidate molecules in the field of drug discovery. In addition, conventional methods for detecting environmental and toxicological threats are usually based on chemical, antibody- or nucleic acid-based assays, which rely on chemical properties or molecular recognition to identify a particular agent. In contrast to these identification assays, cells respond only to biologically active threats. In this regard, primary hepatocytes are the most useful candidate to construct tissue and cell-based biosensors (TBB and CBB). Since isolated hepatocytes are known to readily lose many liver-specific functions during culture, the most crucial issues in hepatocyte-based biosensors are long-term viability and retention of liver-specific functions of cultured hepatocytes In this study, the micropatterning of rat primary hepatocyte hetero-spheroids underlaid with bovine aortic endothelial cells (BAECs), exhibiting high viability and vigorous liver-specific function, was prepared on micro-fabricated glass substrates coated with poly(ethylene glycol) (PEG) brushes. These arrayed spheroids as miniaturized liver may be highly useful for tissue-based biosensor (TBB), offering the promise of sensing drugs and environmental threats through a cellular physiological response.

F5.3
Biodegradable multicomponent silicate-poly(vinyl alcohol) hybrids. Rodrigo L Orefice, Herman S Mansur, Marivalda M Pereira and Wander L Vasconcelos; Department of Metallurgical and Materials Engineering, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

In this work, hybrid biomaterials for tissue engineering applications, based on the combination of inorganic and polymer species at a molecular level, were designed to have: (1) improved mechanical properties; (2) controlled biodegradation kinetics; (3) release of calcium, phosphates and silicate ions during biodegradation that could alter intracellular metabolism; (4) tailorable interaction with proteins. The goal of this work was to determine how the biodegradation of the hybrids and their interaction with proteins can be modified by altering the structure and composition of the materials. Hybrids were synthesized by reacting poly(vinyl alcohol) in acidic solution with tetraethoxy silane. The inorganic phase was also modified by incorporating calcium and phosphate compounds. The kinetics of protein adsorption (IgG) was monitored by UV-Visible spectroscopy. The structure of the hybrids was characterized by swelling experiments, infrared spectroscopy, scanning electron microscopy/microprobe analysis and small angle x-ray scattering. Transparent poly(vinyl alcohol)-silicate hybrid free standing films, having a very broad range of compositions, were produced. Small angle x-ray scattering results showed that broad nano-domains were present in the hybrids. Results obtained from swelling experiments and infrared spectroscopy showed that the crosslink density can be tailored by altering the concentration of the inorganic component. Swelling experiments, performed in aqueous medium, also showed that the obtained hybrids have behaviors that can be varied from fast (hours) to slow degradation by changing the composition of the system or the cross-linking density. Hybrids having high concentration of silica rich components showed slower rates of biodegradation. The kinetics of protein adsorption could also be tailored by modifying the structure and composition of the hybrids.

F5.4
Biodegradable Polymer Scaffolds Fabricated and Processed with Supercritical Carbon Dioxide. Vladimir K. Popov[1], Eugeny N. Antonov[1], Alexander A. Doctorov[1], Elena N. Glukhan[2], Larisa I. Krotova[1], Svetlana I. Tsypina[1] and Alexander I. Volozhin[1]; [1]Institute on Laser and Information Technologies Russian Academy of Sciences, Troitsk, Moscow Region, Russian Federation; [2]State Research Institute of Organic Chemistry and Technology, Moscow, Russian Federation.

Polymer scaffolds, in which biologically active guest species are dispersed throughout porous matrix to encourage dendritic attachment of new tissue forming cells, growth factors and drugs, have widespread applications in tissue engineering. Conventional methods using organic solvents or raised temperature to process the polymer often lead to undesirable thermal and/or solvent induced degradation or changes in its molecular conformations. Here, we report the results of comparative study of bioresorbable mineral-polymer composite scaffolds made by three different routes including mould casting, processing and one-step fabrication in supercritical carbon dioxide (scCO2). We produced poly(D,L)-lactic (PLA) and poly(D,L)lactic-co-glycolic (PLGA) acids samples homogeneously filled with 30wt.% of hydroxyapatite (HA). Initial polymers were cryogenically-milled to make a powder with characteristic particles size 100-200 microns. Synthetic HA with the mean particle size c.a. 1 micron was mixed with the polymer powder in "drunken barrel" type mixer. The first set of monolithic cylindrical samples was fabricated by mould casting. The second set - by processing the part of the first one with scCO2 to make it porous. The third set was made up by one-step fabrication using polymer/HA mixture pressed into Teflon mould placed directly in scCO2 environment. All samples were analysed by Scanning Electron Microscopy, EDAX and Gel Permeation Chromatography to study surface and internal domain morphology, chemical composition, molecular weight (Mw) and polydispersity (Mn) alterations. It was shown that the third method is the most preferable in terms of porous composite scaffold fabrication preventing any changes in Mw and Mn. All sets of samples were also implanted into white rat ("Wistar" line) femoral bone epiphysis for 15, 30 and 60 days to study living tissue response. And again, implants made by the third technique demonstrated the best results in rate of osteointegration and new bone regeneration. Thus, a new fabrication methodology of biodegradable and bioactive composite scaffolds with specific shape and porosity for tissue engineering has been proposed, developed and analysed. Acknowledgements. The authors would like to acknowledge the financial support of International Science and Technology Center, Grant 1530.

F5.5
Conducting Polymers Grown in Hydrogel Scaffolds Coated on Neural Prosthetic Devices. Donghwan Kim[1], Mohammad Reza Abidian[1] and David C. Matin[2,3,1]; [1]Biomedical Engineering, University of Michigan, Ann Arbor, Michigan; [2]Materials Science and Engineering, Univeristy of Michigan, Ann Arbor, Michigan; [3]Macromolecular Science and Engineering, Univeristy of Michigan, Ann Arbor, Michigan.

Conducting polymers including polypyrrole (PPy) were electro-chemically grown in hydrogel scaffolds deposited on surface of microfabricated neural prosthetic devices. Details of the interfacial contact between neural electrodes and brain tissue are important for promoting cell adhesion and facilitating charge transport. Hydrogel coatings provide an additional mechanical buffer layer between the hard silicon-based probe and the soft brain tissue. In addition, the reswelling of the dried gels following implantation anchors the position of the probe in the tissue. In this paper we show that the pyrrole monomer can be grown vertically through the hydrogel layer up to the surface without affecting the adjacent sites on the probes. The electrochemical properties of this conducting polymer-modified hydrogels were studied by impedance spectroscopy and cyclic voltammetry. We also found that the conducting polymers could still be readily grown through the hydrogel after disrupting the microstructure by freeze-drying. Impedance measurements were taken for this novel hydrogel at the biologically important frequency of 1 kHz and the impedance of this polymer-modified hydrogel was found to be 7 kΩ. This is much lower than the impedance of polypyrrole film ($\sim$100 kΩ).

F5.6
Correlation Between Titanium Implant Surface and Calcium

Phosphate Nucleation in the Presence of Hydrogen Peroxide. Julie J. Muyco[1,2], Joanna McKittrick[1,3], John Frangos[3] and Christine Orme[2]; [1]Materials Science and Engineering Program, University of California, San Diego, La Jolla, California; [2]Chemistry and Materials Science, LLNL, Livermore, California; [3]La Jolla Bioengineering Institute, La Jolla, California.

—*3*—h]‘ osseointegration by titanium implants have yet to be fully understood. The aspect of osseointegration we investigate is mineral formation. It can be argued that osseointegration of an implant is dependant upon its ability to develop a well adherent layer of calcium phosphate early in the healing process. Titanium implants are inherently covered with a layer of titanium oxide, titania, that is a stable passivating layer *invivo*. Developments in the chemical and morphological modification of the titania layer have recently shown the sensitivity of osseointegration to the form of titania present. Especially of interest is the use of hydrogen peroxide to alter the surface of titanium implants prior to implantation. Hydrogen peroxide is a highly reactive chemical that can be found in the body under inflammatory conditions. We study the ability of different forms of titanium oxide to nucleate calcium phosphate in the presence of hydrogen peroxide. Correlation between the titania thickness, surface morphology, and phase to the calcium phosphate nucleated on the surface from simulated body fluid will be presented. Methods of characterizing the materials and interface where nucleation occurs include atomic force microscopy (AFM), transmission electron microscopy (TEM), time of flight secondary ion mass spectroscopy (TOF-SIMS), and Raman spectroscopy. This work was performed under the auspices of the U.S. Department of Energy by University of California, Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.

F5.7
Effect of Concentration and Temperature on Hydroxyapatite Morphology. Chandrasekhar Rama Kothapalli[1], Mei Wei[1], Alexandre L Vasiliev[1] and Montgomery T Shaw[2,3]; [1]Department of Metallurgy and Materials Engineering, Institute of Materials Science, Storrs, Connecticut; [2]Department of Chemical engineering, 191 Auditorium Road, Room 204, U-222, University of Connecticut, Storrs, Connecticut; [3]Polymer Program, Institute of Materials Science,, University of Connecticut, Storrs, Connecticut.

Human bone mineral contains calcium-deficient crystalline hydroxyapatite (HA) embedded in collagen fibers. Research over the past two decades has focused on preparing synthetic HA, which closely resembles bone apatite and exhibits osteoconductivity. In this paper we report the synthesis of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$: HA) nano-particles using a wet precipitation method, where in, we varied the concentration of the reactants (0.5, 1.0 and 2.0 g/dL) and the temperature of the reaction (25, 70 and 100 °C). FESEM images of the resulting nano-particles were used to find their size and shape. The length and breadth of the HA particles were found to increase with concentration and temperature as did the aspect ratios. The HA particle size distribution was evaluated by the Weibull distribution. The shape parameters of the distribution lie in the range 0.65-2.76 and the scale parameters in the range 29-152. XRD results of the as-prepared particles showed that pure HA resulted at pH 12, while monetite was formed at a low pH in the range 6-9. HA particles synthesized at each condition were pressed into pellets and sintered at 1200 °C. It was found that HA particles synthesized at 70 °C and concentration 2.0 g/dL had the highest bulk density and biaxial flexural strength. XRD results of the sintered pellets indicated that most of them had slightly decomposed and the decomposition is less than 20 %. The decomposition of the specimens and their surface morphology were closely correlated to their mechanical strength.

F5.8
Formation of eggshells: what do we know so far? Suresh Valiyaveettil, Rajamani Lakshminarayanan, Xian Jun Loh and R Manjunatha Kini; Chemistry, National University of Singapore, Singapore, Singapore.

Avian eggshells are formed at a high rate of calcium carbonate deposition inside the oviduct of the egg laying birds. The composition and architecture of the eggshell is important towards the survial of the embryo inside the egg. Recently, we established a research program to understand the molecular mechanism of biomineralization in whcih eggshells from various species were used as model systems. We have shown that soluble organic materials (e.g. proteins, proteoglycans) extracted from the mineral matrix of the eggshell play a major role towards the nucleation and morphology control of calcium carbonate crystal polymorphs. This presentation will dsicuss our strategy, recent resutls and our hypothesis on the eggshell mineralization, in particular reference to goose eggshells.

F5.9
High-Bandwidth Microrheology Applied to Solutions and Networks of Semiflexible Biopolymers. Karim Munir Addas[1], Jay Tang[3,1] and Christoph Schmidt[2]; [1]Physics, Indiana University Bloomington, Bloomington, Indiana; [2]Physics of Complex Systems, Vrij Universiteit, Amsterdam, Netherlands; [3]Physics, Brown University, Providence, Rhode Island.

Semiflexible protein filament networks are characteristic of the cytoskeleton and the extracellular matrix, and their dynamics over a broad range of time and length scales are at the basis of cellular mechanics. We have developed one and two-particle microrheology, employing micron-sized embedded beads and laser trapping combined with interferometric displacement detection, to study the rheological properties of mono-disperse semiflexible fd-virus solutions. We have tested these relatively new techniques in well-known limits using fd virus as a model polymer. Concentrations tested spanned the dilute, semi-dilute and concentrated regimes allowing comparison with recent theoretical treatments of both the dilute low-frequency regime (rod diffusion) and the high-frequency single filament (internal) dynamics limit. We find good agreement in these limits and we also obtained data on collective dynamics in the entangled regime for which no theoretical model is yet available. Once proven as a reliable technique to characterize the micro-rheological behavior of uniform filament networks, particle tracking may be applied towards detection of subcelluar mechanics, motor activity, granular transport, etc.

F5.10
A Predictive Exploration of the Mechanical Requirements of a Regenerative Solution to Nucleus Pulposus Replacement based on Mimicking the Intradiscal Pressure. Abhijeet Joshi[1], Andrew Karduna[2], Edward Vresilovic[3] and Michele Marcolongo[1,3]; [1]Materials Science and Engineering, Drexel University, Philadelphia, Pennsylvania; [2]Department of Exercise and Movement Sciences, University of Oregon, Eugene, Oregon; [3]Department of Biomedical Engineering, Drexel University, Philadelphia, Pennsylvania.

The origin of the low back pain is often the degenerated lumbar intervertebral disc. In the case of the healthy intervertebral disc, load transfer occurs via generation of a hydrostatic (intradiscal) pressure by the nucleus, which creates tension on the annulus fibers. However, with aging and/or a disease process, the nucleus of the intervertebral disc becomes dehydrated, which alters the normal load transfer mechanism, resulting in debilitating pain to the patient. Solutions to recreating the normal biomechanics of the intervertebral disc include replacing the nucleus with a synthetic polymer and regenerating the nucleus pulposus using a tissue engineered approach. However, the mechanical requirements for these reconstructions are not well understood. In this work, we have investigated the effect of modulus and geometry of a nucleus replacement on the compressive stiffness of the lumbar functional spinal unit (FSU) using finite element modeling. The present study reports a simplified axisymmetric finite element model of the lumbar functional spinal unit (specimen). The model is validated for the human cadaver compression data for an intact and the denucleated specimen, obtained in our lab. The implant was simulated in the denucleated model, to determine the mechanical properties of an implant that would mimic the intradiscal pressure and mechanical behavior of the validated intact model. The geometric parameters of an implant, such as height and diameter, were varied to see the effect on the intradiscal pressure changes in the specimen. The variation of the intradiscal pressure with the implant modulus was also predicted. The finite element model predicts that it is possible to restore the compressive stiffness of the FSU by mimicking the intradiscal pressure of the normal nucleus pulposus. This information gives design parameters for a synthetic or tissue engineered construct required to restore normal compressive behavior to the FSU.

F5.11
Morphology of, Protein Protection in, and Host Tissue Response to Hydrogel Nanoparticle Aggregates. John Vincent St. John[1], Bill Ponder[1], Daniel G Moro[1], Greg Russell-Jones[2], Fiona McDougall[2] and Kirsten McTavish[2]; [1]Hydrogel, Access Pharmaceuticals, Dallas, Texas; [2]Access Pharmaceuticals Australia Pty. Ltd., Chattswood, New South Wales, Australia.

It is often a requirement for the design of a tissue scaffold material that there be drug release concomitant with new tissue growth. Tissue growth can be enhanced with the release of macromolecules (e.g. morphogenetic proteins). Access Pharmaceuticals, Inc. has developed a biocompatible tissue scaffold composed of poly-(2-hydroxyethylmethacrylate) (pHEMA) nanoparticles and copoly-(2-hydroxyethylmethacrylate-methacrylic acid) (pHEMA-MAA) nanoparticles for drug release with tissue growth. These aggregates of non-covalently linked particles can protect proteins from enzymatic cleavage, and allow controlled porosity with protein drug release at physiological pH. In vivo murine biocompatibility studies show a minimal, localized immune response with limited capsule formation. Aggregates composed of pHEMA-MAA nanoparticles or mixtures of pHEMA and

pHEMA-MAA nanoparticles disperse at physiological pH, releasing individual particles. This erosion allows control of the aggregate porosity and mechanical properties, gradually allowing the scaffold to become more porous and softer over time. Changes in porosity can be followed using microscopy by measuring mechanical properties. Aggregates enable the controlled release of trapped proteins through variations in particle size and erosion rate. Studies with protease enzymes show that proteins trapped between particles in the aggregate are protected from enzymatic cleavage, and retain activity after release from the aggregate. Protein activity was monitored using horseradish peroxidase trapped in, and released from, aggregates in the presence of trypsin. In vivo implants show that the degree of tissue response depends on material properties such as percent hydration, and comonomer content. Aggregates composed of particles containing fluorescent tags show cellular infiltration after erosion and pore formation. Particles released from the implant can be found within macrophages adjacent to the implant, within the draining lymphatic system, and in some instances throughout the tissues of the body. Taken together, these results further support the promise of these biocompatible materials to facilitate the delivery of therapeutic agents.

F5.12
Comparison of bulk and microrheology of polyacrylamide hydrogels using the Surface Forces Apparatus (SFA).
Damien Calvet[1], Suzanne Giasson[1] and Joyce Wong[2]; [1]Departement de Chimie, University of Montreal, Montreal, Quebec, Canada; [2]Department of Biomedical Engineering, Boston University, Boston, Massachusetts.

Polyacrylamide hydrogels (PAAm) are widely used as biomaterial scaffolds. Recent studies using PAAm have shown that modulation of the mechanical properties of the substrate can affect cell behavior such as morphology, growth, and migration. In these studies, the bulk mechanical properties are the Young/s modulus determined using a simple tensile test on a piece of gel. However, it is known that the inhomogeneous structure of most hydrogels leads to important deviations in the Young/s modulus measurements. To achieve local measurements of the dynamic properties of hydrogels, we used a modified Surface Forces Apparatus (SFA), including a sliding attachment for shear and friction experiments. PAAm hydrogel ([Monomer] = 8 wt.% and [Crosslinker] = 0.04−0.48 wt.%), are studied in the swelled and unswelled states. In parallel, we measured bulk rheological properties using a rheometer (plate-plate geometry, large 500 mm gap). Our results using the SFA technique demonstrate that the elastic modulus depends on the normal force applied to the hydrogel film. Above a critical force, Fc, G/(ωw) is equivalent to the value measured using bulk rheology, corresponding to a large elastic modulus in comparison to the viscous modulus (G/(ω) = 2500 Pa ≫G//(ω) = 0 − 100 Pa). At Fc, a transition occurs from large G/ to another characteristic modulus value around 200 Pa. A syneresis process (water ejection) can explain this behavior. Quantitative and qualitative assessments of the characteristic dynamic properties of the gels will be discussed.

F5.13
Crosslinked Degradable Biomaterial Networks: Investigation of pH Gradient Formation in Networks Formed from Multifunctional Monomers Via Confocal Microscopy.
Andrew Watkins[1] and Kristi S Anseth[1,2]; [1]Chemical Engineering, University of Colorado, Boulder, Colorado; [2]Howard Hughes Medical Institute, Boulder, Colorado.

Laser scanning confocal microscopy (LSCM) was utilized to create high-resolution three-dimensional images of pH gradients that form in hydrolytically degrading polymer networks. These types of networks are currently being investigated as potential tissue engineering scaffolds and controlled drug delivery devices. For a scaffold, it is critical to correlate the rate of polymer degradation with the rates of tissue growth and wound healing to ensure sufficient support for cellular development in applications such as bone fixation, cartilage repair, and heart valve replacement. To control drug delivery from biodegradable polymer devices, it is imperative to know not only the degradation rate, but also the erosion mechanism to avoid underdosing, which limits treatment efficacy, and overdosing and burst effects, which are potentially harmful to the patient. Finally, hydrolytically degradable polymers often release acidic products during degradation, resulting in the development of a pH gradient in a three-dimensional device due to diffusion limitations. The consequences of this gradient may include reduced biocompatibility of the polymer network, which can lead to an increased inflammatory response and potentially even cell death. In drug delivery devices, the acidic byproducts may have deleterious effects on any encapsulated materials, including therapeutics such as active proteins or nucleic acids. The acidic environment may also alter the rate of degradation such that the total degradation times are unpredictable. The crosslinked polymer networks utilized in this study were formed via photopolymerization of multifunctional monomers. Specifically, LSCM was used to characterize pH gradient formation in networks composed of dimethacrylate terminated poly(ethylene glycol-co-lactic acid) and sebacic anhydride. The polymers in question exhibit a variety of degradation kinetics based on monomer chemistry, degradable unit, and crosslinking density. The pH gradients were imaged using the pH-sensitive, ratiometric fluorescent probes Cl-NERF and DM-NERF. In the materials examined, images demonstrate that pH decreases with depth from the surface during network degradation. In loosely crosslinked systems, images show that pH gradients are not as severe as in highly crosslinked systems.

F5.14
Microfabrication of Spatio-temporally Patterned Biomimetic Polymer-scaffolds for Stem Cell Engineering.
Mary Gazell Mapili[1], Yi Lu[2], Shaochen Chen[2] and Krishnendu Roy[1]; [1]Biomedical Engineering, University of Texas at Austin, Austin, Texas; [2]Mechanical Engineering, University of Texas at Austin, Austin, Texas.

