SYMPOSIUM GG

Polymeric Biomaterials for Tissue Engineering

November 26 – 27, 2001

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* Invited paper

SESSION GG1: PATTERNING BIOMATERIALS
Chairs: David J. Mooney and Jennifer L. West
Monday Morning, November 26, 2001
Gardner (Sheraton)

9:00 AM *GG1.1
PROGRAMMING CELL FUNCTION AT THE MICRO-FABRICATED CELL-BIOMATERIAL INTERFACE. Christopher S. Chen, Johns Hopkins Univ, Dept of Biomedical Engineering, Baltimore, MD.

The dynamic binding interactions between cell surface receptors and local bioactive ligands serves as the principal mechanism by which cells survey their microenvironment and accordingly modulate their behaviors, such as proliferation, differentiation, migration, and suicide. Using conventional and non-conventional microfabrication approaches to engineer well-defined cellular microenvironments, we are examining how cells recognize and respond to different cues in their microenvironment. We will discuss our approaches to control compositional chemistry, mechanical properties, architecture, and geometry of surfaces, and how these factors regulate cells. By developing these approaches to engineering cell-surface interactions, we hope to improve the interconnect between artificial surfaces and living cells.

9:30 AM GG1.2
TOPOGRAPHICAL AND CHEMICAL CONTROL OF HUMAN NEUTROPHIL MOTILITY. Jian Tan and W. Mark Saltzman, School of Chemical Engineering, Cornell University, Ithaca, NY.

Controlling cell response to an implantable material is essential to tissue engineering. Both chemical and topographical properties of a material surface can play a crucial role, because it is the surface that is in direct contact with cells. In this study, parallel ridges/grooves were micropatterned on glass surfaces using photosensitive polyimide to create transparent substrates. The migratory behavior of live human neutrophils on the patterned surfaces was observed using a light microscope with transmitted light source. The width (2 μm) and length (400 μm) of the ridges were kept constant. The height (5 or 3 μm) and the repeat spacing (6 to 14 μm) of the ridges were systematically changed to investigate the effect of microgeometry on neutrophil migration. In addition, the effect of surface chemistry on neutrophil migration was studied by deposition of a thin layer of "inert", biocompatible metal such as Au-Pd alloy and titanium on patterned substrates. More than 95% of neutrophil moved in the direction of the long axis of ridges/grooves regardless of the topographical geometry and chemistry, consistent with a phenomenon termed "contact guidance". Cell migration was characterized using a one-dimensional persistent random walk. The rate of cell movement was strongly dependent on the topographical microgeometry of the ridges. The random motility coefficient μ, 9.8×10^{-9} cm^2/s, was the greatest at a height of 5 μm and spacing of 10 μm, about 10 times faster than on smooth glass surface. The Au-Pd coating did not change neutrophil migratory behaviors on patterned surfaces, whereas titanium decreased cell motility substantially. The results of this study suggested that optimization of both surface chemistry and topography may be important when designing biomaterials for tissue engineering.

9:45 AM GG1.3
CAPILLARY FORMATION IN MICROFABRICATED POLYMER SCAFFOLDS. Jeffrey T. Borenstein, Kevin R. King, Charles Stark Draper Laboratory, Cambridge MA; Hidetomi Terai, Joseph P. Vacanti, Massachusetts General Hospital, Boston, MA.

One of the primary challenges for engineering thick, complex tissues such as vital organs is the requirement for a vascular supply for nutrient and metabolite transfer. Earlier work has shown that Solid Freeform Fabrication techniques such as Three-Dimensional Printing (3DP) are capable of producing biodegradable scaffolds for the subsequent formation of a wide range of tissues and organs. While this approach shows great promise as a method for constructing complex tissues and organs in vitro, the resolution of the process is currently limited to length scales larger than the narrowest capillaries in the microcirculation. In this work, microfabrication technology is demonstrated as an approach for organizing endothelial cells in vitro at the size scale of the microcirculation. Standard process techniques utilized to build MEMS (MicroElectroMechanical Systems) devices include photolithography, silicon and glass micromachining, and polymer replica molding. Photolithography is used to print a model network of blood vessels on silicon wafers; the network is designed to replicate the fluid dynamics of the vasculature of a particular tissue or organ. A reverse image of the channel network is formed either by Deep Reactive Ion Etching (DRIE) of silicon or through the use of a thick negative-polarity photoresist (SU-8.) Polymeric scaffolds are formed by replica molding, using the silicon wafer as a master mold. Microfluidic chambers have been constructed from PDMS and other biocompatible polymers. Initial cell seeding experiments demonstrate that rat lung endothelial cells attach in a single layer to the walls of these structures without occluding them, providing early evidence that MEMS process technology can serve as a method for organizing capillary networks.