One of the fundamental limitations of current efforts in tissue engineering has been our inability to produce multiple tissue types in a pre-designed fashion inside a single scaffold structure. Our goal is to use a laser-based, layer-by-layer stereolithography process to create precise spatio-temporal distribution of bio-factors within 3D scaffolds and study their effects on stem cell differentiation into multiple lineages. We have designed a laser-layered microfabrication system to construct scaffold structures with designed porosities and defined architecture using crosslinkable and functionalized PEGs. Cell differentiation during organ development is guided by spatio-temporal cues of soluble factors and extracellular matrix (ECM) components. We have incorporated controlled-release mircopartícles, in precise, pre-designed spatial patterns to create similar distribution within the microfabricated scaffold structures. Recently much effort has been directed towards mimicking the surface chemistry of extracellular matrix (ECM) onto polymer scaffolds. This provides optimal cell attachments, cell-matrix signaling and prevents diffusion of soluble growth factors thereby creating immobilized spatial patterns of bio-molecules. Using an electrophilic PEG we have conjugated ECM components e.g. heparan sulfate and the integrin-binding peptide RGD, to the scaffold material. Heparan sulfate, a glycoaminoglycan, is critical for spatial immobilization and optimal signaling of growth factors, especially for basic fibroblast growth factor, FGF-2. The incorporation of cell-adhesive RGD peptides would provide us with optimal stem-cell seeding within the microfabricated scaffolds. Our ability to create such spatio-temporally distributed biochemical microenvironments enables us to study the directed, pre-designed differentiation of stem cells into multiple lineages within a single 3D environment.

F5.15
Surface Modification of Poly (3-hydroxybutric acid-co-3-hydroxyvaleric acid) (PHBV) by Type I Collagen Immobilization. Yohannes T Tesema and Dharmaraj Raghavan; Chemistry, Howard University, Washington, DC, District of Columbia.

Cytocompatibility, mechanical performance, and biodegradability characteristics are highly relevant in the selection of a biopolymer for use in tissue engineering scaffolds and implanted devices. Because of the ability to tune the mechanical and biodegradable characteristics of microbially synthesized polyesters, poly (3-hydroxybutric acid-co-3-hydroxyvaleric acid) (PHBV) has emerged as a promising biopolymer candidate. The objective of this study is to investigate the factors that can enhance cytocompatibility of PHBV film. To enhance the cyctocompatability of PHBV film, we modify the surface by ozone treatment, then graft methacrylic acid and perform chemical covalent immobilization of Type I collagen using a condensing agent, 1-ethyl-3-(3-dimethylamine propyl) carbodiimide. We determined using the iodide method that the maximum density of surface peroxide is 11 nmol/cm^2 when ozone oxidation was performed for 2 hours at the flow rate of 2.2 g/h of ozone gas. Upon treating the oxidized surface with an aqueous solution of MAA 10% (w/w) for 60 min at 60°C, we found that the surface density of the carboxyl group grafted on the treated PHBV surface was 84 nmol/cm^2. The surface topography of Type I collagen immobilized on the PHBV-g-PMAA surface was characterized using AFM, and the amount of collagen immobilized on the surface was found to be 0.35 μg/cm^2. The duration of ozone exposure and monomer concentration strongly influenced the amount of collagen immobilized on the PHBV film. Acknowledgement: The Keck foundation for the financial support.

F5.16
Surface modification of neural prosthetic devices by electrochemical deposition of conducting polymers through electrospun nanofibrous templates. Mohammad Reza Abidian[1], David Y. Lin[2], Donghwan Kim[1] and David C. Martin[1,2,3]; [1]Biomedical Engineering, University of Michigan, Ann Arbor,

Michigan; [2]Macromolecular Science and Engineering Center, University of Michigan, Ann Arbor, Michigan; [3]Material Science and Engineering, University of Michigan, Ann Arbor, Michigan.

The electrode host interface is a significant problem affecting the long-term use of neural prosthetics in vivo. Problems include mechanical failure and inflammation at the implantation site, leading to a loss of signal transmission from neurons. The engineering of bioactive electrode coatings has been investigated for its potential to promote in-growth of neural tissue, reduce shear stress, and enhance signal transport from electrons to ions at the electrode-host interface. We have found that films of electrospun nanofibers can be deposited on the surface of these devices, followed by electrochemical polymerization of conducting polymers such as polypyrrole and PEDOT. After polymerization, the nanofibers can be dissolved, leaving tiny channels and pores in the conducting polymer that facilitate efficient signal transport and communication with the neural tissue. The electrical properties of the functionalized probes were examined with impedance spectroscopy, and the structure examined by optical and electron microscopy.

F5.17
A Novel Technique for Fabrication of Hydroxyapatite Scaffolds for Bone Tissue. Hassna Rehman Ramay and Miqin Zhang; Materials Science and Engineering, University of Washington, Seattle, Washington.

Musculoskeletal disorders have become one of the major health concerns in the United States as a result of aging population and increased occurrence of sports related injuries, trauma or tumors. The implantation of patient-derived bone marrow cells onto a man-made, temporary, biodegradable porous scaffold to create natural new bone is a potential therapy in tissue engineering. One of the major challenges in this approach is to construct a scaffold that is sufficiently strong for weight- or load-bearing while retaining its high porosity because the mechanical strength reduces as the porosity increases. We report a novel technique that integrates the gel casting technique with polymer sponge method to prepare HA porous scaffolds with controllable porous structure and superior mechanical strength close to natural cancerous bone. The macroporous structure of the produced scaffold is the replicate of the polymer sponge template; thus, the pore size and shape can be easily controlled, and the scaffolds of complex shapes can be fabricated. The scaffolds are formed through in situ polymerization, thus reducing the defects on the pore walls and avoiding the sedimentation of HA slurry at the bottom of the scaffold. As a result, a highly homogenous microstructure is produced. The pore morphology, size, and size distribution are characterized by scanning electron microscopy (SEM). X-ray diffraction (XRD) and infrared spectroscopy are used to characterize the crystal structure and chemical composition, respectively. Energy dispersive spectroscopy (EDS) is used to obtain the elemental composition of scaffolds. Mechanical compression tests will be performed using an Instron mechanical tester to evaluate the yield strength and elastic modulus. The technique introduced can be applied to the development of other bioceramics such as beta-TCP and biphase of HA/beta-TCP with enhanced mechanical strength for load-bearing tissue engineering applications.

F5.18
3D Cell and Tissue Distribution Patterns Characterized by Micro-CT. Amy J Wagoner Johnson[1], Jennifer Dellinger[2] and Russ Jamison[2]; [1]Mechanical and Industrial Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois; [2]Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois.

Synthetic bone scaffolds are being developed to replace allograft and autograft bone, for which the risk of disease or other complications is significant. In this study, hydroxyapatite scaffolds are fabricated using a directed colloidal assembly technique called "robocasting," which allows for controlled porosity on the meso- to nano- length scales. Specific features and length scales that are best suited for optimizing cell attachment and proliferation and tissue mineralization are being identified. In order to ultimately understand the tissue integration process in vivo, these in vitro scaffolds must be carefully characterized. While several techniques are employed for full characterization including scanning electron microscopy and histology, all are destructive in nature and can only represent the cellular activity in two dimensions. Furthermore, preparation of such samples for histology is time consuming and labor intensive. A non-destructive evaluation technique called X-ray Micro-computed Tomography was used for the first time to characterize cell and tissue distribution patterns in hydroxyapatite scaffolds with a resolution up to 5 microns. In this study, scaffolds were seeded with mesenchymal rat stem cells and cultured for periods ranging from 1 day to 4 weeks. Cells and extracellular matrix were fixed using OsO4, which attenuates x-rays more than the CaP scaffolds, and allows them to be viewed using the x-rays. Cell clusters can be identified and their position determined after as little as one day. By three weeks, tissue is observed to span between rods at the interior of the scaffold. Data is viewed as two dimensional "slices" or as a three dimensional object using ANALYZE software and volumes of tissue can be estimated. This work was carried out at the 2BM at the Advanced Photon Source at Argonne National Laboratory.

F5.19
Effects of Coupling Agents on Local Mechanical Properties of Bioactive Composite by Nano-indentation Technique. Emily Y Ho and Michele Marcolongo; Materials Science and Engineering, Drexel University, Philadelphia, Pennsylvania.

Nano-indentation has been applied to analyze the local modulus and hardness of fiber reinforced bioactive ceramic / polymer composites. This test can continuously generate loading-unloading cycle with a very small load (10mN) while providing information on the degree of energy absorbed, the elastic modulus and hardness of the small contact surface on a composite. But the details of the indent marks and the local fracture mechanism of the composite are not yet understood due to the lack of the capability of clearly imaging the mark at 3000 to 5000 magnification. In our research, we have investigated a bioactive composite, a hydroxyapatite / polymethylmethacrylate scaffold, as a mandible replacement. We introduced different coupling agents to improve the adhesion properties at the hydroxyapatite-polymethylmethacrylate interface. Via the application of nano-indentation onto various positions of the cross section of composite, we were able to determine the influence of each coupling agent on the local mechanical properties of the system. We observed that with the addition of coupling agents, the local hardness and Young's modulus of the hydroxyapatite particles has been reduced due to the presence of the pop-out and elbow events during loading. These events indicate that there is a shift of material behavior from elastic to plastic response, as the applied load increases. Also under field emission environmental scanning electron microscopy, we analyzed each indent mark up to 5000x magnification. This imaging enhanced the evaluation of small indentation (less than 1 micron in each dimension). Coupling the microscopical analysis with the load-displacement curves allowed us to perform a more comprehensive analysis than has previously been accomplished for bioactive composite materials.

F5.20
A Bioactive, Hyaluronic Acid-Based Biomaterial System for Neural Tissue Engineering Applications. Michael A Beaumont[1], Glenn D Prestwich[2] and Alyssa Panitch[1]; [1]Harrington Department of Bioengineering, Arizona State University, Tempe, Arizona; [2]Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah.

Repair of the central nervous system (CNS) is one of the most daunting tissue engineering challenges. The inability of the CNS to heal has been attributed to the lack of an extracellular environment conducive to neurite regeneration. To address this, we have developed a bioactive hyaluronic acid (HyA)-based material that incorporates peptide sequences, from the extracellular matrix adhesion molecules N-cadherin (CHAVDI and CINPISG) and fibronectin (CRGDS), to improve neurite outgrowth. A thiolated version of HyA was chosen as a base material due to the ubiquitous presence of HyA during embryonic development and expression of a variety of hyaladherin receptors on neural cells. Multi-arm poly(ethylene glycol) (mPEG) was added to the system as an augmentation to provide increased capacity for the inclusion of bioactive factors. The mPEG was chemically functionalized with vinyl sulfone moieties to facilitate the conjugation of the peptides to the polymer (via Michael addition between the vinyl-sulfone moieties on the mPEG arms and the N-terminal cysteine of the peptides) and the crosslinking of the peptide-mPEG conjugates to the HyA (via Michael addition between the free vinyl-sulfone moieties on the unconjugated mPEG arms and the thiol groups on the base material). The peptides were attached to mPEG on a 3:1 molar ratio, leaving an average of two polymer arms free for crosslinking of the conjugates into the HyA. Day 9 embryonic chick dorsal root ganglia (DRGs) were cultured for 48 hours within HyA matrices containing various combinations and concentrations of the peptide-mPEG. Images of the cultures were analyzed by delineating the outer edge of the DRG body and the 'halo' of extended neurites. The radius of the annulus formed from these relative demarcations was used as a measure of neurite growth. The gels containing the peptide-mPEG showed up to a 250% increase in mean neurite length versus unmodified HyA, along with a notable increase in neurite density. These data show that this bioactive material system provides a permissive environment for neurite outgrowth. Continued work is planned to expand the repertoire of included molecular factors to further enhance the neurogenic capabilities of the system.

F5.21

Electron-Beam Patterned Poly(ethylene glycol) for Spatial Control of Surface Bioactivity. Peter Krsko[1], Svetlana Sukhishvili[1], Marc Mansfield[1], Robert Clancy[2] and Matthew Libera[1]; [1]CBME, Stevens Institute of Technology, Hoboken, New Jersey; [2]Hospital For Joint Disease, NYU, New York, New York.

This research describes a method to create microscopic hydrogels from poly(ethylene glycol) [PEG 6800] and poly(ethylene oxide) [PEO 200K] using spatially resolved radiation from a scanning electron microscope with an approach similar to that used in the electron-beam patterning of polymeric photoresists. We demonstrate that, indeed, PEG hydrogels with micrometer and submicrometer feature sizes can be created by this approach, and we call these microhydrogels. Using solvent-free PEG 6800 and PEO 200K films 50-100 nm thick, we have identified sets of irradiation conditions where sufficient cross-linking occurs so that the exposed patterned polymer remains while the unexposed polymer dissolves during a post-irradiation solvent rinse. Arbitrary spatial patterns can be made. We have generated patterned dots with diameters below 200 nm. Using atomic force microscopy, in air and water, to study 5x5 μm PEG and PEO pads on silicon, we show that the patterned features generated by electron-beam cross-linking swell when exposed to water. The extent of swelling depends on the incident electron dose. Maximum swelling ratios of 14-16 have been observed. The swelling ratio decreases with increasing dose toward a limit of unity at the highest doses studied. Because of the significance of PEG in biomaterials applications, we examined the adsorption of fibronectin [Fn] cell-binding fragments onto the PEG microhydrogels using immunofluoresence optical microscopy. Undetectable Fn levels are observed on microhydrogels subjected to the lowest radiative exposure conditions where maximum swelling occurs. Fn adsorption increases with increasing dose and reaches a maximum at the highest doses where swelling ratios of unity are observed. This approach opens a new means for arbitrarily patterning the spatial distribution of proteins on surfaces and may be useful for controlling surface bioactivity.

F5.22

Microfabrication of biodegradable polymeric structures for guided tissue engineering. Giovanni Vozzi[1,2], Gianluca Ciardelli[2], Arti Alhuwalia[1], Paolo Giusti[2], Antonino Previti[1], Cristina Cristallini[3] and Nicoletta Barbani[2]; [1]Centro Interdipartimentale di Ricerca"E. Piaggio", university of PIsa - Faculty of Engineer, pisa, Italy; [2]Chemical Engineer, University of Pisa - Faculty of Engineer, pisa, Italy; [3]3Istituto per i Materiali Compositi e Biomedici, CNR, pisa, Italy.

Designing new polymeric materials with chemical and physical characteristics and manufacturing them into 3D, highly porous scaffolds appears to be the most promising approach to get structures able to guide tissue regeneration. To achieve this goal two new microfabrication techniques were experienced: 1. pressure assisted microsyringe (PAM), an automated system using a microsyringe and a stage controller.(2) 2. selective laser sintering (SLS), that utilizes the heating energy of a laser beam to sinterize powder particles in a desired architecture. Tri-block poly-(ϵ-caprolactone)-poly-(oxyethylene)-poly-(ϵ-caprolactone) copolymer (85 wt. % ϵ-caprolactone) (Mn 35000), synthesized by our group, was used to realise 2- and 3D patterns scaffolds with well defined geometry. 3T3 mouse fibroblasts were seeded on the scaffolds, on spin coated films and on a layer of gelatin. The ratio between cell density on scaffolds and on the gelatin layer was taken as an index of cell adhesion efficiency. Cells displayed a better adhesion to microstructures realised than to film-shaped scaffolds, both in terms of cell density and distribution. On microfabricated scaffolds cell distribution became more uniform with increasing culture time, until the entire scaffold was covered by cells. On the contrary, cell density on films does not change with time while distribution worsened, because of cell migration to the edges, where adhesion protein concentration is higher due to the irregular surface. In conclusion our study demonstrate that: - The use of PAM system and SLS offer a broad range of variable operating conditions for the realisation of 2D and 3D structures with tailored characteristics to support the growth of different cell types - cell adhesion appears to be enhanced by a scaffold with a well defined geometry and a flexible, speed and easy technique such as PAM can help in finding such an architecture.

F5.23

Biodegradable Silicon/Polymer Composites for Orthopedic Tissue Engineering. Jeffery L Coffer[1], Melanie Whitehead[1], Priyabrata Mukherjee[1] and Leigh T Canham[2]; [1]Department of Chemistry, Texas Christian Universiity, Fort Worth, Texas; [2]PSi Medica Ltd, Malvern, United Kingdom.

A plethora of both natural and synthetic materials are currently in use as tissue engineering scaffolds, with the necessity of ultra-high porosity an oft-cited factor in achieving proper vascular & neurological growth. Unfortunately, however, most structures to date are insulating in character, thereby limiting their performance in electronically-responsive applications. The development of new composite materials designed to incorporate semiconducting, bioactive silicon nanostructures with erodible biocompatible polymers is currently underway in our laboratories. Through a broad-based strategy, a platform of diverse scaffolds is possible with tunable properties that can be controlled not only by the structure of the composite but also by the electronic response of the semiconductor present as interconnecting networks. Such properties include mechanical strength, controlled release of useful substances to living tissue, and rate of resorption of the composite to the host surroundings. We have recently succeeded in producing porous sponges existing as a composite of bioactive porous silicon (BioSilicon) with polymers such as poly-caprolactone (PCL). In this presentation the fabrication of a range of composites of varying shape, size, and porosity, prepared by both salt leaching and microemulsion techniques, will be discussed. Particular attention to the influence of Si content in the composite on in vitro calcification assays will be assessed. For each system, bio-compatibility and cellular infiltration can explicitly evaluated through animal cell culture assays.

F5.24

The Effect of Polymer Content on the Swelling and Mechanical Behavior of PVA/PVP Hydrogels in a Protein-Containing and Protein-Free Immersion Media. Jonathan Thomas[1], Justin Scanlon[1], Anthony Lowman[2] and Michele Marcolongo[1]; [1]Materials Science and Engineering, Drexel University, Philadelphia, Pennsylvania; [2]Chemical Engineering, Drexel University, Philadelphia, Pennsylvania.

We have proposed a replacement of the nucleus pulposus of the intervertebral disc with a hydrogel blend of polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP). These hydrogels have been shown to be stable in buffered saline solution due to physical crosslinks consisting of intramolecular hydrogen bonds within PVA crystallites and intermolecular hydrogen bonds between PVA and PVP. Here we examine the effects of polymer content on the swelling, dry mass %, and compressive stiffness of PVA/PVP blends immersed in protein-containing (DeBucco's growth media with 10% fetal bovine serum (FBS)) and protein-free (simulated body fluid (SBF)) immersion media. Blends were processed with 5, 10, and 15% polymer content. Swelling ratios, dry mass %, and compressive modulus were determined after 1, 2, 4, 7, 14, 35, and 70 days of immersion in either FBS or SBF. The compressive modulus of each blend was found to increase over immersion time in both media. Swelling was dependent on the polymer content of the blends and stabilized after 35 days of immersion in either media. The swelling ratios of each polymer blend were independent of the immersion media. Upon drying the blends of each polymer content, the dry mass % was found to be greater for the blends immersed in SBF than FBS. EDAX was used to determine there was a higher uptake of salts in the hydrogels immersed in SBF which led to an increase in dry mass over immersion time. Protein shielding could possibly explain why there was less uptake of ions from FBS into the hydrogel network.

F5.25

Measurement of Protein Adsorption and Cell Adhesion onto Polymer Surfaces using Immuno-Fluorescence Assays (IFA) and the QCM-D technique: A Comparative Study. Norbert Weber[1,2], Carlos E Caicedo-Carvajal[1], Corinne Nardin[1] and Joachim Kohn[1,2]; [1]Chemistry, Rutgers University, Piscataway, New Jersey; [2]New Jersey Center for Biomaterials, Rutgers University, Piscataway, New Jersey.

Protein adsorption onto polymeric surfaces plays a central role in the adhesion/activation of cells and these events can affect the biocompatibility of artificial surfaces. Thus, there is a need for the analysis of the patterns of adsorbed proteins onto polymer surfaces. We have developed a unique method (IFA), based on the use of 384-well microtiter plates, to analyze protein adsorption (fibrinogen, fibronectin) and cell adhesion (fibroblasts) onto various polymer surfaces. The different polymers were chosen from the combinatorial libraries of polycarbonates and polyarylates available in our laboratory. Our results indicate that the polymer surface chemistry affects the adsorption of fibrinogen and fibronectin. Further, we can show that the selective adsorption of fibrinogen and fibronectin onto polymer surfaces followed by incubation with fluorescently labeled cells facilitates the study of cell adhesion under static conditions. The use of Immuno-Fluorescence methods to detect adsorbed proteins and attached cells is ideally suited for an initial rapid screening of a large number of different polymer surfaces. The Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) has been used to investigate the adsorption of fibronectin, subsequent antibody binding and cell adhesion onto a sub-set of polymer surfaces in real time. The results show good agreement between the two techniques and similar sensitivity. The overall advantage of the QCM-D technique lies in the

real time detection of adsorbing proteins and adhering cells. Moreover, the QCM-D technique provides additional information about the physico-chemical properties of the adsorbed protein layer (viscosity, elasticity and thickness) that can be correlated to the protein functionality (conformation and dynamic). Thus, the combination of the IFA with the QCM-D technique can enable a more efficient exploration of the biological processes involved in cell-material interactions.

F5.26

Electric Field Assisted Patterning and Characterization of Live Neuronal Networks for the Study of Brain Functions. Shalini Prasad[1], Xuan Zhang[2], Mo Yang[2], Cengiz Sinan Ozkan[2] and Mihri Ozkan[1,3]; [1]Electrical engineering, University of California Riverside, Riverside, California; [2]Mechanical Engineering, University of California Riverside, Riverside, California; [3]Chemical and environmental Engineering, University of California Riverside, Riverside, California.

Experimental investigation of the dynamics of neuronal networks is a fundamental step towards understanding how the nervous system works. Activity-dependant modification of synaptic strength is widely recognized as the cellular basis of learning, memory and developmental plasticity. Understanding memory formation and development thus translates to changes in the electrical activity of the neurons. To map the changes in the electrical activity, it is essential to conduct in-vitro studies on individual neurons. Hence there is an enormous need to develop novel ways for the assembly of highly controlled neuronal networks. To this end, we used a 3x3 microelectrode array system and isolate and spatially position single neurons over individual electrodes using dielectrophoretic traps. The process growth was directed and controlled using DC field. Electrical activity from a single non-synaptic axon, and a synaptic axon was recorded and analyzed. The direction and speed of electric current propagation due to neuron stimulation was mapped and characterized. Modification in a single cell electrical activity due to direct as well as indirect electrical stimulation is determined and the associated memory development at the cellular level is analyzed. Electrical activity modification at the cellular level provides an insight into the development and variation of higher level functions in the nervous system.

F5.27

Single Cell Based Bio-Sensor. Xuan Zhang[1], Shalini Prasad[1], Mo Yang[1], Mihri Ozkan[1] and Cengiz S. Ozkan[1]; [1]Mechanical Engineering, University of California, Riverside, Riverside, California; [2]Electrical Engineering, University of California, Riverside, Riverside, California; [3]Mechanical Engineering, University of California, Riverside, Riverside, California; [4]Electrical Engineering, University of California, Riverside, Riverside, California; [5]Mechanical Engineering, University of California, Riverside, Riverside, California.

There is a need to develop small, highly sensitive, accurate, portable biosensors that can be used in real time situations which has the ability to distinguish between various chemical agents. Current methods rely on chemical properties or molecular recognition to identify a particular agent. These are limited in their capability to detect large number of possible agents both known and unknown, characterize the functionality of the known agents and predict human performance decrements. There is still a major gap in performing functional assays in the laboratory and implementing this concept in the field. This can be overcome by using cell based bio-sensors (CBB). We have designed and implemented a novel CBB that achieves single cell sensitivity and single agent selectivity using dielectrophoresis. We show here the capability of this sensor to accurately identify specific chemical agents from an environment containing a mixture of chemical agents. The chemical identification was performed by frequency domain analysis of the extracellular signal which generated a unique signature pattern vector corresponding to specific chemical agents. A time domain analysis was performed to determine the speed of response. The goal of the research is to develop a single cell sensor applicable in real time field conditions. We explore the ability of the sensor to identify unknown chemical agents.

F5.28

Characterisation of Hydroxyapatite Formation Induced in Chitosan Solution. I. Manjubala[1,2], C. Bhavani[1] and T. P. Sastry[1]; [1]Bio-Products lab, Central leather Research Institute, Chennai 600025, India; [2]Friedrich-Schiller-Universitat-Jena, Institute of Materials Science and Technology, Jena, Germany.

Chitosan, a natural polysaccharide biopolymer, is an attractive biomaterial due to its excellent biocompatible and biodegradable property. Its potential applications include wound dressing, burn treatment and as haemostatic agent. Synthetic hydroxyapatite (HA) is used extensively as bone implant material due to its similar chemical composition and good biocompatibility with natural bone. A composite material of hydroxyapatite and chitosan is expected to show increased osteoconductivity and biodegradation together with sufficient mechanical strength for orthopaedic use. In this present study, biomimetic apatite composites were prepared by inducing HA crystals formation in chitosan solution. The highly crystalline chitosan solution was calcified and nanocrystals of HA was produced in it, with and without cross-linking agent. The SEM analysis shows that the polymer backbone is coated with deposits of HA on it. The XRD analysis shows that stoichiometric HA is formed and the calculated lattice parameter were comparable with standard values. The FTIR result clearly shows the peaks due to amide groups and phosphate groups along with hydroxyl peaks. The elemental analysis and EDAX analysis also confirms the formation of stoichiometric HA. The HA content was more in non-cross-linked composite due to more calcium affinity of chitosan chelation effect. This composite material is expected to have better mechanical properties and osteogenic properties. This method of producing HA crystals in chitosan solution paves a new way to analyse the biomimetic HA formation in biopolymers.

F5.29

In Situ Biodendrimers for Tissue-Engineered Articular Cartilage Repair. Mark Grinstaff[1], Serge Sontjens[1], Michael Carnahan[1], Dana Nettles[2], Tad Vail[2] and Lori Setton[2]; [1]Department of Biomedical Engineering and Chemistry, Boston University, Boston, Massachusetts; [2]Department of Biomedical Engineering, Duke University, Durham, North Carolina.

Degeneration of cartilage in the meniscus, intervertebral disk, or joint by osteoarthritis or trauma can lead to severe and debilitating pain in patients. Because of the low regenerative power of native cartilage, such an injury is generally present for long times and can eventually lead to more severe secondary damage. At present, the available treatments are chronic use of anti-inflammatory drugs, total joint replacement, transplantation, discectomy, or joint fusion. Alternative treatments based on tissue engineering concepts may significantly improve patient care. We are studying a class of photocrosslinkable dendritic macromolecules that are composed of biocompatible building blocks. Specifically, we have prepared ABA tri-block dendritic-linear macromolecules composed of glycerol, succinic acid, and PEG. In a rabbit model of femoral cartilage defect, we observed partial regrowth of cartilaginous tissue at four weeks using a gel prepared from a G1 dendritic macromolecule. Additional studies are underway to evaluate this macromolecule as a temporary scaffold for cartilaginous tissue repair.

F5.30

Design of Oriented Electrospun Nanofibrous Scaffolds for Nerve Contact Guidance. David Y. Lin[1], Mohammad R. Abidian[3], Jeff L. Hendricks[3] and David C. Martin[1,2,3]; [1]Macromolecular Science and Engineering Center, University of Michigan, Ann Arbor, Michigan; [2]Materials Science and Engineering, University of Michigan, Ann Arbor, Michigan; [3]Biomedical Engineering, University of Michigan, Ann Arbor, Michigan.

Scaffolds of poly(lactic acid) (PLA), poly(vinyl alcohol) (PVA), and poly(lactide-co-glycolide) (PLGA) made by electrospinning are being studied as nerve contact guidance devices. Electrospinning is known to generate nanometer diameter fibers using various polymer/solvent combinations. By rotating the substrate at extremely high speeds during electrospinning, these fibers can be collected as aligned filamentous scaffolds. These oriented scaffolds are then rolled into tubes and used as nerve guidance channels. The average diameter of the fibers is varied by changing the concentration of the polymer solution. The degree of filament orientation can be improved by increasing the speed of the take-up wheel. The influence of the morphology of the oriented, nanofibrous scaffolds on neural outgrowth will be discussed.

F5.31

Affinities of Multifunctional Peptide Coatings for Polymeric and Metallic Surfaces. Elisabeth Walsh[1], Woo Lee[3], Stefan Zauscher[3], Daniel Kenan[2] and Mark Grinstaff[4,5]; [1]Chemistry, Duke University, Durham, North Carolina; [2]Pathology, Duke University Medical Center, Durham, North Carolina; [3]Mechanical Engineering and Materials Science, Duke University, Durham, North Carolina; [4]Chemistry, Boston University, Boston, Massachusetts; [5]Biomedical Engineering, Boston University, Boston, Massachusetts.

Interfacial biomaterials (IFBMs) are macromolecules able to secure or separate two or more similar or dis-similar surfaces. In this report, we describe the characterization and affinity measurements of specific peptide adhesion domains which comprise these materials. The adhesion domains were originally isolated from a type m13 peptide phage display library. Peptides with high affinity to a polystyrene or a titanium surface were identified. Binding affinities were determined by a modified ELISA procedure; at physiological pH, micromolar to

nanomolar affinities were observed. We can systematically vary binding affinity by external conditions or by chemical manipulation of the isolated peptides, such as tandem domain synthesis or disulfide networking. Pull-off forces were measured by contact mode atomic force microscopy (AFM); for example, FFPYSHLGVLSSGC, a high affinity polystyrene binder, yielded an adhesion force of approximately 300 pN. LLFFDDYFKSAGR adhesion to a titanium surface was confirmed by x-ray photoelectron spectroscopy (XPS), micro-patterning, and light microscopy. In summary, we have confirmed binding activity of small peptides on polymeric and metallic surfaces for use in interfacial biomaterials. Such materials may provide a strategy to mediate cell behavior on a synthetic surface and be useful in a range of tissue engineering applications, from orthopedics to soft tissue reconstruction.

F5.32
Dispersion Properties of Hydroxyapatite Synthesized by Mechanochemical-Hydrothermal Methods. Chunwei Chen[1], Richard E Riman[1], Kelly Brown[2], Victor F Janas[2] and Kevor S TenHuisen[2]; [1]Rutgers University, Piscataway, New Jersey; [2]Johnson & Johnson Ctr for Biomaterials & Advanced Technologies, Somerville, New Jersey.

The dispersion of hydroxyapatite [HA, $Ca_{10}(PO_4)_6(OH)_2$], an important component in biomaterials, was studied with the objective of making stable colloidal HA suspensions. Our studies utilized either pure HA or Mg-substituted HA (HAMg) that were prepared by mechanochemical-hydrothermal (M-H) methods. This approach produced nanostructured agglomerated powders with 20-30 nm primary crystallites. Dynamic light scattering (DLS) methods were used to find effective conditions for particle deagglomeration in the dispersion medium. The following variables were investigated in this study: method of deagglomeration, washing process, drying method, dispersion medium, pH and use of dispersants. An ultrasonic bath, micro-tip ultrasonicator and high-shear-rate mixer were used to deagglomerate HA powders in dispersion media for comparison purposes. The correlation between dispersion quality and duration time was studied. DLS measurements revealed that the mass average diameter of HA agglomerates in deionized water was ca. 4.5 μm. The agglomerate size decreased remarkably with increasing pH. The mass average diameter of HA agglomerates decreased to 360 nm at pH 11, which was attributed to improved electrostatic stabilization. Ionic surfactants and polyelectrolytes were also found to be suitable to deagglomerate HA particles in water. However, ethanol was found to be the ideal dispersion medium. The mass average diameter of HA agglomerates decreased to 220 nm. The dispersion properties are also affected by the chemical composition of HA particles. Due to the adsorption of unreacted $Mg(OH)_2$ and some Mg-containing amorphous species on the surface of primary crystalline particles, the mass average diameter of the as-prepared HAMg agglomerates was 1.1 μm. By removing the unreacted $Mg(OH)_2$ and Mg-containing amorphous species with ammonium citrate washing, the mass average agglomerate size decreased to 770 nm. The mass average diameter of HAMg agglomerates was reduced by 35% when HAMg particles were freeze dried instead of dried by conventional ovens.

F5.33
Protein Resistant Polymer Substrates for Bioseparations. William Kuhlman[1], Eugene W Chan[2], Linda G Griffith[2] and Anne M Mayes[1]; [1]Materials Science, MIT, Cambridge, Massachusetts; [2]Biological Engineering, MIT, Cambridge, Massachusetts.

Substrates based on amphiphilic poly(methyl methacrylate)-g-poly(ethylene oxide) graft copolymers (PMMA-g-PEO) have been examined in previous studies as materials for tailoring the biological activity of surfaces. These copolymers demonstrate excellent resistance to protein adsorption and cell attachment, effectively producing biologically inert surfaces on a variety of materials. Where specific biological interactions are desired, these polymers have been functionalized with small molecules such as adhesion peptides or growth factors. In the present study, we extend this work to apply PMMA-g-PEO copolymers to produce surfaces suitable for separation of biological molecules. Antibodies have been immobilized on PMMA-g-PEO substrates using biotin-avidin chemistry. These antibodies retain their functionality and specificity following immobilization as demonstrated using a model antibody-antigen system. Further studies using surface plasmon resonance demonstrate that immobilization of these antibodies does not compromise the inherent protein resistance of the substrate, providing substrates that bind proteins with the specificity of an antibody with little non-specific protein adsorption.

F5.34
Selective Laser Sintering of 3-D Biodegradable Scaffolds for Tissue Engineering. Vladimir K. Popov[1], Eugeny N. Antonov[1], Victor N. Bagratashvili[1], Alexey N. Konovalov[1] and Steven M. Howdle[2]; [1]Institute on Laser and Information Technologies Russian Academy of Sciences, Troitsk, Moscow Region, Russian Federation; [2]School of Chemistry, Nottingham University, Nottingham, United Kingdom.

Selective Laser Sintering (SLS) is an innovative rapid prototyping technique allowing production of very complicated accurate 3-D polymer replicas directly from a computer image. The major drawback in this technology for biomaterial applications has been that the processes of sintering limit the type of polymer materials from which the 3-D object can be made. These materials (specific wax, carbonate or nylon based polymers) are neither biocompatible nor biodegradable and, hence, not appropriate for implantation or tissue engineering. In conventional SLS volumetric absorption of the laser radiation by polymer leads to melting of the whole particle and fusion. This exposes the polymer powders to high temperatures which are prohibitive to many biodegradable materials and bioactive species. Our studies of SLS process of biodegradable polymer powders lead to development of a novel Selective Laser Sintering technique enable us to extend the range of polymers that can be used. Optimization of polymer powder composition and the processing parameters such as cw-diode laser intensity and laser beam scanning speed has enabled reproducible fabrication of 3-D biodegradable scaffolds of specific shape and internal structure with a spatial resolution ~0.2mm. We have demonstrated that composite scaffolds containing 30wt.% of calcium hydroxyapatite (HA) can also be effectively produced by this method. All samples were analysed by Scanning Electron Microscopy, Fourier Transform Micro Raman Spectroscopy and Gel Permeation Chromatography. No significant changes in polymer molecular weight, polydispersity or crystallinity were detected. As a result, new SLS methodologies for preparing biodegradable composite scaffolds that incorporate a high concentration of bioactive particles of a second component (e.g. HA) into the 3-D structure with the desired architecture has been developed. Acknowledgements The authors are grateful to John Barry and Daniel Bratton for their help in SEM and GPC analysis of the samples. The authors would like also to acknowledge the financial support of The Wellcome Trust for Collaborative Research Initiative Grant 062760. SMH is a Royal Society Wolfson Research Merit Award Holder.

F5.35
Abstract Withdrawn

F5.36
PVA Hydrogel/PLGA Microsphere Composite Coatings to Promote Wound Healing and Control Inflammation at Biosensor Implant Site. Siddhesh D Patil[1], Misiya A Norman[1], Izabela Galeska[2], Fotios Papadimitrakopoulos[2] and Diane J Burgess[1]; [1]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut; [2]Nanomaterials Optoelectronic Laboratory, Department of Chemistry, Polymer Program, Institute of Material Science, University of Connecticut, Storrs, Connecticut.

Control of wound healing at the site of biosensor implantation is critical to the success of implantable devices such as biosensors, in order to control inflammatory response and prevent consequent device malfunctioning and eventual failure. We have developed tissue-compatible poly(vinyl alcohol) (PVA) hydrogel/ poly(lactic-co-glycolic) acid (PLGA) microsphere composite coatings containing dexamethasone to control the inflammatory response due to biosensor implantation and VEGF to promote neovascularization. In the present study, release of dexamethasone and its pharmacodynamic effect at the implant site in controlling inflammation were investigated. Composite coatings were inserted into the interscapular subcutaneous tissue of anesthetized rats. In vivo drug release [pharmacokinetics (PK)] and tissue response [pharmacodynamics (PD)] were determined through serial sacrifice (days 1, 3, 7, 14, 21 and 28). Histological evaluation of excised tissue samples revealed that dexamethasone released from these composite coatings was successful in suppressing both acute (1 - 5 days) and chronic (5, 7, and 14 days) inflammation. Dexamethasone also minimized fibrotic band formation (21 and 28 days) at the implantation site. VEGF was shown to enhance neovascularization. The reported hydrogel layer coating is able to control many of the negative tissue responses (inflammation and fibrosis) and promote wound healing. The use of this coating approach has the potential to advance biosensor technology. This work was supported by the Department of Defense, US Army Medical Research Grant # DAHD17-02-1-0713.

SESSION F6: New Technologies
Chair: Christopher Chen
Thursday Morning, December 4, 2003
Back Bay A (Sheraton)

8:30 AM F6.1
The Development and Characterization of a Novel

BIORESIST for the Spatial Organization of Mammalian Cells. Wei He[1], Kenneth E. Gonsalves[2] and Craig R. Halberstadt[3]; [1]Chemistry & Institute of Material Science, University of Connecticut, Storrs, Connecticut; [2]Chemistry & Cameron Applied Research Center, University of North Carolina, Charlotte, North Carolina; [3]General Surgery Research, Carolinas Medical Center, Charlotte, North Carolina.

An important challenge in tissue engineering is how to achieve and control spatial organization of cells. The formation of tissue is reliant on the formation of cell-cell contacts in a defined pattern. This could be achieved by conventional lithography. Photolithography is the current workhorse for the microelectronic industry. It is a well-developed patterning process. Researchers have studied the application of photolithography in patterning molecules onto the surface, such as proteins. However, such applications are limited due to the use of organic solvents in the pattern development process, which can denature the biomolecules. To address this problem, novel biocompatible photoresists (BIORESIST) based on 3-(t-butoxycarbonyl)-N-vinyl-2-pyrrolidone (TBNVP) were prepared and in vitro fibroblast cell growth on these resists was studied. Cell attachment and cell proliferation on these BIORESISTs were investigated. In addition, patterns of 25 micron by 25 micron lines were obtained by chemically manipulating the surface of photoresist using UV lithography without any solvent development. Fibroblast cells were observed to align on the patterned surface. By incubation in serum versus serum-free medium, such alignment could be contributed to the preferential deposition of serum proteins along the patterns. With this BIORESIST, we were able to pattern a 2-in glass substrate. At the end of 2 weeks in culture, a well-defined collagen matrix deposition was observed to be oriented along the patterns that were not observed in the control sample. These BIORESISTs would be suitable candidates for the application of conventional lithography in cell and protein patterning. It would allow the fabrication of large biomaterial structures with defined structural patterns in order to guide the growth and development of new tissues.

8:45 AM F6.2

Multi-array formation of hepatocyte hetero-spheroids on micro-fabricated PEG-brush surface. Hidenori Otsuka[1], Tomomi Satomi[1], Jun Itadani-Harada[1], Yukio Nagasaki[2], Yasuhiro Horiike[1], Teruo Okano[4,1] and Kazunori Kataoka[3,1]; [1]Biomaterials Center, National Institute for Materials Science, Tsukuba, Ibaraki, Japan; [2]Department of Materials Science and Technology, Science University of Tokyo, Noda, Chiba, Japan; [3]Department of Materials Science and Engineering, The University of Tokyo, Bunkyo-ku, Tokyo, Japan; [4]Institute of Advanced Biomedical Engineering and Science, Tokyo Womens Medical University, Shinjuku-ku, Tokyo, Japan.

Microarray technology allows the simultaneous analysis of thousands of parameters within a single experiment. Microspots of capture molecules are immobilized onto a solid support and exposed to samples containing the corresponding binding molecules. Such miniaturized and parallelized binding assays can be a useful tool to characterize gene and protein expression patterns in human disease processes in order to identify novel molecular targets for therapy. Intensive investigation of proteins and chemical networks that comprise the cells and tissues of an organism, and the specific roles of proteins in these networks, will be a necessary next step to understand cellular functions in healthy and diseased states. Tissue and cell-based biosensors (TBB and CBB) will facilitate clinical and pharmaceutical analysis of molecular targets, because living cells can monitor the targets through their physiological changes that are induced by exposure to drugs and environmental perturbations, such as toxicants, pathogens or other agents. Primary hepatocytes are the most useful candidate to construct TBB and CBB. Since isolated hepatocytes are known to readily lose many liver-specific functions, the most crucial issues in hepatocyte-based biosensors are long-term viability and retention of liver-specific functions of cultured hepatocytes. In this study, the micropatterning of rat primary hepatocyte hetero-spheroids underlaid with bovine aortic endothelial cells (BAECs), exhibiting high viability and vigorous liver-specific function, was prepared on micro-fabricated glass substrates coated with poly(ethylene glycol) (PEG) brushes. These arrayed spheroids as miniaturized liver may be highly useful for TBB, offering the promise of sensing drugs and environmental threats through a cellular physiological response. Furthermore, the isolated spheroids from the array may have a potential utility in constructing a tissue-engineered liver by seeding spheroids into three-dimensional scaffolds. This technique is useful as a tool to obtain insights into the mechanism of cell-cell interaction, a central research topic in cell biology.