10:30 AM GG1.4
CHARACTERIZATION OF CHEMICALLY AND TOPOGRAPHICALLY MODIFIED SILOXANE ELASTOMER FOR CONTROLLED CELL GROWTH. Amy L. Gibson, Leslie H. Wilson, Adam W. Feinberg, Wade R. Wilkerson, Charles Seegert, Ronald Baney, Anthony B. Brennan, Biomedical Engineering Program, Department of Materials Science and Engineering, University of Florida, Gainesville, FL.

A main limitation of biomedical devices is the inability to start, stop and control cell growth making it crucial to develop biomaterial surfaces that induce a desired cellular response. We have developed a process to engineer the surface energetics of polydimethylsiloxanes by manipulating the topography, mechanical properties and surface energy. Micropatterns of ridges and pillars were created in a siloxane elastomer (Dow Corning) by casting against polystyrene and epoxy replicates of a micromachined silicon wafer. Silicone oils were incorporated to determine the change in modulus and surface energy caused by these additives. AFM, SEM and white light interference profilometry verified that the micropatterning process produced high fidelity, low defect micropatterns. Mechanical analysis indicated that varying the viscosity, weight percent and functionality of the added silicone oil could change the elastic modulus by over an order of magnitude. As a self-wetting resin, silicone oils migrate to the surface, hence changing the surface properties from the bulk. Both topographical and chemical features define the surface energy, which in combination with elastic modulus, dictate biological activity. Evaluation of the surface energy with dynamic contact angle analysis showed measurable change between smooth and microtextured domains. The results imply that the morphology, mechanical properties and surface energy of the siloxane elastomer can be modified to elicit a specific cell response as a function of engineered topographical and chemical functionalization.

10:45 AM GG1.5
TWO-DIMENSIONAL PATTERNING OF CELL ADHESION PROTEINS VIA COLLOIDAL ASSEMBLY: PROTEIN ORGANIZATION DIRECTS CELL BEHAVIOR. Eileen M. Higham, Nathaniel J. Gleason, Ilhan A. Aksay, Jeffrey D. Carbeck, Princeton Univ, Dept of Chemical Engr, Princeton, NJ; Jean E. Schwarzbauer, Princeton Univ, Dept of Molecular Biology, Princeton, NJ.

This talk describes the two-dimensional patterning of cell adhesion proteins on sub-cellular length scales and the effects of patterning and surface topology on cell organization and behavior. Arrays of protein coated colloidal particles are used to pattern cell adhesion proteins on three length scales: the size of individual particles (500 nm 2 microns), the length scale of micropatterns of colloidal particle arrays produced via self-assembly and soft lithography (10 100 microns), and microdomains of particles formed in binary colloidal arrays, the length scale of which is between individual particles and micropatterns. Using different techniques for the deposition of particles we have produce surfaces that vary from random distributions of isolated particles to close-packed, two-dimensional crystalline arrays. Once particles or particle arrays are deposited on a surface, the area around particles are made non-adhesive to other proteins and, thereby, cells by blocking these surfaces with bovine serum albumin, or polyethylene glycol. Interfaces produced in this way show that the organization of the cell adhesions protein fibronectin can directly affect cell adhesion, stress fiber formation, membrane texture, cell shape and mechanism of cell spreading. In particular, by varying the density of particles we were able to switch fibroblast cells from a morphology consistent with a static, adhesive state to a morphology consistent with a dynamic, migratory state, similar to that observed in wound healing. This change in cell morphology was coupled with distinct changes in the organization of the cytoskeleton and number of fillapodia. For the first time we show that these changes in cell behavior can be directed through protein organization on surfaces; in the past, such changes had only been seen in response to changes in the composition of proteins on surfaces.