9:00 AM F6.3

Ink Jet Deposition of Human Cells. Rachel Saunders[1], Nuno Reis[2], Julie Gough[1] and Brian Derby[1]; [1]Manchester Materials Science Centre, UMIST, MANCHESTER, United Kingdom; [2]Department of Engineering Materials, Instituto Superior Tecnico, Lisbon, Portugal.

Ink jet printing can be used for the selective deposition and incorporation of cells within tissue engineering scaffolds. During the droplet formation process the liquid experiences shear strain rates close to 10^4 s^{-1} and similar strain rates occur during droplet impact on a surface. These strain rates will subject cells in suspension to large stresses and deformation and the response of cells to these conditions is unclear. A commercial piezoelectric ink jet printer has been used to deposit a range of cell lines and primary cells onto polymer substrates suitable for use in tissue engineering scaffolds. Cells have been characterised and assayed for viability in culture after deposition. A range of printing conditions have been studied by changing the characteristics of the excitation pulse used to drive the piezoelectric actuator. This results in a range of droplet sizes and velocities of deposition. Cells are found to show a robust response to ink jet printing and the technique is a viable method of cell deposition and patterning.

9:15 AM F6.4

Bone Marrow Tissue Engineering on the basis of Inverted Colloidal Crystals. Nicholas Alexander Kotov[6,1], Shaopeng Wang[2], Yuanfang Liu[1], Joan Nichols[3], Joaquin Cortiella[5], Massoud Motamedi[4], Mohammad Eghtedari[4] and Gracie Vargas[4]; [1]Chemistry, Oklahoma State University, Stillwater, Oklahoma; [2]Nanotechnology, Nomadics Inc, Stillwater, Oklahoma; [3]Internal Medicine, University of Texas Medical Branch, Galveston, Texas; [4]Biomedical Engineering, University of Texas Medical Branch, Galveston, Texas; [5]Anestesiology, University of Texas Medical Branch, Galveston, Texas; [6]Chemical Engineering, University of Michigan, Ann Arbor, Michigan.

Stem cell cultures require different ex-vivo scaffolds than the currently used supports for cells from developed organs. Adhesion of the stem cells and uniformity of metabolic conditions need to be significantly improved. Additionally, the scaffolds should be tailored for a particular differentiation route selected for the culture. Some of these challenges can be met by taking advantage of nanoscale processing of the surface supporting cell growth. The new type of scaffolds was made on the basis of inverted colloidal crystals. Their surface was modified with biologically active compounds following the layer-by-layer deposition protocol. Excellent adhesion and viability of stem cells and stromal cells were obtained. The network of voids and channels in the inverted colloidal crystals creates bone marrow-like environment for stem cell development. The functional similarity of the inverted colloidal crystals with stromal cell culture on them was demonstrated by cell marker assays and microscopy data. Ordered nature of the crystals and uniform layer-by-layer assembled coating of the scaffold walls standardize the differentiation conditions in each spherical compartment. The production of T and B cells from CD34 stem cell on these scaffolds is demonstrated.

9:30 AM F6.5

Electrospinning Tissue Engineering Scaffolds. Gary E. Wnek[2], Eugene Boland[1], David Simpson[3] and Gary Bowlin[1]; [1]Biomedical Engineering, Virginia Commonwealth University, Richmond, Virginia; [2]Chemical Engineering, Virginia Commonwealth University, Richmond, Virginia; [3]Anatomy, Virginia Commonwealth University, Richmond, Virginia.

Since the inception of the field of tissue engineering there has been a considerable effort to develop an "ideal" tissue-engineering scaffold. Biocompatibility, mechanical stability and bioresorption/degradation are the defining assets of an "ideal" scaffold yet this combination has continually eluded researchers. Electrospinning is proving to be able to overcome many of the historical limitations due in particular to the ability to produce fibrous scaffolds that can mimic the composition and architecture (fiber size and orientation) of the native extracellular matrix. Our laboratory has demonstrated that nano- to micro-scale fibrous scaffolds (various geometries) can be reproducibly electrospun from biopolymers including collagen type I, II, and III, elastin, fibrinogen, poly(glycolic acid), poly(lactic acid), and polycaprolactone either individually or in combinations tailored to a specific application. Fiber diameters are controllable via electrospinning solution concentration and mechanical properties of the scaffolds are controllable via fiber orientation. Cellular/tissue interactions have been shown to be dependent on the characteristics of the electrospun scaffolds with composition and fiber diameter as the dominant variables in the process. Thus, electrospinning provides a means to produce scaffolds that address the fundamental needs of tissue engineering scaffolds for a wide variety of tissues and organs. Examples to be presented in support of the electrospun scaffolding's potential will include the engineering of blood vessels, cartilage, bone and skeletal muscle.

9:45 AM F6.6

Electrospinning of Nanofibrous Scaffolds for Tissue Engineering. S S Chia[2], Y Z Zhang[1], S Ramakrishna[2,1] and C.T. Lim[1,2]; [1]Division of Bioengineering, National University of

Singapore, Singapore, Singapore; [2]Department of Mechanical Engineering, National University of Singapore, Singapore, Singapore.

Electrospinning provides a direct method to produce fibers with diameters ranging from a few nanometers to microns. Recently electrospun fibers have been used as scaffolds for tissue engineering. In this paper, an electrospinning apparatus, which is able to produce both aligned and randomly oriented nanofibrous mats in a high-throughput way will be presented. The parameters affecting nanofiber morphology have also been studied. Our investigation shows that nanofiber diameter increases with polymer concentration. Also, at higher concentration, smoother fibers are formed. However, fibers with beads are found to form at higher applied voltage and lower tip to collector distance. Cylindrical frame structure is a convenient and effective method to obtain aligned nanofibers. It is found that the frame material, dimensions of the frame and rotational speed of the frame have effect on fiber alignment. Based on the simulation of electric field, a fiber alignment mechanism is proposed. Nanofiber productivity can be increased by using a multiple spinneret system. There is no difference in nanofiber morphology for fibers obtained using either single or multiple spinneret system. To improve the performance of the electrospinning apparatus, this apparatus should be operated in a Faraday cage to minimize the effect of surrounding electric field. A heated spinneret system can also be implemented to electrospun polymer melt.

10:30 AM F6.7
Aligned Axonal Outgrowth for Neurons Cultured on Nanophase Carbon Deposited on Porous Silicon Templates. Janice L McKenzie[1], Marisa A Sambito[2], Nader M Kalkhoran[2] and Thomas Jay Webster[1]; [1]Biomedical Engineering, Purdue University, Lafayette, Indiana; [2]Spire Biomedical Corporation, Bedford, Massachusetts.

Materials used as central nervous system implants often lack the surface properties necessary to limit unwanted glial-scar tissue formation and to promote interactions with neurons. Previous research has shown that among other attractive properties for nervous system tissue-engineering applications such as high conductivity, carbon nanofibers have excellent cytocompatiblity properties pertinent for neural implantation. Studies have shown these properties result directly from their biologically-inspired nano-structured dimensions (less than 100 nm). However, carbon nanofibers do not inherently strongly bond to each other and, thus, cannot be used alone as an implant material. As a possible solution to this problem, the present study sought to deposit carbon nanofibers on a silicon matrix in a controlled manner to direct axonal outgrowth in neurons. To accomplish the directed formation of carbon nanofibers, silicon substrates were anodically etched to form a porous nanostructured silicon matrix on the surface as a template for subsequent deposition of carbon. Scanning electron microscopy provided evidence of a well-controlled deposition of carbon along regions of high stress on the silicon matrix. Results from cell studies demonstrated enhanced interactions of neurons along deposited carbon nanofibers. Specifically, increased numbers of neurons and axons were observed along carbon nanofibers on the silicon matrix. Decreased interactions of astrocytes (cells that contribute to undesirable glial scar tissue formation) were also observed on deposited carbon nanofibers. Based on these results, the present study demonstrated the controlled deposition of carbon on a silicon matrix that could be used to direct axonal outgrowth in neurons as well as to decrease functions of astrocytes; such criteria are critical for the development of the next-generation of neural implants. This work was supported by NSF Grant No. DMI-023259.

10:45 AM F6.8
Bioactive Organosilicate Nanoparticles as Gene Delivery Vehicles for Bone Cells. Suniti Moudgil and Jackie Y Ying; Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Although normal bone has a substantial capacity to regenerate itself, fracture healing is extremely difficult in the following cases: fractures resulting in extensive tissue loss, fractures in sites of low vascularity, and fractures caused by bone fragility syndromes such as osteoporosis. In such cases, a tissue engineering approach is desirable to accelerate bone growth and restore function. This can be done through the use of bioactive materials that encourage osteoblast proliferation, as well as through the intracellular delivery of genes that encode for proteins that aid bone cell growth and differentiation. We have taken both approaches by synthesizing bioactive organosilicate nanoparticles with compositions similar to Bioglass®, a doped silicate that has been recognized in orthopedic medicine for its ability to bond quickly and strongly to bone. Monodisperse organosilicate particles of 10 nm - 1 μm have been successfully achieved, as indicated by Dynamic Light Scattering (DLS) and electron microscopy. Certain nanoparticles, especially those containing calcium, significantly increased bone cell proliferation in cell culture experiments. Investigation with transmission electron microscopy (TEM) showed that these nanoparticles entered the cell cytoplasm. Particle uptake was shown to be dependent on particle size and composition. Because of their ability to be selectively ingested by bone cells, these nanoparticles have been explored as potential gene delivery vehicles for bone. Plasmid DNA which express green fluorescent protein upon transfection were adsorbed onto the surface of certain nanoparticles to form nanoparticle-DNA complexes. Atomic force microscopy (AFM) showed that the size of these complexes affected their ability to effectively transfect bone cells. The synthesis, characterization, and transfection efficiency of these nanoparticle-DNA complexes will be discussed in this presentation.

11:00 AM F6.9
Lymphoid Scaffolds Prepared by Colloidal Crystal Templating. Agnieszka Stachowiak[1] and Darrell J Irvine[2]; [1]Dept. of Materials Science & Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]Dept. of Materials Science & Engineering/Division of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

The primary immune response occurs in the T-cell rich paracortex (T zone) of the lymph node, where antigen-presenting dendritic cells activate naive T cells. The T zone extracellular matrix provides a uniquely high surface area scaffold to support cell migration and interactions, and we hypothesize that the unique structure of the T zone is one factor that augments and directs the primary immune response. We have developed a novel scaffold fabrication method based on colloidal crystal templating, which allows us to mimic T zone architecture in an *invitro* model by making ordered porous hydrogel scaffolds with high pore interconnectivity. Peptide-modified poly(ethylene glycol)dimethacrylate was photopolymerized atop a colloidal template consisting of poly(methyl methacrylate) particles, after which the particles were selectively removed by dissolution. The physical and mechanical properties of scaffolds made by this method are readily varied. The pore size of the scaffold was varied from 20±0.3 – 60±1 μm by using appropriate PMMA particles. Copolymerization of the peptide-modified PEG dimethacrylate with 0.1-5% (w/vol) bisacrylamide allowed the stiffness of the scaffold to be varied. To facilitate recovery of cells from scaffolds for analysis, selective degradation of PEG-peptide scaffolds was achieved by incorporation of enzyme-sensitive peptides such as APGL; collagenase treatment of APGL-containing scaffolds caused their complete enzyme-specific disintegration. We have used these novel hydrogel scaffolds to study the interactions of labeled T cells and dendritic cells using time-lapse 3D fluorescence microscopy. The scaffolds are optically transparent and show minimal autoflourescence, allowing for imaging of several cell layers within the scaffold. We have also determined that lymphoid stromal cells respond to RGD-presenting scaffolds by spreading, and are viable for at least three days. This lymphoid tissue model provides an *invitro* platform for studies of the immune response and may be developed into a tissue engineering construct for immune therapeutic purposes, or an *exvivo* device for testing human immune responses to vaccines or other biological agents.

11:15 AM F6.10
Dielectrophoretic Cell Patterning Within Hydrogel Scaffolds. Dirk R Albrecht, Robert L Sah and Sangeeta N Bhatia; Bioengineering, University of California, San Diego, La Jolla, California.

Microfabrication tools have been extensively utilized to define and quantify the role of the cellular microenvironment on cell fate and function on two-dimensional (2D) surfaces. Exploring these relationships for cells that are organized in 3 dimensions (3D) in tissues, or for cells that are cultured within hydrogel materials, will require novel patterning tools. Our aim was to develop a versatile 3D patterning tool compatible with many biomaterials and cell types. Here, we present the development and characterization of a model system that utilizes dielectrophoretic (DEP) forces to rapidly organize cells within firm hydrogel slabs. High frequency excitation of micropatterned electrodes resulted in positioning of cells within minutes in a photosensitive polymer solution. Subsequent light-induced crosslinking generated a stable hydrogel. Patterning was achieved with a resolution (<10 μm) at the length scale of cells. In particular, lines and arrays of cells or cell clusters were created using different electrode arrays that were rapidly fabricated by a single-step lithography process. Embedded cells of various types remained largely viable for weeks. Using cartilage as a model tissue, patterned chondrocytes maintained differentiated function over long periods of time, in contrast to 2D cultures. These cell patterns can be designed to specifically modulate cell-cell, cell-matrix, and paracrine signaling interactions within a supportive 3D hydrogel. Notably, DEP patterning is compatible with many novel "active" biomaterials that incorporate adhesion and degradation sites, tethered growth factors, and engineered mechanical and diffusive properties. By simultaneously

controlling cell patterns and scaffold material properties, this technique may become a useful and versatile tool for investigating structure/function relationships in tissues with complex 3D cellular architecture.

11:30 AM F6.11
Novel Seeding Mechanisms to Form Multilayer Heterogeneous Cell Constructs. Brad Richard Ringeisen, Jason A. Barron and Barry J. Spargo; Biological Chemistry Section, Naval Research Laboratory, Washington, District of Columbia.

Methods to print living mammalian cells into layers of tissue scaffolding are gaining momentum as novel alternatives to traditional cell seeding techniques. One such method, termed BioLP$^{©n}$ for biological laser printing, is a technique capable of precisely embedding patterns of cells into three-dimensional tissue scaffolds. The resolution of this cell seeding apparatus is controlled by the size and energy of a focused laser pulse and has the demonstrated ability to print single cells onto a substrate. Studies show that the cells embedded into scaffolding layers by BioLP$^{©n}$ retain 100% viability, are able to differentiate, and maintain normal genotype and phenotype. By placing different cell types at defined locations throughout a multi-layer scaffold and by controlling the physical and chemical characteristics of the non-cellular construct (porosity, channeling, multi-material scaffolding), BioLP$^{©n}$ enables unique tissue engineering studies to be performed. We will discuss our investigations into several aspects of heterogeneous, three-dimensional cell constructs including cell growth and migration, cell-cell interactions, and cell viability as a function of time, construct size, and characteristics of the scaffolding.

11:45 AM F6.12
Laser Fabrication of Three Dimensional Tissue Structures Cell-By-Cell. Douglas Brian Chrisey, Rohit Modi, Raymond C.Y. Auyeung and Huey-Daw Wu; Naval Research Laboratory, Washington, District of Columbia.

We have developed a novel laser forward transfer process, termed MAPLE DW for matrix assisted pulsed laser evaporation direct write, that enables the layer-by-layer and even the cell-by-cell deposition of mammalian cells and organic and inorganic biomaterials. This is a CAD/CAM approach that utilizes a 193 nm ArF excimer laser and it allows the fabrication of tissue constructs that more closely approach the heterogeneous structure of natural tissue than do other more conventional approaches to tissue engineering. We have successfully deposited several different types of mammalian cells including mouse muscle cells and pluripotent cells, human osteoblasts, and hamster ovary cells. When transferred as single layers, the cells spread out and multiply, but when deposited on top of one another they assemble and grow slowly together behaving more like natural tissue. We have done genetic comet testing on the pluripotent cells which are most sensitive to the transfer process to determine that there is minimal to no double strand breaks in the laser-transferred cells. In this presentation, we will show tissue engineering results using differential adherence on two dimensional cell layer alignment and growth into three dimensional myotubes, the direct MAPLE DW fabrication of three dimensional myotubes, and the fabrication of a vascularization network; in all cases, the CAD/CAM program used in the MAPLE DW process predetermines the size and pattern. Finally, a discussion of the potential of the
MAPLE DW approach and critical areas for improvement will be given.

SESSION F7: Surface Anaylsis and Engineering
Chair: Anne Plant
Thursday Afternoon, December 4, 2003
Back Bay A (Sheraton)

1:30 PM F7.1
A Quartz Crystal Microbalance Study of the Isolation of Cell Free Endothelial Cell Extracellular Matrix and Its Properties. Kenneth A. Marx, Tiean Zhou, Donna McIntosh and Susan J. Braunhut; Center for Intelligent Biomaterials, Departments of Chemistry and Biological Sciences, University of Massachusetts, Lowell, Massachusetts.

We have previously studied the attachment and spreading of large and microvessel endothelial cells (ECs) and other cell types on the hydrophilic gold surface of a Quartz Crystal Microbalance (QCM) under normal culture conditions (1, 2). After 24 hrs, when fully spread on the QCM gold surface, ECs achieve their steady state, and can then serve as an effective whole cell biosensor capable of detecting the intracellular action of cytoskeleton disrupting drugs, such as nocodazole, down to low nM concentrations in a dose dependent fashion (3,4). In the current study, we used ECs to create a whole cell biosensor system. Using non-proteolytic conditions, the biosensor was then used to detect the complete release of intact adherent cells from the surface and to document the presence of the underlying extracellular matrix (ECM) which remained after all cells were removed. We followed this process continuously by measuring the QCM oscillating quartz crystal frequency, f, and motional resistance, R, values as we carried out a 3-step removal of the cells using successive 2 hr incubations with 2.5 mM EGTA in Ca++, Mg++ free PBS. Changes in f and R magnitude with time were in agreement with the quantitative release of cells from the QCM surface measured by electronic cell counting and also as measured at similar times in tissue culture simulations of the release process. A novel transient reversible change in R was observed during the first release step. We characterized the per cell normalized shifts in f and R over a varying cell density range. Finally, we demonstrate that the ECM remaining on the QCM surface following cell removal does not exhibit any significant level of energy dissipation as do the cells when they were attached to their matrix. In addition, we demonstrate that the ECM is available for further electrochemical processing or stimulation steps-a procedure that has potential applications to tissue engineering and drug delivery. 1. Zhou, T., Braunhut, S., Medeiros, D., Marx, K.A., MRS: Tissue Eng. Symp., 1999, 550, 177-182 2. Zhou, T., Marx, K.A., Warren, M., Braunhut, S. Biotech. Prog., 2000, 16, 268-277. 3. Marx, K.A., Zhou, T., Schulze, H., Montrone, A., Braunhut, S., Biosensors & Bioelectronics, 2001, 16, 773-782. 4. Marx, K.A., Zhou, T., Montrone, A., Braunhut, S., MRS: Adv. Biomaterials-Char., Tissue Eng. & Complexity, 2002, 711, 125-132.

1:45 PM F7.2
Real-Time Measurements of Fibronectin-Integrin Interactions on Polymers Using a Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D). Norbert Weber[1,2] and Joachim Kohn[1,2]; [1]Chemistry, Rutgers University, Piscataway, New Jersey; [2]New Jersey Center for Biomaterials, Piscataway, New Jersey.

The hemocompatibility of artificial surfaces is highly dependent on the adhesion and activation of platelets. The integrin GPIIb-IIIa is the major cell surface protein in platelets and functions as receptor for certain adhesive proteins (fibronectin, fibrinogen, von Willebrand factor and vitronectin). So far, this receptor has only been detected on platelets and megacaryocytes. Because surface-adsorbed fibronectin has been shown to support platelet adhesion we investigated the selective adsorption of fibronectin to different biomaterials and the subsequent binding of purified GPIIb-IIIa by real-time measurements using the recently developed QCM-D technology (Q-Sense AB, Sweden). This device can measure the adsorbed mass of proteins binding to a polymer-coated crystal and the change of dissipation of the surface. Dissipation is a measure of how the adhered material dampens the surface vibrations and gives information about the viscosity, elasticity and thickness of adsorbed protein layers. Results show that concentrations of adsorbed fibronectin on test polymers, such as tyrosine-derived poly(DTE carbonate) and poly(lactide-co-glycolide) (PLGA), correlate with the binding capacities for GPIIb-IIIa. Modification of poly(DTE carbonate) by copolymerization with 5% poly(ethylene glycol) (PEG) resulted in a decreased binding of fibronectin followed by a reduced binding of GPIIb-IIIa. The binding of GPIIb-IIIa to surface-adsorbed fibronectin was inhibited completely by blocking GPIIb-IIIa with synthetic inhibitor peptides (GRGDSP). These peptides are based on the cell-attachment sequence (RGD) of fibronectin. Inactive control peptides (GRADSP) did not inhibit the receptor-ligand reaction showing the specificity of the binding reaction. In addition to the changes in the adsorbed mass to the polymers, the changes in the dissipation may give insights into the structure, orientation and binding of the receptor to surface-adsorbed plasma proteins.

2:00 PM F7.3
Bioengineered Polymeric Substrata to Probe Vascular Smooth Muscle Cell Behavior. Joyce Y Wong, Biomedical Engineering, Boston University, Boston, Massachusetts.

Restenosis remains a major problem in treatments of arterial occlusive disease. Vascular smooth muscle cells play a major role in vascular remodeling, and local control of their cellular phenotype would greatly enhance efforts to reduce the occurrence of restenosis. A common result of vascular injury is excessive remodeling of the extracellular matrix, which becomes rich in collagens and proteoglycans. While this leads to changes in biochemical properties, it also significantly alters biomechanical properties. We have been developing bioengineered polymeric substrata that recapitulate the biomechanical environment during vascular remodeling with the long-term goal of to identifying key relationships between substrate physical properties and vascular smooth muscle cell phenotypes associated with restenosis. Our model bioengineered substrata are designed to exhibit a systematic variation in their properties ranging from the microscopic to macroscopic length scales. We have focused on modulating the mechanical properties of the substrate and find that vascular smooth muscle cell attachment, spreading, migration, and proliferation is affected by the mechanical

properties of the substrate. We report our findings on hydrogel and polydimethylsiloxane substrata. We used tools from polymer science and engineering and microfabrication and microfluidics technologies to create bioengineered substrata that exhibit a systematic variation in mechanical properties. The mechanical properties of our substrata vary from very low moduli ($\sim$ 3 kPa) that are comparable to the moduli measured for soft collagen gels to high moduli ($\sim$ 2 MPa) that correspond to the moduli measured in actual blood vessels. On substrata with gradients in mechanical properties, we found that smooth muscle cells prefer to migrate toward the regions of the substrata that have higher elastic modulus. Thus, substrate mechanical properties can be used to direct smooth muscle cell migration. We also find that the rate of vascular smooth muscle cell proliferation on stiff substrata is only 66% of the rate found on soft substrata. Our results show that vascular smooth muscle cells are capable of sensing and responding to changes in mechanical properties of the substrate in a range that is physiologically relevant.

2:15 PM F7.4
A Method for Fabricating Microtextured Basal Lamina Analogs with Submicron Porous Architecture. Angela Throm[1,2] and George D Pins[1]; [1]Biomedical Engineering, Worcester Polytechnic Institute, Worcester, Massachusetts; [2]Graduate School of Biomedical Sciences, University of Massachusetts Medical School, Worcester, Massachusetts.

The ability to fabricate microtextured basal lamina analogs with microstructures that incorporate controlled submicron porosities will enhance the long-term maintenance of cell morphology, differentiation and function on the surfaces of these scaffolds. In this study, a combination of membrane synthesis techniques was used to produce microtextured collagen membranes with submicron pore structure. To form these membranes, a solution of acid-soluble type I collagen was processed to facilitate fiber formation and to control porosity. Freeze-dried collagen extracted from rat tail tendons was dissolved in a hydrochloric acid solution and was applied to a polydimethyl siloxane (PDMS) negative replicate of a micropatterned wafer. Self-assembly of collagen fibrils was initiated by submerging the PDMS template and collagen solution into a fiber formation buffer. These fibrillar collagen scaffolds were subsequently rinsed with distilled water, and air-dried. Dry collagen membranes were rehydrated for various lengths of time, frozen at several cooling rates under controlled conditions and lyophilized. Scanning electron microscopy (SEM) analyses indicate that there is a correlation between the membrane processing conditions and porous architecture. Our initial results show that these membranes contain collagen fibers with diameters ranging from 200 to 900 nm in diameter and pores ranging from 400 nm to 5 μm in diameter. Ultimately, the ability to regulate the porosity of microtextured basal lamina analogs will improve the design of tissue engineered scaffolds by enhancing the ability to control molecular transport, guide cell growth and modulate cellular function.

2:30 PM F7.5
The Influence of Collagen Membrane Microtopography on Keratinocyte Proliferation and Differentiation.
Brett R. Downing[1], Mehmet Toner[2] and George D. Pins[1]; [1]Biomedical Engineering, Worcester Polytechnic Institute, Worcester, Massachusetts; [2]Center for Engineering in Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts.

The design of future bioengineered skin substitutes must focus on understanding the mechanisms by which the three-dimensional microarchitecture of tissue scaffolds modulates keratinocyte function. Microtextured basal lamina analogs were developed to analyze the relationship between the characteristic topography found at the dermal-epidermal interface of native skin and keratinocyte proliferation and differentiation. Microtextured collagen membranes and dermal analogs with varying topographic features were produced using microfabrication strategies. Photolithography was used to develop master patterns with ridges and channels of length scales (50 – 400 μm) similar to the invaginations found in native basal lamina. Negative replicates of the microfabricated patterns were produced with polydimethylsiloxane silicone elastomer. Thin collagen membranes created from these replicates consisted of a series of transverse channels and ridges that recapitulated the features of the master pattern. The membranes were laminated onto type I collagen sponges and seeded with cultured human epidermal keratinocytes to form skin equivalents. To determine how the microenvironment created by the basal lamina analogs affected keratinocyte function, we performed histological analyses of sections of the skin equivalent after 7 days of tissue culture at the air/liquid interface. Hematoxylin and eosin staining revealed that deep channels enhanced stratification of the epidermis by promoting keratinocyte differentiation. Immunohistochemistry staining for β1, Ki67, and involucrin was analyzed to determine how topography affects the mitotic activity and distribution of the cells. The deeper and wider channels promoted larger clustering of epidermal stem cells and a thicker region of transient-amplifying cells. Ultimately, understanding the relationships between the microtopographic features of basal lamina analogs and keratinocyte function will enhance the performance of bioengineered skin substitutes.

2:45 PM F7.6
Protein Stability at the Interfaces of Polymeric Biomaterials.
Ananthakrishnan Sethuraman[1], Mina Han[1], Ravindra Kane[1], Taiji Imoto[2] and Georges Belfort[1]; [1]Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York; [2]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan.

Preconditioning of biomaterials through protein adsorption will affect their ability to support cell growth under physiological conditions and could limit their function. Here, we focus on such preconditioning protein layers and investigate the influence of the underlying chemistry on protein adhesion and conformational stability. How proteins behave at interfaces is also of concern to the non-medical community (chromatography, synthetic membrane filtration, fouling of ship surfaces and other marine structures). Despite the large number of studies devoted to protein adsorption, little is known about adsorbed protein activity, orientation and conformation on different polymeric, or with biological (chaperone) substrates. Attenuated total reflection Fourier-transformed infrared (ATR/FTIR) spectroscopy together with an optimization algorithm for predicting the content of secondary structure motifs is used to determine the secondary structure and amount of adsorbed hen egg lysozyme at solid surfaces. Lysozyme unfolds fast and the slowly at solid interfaces. The slow process involves a secondary structural conformational transition from α-helix to β-sheet, turns and unordered with increasing surface hydrophobicity. Three independent variables, surface wettability, solution concentration, and time for adsorption, were used to follow the fractional structural changes of lysozyme. The results suggest that lateral interactions between proteins drive the transition process. The kinetics of this transition for adsorbed protein is orders of magnitude slower than those reported for free solution. The time-dependent rate constants for the structural transitions are obtained from a fit of a relatively simple kinetic model. These results demonstrate that, the chemistry of solid substrates, characterized by their wettability, can perturb the contents of the native secondary structure of the protein and produce alternate structures rich in β-sheets. This is affected by the surface chemistry of the solid substrate either directly on the protein structure or indirectly due to increased lateral interactions resulting from the increased adsorbed amount.

3:30 PM F7.7
Molecular Structures of Proteins at Interfaces Detected Using Sum Frequency Generation Vibrational Spectroscopy. Jie Wang, Zoltan Paszti, Matthew L Clarke, Xiaoyun Chen and Zhan Chen; Chemistry, University of Michigan, Ann Arbor, Michigan.

Although for decades excellent studies on the interactions between biomaterials and proteins have been performed by a myriad of surface-sensitive methods, molecular level interrogations of these interactions in situ have not yet been achieved. We have investigated structures of various protein molecules at the protein solution/polymer interfaces at the molecular level and in situ with a surface sensitive nonlinear optical technique, sum frequency generation (SFG) vibrational spectroscopy. Polymers that have been investigated include polyurethane, polystyrene, polymethacrylates, polyethylene, and fluorinated polymers. Proteins studied include albumin, fibrinogen, ubiquitin, collagen, and factor XII (FXII). SFG results show that protein molecules have different structures at different polymer/protein solution interfaces, providing direct evidence of different conformations or structural changes of protein molecules at different interfaces. A thin film model has been proposed and it successfully interprets different SFG spectra from these interfaces. Our SFG studies also indicate that adsorbed protein molecules exhibit different structural changes when exposed to different chemical environments, including air, water, and hydrophobic solvents. The SFG studies on blood coagulation initiator FXII adsorption on various polymers reveal kinetics of FXII autoactivation on different surfaces, which provides new insight into blood clotting on foreign surfaces. We also elucidated how plasticizers on the surface mediate protein adsorption on polyurethane surfaces. Using randomly isotope labeled proteins, we showed how polymer surfaces change structure upon protein adsorption, and demonstrated that protein adsorption is a dynamic equilibrium. Using selectively isotope labeled proteins, we illustrated that it is feasible to acquire quantitative structural information of interfacial proteins at the solid/liquid interface by SFG. We are developing SFG into a new sensitive bio-analytical technique to study biocompatibility of polymer materials through studies on interactions between protein molecules and polymer surfaces at the molecular level in situ.

3:45 PM F7.8
Fibronectin Adsorption on surface-activated polydimethylsiloxane and its effect on cellular function.
George K. Toworfe[1], Russell J Composto[2,1,3], Christopher S Adams[3,1], Irvin M Shapiro[3,1] and Paul Ducheyne[1,2,3]; [1]Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania; [2]Materials Science & Engineering, University of Pennsylvania, Philadelphia, Pennsylvania; [3]Orthopaedic Research, Thoma Jefferson University, Philadelphia, Pennsylvania.

Cell adhesion and proliferation on biomaterials may depend on adsorbed protein concentration as well as protein conformation. To understand the interplay between these two effects, fibronectin (Fn) was physi-sorbed on smooth, activated polydimethyl siloxane (PDMS) films spin cast on silicon wafers. The nanoscale roughness and thickness of these films were characterized by contact angle goniometry, ellipsometry, atomic force microscopy and Rutherford backscattering spectrometry. The PDMS films were activated by exposure to 30 min ultraviolet ozone (UV/Ozone) conditioning. Contact angle measurements indicated that the hydrophobicity of PDMS was higher (105 ± 3o) compared to the activated surfaces (63 ± 2 o). Tapping mode AFM surface scans indicated that the activation process produced a slightly rougher PDMS surface, with Ra increasing from 0.25 nm to 0.55 nm. Using a Fn concentration of 20μg/mL, the surface density of Fn was higher on the PDMS (1600 ± 200 ng/cm^2; multi-layer coverage) surface compared to the activated PDMS due to favorable hydrophobic interactions between PDMS and Fn. MC3T3-E1 osteoblast-like cells seeded on these substrates and incubated in serum-free medium for 15 min at 37oC in 5 % CO2 indicated greater attachment on Fn-mediated and activated PDMS substrates than on non-activated substrates. Cell spreading and cytoskeleton organization after 72 h was clearly favored on the Fn-mediated and the activated PDMS surfaces. The increase in cell coverage by a factor of two from 9000μm2 to 17000μm2 is attributed to changes in Fn conformation. Improved cell-biomaterial interaction therefore is dependent on a combination of factors although our data suggest strongly that surface activation of Fn-mediated PDMS substrate produces a mostly favored Fn conformation and cell function.

4:00 PM F7.9
Biomimetic Thin Films for Inducing Cell Response. John T. Elliott, John T Woodward, Kurt J. Langenbach and Anne L. Plant; Biotechnology, NIST, Gaithersburg, Maryland.

Thin film mimics of the fibrillar form of the ECM protein, type I collagen, are constructed by allowing native collagen to polymerize on a hexadecanethiol monolayer. The resulting thin films are approximately 30nm thick, and are composed of collagen fibrils that are as large as several hundred nanometers in diameter and microns in length. These films have unique advantages for allowing observation of the interaction of smooth muscle cells, in real time, with collagen fibrils. Cell response on thin films of collagen fibrils is different from their response to films of nonfibrillar collagen. On nonfibrillar collagen, cells are larger, have a better developed cytosketon, proliferate at a higher rate, and produce more of the ECM protein, tenascin, then do cells on fibrillar collagen. By controlling the flexibility of collagen fibrils, we show that the mechanical nature of collagen fibrils is an essential determinant of cellular response. These data suggest that it is the ability of cells to mechanically manipulate the fibrils that apparently causes the cells to assume a small morphology and remain in a non-proliferative phenotype.

4:15 PM F7.10
Dynamic Impedance Spectroscopy of Cell/Surface Interaction. Paul Takhistov, Rutgers University, New Brunswick, New Jersey.

Impedance Spectroscopy provides a powerful tool for investigating a variety of dielectric processes for both electrical and non-electrical applications. In dielectric spectroscopy the current flowing through a sample cell containing a nano-scale patterned bio-interface and the voltage across this cell are measured as a function of frequency. From this data one can obtain the impedance of the system as a function of frequency. An oscillatory field applied to a microorganism adhered to the surface changes the distribution of ions in the electrostatic double layer, as well as the neutral region just outside of the double layer. The applied field polarizes the double layer when time scales of ionic transport processes are fast compared with the period of the oscillatory field. High polarization is manifested as a relative dielectric permittivity that may be much greater than that of the suspending medium. This process, dielectric relaxation, can therefore indicate the time scales of ionic transport processes near of the nano-scale surface constraints. Dielectric spectroscopy characterizes the dynamics of double layer relaxation and yields more information per measurement than static methods such as electrophoresis. Full interpretation of dielectric models requires the use of colloidal electrodynamics. These models usually rely upon electrostatic parameters that are obtained through electrokinetic methods. Thus the availability of both electrokinetic and dielectric techniques offers an advantage for reconciling and interpreting measurements of particle surface structure and electrochemistry. This research is devoted to the characterization of the passive electrical parameters of biological cells adhered to the nano-patterned surface by means of dielectric spectroscopy measurements. These parameters, mainly the membrane conductivity and the membrane permittivity, describe the structural and transport properties of the membrane/nano-patterns system.

4:30 PM F7.11
Attachment and Immune Reponses to Nanostructured Carbon Films. V M Ayres[1], S S Grabski[2] and M S Schindler[2]; [1]Department of Electrical and Computer Engineering, Michigan State University, East Lansing, Michigan; [2]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan.

Nanostructured carbon films have important properties which may make them suitable as biological implant materials. There are multiple considerations involved in the design of a biocompatible material. In this work we consider two aspects: (1) the ability of the material to promote cell attachment and growth, and (2) the ability of the material not to trigger an immune response. These aspects are explored through studies of (a) attachment protein and (b) cell response to a series of carbon nanofiber, carbon nanotube and fullerene films; also diamond and diamond like carbon films, to correlate the new studies with our previous results1. Our initial results have indicated less of a dependence on morphology than on the crystalline versus disordered surface content of the film, leading to attachment versus benign non-attachment behaviour on the part of the fibroblast cells studied. In the present studies, the work is extended and correlated with individual attachment protein response, and with the effects of hydrophobicity and hydrophilicity. The cell chemical responses, which would indicate an immune, or alternate, response, are also investigated. 1. V.M. Ayres, S. Grabski and M.S. Schindler, "Fibroblast response to DLC, polycrystalline and Ib diamond films", Diamond 2002, Granada, Spain, September 9-13, 2002, Abstract Book 03.3 (2002), Elsevier Publishing

4:45 PM F7.12
Effects of well-defined micro- and nanostructured substrates on cell behavior. Paul F Nealey[1] and Christopher J Murphy[2]; [1]Chemical Engineering, University of Wisconsin, Madison, Wisconsin; [2]School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin.

Cells receive chemical and physical signals from neighboring cells, the surrounding fluid and extracellular matrices (ECMs). Cells integrate these various stimuli and interpret them to generate appropriate cellular responses. In vivo, epithelial cells adhere to specialized ECMs called basement membranes. Basement membranes possess a complex felt-like topography with feature dimensions in the nanoscale range. The nanostructured nature of the basement membrane motivated the hypothesis explored in this work- that nanoscale substrate topography, independent of chemistry, is a relevant cellular stimulus. Model surfaces with patterns of grooves and ridges with well-defined dimensions and uniform chemistry were used to study the effects of nanoscale substrate topography on cell behaviors. These patterns are naturally anisotropic and many cell types have been reported to respond to substrate anisotropies by aligning along the topographic features. Therefore, cellular responses could be assessed using assays based on optical microscopy and image analysis of cells. We have demonstrated that human corneal epithelial cells (HCECs) sense and react to substrate topographic features of nanoscale dimensions. Cells cultured in DMEM/F12 medium elongated and aligned along patterns of grooves and ridges with feature dimensions down to 70 nm, whereas on smooth substrates cells were mostly round. The percentages of aligned cells were constant on substrates topographies with lateral dimensions ranging from the nano- to the micronscale, and increased with groove depth. The context in which topographic stimuli were presented to cells had a great impact on the cellular responses. The composition of the culture medium alone determined whether nanotopographic cues elicited parallel or perpendicular cell alignment. Cells cultured in Epilife medium aligned preferentially in the direction perpendicular to nanoscale grooves and ridges. Additionally, cell alignment in Epilife medium was dependent on pattern pitch. Cells switched from perpendicular to parallel alignment when the pitch was increased from 400 to 4000 nm. Finally, cellular responses to substrate topographies were dependent on the cell type. Human keratocytes were more responsive to substrate topographic features than human corneal epithelial cells. This work documents that biologic length-scale topographic features that model features encountered in native basement membrane profoundly affect cell behaviors in a context and cell type dependent manner.

SESSION F8: Poster Session: Biomaterials for Tissue Engineering II
Chair: Lonnie Shea
Thursday Evening, December 4, 2003
8:00 PM
Exhibition Hall D (Hynes)

F8.1
Cell Patterning in 3-Dimensions: Microtextured Tissue Scaffolds. James Norman and Tejal Desai; Biomedical Engineering, Boston University, Boston, Massachusetts.

Scaffolds of both natural and synthetic materials have been widely utilized to provide a three dimensional environment for cell growth. These scaffolds can provide both physical as well as biochemical support for cell proliferation and differentiation. The local response of each cell to its microenvironment determines the overall physical arrangement of the engineered tissue. Since most scaffolds are made from amorphous polymers they are not able to provide a prescribed microarchitecture to individual cells at different layers within the construct. Recently microfabrication techniques such as photolithography and soft lithography have been used to design 2-D tissue culture substrates in order to study the effects of microscale features on cell growth. These techniques have been used to guide single cells as well as monolayers of cells in culture into more physiological arrangements. It is this type of prescribed microscale environment that is missing when trying to culture cells in 3-D polymeric scaffolds with the goal of building morphologically correct tissue constructs. We are developing a technique for embedding internal microscale structures that run though the full height of a 3-D tissue engineering scaffold. Parallel channels are created in PDMS using standard photolithography and soft lithography techniques to create an internal skeleton for polymeric scaffolds. The PDMS mold acts as an internal skeleton to a polymeric scaffold to provide contact guidance in 3-D. Fibroblasts are mixed into either collagen gel or poly(ethylene glycol) diacrylate (PEGDA), a photo-crosslinkable hydrogel, and molded around the PDMS features. Surface modification of the PDMS mold with silane A-174 is needed to stabilize the PEGDA scaffold on the internal structure. Silane A-174 will cross-link with the PEGDA under UV-irradiation. By providing contact guidance to every cell in the scaffold, alignment of multiple layers of fibroblasts can be achieved.