11:00 AM GG1.6
POLYMERIC TISSUE CULTURE SUBSTRATES PATTERNED BY UV IRRADIATION. Alexander Welle, Eric Gottwald, Karl-Friedrich Weibezahn, Hermann Dertinger, Forschungszentrum Karlsruhe GmbH, Institute for Medical Engineering and Biophysics, Karlsruhe, GERMANY.

We studied the physico/chemical effects of deep UV irradiation of polystyrene, PMMA and polycarbonate with respect to cell adhesion and protein immobilization. Photochemical modifications of the polymer surfaces yielded peroxides and mainly carboxylic acid groups

which were identified by X-ray photoelectron spectroscopy, dye binding and contact angle titrations. Masked irradiations opened a simple, fast, and economical route to obtain chemically patterned polymeric substrates. This procedure is advantageous as compared to silane or thiol based patterning by micro contact printing or other techniques due to the availability of the polymeric substrates, the elimination of any chemical treatment, the clean room compatibility and the small size of achieved structures. It was observed that hepatocytes (HepG2) and fibroblasts (L929) adhered in the presence of serum proteins in the culture medium on the irradiated regions of the substrate without any further treatment. Beyond a spontaneous formation of a patterned protein adsorbate from a multi component mixture like foetal bovine serum, defined adsorbates can be realized by coupling peptides or proteins to the carboxylic groups with carbodiimid activation. We have immobilized horseradish peroxidase, other enzymes and antibodies. Phaeochromocytoma cells (PC-12) required a two step adsorption of albumin and collagen or laminin to adhere. Together with supplemented nervous growth factor the collagen matrix stimulated the formation of the neuronal phenotype. The described patterning technique may become a useful tool for the study of a variety of defined co-cultures (for example hepatocytes and fibroblasts), neuronal networks, intercellular communication, organogenesis and for applications like biosensors or engineered highly functional tissues and implants as bioartifical organs.

11:15 AM GG1.7
DEVELOPING INTERFACIAL BIOMATERIALS VIA PHAGE DISPLAY TECHNOLOGY. Elisabeth B. Walsh, Mark W. Grinstaff, Duke Univ, Dept of Chemistry, Durham, NC; Daniel J. Kenan, Dept of Pathology, Duke Univ Medical Center, Durham, NC.

11:30 AM GG1.8
ENGINEERING MICRON AND NANOMETER SCALE FEATURES IN POLYDIMETHYLSILOXANE ELASTOMERS FOR CONTROLLED CELL FUNCTION. Adam W. Feinberg, Charles A. Seegert, Anthony B. Brennan, Univ of Florida, Biomedical Engineering Program, Dept of Materials Science and Engineering, Gainesville, FL.

Cell movement, differentiation and metabolic function must be controlled in precise ways to produce both regenerated tissues such as bone and functional tissue equivalents such as immuno-isolated islet cells. Close examination of extracellular matrix (ECM) reveals structures on the micron and nanometer scale that are shown to influence these factors and therefore we hypothesize that cells will move based on topographical cues in the scaffold. We have engineered siloxane elastomer surfaces that mimic the ECM by combining micron and nanometer scale topographic features. Micron scale pillars and ridges ranging in height from 1.5 to 5 microns and separated by 5, 10 and 20 microns were fabricated in a silicon wafer using micro-processing techniques and replicated in PDMS elastomer. Nanometer scale pillars, ridges and more complex shapes ranging in height from 12 to 300 nanometers were superimposed on the micron scale features using nanolithography. This was achieved by using a tapping mode tip in the atomic force microscope (AFM) to plastically deform the substrate surface. The AFM enabled nano-features to be placed on sloped surfaces and added directly to the PDMS elastomer surface. Surface topography was examined using scanning electron microscopy, atomic force microscopy and white light interference profilometry to verify surface modifications and fidelity of the replication process. Results indicate that it is possible to create spatially engineered surface textures from 10-5 m to 10-8 m in size, in specified patterns, by using a combination of microprocessing and nanolithography techniques. As better understanding of ECM function and design is gained, the processing methods outlined here will assist in fabricating tissue engineering scaffolds optimized at the nanometer and micron scale.

11:45 AM GG1.9
BIOCOMPATIBLE POLYMERS FOR REMOTE MANIPULATION. Eric Pape, Julie Kornfield, Jagdish Jethmalani, Christian A. Sandstedt, Robert H. Grubbs, Dan Schwartz.