F8.2
Transparent Hydrogels For Ophthalmic Surgeries. Mark Grinstaff and Michel Wathier; Departments of Biomedical Engineering and Chemistry, Boston University, Boston, Massachusetts.

Hydrogels have wide-spread uses in tissue engineering and wound management. Recently, we have had success in repairing large corneal lacerations using photocrosslinked polysaccharide and biodendrimer based hydrogels. These polymers are applied to the wound and then are photocrosslinked using an argon ion laser to form a hydrogel. The use of light has many advantages, but there are particular applications where this mode of hydrogel formation is not desired. Consequently, we are exploring alternative approaches to hydrogel formation that do not require light for formation. We have synthesized a dendron containing four cysteine residues and studied the hydrogel formation reaction of this dendron with the a PEG di-succinimide (PEG SPA) (MW 3400). The dendron with four cysteine residues was obtained by coupling of four protected cysteine with the LysLys(Lys)OMe. The pentafluoro ester strategy was used for the synthesis of the ZLys(Z)Lys((Z)Lys(Z))OMe in a high yield (98%). The deprotection of the Z groups by hydrogenolysis (Pd/C, H2) followed by the salt formation gave the expected product (structure confirmed by NMR spectrum and mass spectrum). The final step was performed in a DMF/DMSO mixture with the pentafluoro phenol ester of the protected cysteine and lysine dendron to afford the cysteine dendron. A gel is rapidly formed at pH 7 in aqueous solution upon mixing the cysteine dendron and PEG(succinimide)2.

F8.3
Interfacial Biomaterial Coatings for Engineered Human Blood Vessels. Mark Grinstaff[1], Xin Huang[2], Amy Solan[2], Laura Niklason[2] and Daniel Kenan[3]; [1]Departments of Biomedical Engineering and Chemistry, Boston University, Boston, Massachusetts; [2]Department of Biomedical Engineering, Duke University, Durham, North Carolina; [3]Department of Pathology, Duke University Medical Center, Durham, North Carolina.

A current challenge in tissue engineering is the construction of small diameter human blood vessels. One of the major hurdles is endothelial cell adhesion to the vessel scaffold. We are synthesizing functional coatings termed interfacial biomaterials (IFBMs) that are designed to improve cell adhesion to a synthetic PGA scaffolding. These IFBMs posses two adhesion domains, one for PGA and a second domain for endothelial cells. Peptide binding domains were identified using M13 phage display combinatorial libraries. Three rounds of phage display panning yielded 26 strong PGA binders, as confirmed by ELISA. The best PGA binder was isolated for use in an IFBM containing an endothelial cell binding domain. When coated on a PGA scaffold, the IFBM improved cell adhesion over controls. DNA assays, western analysis, and SEM images confirmed these results. In summary, we have developed and evaluated a functional coating for use in human blood vessel engineering.

F8.4
In Situ Mineralized Hydroxyapatite for use in Composite Scaffolds for Bone Tissue Engineering. Kalpana S Katti, Phanikumar Turlapati and Rahul Bhowmik; Civil Engineering, North Dakota State University, Fargo, North Dakota.

The role of synthetic macromolecules in controlling the mineralization of hydroxyapatite (HAP) plays a critical role on mechanical responses of the mineral-polymer nanocomposite material systems. We analyzed mechanical responses of the in situ composite after soaking in a simulated body fluid. Scanning electron microscopy, x-ray diffraction and infrared spectroscopy are utilized for this purpose. In general, superior response of the composites is observed as compared to soaking in water. Crystallographic investigation indicated presence of secondary phosphates. These microstructural and molecular interpretations are investigated in the context of resulting stress-strain behavior of the composite. Small changes in macromolecular concentration in the in situ preparation results in large differences in mechanical responses of the composite. Fourier transform infrared attenuated total reflectance (FTIR ATR) spectroscopic experiments indicate the association of polymeric chains with HAP during mineralization processes. The insitu mineralized HAP minerals are used to stiffen scaffolds fabricated with polyglycolic acid using a rapid prototyping instrument. Spectroscopic investigations on the role of the insitu mineralized HAP on failure mechanisms of the scaffolds is also presented. Molecular models of the interactions between HAP and polymeric additives are also presented that relate to the experimental spectroscopic evidence of the interactions. The molecular mechanics simulations include the development of models of HAP that incorporate the part-ionic and part-covalent nature of the bonds in the mineral. This work presents experiments and theoretical studies on polymer-HAP interactions for design of mechanical strong composite scaffold systems for potential applications for bone tissue engineering.

F8.5
Fabrication of submicron continuous chitosan fibers for use in cartilage tissue engineering. Anu Subramanian, David Vu, Hsin Yi-Lin and Gustavo Larsen; Chemical Engineering, University of Nebraska, Lincoln, Nebraska.

Tissue engineering concepts and methodologies that employ biocompatible matrices or scaffolds have the potential to meet needs encountered in the repair of defects in bone and cartilage. To date, biomedical engineers and medical device designers have employed almost exclusively degradable polyesters as poly(glycolic acid), poly(lactic acid), polydioxanone or various copolymers thereof, which are the only synthetic, degradable polymers with an extensive regulatory approval history in the United States. On the other hand, natural biopolymers like chitosan, collagen, and keratin possess the inherent physical and biological characteristics that may render them useful as a component in a biomaterial and appears to be suitable for incorporation into scaffold fabrication for use in cartilage- or bone-tissue engineering. Development of a chitosan-based material that can support chondrogenesis may be significant not only in terms of the quantity of neocartilage produced, but also in terms of the ability of that tissue to integrate with the host matrix. Thus, a need exists to develop a reliable method of producing chitosan-based scaffolds with the appropriate size, density, porosity, and biomechanical property for use in tissue engineering applications. The long-term goal of our research initiative is to design, develop and test various methodologies and techniques for the fabrication of unique porous scaffolds, using a blend of chitosan and synthetic or natural biopolymers. Furthermore, the scaffolds prepared will be evaluated in the paradigm of cartilage tissue engineering. We will employ a variation of the method of electrospinning to primarily fabricate the scaffolds with controlled pore architecture, interconnectivity and orientation. Specifically, we will test the ability of these new scaffolds produced in this study to support the growth and proliferation of chondrocytes. Preliminary results are very encouraging in that we have succeeded in making for the first time, (based on an extensive literature search) submicron continuous chitosan fibers with an excellent degree of orientation. Physiochemical and biomaterial characterization results will be reported. Our research is poised to ultimately develop a novel chitosan-based biocompatible tissue scaffold with controllable mechanical properties.

F8.6
Scanning Probe Recognition Microscopy Investigation of Cells

on Scaffolding. Q Chen[1], L Udpa[1], M S Schindler[2] and V M Ayres[1]; [1]Department of Electrical and Computer Engineering, Michigan State University, East Lansing, Michigan; [2]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan.

Direct investigation of and interaction with biological objects at the macromolecular level will provide insight into multiple physical regulatory processes. Scanning probe microscope techniques have the potential to provide a direct interaction with living specimens at the macromolecular level. Present implementations of SPM techniques rely on a raster scan of an area around the object of interest and a directive interaction with a human operator. In our recent development of scanning probe recognition microscopy, the machine is taught, by way of image processing techniques, to identify sites by their "feel". A scan plan for investigation is then generated based on the shape of a particular type of object. Adaptive learning techniques allow for dynamic variations in object shapes. Scanning probe recognition microscopy has the potential to extend site specific investigation to the resolution of the scanning probe microscope which is nanometer (macromolecular) level for atomic force microscope and angstrom (atomic) level for scanning tunneling microscopy. We will report recent results using recognition schemes which have allowed us to distinguish tubular from globular biological objects1 and their application in a study of cell attachment with carbon nanofiber tissue scaffolding. In these studies we investigate cell attachment and guiding as a function of diameter, composition and porosity along the length of the nanofibers, over the cell surfaces, and at the site-specific attachment points. 1. B. Goolsby, Q. Chen, L. Udpa, Y. Fan, R. Samona, B. Bhooravan, F. M. Salam, D. H. Wang, and V. M. Ayres, "Scanning Probe Microscopy with Landmark Referenced Control For Direct Biological Investigations", accepted for publication, J. Nanosci. Nanotech., Vol. 3 (2003).

F8.7
Characterizing Cell Culture Substrates with Molecular Recognition Force Microscopy. John T. Woodward and John T. Elliott; Biotechnology, NIST, Gaithersburg, Maryland.

As part of a program to develop measurement tools and reference materials for tissue engineering, we have been developing a molecular recognition force microscope (MRFM) to characterize cell culture substrates. The goal is to develop well-characterized, homogeneous surfaces that yield reproducible responses from cells in culture. Our approach is to use thin films of extracellular matrix (ECM) proteins as substrates. Advantages of thin ECM films include the use a quantitative microscopy to assess cell response and the ability to use surface sensitive techniques for characterization. For native collagen surfaces the quaternary structure, in the form of collagen fibrils, is indicative of the chemical functionality of the surface and correlates well with cellular response to the surface. This allows the topography of the surface to serve as a proxy for the biofunctionality of the surface. For other ECM protein surfaces, such as fibronectin, we do not observe a similar structural proxy that correlates to cell response. Thus we have also developed MRFM for probing the identity and accessibility of ECM proteins on a culture surface. The MRFM is operated in pulsed force mode and uses a tip coated with an antibody that recognizes the ECM protein. This allows us to quantify the surface density of ECM protein epitopes known to be involved in cell signaling and correlate this with cell response.

F8.8
Characterizing Surface Modifications of Basal Lamina Analogs to Enhance Keratinocyte Function and Tissue Regeneration. Sarah E. Walsh, Brett R. Downing, Sarah L. Tressel, Kristin A. Coughlan, Kristen L. Billiar and George D. Pins; Biomedical Engineering, Worcester Polytechnic Institute, Worcester, Massachusetts.

The creation of basal lamina analogs presenting extracellular matrix (ECM) cues that mimic the cellular microenvironment of the dermal-epidermal junction may enhance keratinocyte function and subsequently improve tissue regeneration on the surfaces of bioengineered skin substitutes. This study was undertaken to establish the relationship between the ECM composition of the surfaces of collagen-glycosaminoglycan (GAG) membranes and keratinocyte adhesion. To this end, a screening device was developed to perform high-throughput assays for characterizing keratinocyte adhesion on collagen-GAG membranes that were conjugated with varying concentrations of basal lamina proteins. The device consists of a crosslinked collagen-GAG membrane pre-wet with ethanol and dried on a supporting layer of polydimethyl siloxane (PDMS). Customized polycarbonate templates were affixed to the surfaces of the membranes to create a multiwell assay system. To modify the surfaces of the collagen-GAG membranes, ECM molecules known to enhance keratinocyte adhesion, including type I collagen, fibronectin, laminin, were adsorbed to the surfaces. After seeding each well with keratinocytes, an MTT assay was used to determine the numbers of cells anchored to the membrane. Our initial studies indicated that increasing concentrations of fibronectin or laminin adsorbed to the surfaces of these membranes significantly enhanced keratinocyte adhesion to the surfaces of these collagen-GAG membranes. These findings suggest that membranes conjugated with fibronectin or laminin will enhance the performance of bioengineered skin substitutes by improving the rate of keratinocyte proliferation and stratification, as well as by promoting the rapid regeneration of an epidermal layer on the surfaces of basal lamina analogs.

F8.9
Abstract Withdrawn

F8.10
Preparation of Polyphosphazene Non-Woven Nanofiber Mats By Electrospinning. Cato Laurencin[2], Subhabrata Bhattacharyya[1], S Lakshmi[2], J Bender[3] and H R Allcock[3]; [1]Department of Chemistry, The University of Virginia, Charlottesville, Virginia; [2]Department of Orthopaedic Surgery, College of Medicine, The University of Virginia, Charlottesville, Virginia; [3]Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania.

Polyphosphazenes form novel class of polymers having an inorganic back bone of phosphorous and nitrogen atoms with two organic groups attached to each phosphorous atom. The physico-chemical properties of polyphosphazenes can be effectively tuned by varying the nature/ratios of the side group substituents. This synthetic versatility coupled with their excellent biocompatibility make polyphosphazenes attractive candidates for biomedical applications. Poly[bis(carboxylato phenoxy)phosphazene] (PCPP) has been investigated as a matrix for encapsulating hepatocytes as well as matrix for controlled drug delivery due to its ability to form ionotropic gels in the presence of calcium ions under mild conditions$_1$. The present study evaluates the potential of electrospinning PCPP to develop microporous, non woven mat like structures that may find applications in tissue engineering and drug delivery. PCPP was synthesized by nucleophilic substitution of poly dichlorphosphazene as reported earlier$_2$. The polymer used has a molecular weight of 152,000 g/mol and the structure of the polymer was confirmed by nuclear magnetic resonance (NMR) spectroscopy. The polymer dissolved in a mixture of tetrahyrofuran (THF) and N,N'dimethyl formamide (DMF) in the ratio 1:4 was used to electrospun fibers of submicron diameters. The morphology and the diameter of the nonofibers obtained by electrospinning was determined using scanning electron microscopy (SEM). The effect of electrospinning parameters such as polymer solution concentration, orifice diameter and electrospinning voltage per unit length on the morphology and diameter of the fibers were determined. Electrospinning of polymer solutions having concentrations lower than 0.06 g/mL showed the characteristic bead formation along with nanofiber formation. The bead formation decreases with increase in solution concentration and at a concentration of 0.09 g/mL nanofibers with diameters <75 nm were obtained. The study demonstrated the feasibility of developing polyphosphazene nanofibers which may find wide applications in the biomedical field. References: 1. S. Lakshmi, D.S. Katti, C.T Laurencin Biodegradable polyphosphazenes for drug delivery applications. Adv Drug Delivery Rev 2003;55:467. 2. H.R Allcock, S. Kwon An ionically cross-linkable polyphosphazene poly[(bis(carboxylato phenoxy)phosphazene] and its hydrogel and membranes. Macromolecules 1989;22:75. Acknowledgements: The authors acknowledge NIH grant #46560 for financial support.

F8.11
Conformational Changes of Water Stable Silk (*Bombyxmori*) Films by Mechanical Treatment. Jaehyung Park[1], Hyoung-Joon Jin[1,2], Ung-jin Kim[1] and David L. Kaplan[1]; [1]Department of Chemical and Biological Engineering, Tufts University, Medford, Massachusetts; [2]Department of Polymer Science and Engineering, Inha University, Incheon, South Korea.

Elastic and water stable silk-protein films were formed and physically stretched to control structure. These water stable films were formed from regenerated concentrated aqueous silk fibroin solutions (>8%) without crystallization or formation of silk II (β-sheet) structure. The films were cast and annealed in water vapor at room temperature, resulting in the formation of hydrogels that were highly elastic based on swelling in water. The swelled films could be stretched up to 3 times original length. Conformational changes in the films were assessed by FTIR-ATR and XRD. Cast films were mostly amorphous with some silk I structure. After swelling in water the films were predominately silk I in structure. With methanol treatment and stretching of the films the silk II structure appeared. Stretched films had increased tensile strength that correlated with increased crystallinity due to transitions to silk II (β-sheets). The water-stability of the silk films in the absence of silk II was due to the content of silk I structure formed during processing the silk fibroin aqueous solution to high concentration, combined with the slow evaporation of water. Mechanical properties and topographical images

according to structural changes of fibroins on the films' surface are under study. In conclusion, with the ability to concentrate fibroin in water to high concentrations, mimicking the native process in silkworm and spider glands, water stable and elastic silk fibroin films can be formed even in the absence of β-sheet formation.

F8.12
The Behavior of Smooth Muscle Cell on Aligned P(LLA-CL) Nanofiber, a Potential Scaffold for Vascular Engineering.
Chengyu Xu[1,2] and Seeram Ramakrishna[2,1,3]; [1]Mechanical Engineering, National Univ. of Singapore, Singapore, Singapore; [2]Nanoscience and nanotechnology initiative, National University of Singapore, Singapore, Singapore; [3]Division of Bioengineering, National University of Singapore, Singapore, Singapore.

Substantial effort is being invested by the bioengineering community to develop biodegradable polymer scaffolding suitable for tissue engineering application. An ideal scaffold should mimic the structural and purposeful profile of materials found in the natural extra cellular matrix architecture. To accomplish this goal, a unique biodegradable nanofibrous structure, aligned poly(L-lactide-co-epsilon-caprolactone) [P(LLA-CL)] (75:25) copolymer nanofibrous scaffold was produced by a simple yet effective method using electrospinning. The diameter of the generated fibers was around 500nm with an aligned topography which mimics the circumferential orientation of cells and fibrils found in the medial layer of a native artery. A favorable interaction between this scaffold with human coronary artery smooth muscle cells (SMCs) was demonstrated via MTS assay, phase contrast light microscopy, scanning electron microscopy, immunohistology assay and laser scanning confocal microscopy separately. Tissue culture polystyrene and plane solvent-cast P(LLA-CL) film were used as controls. The results showed that, the SMCs attached and migrated along the axis of the aligned nanofibers and expressed a spindle-like contractile phenotype; the distribution and organization of smooth muscle cytoskeleton proteins inside SMCs were parallel to the direction of the nanofibers; the adhesion and proliferation rate of SMCs on the aligned nanofibrous scaffold was significantly improved than on the plane polymer films. The above results strongly suggest that this synthetic aligned matrix combines with the advantages of synthetic biodegradable polymers, nanometer scale dimension mimicking the natural ECM and a defined architecture replicating the in vivo-like vascular structure, may represent an ideal tissue engineering scaffold, especially for blood vessel engineering.

F8.13
Fabrication of Nano-structured PLLA Fibers by Electrospinning Intended for Nerve Tissue Engineering.
Fang Yang[1], Seeram Ramakrishna[1] and Shu Wang[2]; [1]Faculty of Engineering, National University of Singapore, Singapore, Singapore; [2]Institute of Bioengineering and Nanotechnology, Singapore, Singapore.

Neural tissue engineering (NTE) is one of the most promising methods to restore central nerve systems (CNS) in human health care. Permissive matrices which can guide neurite outgrowth are of clinical significance for NTE. In this study, an attempt was made to develop polymeric nano fibrous scaffold using a biodegradable poly(L-lactic acid) (PLLA) for in-vitro culture of nerve stem cells (NSCs). The processing of PLLA scaffold has been carried out by electrospinning method. The physico-chemical properties of the scaffold were fully characterized by using differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). The effects of processing parameters, such as solution concentration, applied voltage, feeding rate and salt addition, on the average fiber diameters were studied. As our nano structured PLLA scaffold mimics natural extracellular matrix (ECM), we have intended this biodegradable scaffold as cell carrier in NTE. The in-vitro performance of NSCs seeded on nano fibrous scaffold is also addressed in this study. The cell cultural tests showed that the fabricated nano fibrous scaffolds could provide NSCs differentiation and support neurite outgrowth. These results suggested that the nano structured porous PLLA scaffold is a potential cell carrier in NTE.

F8.14
In Situ Cartilage and Bone Tissue Engineering Using Phosphoester-containing Hydrogels. Dong-an Wang, Christopher Williams, Syukjin Lee, Blanka Sharma and Jennifer Elisseeff; Biomedical Engineering, Johns Hopkins University, Baltimore, Maryland.

A phosphoester-containing photo-crosslinkable polymer, "PhosPEG-dMA" was synthesized. As a water-soluble macromer, PhosPEG-dMA is suitable for in situ injection and cell-encapsulation by light-induced gelation to produce degradable hydrogels for cartilage and bone tissue engineering. Goat mesenchymal stem cells (gMSC) were photo-encapsulated in PhosPEG gels and remained viable. The gMSC-polymer constructs were incubated in osteogenic and chondrogenic conditions. For osteogenesis, the results from 3-week cultivation indicated that small amounts of phosphor in hydrogels were important for gMSC osteogenic differentiation that was determined by the osteogenic gene markers of alkaline phosphatase, osteonectin, and type I collagen. The presence of phosphor also promoted both gel mineralization and cell-related calcification. For cartilage regeneration, PhosPEG gels were implanted into cartilage explants in vitro and cultivated in chondrogenic medium for 2 weeks. The clinically significant problem of hydrogel integration to surrounding/adjacent cartilage tissue was resolved by a novel approach of "tissue-initiated photopolymerization" to bond the cartilage explants and hydrogel. The strategy for this covalent integration was summarized as: (1) clearance of the proteoglycans in cartilage to expose the collagen network; (2) in situ generation of tyrosyl radicals by photo-oxidation of tyrosine residues on collagen with H2O2; and (3) introduction of a macromer solution and in situ photo-gelation via tyrosyl radical initiation and UV-excitation. Since the reaction is initiated by components of the tissue, naturally the hydrogel was covalently grafted onto the tissue. Mechanical and chemical testing confirmed the enhanced interface for integration, and the cytotoxicity testing suggested the biocompatibility of the method. The characterization for the implantation and regeneration was performed by measuring the component variation in hydrogel using NMR imaging and quantification (MRI). A combined behavior of cartilage-related matrix production and hydrogel degradation was observed.

F8.15
Design And Computer-Aided Preparation Of Scaffolds With Hierachical Structures For Bone Tissue Engineering.
Zhuo Xiong, Yongnian Yan, Xiaochen Fei, Renji Zhang and Feng Lin; Department of Mechanical Engineering, Tsinghua University, Beijing, China.

The digital extrusion/jetting based rapid prototyping (RP) technologies were applied to make bone tissue engineering scaffolds. A hierachical structure of bone tissue engineering scaffolds was designed. A new computer-aided process and a new RP system carrying out the process were developed to form the hierachical structure. According to the functional requirements of the scaffolds and the formability requirements of the scaffold materials, the composite of aliphatic polyesters and calcium phosphates was adopted as the scaffold material. The microstructures, the porous structures of the composite and the hierachical structure of the scaffolds were designed. A new forming process named low-temperature deposition manufacturing (LDM) was designed to form the hierachical structure of the scaffolds without destroying the biological properties of materials. It integrated the multi-nozzle extrusion/jetting process and the thermally induced phase seperation process of the materials. A LDM system was developed. The influences of process parameters on the macro-porous structures, micro-porous stuctures, porosity and mechanical properties of the scaffolds were also lucubrated. The PLGA/TCP scaffolds prepared by LDM process were loaded with rhBMP-2 and then implanted into rabbit radius large-segmental defects to evaluate their biology properties. The implants successfully repaired the defects, and the regenerated bone has similar properties to the healthy bone. The results of these implantation experiments showed that the implants have good bone-inductive property and bone-conductive property, and the scaffolds have good tissue compatibility and biodegradation property.

F8.16
Electropolymerization of Copolymer Biomimetic Films Containing an RGD Peptide Recognition Element That Promotes Endothelial Cell Adhesion and Spreading.
Tiean Zhou, Kenneth A. Marx, Donna McIntosh and Susan J. Braunhut; Center for Intelligent Biomaterials, Departments of Chemistry and Biological Sciences, University of Massachusetts, Lowell, Massachusetts.

Endothelial cells (ECs) and other cell types attach and spread on the hydrophilic gold surface of a Quartz Crystal Microbalance (QCM) under normal culture conditions (1, 2). When fully spread (24 hr), ECs achieve their steady state and can then serve as an effective whole cell biosensor detecting cytoskeleton disrupting drugs, such as nocodazole, down to low nM concentrations in a dose dependent fashion (3,4). In this study, we modified the gold surface with an electropolymerized film that enables attachment of capillary ECs via cell surface integrins' binding of specific recognition peptides. The film is electropolymerized on the gold QCM surface, which is the working electrode in a 3-electrode cell. In every case, we electropolymerized 2 monomers, both of which contained phenolic rings, via a free radical mechanism to form covalent ortho or meta C-C bonds between adjacent phenolic rings. One of the monomers is the peptide Arg-Gly-Asp-Tyr (-RGDY-) which is electropolymerized covalently into the film via the pendant amino acid tyrosine (Y). Thus, the recognition peptide, RGD, found within fibronectin and vitronectin,

will be available to bind the alpha(v)beta(3)/alpha(v)beta(5) integrins on the EC surface. We followed the film formation process, then studied the peptide based enhancement of EC attachment via measured QCM f and R changes. As a result of RGD incorporation, there was a clear increase in the f and R changes and an enhancement of the number of trypsinizable ECs adhering to the QCM surface. To confirm the cell attachment and their normal attached appearance, we carried out light microscopy of stained ECs following their attachment to electropolymerized films formed in a different electrochemical device on thin gold electrodes (ECIS; Applied Biophysics). ECs cultured at the identical cell densities upon films formed without the RGDY peptide monomer had abnormal spindly neuronal morphology. However, ECs at the same cell density cultured upon films incorporating RGDY, formed normal cobblestone monolayers resembling those in tissue culture plasticware. We believe that electropolymerized films, incorporating specific peptide recognition sequences, have important applications in tissue engineering, possibly facilitating selective surface attachment of specific cell types from a complex cell mixture during the tissue formation process. 1. Zhou, T., Braunhut, S., Medeiros, D., Marx, K., MRS: Tissue Eng. Symp., 1999, 550, 177-182 2. Zhou, T., Marx, K., Warren, M., Braunhut, S. Biotech. Prog., 2000, 16, 268-277. 3. Marx, K., Zhou, T., Schulze, H., Montrone, A., Braunhut, S., Biosensors & Bioelectronics, 2001, 16, 773-782. 4. Marx, K., Zhou, T., Montrone, A., Braunhut, S., MRS: Adv. Biomaterials-Char., Tissue Eng. & Complexity, 2002, 711, 125-132.

F8.17

Evidence for Cell-Cell Cooperativity in Initial Adhesion and Spreading of Endothelial Cells on the Gold Surface of a Quartz Crystal Microbalance. Kenneth A. Marx, Tiean Zhou and Susan J. Braunhut; Center for Intelligent Biomaterials, Departments of Chemistry and Biological Sciences, University of Massachusetts, Lowell, Massachusetts.

We previously studied the attachment and spreading of large and microvessel endothelial cells (ECs) and other cell types on the hydrophilic gold surface of a Quartz Crystal Microbalance (QCM) (1). Also, ECs, when fully spread on the QCM gold surface at their steady state after 24 hrs, serve as an effective whole cell biosensor that can detect the intracellular action of a cytoskeleton disrupting drug, nocodazole, down to low nM concentrations in a dose dependent fashion (2,3). Due to the sensitivity of the QCM to changes in attached mass and the viscoelastic properties of attached mass, we decided to study the process of EC attachment. The measured QCM f and R changes observed for varying numbers of attached ECs at early (1 hr) and steady state (24 hr) times were found to follow different functional dependencies. At 1 hr, there was a sigmoid relationship between either the f or R change magnitude and the number of trypsinizable ECs adhering to the QCM surface causing those changes. However, after 24 hr, there was a hyperbolic relationship between these quantities. We interpret the sigmoid relationship at initial attachment times as revealing that cell-cell cooperativity exists in the initial attachment process (4). To corroborate this idea, we carried out a fluorescence light microscopy simulation of the attachment process in cell culture plates at the identical times and varying cell densities of our QCM experiments. We observed a 10 min lag between the time the cells contacted the surface and when the f and R values began to change. Under the initial adhesion time sigmoid condition, we found the cells to be nearly perfectly rounded and highly refractile under phase microscopy at the lowest cell densities. However, at higher cell densities, the ECs extended multiple cellular processes indicating more rapid spreading in the same time interval. These simulation data corroborate the cooperativity interpretation of our QCM results. Clearly, through a process of cell-cell contact via processes or through sensing of diffusible factor(s), the ECs behave differently at different densities. We believe that these data and their interpretation in terms of cell-cell cooperativity in the initial attachment process have important future implications in tissue engineering where the choice of cell density during the engineering process is a critical consideration. 1. Zhou, T., Marx, K., Warren, M., Braunhut, S. Biotech. Prog., 2000, 16, 268-277. 2. Marx, K., Zhou, T., Schulze, H., Montrone, A., Braunhut, S., Biosensors & Bioelectronics, 2001, 16, 773-782. 3. Marx, K., Zhou, T., Montrone, A., Braunhut, S., MRS: Adv. Biomaterials-Char., Tissue Eng. & Complexity, 2002, 711, 125-132. 4. Marx, K., Zhou, T., Warren, M., Braunhut, S., Biotech. Prog., 2003, 19, 987-999.

F8.18

The Control of Beta-TCP Formation and Sintering Characteristics of Hydroxyapatite Nanopowder. Yun-Mo Sung[1], Jung-Chul Lee[1], Dae-Hee Kim[1], Jin-Kyung Lee[1] and Hoon Sung[2]; [1]Materials Sci. & Eng., Daejin University, Pochun-koon, South Korea; [2]YDC Inc., Yang-Joo.

Hydroxyapatite (HAp) nanopowders were synthesized using two different chemical precipitation routes. Dried HAp gel powders were almost amorphous in nature with very low crystallinity and showed different particel sizes depending upon the chemical routes. The particel size difference induced the difference in density and morphological features of sintered powders. The HAp powders heated at 1000C showed different amount of beta-TCP formation most probably due to the difference in chemcial homogeneity of precipitated particles. The Ca/P ratio in powder preparation was changed and the amount of HAp and beta-TCP phases was analyzed using quantitative x-ray diffraction (Q-XRD). The powder with Ca/P ratio of 1.70 showed formation of the lowest beta-TCP content, while that of 1.75 showed formation of CaO as well as high beta-TCP. By using different chemical routes and changing chemical composition sintering and beta-TCP formation behaviors of HAp powder could be successfully controlled for the artificial hard tissue applications.

F8.19

Developing Carbohydrate-Based Side-Chain Polyethers as New Biomaterials for Protein Resistance and Tissue Engineering. Zhibin Guan, Mark Metzke and Jane Z. Bai; Chemistry, University of California, Irvine, California.

The ability to resist nonspecific protein adsorption is a major indicator of a materials biological inertness or biocompatibility. Applications of protein resistant materials include prostheses, sensors, substrates for enzyme-linked immunosorbent assays (ELISAs), materials for use in contact lenses, and implanted devices. More recent applications include systems for patterned cell cultures, tissue regeneration, microfluidic systems, drug delivery, and systems for high-throughput screening of proteins or cells. Poly(ethylene glycol) (PEG) is among the most commonly used biomaterials because of its exceptional biocompatibility. Although PEG shows unsurpassed resistance to nonspecific protein binding, as a simple main-chain polyether, it has several limitations. Two of those limitations are: (i) PEG can only be functionalized at the chain ends; and (ii) PEG is not biodegradable. For many biomedical applications, biodegradability and flexibility to incorporate desired functionalities are critical. Recently in our lab, a novel carbohydrate-derived side-chain polyether was synthesized as a new biomaterial by condensation polymerization of monomers derived from natural occurring carbohydrates (J. Am. Chem. Soc. 2003, 125, ASAP). Surface plasmon resonance spectroscopy studies demonstrated that this side-chain polyether has excellent resistance to non-specific protein adsorption. The protein resistant capability of the side-chain polyether is comparable to that of polyethylene glycol, a main-chain polyether that is, to date, the best protein resistant material. In addition to the excellent biocompatibility, the new polymer also combines biodegradability and functionalizability. With these combined good properties, this side-chain polyether is envisioned as a new biomaterial for many potential biomedical applications including as matrices for tissue engineering. This presentation will discuss the synthesis, protein resistance, and cell culture studies of this new family of biomaterials.

F8.20

Determination of Solute and Solvent Permeability Parameters in Native or Artificial Tissue Sections: An Inverse Approach. He Yimeng and Ram V Devireddy; Mechanical Engineering, Louisiana State University, Baton Rouge, Louisiana.

The objective of this study is to develop a generic numerical model to simulate the coupled solute and solvent transport process during the addition and removal of cryoprotective agents (CPAs) in native or artificial biological tissue systems. The model accounts for the axial and radial diffusion of the solute as well as axial convection. In addition, the model also accounts for the radial movement of the solvent (water) into the vascular spaces. And finally, the model developed has the capability to simulate the radial diffusion of both the solute and the solvent in the interstitial spaces as well. Volumetric responses of the tissue cells are simulated by the numerical model with different solute permeability coefficients (w), water permeability coefficients (Lp), reflection coefficients (s) and the diffusion coefficients of the solute in the vascular space (D). The present model differs from previously published models in that it includes the presence of interstitial space and a variable vascular space. The model developed in this study has the unique capability to predict the osmotic or volumetric response of individual tissue cells within a native or artificial tissue slice exposed to different concentration CPAs. In addition, preliminary data indicates that we can calculate, for the first time, the individual tissue cell membrane values w, Lp and s, by fitting our model results to experimentally obtained osmotic response at various locations in a native biological tissue system. ACKNOWLEDGEMENTS: This study is supported by a grant from the Louisiana Board of Regents [LEQSF (2002-05)-RD-A-03].

F8.21

Membrane Permeability Parameters of Irregularly Shaped Biological Cells During Freezing: The Use of Differential Scanning Calorimetry. Ram V Devireddy[1], Brooke Fahrig[2,3],

Robert Godke[2] and S. Leibo[3,4]; [1]Mechanical Engineering, Louisiana State University, Baton Rouge, Louisiana; [2]Animal Science, Louisiana State University, Baton Rouge, Louisiana; [3]Biological Sciences, University of New Orleans, New Orleans, Louisiana; [4]Audubon Center for Research of Endangered Species, New Orleans, Louisiana.

Incomplete understanding of the water permeability parameters during freezing in the presence of extracellular ice and cryoprotective agents (CPAs) is one of the main limiting factors in reconciling the dichotomy between the numerically predicted and experimentally determined optimal rates of freezing in boar (and in general mammalian) gametes. In the present study a well established shape independent Differential Scanning Calorimeter (DSC) technique will be used to measure the water transport during freezing of boar sperm cells. Water transport during freezing of boar sperm cell suspensions was obtained at cooling rates of 5 and 20 C/min in the presence of extracellular ice and 6% (v/v) glycerol. Using previously published values, the boar sperm cell was modeled as a cylinder of length 80.1 micrometers and a radius of 0.31 micrometers with an osmotically inactive cell volume, Vb, of 0.6Vo, where Vo is the isotonic cell volume. By fitting a model of water transport to the experimentally obtained data the best fit water transport parameters (Lpg and ELp) were determined. The 'combined best fit' parameters at 5 and 20 ?C/min for boar sperm cells in the presence of extracellular ice are: Lpg = 3.6 x 10-15 m3/Ns (0.02 micrometer/min-atm) and ELp = 122.5 kJ/mole (29.3 kcal/mole) (R2 = 0.99); and the corresponding parameters in the presence of extracellular ice and glycerol are: Lpg[cpa] = 0.90 x 10-15 m3/Ns (0.005 micrometer/min-atm) and ELp[cpa] = 75.7 kJ/mole (18.1 kcal/mole) (R2 = 0.99). The water transport parameters obtained in the present study are significantly different than previously published parameters for boar and other mammalian sperm obtained at suprazero temperatures and at subzero temperatures in the absence of extracellular ice. The theoretically predicted optimal rates of freezing using the new parameters (approximately 30 C/min) are in close agreement with previously published but empirically determined optimal cooling rates. Thus, reconciling a long standing dichotomy between theoretically predicted and empirically determined optimal cooling rates.

F8.22
Single Cell Based Sensing. Cengiz Sinan Ozkan, Mechanical Engineering, University of California at Riverside, Riverside, California.

The broad-spectrum sensitivity of cell based biosensors offers the capability for detecting known and unknown chemical/biological agents. One cellular parameter that is often measured is the extracellular potential of electrically active cells. Membrane excitability in osteoblasts plays a key role in modulating the electrical activity in the presence of chemical agents. However, the complexity of this signal makes interpretation of the cellular response to a chemical agent difficult to interpret. By analyzing shifts in the signals power spectrum, it is possible to determine a frequency spectrum also known as signature pattern vectors (SPV) specific to a chemical. It is also essential to characterize single cell sensitivity and response time for specific chemical agents for developing detect-to-warn biosensors. To determine the real time sensing capability of single osteoblast sensors multi-chemical sensing also termed as cascaded sensing is performed and the performance of the sensor is evaluated. A system is described for the measurement of extracellular potentials from cells isolated onto planar microelectrode arrays. We used a 5x5 multiple microelectrode array system to spatially position osteoblast cells, by using a gradient AC field. Fast Fourier Transformation (FFT) and Wavelet Transformation (WT) analyses were used to extract information pertaining to the frequency of firing from the extracellular potential. Quantitative dose response curves and response times are also obtained with the cultured single cell systems using local time domain characterization techniques. Future applications of this technique are also discussed.

F8.23
RGD and YIGSR Peptides Micropatterned on PMMA to Direct Schwann Cell Attachment and Proliferation. MinJung Song[1], Kristine Schmalenberg[2] and Kathryn Uhrich[2,1]; [1]Biomedical Engineering, Rutgers University, Piscataway, New Jersey; [2]Chemistry Department, Rutgers University, Piscataway, New Jersey.

Microcontact printing is a convenient method to create patterns on a variety of surfaces that can then be used to induce cell adhesion and proliferation. The binding sequences, YIGSR and/or RGD, were patterned onto poly(methyl methacrylate) (PMMA) to create striped patterns on the polymer surface. The YIGSR sequence is derived from laminin and promotes cellular adhesion, thus is an important extracellular matrix protein for neurons. RGD is the cell adhesive sequence, also derived from laminin, that supports attachment and proliferation of a variety of cells, including neurons. Primary rat Schwann cells were cultured on PMMA substrates patterned in three ways - RGD, YIGSR and RGD/YIGSR (1:1) peptides. The biological functionality of the peptide patterns was evaluated by immunostaining using a Zeiss confocal laser scanning microscope and Image Pro software. Pattern adherence was evaluated by comparing the number of cells attached to the peptide-patterned regions relative to unpatterned (i.e., no peptides) regions. Cell spreading will be evaluated by a qualitative comparision of the patterned against unpatterned areas. In addition, cell proliferation will be evaluated by Calcein AM staining. By stamping on PMMA surface the cell adhesive sequence, we would able to guide cellular attachment, alignment and proliferation.

F8.24
Ligament Cells Grown on a Microgrooved Substrate Increase Intracellular Calcium Levels in Response to a Mechanical Stimulus. Bertina F Jones[1,2], Albert J Banes[2,3,1], Michelle E Wall[3,2], Sean Washburn[4,1] and Richard L Carroll[4]; [1]Curriculum of Applied and Materials Science, University of North Carolina-Chapel Hill, Chapel Hill, North Carolina; [2]Department of Orthopaedics, University of North Carolina-Chapel Hill, Chapel Hill, North Carolina; [3]Biomedical Engineering Department, University of North Carolina-Chapel Hill, Chapel Hill, North Carolina; [4]Department of Physics and Astronomy, University of North Carolina-Chapel Hill, Chapel Hill, North Carolina.

Injury to the medial collateral and anterior cruciate ligaments (MCL and ACL) accounts for the majority of all knee-ligament trauma. Immobilization of the damaged tissue during healing has been shown to contribute to a decrease in mechanical strength. In contrast, mechanical stretch during the healing stages leads to an increase in strength. In this study, an in vitro model was used to investigate the effect of mechanical stimuli on the level of intracellular calcium ([Ca2+]ic) signaling in MCL cells. This model used a microgrooved membrane to align cells in parallel fashion, creating an in vitro spacing environment closely resembling that of cells in native tissue. The objective of this study was two-fold. The first was to determine if cells that were aligned would elicit a greater increase in [Ca2+]ic response (i.e., number of cells participating in signaling cascade) to a mechanical stimulus than cells that were randomly oriented. The second was to test the hypothesis that aligned ligament cells subjected to mechanical stretch would elicit a greater increase in [Ca2+]ic than non-stretched aligned cells. Cells from the MCL of Sprague-Dawley rats were isolated and grown to confluence in DMEM with 10% fetal bovine serum. Microgrooved substrates were fabricated from PDMS using soft lithography techniques. MCL fibroblasts, cultured in a six-well bioreactor containing rectangular patterned substrates were loaded with Fura-2, a fluorescent dye, and stimulated using a 10-μm micropipette tip. To test the hypothesis that stretching ligament fibroblasts elicits a greater signaling response, a 3.5 %, 1 Hz stretch regimen was applied for 2 hours. Cells were rested 24 hours before stimulation and a ratio-imaging fluorescence technique was used to quantitate the [Ca2+]ic. In the non-stretched group, aligned MCL cells increased their [Ca2+]ic from a basal level of 50 nM to a mean peak level of over 390 nM, with 1 neighboring cell signaling in response to the stimulation of one target cell. In the same group, cells that were randomly oriented increased from 45nM to over 240 nM, with 2 neighboring cells signaling in response to the target cell stimulation. In contrast, cells that were aligned and stretched increased their [Ca2+]ic from 50 nM to over 150 nM, with 6 neighboring cells signaling. The stretched control cells increased from 55 nM to over 270 nM, with 3 neighboring cells signaling. The microgrooved membranes controlled the shape, alignment, and loading conditions of ligament fibroblasts. For cells that were stretched, the calcium signal propagated to a greater number of adjacent cells than those receiving no stretch, and a greater number of the cells that were stretched and aligned signaled compared with those randomly oriented. From this study, it can be concluded that ligament fibroblasts that are mechanically loaded and aligned recruit more cells in a signaling response than non-loaded cells.

F8.25
Monolayer Formation of Endothelial Cells on Microporous Films of Biodegradable Polymers. Takehiro Nishikawa, Junko Hayashi, Takuya Ohzono, Masahiko Hara and Masatsugu Shimomura; Spatio-Temporal Function Materials Research Group, The Institute of Physical and Chemical Research, Wako, Saitama, Japan.

Honeycomb films are microporous films of polymers which are formed spontaneously by applying moist air to a spread polymer solution. The regular array of micropores of the porous film can work as a micropatterned surface which can control cell adhesion. We report the monolayer formation of endothelial cells (ECs) on self-supporting honeycomb films. The tissue formation was studied in regard to cell-matrix adhesion, proliferation, and movement. Honeycomb films were prepared from mixtures of biodegradable polymers (poly(L-lactic acid) (PLLA) and poly(ϵ-caprolactone) (PCL)) and amphiphilic polymers (poly(alkylacrylamide)). Adhesion behavior of ECs was

characterized by formation of stress fiber of actin filaments and localization of focal adhesion proteins at the interface between cells and culture substrate. ECs did not form focal adhesions on self-supporting microporous films. The modulated cell adhesion on the microporous films influenced cell-division cycle of ECs. The doubling time in the cell-division cycle of ECs was estimated from the proliferation curves. The average doubling time was 20 hrs on flat cast film of PCL and 27 hrs on microporous films of PCL. The micropores of the honeycomb film can be considered to be pathways connecting two sides of a self-supporting honeycomb film of PLLA. Can cells migrate through the micropores? ECs were seeded onto a top side of a honeycomb film having an average poresize of 5 μm and an average thickness of 3 μm. At the day 11 of culturing, the cell culture was observed by confocal microscopy after staining filamentous actin of ECs and a honeycomb film with fluorescent dyes. Monolayer of ECs was confirmed at each side of the honeycomb film. This suggests that ECs attached onto the top side pass through the micropores, appear on the bottom side of a honeycomb film, grow, proliferate, and finally cover the both sides of the honeycomb film.

F8.26
Modeling of Elastic Modulus Evolution of Cirrhotic Human Liver. Lizhi Sun, H.M. Yin, G. Wang and Michael Vannier; University of Iowa, Iowa City, Iowa.

A micromechanics-based composite model is developed for the elastic behavior and its modulus evolution of cirrhotic human liver correlated with different pathological stages. Microstructurally, the cirrhotic liver is hypothesized to be pathologically elastic nodules embedded in the soft tissue matrix whose hyperelastic behavior is controlled by Veronda-Westmann model. Under finite deformation, the total strain energy of the liver is collected through the combination of that in nodule particles and that in the tissue matrix. The overall elastic constitutive relation of the pathological liver can further be established through the nonlinear hyperelasticity theory. Predictions of the elastic modulus and its pathological evolution are compared with available experimental data.

F8.27
Surface Characterization of Aged, Oxygen Plasma-Treated Polymeric Substrates Used for Directed Nerve Cell Growth. Bryan A. Langowski and Kathryn Uhrich; Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, Piscataway, New Jersey.

Protein patterns have been shown to direct the attachment and outgrowth of nerve cells. Currently, many patterning techniques, including microcontact printing, have been used to produce protein patterns on various inorganic substrates. Microcontact printing has also been used to pattern polymeric substrates and demonstrated to direct nerve cell attachment and outgrowth. However, it is necessary to first activate the substrate surface with oxygen plasma for the polymer to accept ("patternability") and maintain the protein pattern for an extended period ("pattern stability"). Because polymer surfaces are dynamic systems that have the ability to reorient themselves in response to their surroundings, chemical changes may occur at the surface that could potentially affect a polymer's ability to either initially accept a protein pattern or stabilize the proteins once patterned. Polymers stored in different environments (hydrophobic vs. hydrophilic) are expected to exhibit different degrees of patternability and pattern stabilization. Oxygen plasma-treated samples of polyethylene and poly(methyl methacrylate) stored in wet and dry environments for 8 weeks have been evaluated by x-ray photoelectron spectroscopy (XPS), contact angle measurements, scanning electron microscopy (SEM) and atomic force microscopy (AFM). Storage- and time-dependant changes to polymer surfaces following plasma treatment were measured and their effects on polymer patternability and pattern stability were evaluated to maximize protein pattern attachment and stabilization.

F8.28
The effect of pore size on cell adhesion in collagen-GAG scaffolds. Brendan A. Harley[1], Fergal O'Brien[2], Ioannis Yannas[1] and Lorna J. Gibson[3]; [1]Mechanical Engieneering, MIT, Cambridge, Massachusetts; [2]Anatomy, Royal College of Surgeons of Ireland, Dublin, Ireland; [3]Materials Science and Engineering, MIT, Cambridge MA, Massachusetts.

The biological activity of an extracellular matrix analog (scaffold) depends sensitively on the density of available ligands, which, in turn, depends on the composition of the scaffold and its specific surface area (SA/V). Collagen-GAG (CG) scaffolds were manufactured using a lyophilization technique, varying the final temperature of freezing (Tf = -10, -20, -30, -40 degrees C) to produce a homologous series of scaffolds with a constant composition and solid volume fraction (0.005) but with four different pore sizes and specific surface areas. MC3T3-E1 mouse clonal osteogenic cells were seeded onto the four scaffolds and maintained in culture for 24 and 48 hours. At the end of this period, the remaining viable cells were counted to determine the percent cell attachment at the two time-points. Table 1 shows the effect of freezing temperature on the average pore size and the specific surface area of the scaffolds as well as the percent cell attachment observed at 24 and 48 hours. Statistically significant differences ($P<0.05$) were observed in the average pore size of each of the four scaffolds. There was a significant difference in cell attachment in the different scaffolds ($P<0.05$), but there was no significant change in cell attachment between 24 and 48 hours for any of the scaffolds ($P>0.05$). The fraction of cells attached to the CG scaffold decreases with increasing average pore diameter, and increases linearly with specific surface area (R2 = 0.95, 0.91 at 24, 48 hours). These results are consistent with an increase in ligand binding site density being responsible for increased cell attachment. Table 1 Average pore size and specific surface area of homologous series of CG scaffolds and percent MC3T3 cell attachment at 24 and 48 hours post-seeding.

F8.29
An Investigation of Nano-Structured, Three-Dimensional Polymers As Bladder Tissue Constructs. Megan Pattison, Karen M Haberstroh and Thomas J Webster; Biomedical Engineering, Purdue University, West Lafayette, Indiana.

Many treatments for bladder diseases or disorders, such as bladder cancer and bladder outlet obstruction, require resection of the bladder wall. When this is necessary, biomaterials are needed as bladder wall replacement materials. For these reasons, the objective of the present *invitro* research was to construct a synthetic polymer scaffold that has nano-dimensional surface features similar to what cells experience in the bladder. Three-dimensional polyurethane scaffolds were constructed using solvent casting and salt leaching processes. These scaffolds were then manipulated to possess nano-dimensional surface features by soaking in nitric acid at select concentrations for various periods of time. In order to negate the effects of chemistry changes, a poly(methylmethacrylate) (PMMA) mold of the scaffolds was fashioned. Catalyzed liquid methylmethacrylate monomer was poured onto the scaffold and allowed to cure. Next, the original scaffold was removed from the mold by soaking in dimethylacetamide. Finally, a polyurethane solution was poured into the mold and cured, and the PMMA mold was removed by soaking in acetone. Human bladder smooth muscle cells were seeded into the scaffolds at a density of 50,000 cells per scaffold to perform cytocompatibility studies. Adhesion and proliferation experiments were performed for 4 hours, and 1, 3, and 5 days respectively. Results of this study provide evidence that porous, nano-dimensional polymer scaffolds can be constructed using these methods. Increased bladder smooth muscle cell function has been previously observed on two-dimensional nano-structured polyurethane films compared to conventional (micro-dimensional) polyurethane films. For this reason, cytocompatibility studies on three-dimensional polyurethane scaffolds will be presented.

F8.30
Porous Hydroxyapatite Networks for Synthetic Bone Material by Spinodal Decomposition. Michael P. Sansoucie and Robert W Hyers; Mech and Ind Eng, University of Massachusetts Amherst, Amherst, Massachusetts.

Natural bone consists mainly of minerals and collagen fibers, with approximately 69% by weight being hydroxyapatite (HAp, Ca10(PO4)6(OH)2). Previous research has shown that natural bone grows into porous, synthetic HAp producing a strong bond. Additionally, the porous HAp is resorbable, meaning it will gradually be broken down and replaced with new, natural bone. The synthetic bone provides support and shape until it is replaced by the body. Current methods for the production of hydroxyapatite typically involve sintering at high temperatures. However, HAp decomposes at the sintering temperatures, approximately 1000°C. Furthermore, sintering at lower temperatures produces weak bonds while sintering at higher temperatures causes low porosity and poor phase purity (due to decomposition). This makes sintering an inadequate method for producing good quality HAp implant material for structural bone replacement. Formation of hydroxyapatite through spinodal decomposition will be analyzed. Spinodal decomposition involves a thermodynamic separation of a single-phase liquid into an interconnected, two-phase liquid with a continuous network. This second phase may be dissolved, leaving a porous, continuously-connected structure of a mixture of HAp and tricalcium phosphate (TCP, Ca3(PO4)2). The porosity can be modified by varying the initial composition, while the pore size can be changed by varying the time-temperature history. Furthermore, TCP resorbs faster than HAp; therefore, the resorption rate may be controlled by altering the HAp/TCP ratio through solution treatment after the second phase is removed. Control of these process variables would allow the implant material to be tailored to the specific application.

F8.31
Structural and Morphological Map of the Human Fallopian Tube. Frank Kellogg[1], Shahab Minassian[2], Mark Woodland[2] and Michele Marcolongo[1]; [1]Materials Science and Engineering, Drexel University, Philadelphia, Pennsylvania; [2]College of Medicine, Drexel University, Philadelphia, Pennsylvania.

An ectopic pregnancy occurs when a fertilized ovum embeds and develops outside of the uterus. Approximately 2 percent of all pregnancies in the United States, both natural and assisted, are ectopic; 95 percent of which occur in the Fallopian tubes. An ectopic pregnancy (a tubal pregnancy) in the Fallopian tubes (oviducts) results in miscarriage of the fetus (either spontaneously or through surgical means) and can lead to tubal rupture, hemorrhaging and infertility. While fertility and ectopic pregnancy has been investigated in the biological community, little engineering or materials science efforts have been made toward elucidating mechanisms responsible for the ectopic implantation. To start to develop methods of treating diseased or damaged oviducts to prevent this occurrence, it is necessary to first understand the structural map of the tissue of the oviduct. This is an area of conflict in the present literature. The classical description of the muscularis of the ampulla and isthmus regions of the oviduct shows that there is an organized, layered fibrous architecture in the wall of the tube. However, more recent microscopy analysis has showed a more amorphous structure of the muscularis. We have examined the muscularis of the ampulla and isthmus using environmental scanning electron microscopy for dimensional, structural and morphological characterization of the oviduct every 0.5 mm along the length. This information is critical to modeling fluid flow through the Fallopian tube, understanding mechanical behavior and structure/properties relationships of this tissue. With a more thorough understanding of these engineering relationships, potential solutions to ectopic pregnancy may be posed and investigated.

F8.32
A comparison of elasticity of the crosslinked hyaluronan films and hydrogels. Kaustabh Ghosh[1], Xiao Zheng Shu[3], Robert Mou[4], Jack Lombardi[4], Miriam Rafailovich[2,1], Glenn D. Prestwich[3] and Richard A.F. Clark[1]; [1]Biomedical Engineering, State University of New York at Stony Brook, Stony Brook, New York; [2]Material Science and Engineering, State University of New York at Stony Brook, Stony Brook, New York; [3]Medicinal Chemistry, University of Utah, Salt Lake City, Utah; [4]Physical Chemistry Laboratory, Estee Lauder Research, Melville, New York.

Cell growth and development requires detection of the elasticity of underlying substrata. To optimize tissue engineering materials, the elasticity of biological scaffolds must be controlled. Hyaluronan (HA) materials are used in wound healing and tissue repair, but many lack suitable mechanical properties. However, HA can be functionally derivatized and then chemically cross-linked to obtain substrates for biological applications. The nature and degree of cross-linking determines the mechanical properties and the residence time of HA in vivo. Hydrogels were prepared by crosslinking thiol-functionalized 3% (w/v) HA (HA-DTPH) with poly (ethlylene glycol) diacrylate (PEGDA), and gels were air-dried to obtain thin films. Different formulations of hydrogels and films were prepared by either varying the molecular weight (Mw) or concentration (ϕ) of PEGDA. The elastic properties of the air-dried films were measured using a Mettler-Toledo Dynamic Mechanical Analyzer and a Tytron 5540 Instron tensile tester. The viscoelastic properties of the hydrogels were determined using an Aries 2000 Rheometer. We found that the bulk elasticity of the hydrogels scaled linearly with ϕ and were not dependent on the Mw of PEGDA. In contrast, for dry films, both ϕ and Mw had a comparable influence on the elastic modulus. These results demonstrate that the elasticity in both cases is determined by the most flexible component. For flexible HA hydrogels, the elasticity is determined by the number of or spacing between the cross-linking junctions. For the stiffer HA films, the elasticity is also sensitive to the flexibility of the crosslinking junctions, which can be controlled by varying the Mw of the PEGDA.

F8.33
Morphology Control of Electrospun Fiber Membrane Using NaCl Crystallization. Hyun-Suk Kim, Kwan-Young Kim, Hyoung-Joon Jin and In-Joo Chin; Department of Polymer Science and Engineering, Inha University, Incheon, South Korea.

Electrospun fibers have been explored for applications such as high performance filters and biomaterials for vascular grafts, wound dressings or tissue engineering scaffolds. Electrospinning offers an interesting means to form protein fibers of nanometer scale diameters with large surface areas while maintaining high porosity. Not only biodegradable synthetic polymers but also natural polymers like silk and collagen have been electrospun to generate nanofibers. However, the pore size of the typical electrospun membranes was too small for the fibroblast or stem cell to penetrate through the membrane and the membrane surface was too smooth to be applied onto epidermis as artificial skin. In the present study, we explored the preparation of the poly(lactide-co-glycolide) (PLGA) membrane by the modified electrospinning process in which a NaCl solution reservoir was used as the collector. Surface morphology of the membrane was controlled by varying the concentration and the crystallization time of NaCl. Rough surface of the electrospun membrane simulating the rete ridge morphology of epidermis was obtained by dissolving NaCl in water and the hydrophobicity of PLGA was expected to be reduced. The structure and morphology of the membranes were investigated by field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD), atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS).

F8.34
Tensile Testing of a Single Electrospun Ultrafine Polymeric Fiber. C N Goh[2], C H Sow[3] and C T Lim[1,2]; [1]Division of Bioengineering, National University of Singapore, Singapore, Singapore; [2]Department of Mechanical Engineering, National University of Singapore, Singapore, Singapore; [3]Department of Physics, National University of Singapore, Singapore, Singapore.

One of the emerging trends in biomedical engineering is the use of polymeric nanofibers for tissue engineering. This involves seeding living cells on a nanofibrous scaffold comprising individual nanofibers. As such, mechanical characterization such as tensile testing of individual nanofibers is important in determining their structural integrity. However to date, little is done in this area due to difficulty in handling the ultrafine fibers and measuring the very small tensile force and displacement involved. A novel approach in the tensile testing of a single strand submicron sized fiber is proposed. Here, an optical microscope is used as an optical means to monitor the process of the tensile test. Manipulation of the tested fiber is made possible using micro-manipulators. To conduct the tensile test, a portion of the fiber to be tested is glued to a glass fiber attached to a piezo-resistive Atomic Force Microscope (AFM) cantilever tip. This cantilever functions as a force transducer for force measurements. Elongation of the fiber is determined from the displacement of the optical microscope stage, to which one end of the tested fiber is rigidly attached. As the stage is displaced, the tested fiber is stretched and this causes a deflection of the piezo-cantilever. This deflection results in a change in resistance of the piezo-cantilever which is recorded with a sensitive multimeter. Calculations are then performed to determine force applied during the tensile test. From the data collected, the mechanical properties of the tested fiber can be deduced. Results obtained show the experimental setup performs satisfactorily in determining the mechanical properties of single strand nanoscale fibers. For a single-strand PEO fiber with an average diameter of about 700nm, the Young's modulus is found to be 45 MPa over strains of 0.07 to 0.4.

F8.35
Adhesion of Fibroblasts to Photocurable Polymer Substrates. Suphasinee Limpanuphap and Brian Derby; Manchester Materials Science Centre, UMIST, MANCHESTER, United Kingdom.

Photocurable polymers are attractive candidates as substrate materials for scaffolds in tissue engineering applications. There are a number of freeform fabrication techniques that use photocurable materials and can be developed to produce complex 3-dimensional architectures required for scaffolds. PEG diacrylate and PEG dimethacrylate polymers each of two different PEG lengths have been used with UV curable photoinitiators to produce highly crosslinked polymer matrices after photocuring. Immortalised fibroblast-like cell lines have been used for cell adhesion studies. Cells are found to adhere and spread on all four substrates to confluence after four days. However, cell adhesion and spreading occurs more rapidly on the polymers containing shorter PEG chain lengths for both the diacrylate and dimethacrylate functional groups. This result is found despite the shorter chain length materials showing larger wetting angles (less hydrophillic) than were found with longer PEG lengths. Fluorescence assays have been used to identify the presence of focal adhesion centres and determine the onset of cell adhesion on each of the four substrates studied.

F8.36
Selective Protein Adsorption on PTFE Surface with ArF Laser Induced Photochemical Modification. Yuji Sato and Masataka Murahara; Electrical Engineering, Tokai Univ., Hiratsuka, Japan.

Hydrophilic groups were substituted photo-chemically on poly-tetrafluoroethylene [PTFE] surface using ArF Laser. Biocompatible and affinity implant material has been developed for the tissue. The PTFE has widely used such as a vessel catheter or implanted devices for ophthalmology because it has chemical resistance, weatherproof and repellency. When it is implanted in

human body, protein is rapidly absorbed on PTFE, and a fibroblast was adhered on it, forming the fibrous tissue. However, a bonding power of PTFE and the tissue is weak because of its repellency. Therefore we substituted hydrophilic group on the PTFE surface in order to improve affinity for the tissue. Our previous studies have found that B, Al and H atoms are effective for the defluorination of fluorocarbon. Furthermore, H_2O was employed as a defluorination agent in order to inhibit rejections. In this study, hydrophilic group was substituted on the PTFE surface using water as reaction liquid. The water is sandwiched with a fused silica glass by a capillary phenomenon. In this condition, an ArF laser was vertically irradiated. Thus, OH groups were substituted photo-chemically on the PTFE surface. FT-IR analysis was carried out to investigate the hydrophilic groups that had been substituted on the PTFE. As the result, OH absorption band was observed at 3300 cm^{-1}. Moreover protein adsorption test was carried out with bovine serum albumin [Alb] and fibrin using SEM and FT-IR. The Alb and fibrin sticking rate on the non-treatment sample was low. However, as the OH substitution density was increased, the protein-sticking rate was increased. With the laser fluence of 15 mJ/cm^2, shot number of 2000, the Alb sticking rate of the treated sample became maximum, which was 2.3 times as high as that of non-treatment sample. In conclusion, our study indicated the possibility that this material has high affinity for the tissue.

F8.37
Cross-linked polyelectrolyte multilayer films : influence on cell adhesion properties. Catherine Picart, INSERM U595, Strasbourg, France.

Poly(L-lysine)/hyaluronane (PLL/HA) multilayered films were chemically cross-linked with a water soluble carbodiimide (EDC) in combination with a N-hydroxysulfo-succinimide (NHS) to induce amide formation. Fourier transform infrared spectroscopy confirms the conversion of carboxylate and ammonium groups into amine bounds. Quartz crystal microbalance evidences a change in the viscoelastic properties of the films after the cross-linking. The zeta potential of the cross-linked films becomes negative, whether cross-linking has been performed on positive or negative ending films. The diffusion of PLL-FITC within a cross-linked film is dramatically reduced compared to a non-crosslinked film, as shown by Confocal Laser Scanning Microscopy. Indeed, these films are very stable in water, in ethanol solutions at increasing concentrations, and they are highly resistant to hyaluronidase, an enzyme that naturally degrades hyaluronan. As a consequence of the modication of the film properties by cross-linking, primary chondrocytes and chondrosarcoma cells do adhrere and spread nicely on the cross-linked films whereas the non-crosslinked films are highly anti-adhesive (terminating either with HA or PLL). This proves the high biocompatibility of the (PLL/HA) films and opens applications in the coating of biomaterials.