Due to the unpredictability of wound healing, there is a need for materials that enable non-invasive manipulation of an implant after wound healing has stabilized. Here we describe materials in which physical and optical properties can be altered in a spatially resolved way using light. Selective photopolymerization of a macromer in an elastomeric matrix is used to produce the desired change. In the irradiated regions, macromer polymerizes; depletion in macromer concentration drives diffusion of macromer into this region. The redistribution of macromer produces corresponding changes in properties. As an example, polydimethylsiloxane is used because it is biocompatible, has high diffusion rates, and is optically transparent for visible light. We use a bismethacrylate endcapped siloxane macromer and near-UV photo-initiator contained in a polydimethyl-siloxane (PDMS) matrix. Spatially patterned irradiation with a HeCd laser induces polymerization of the macromer in irradiated regions. We characterize the effects of macromer molecular weight, percent macromer in the material, and irradiation dose on the rate and magnitude of the change. Forced Rayleigh scattering is used to calculate macromer diffusion rate and effective time scale for changes in properties.

SESSION GG2: DEGRADATION OF BIOMATERIALS
Chairs: Kristi S. Anseth, Amy K. Burkoth Poshusta and David J. Mooney
Monday Afternoon, November 26, 2001
Gardner (Sheraton)

1:30 PM *GG2.1
POLYANHYDRIDES AS POLYMER-BASED DRUGS. Kathryn Uhrich, Rutgers University, Dept. of Chemistry, Piscataway, NJ.

Poly(anhydride-esters) are biocompatible polymers that predominantly degrade into salicylic acid, the degradation product of aspirin. These polymers were designed to influence the tissue response when implanted. Preliminary animal studies indicated that poly(anhydride-ester) degradation induced a pronounced, localized reduction in inflammation and also promoted new bone growth. We are currently evaluating these degradable polymers for controlling and preventing periodontal disease as well as orthopedic applications. Based on the success of the poly(anhydride-esters), several new polymer compositions were synthesized using various other salicylates as the polymer backbone. For example, aminosalicylates are effective anti-inflammatory agent in treating inflammatory bowel disease. Because current aminosalicylate delivery systems cause adverse side effects, we prepared several polymers that degrade into amino-salicylates including poly(anhydride-esters), poly(azo-anhydrides) and poly(anhydride-amides). As a two-pronged approach to drug delivery, antibiotics (e.g., tetracycline) were physically admixed with the polymers such that two drugs are released - tetracycline via polymer erosion and salicylate via polymer degradation. The antibiotic-laced polyanhydrides were prepared as both fibers and membranes, and their relative degradation examined. Fibers with biologically relevant diameters were made of the various polymer compositions and the degradation rates studied over a wide pH range.

2:00 PM GG2.2
CONTROLLED GROWTH FACTOR DELIVERY BY MECHANICAL STIMULATION. Kuen Yong Lee, Martin Peters, Kenneth Anderson, David Mooney, University of Michigan, Dept of Biologic & Materials Sciences, Chemical Engineering, Biomedical Engineering, Ann Arbor, MI.

The delivery of growth factors using polymeric matrices is one exciting approach to regenerate tissues. Most growth factor delivery systems, however, have been designed to operate under static conditions, while many tissues including bone, muscle, and blood vessels, exist in a mechanically dynamic environment. Considering the dynamic environment of our body, mechanical stimulation is an important signal that could be readily exploited. We hypothesize that polymeric matrices, releasing growth factors in response to mechanical stimulation, could provide a novel approach to engineer tissues in mechanically stressed environments. Critical design parameters for

this type of system include the ability to allow the repeated deformation of polymeric matrices, and a reversible binding of protein growth factors to the matrices in response to repeated stimulation. We report here a model system, comprised of alginate hydrogel and vascular endothelial growth factor (VEGF), which enhances the release of the growth factor in response to mechanical stimulation (e.g., cyclic compressional loading) and subsequently promotes blood vessel formation in animals. This approach may find a number of utilities in tissue engineering applications, as well as in drug delivery applications.

2:15 PM GG2.3
SYNTHESIS, CHARACTERIZATION AND APPLICATIONS OF POLY(ETHER-ESTER) AND POLYESTER BIODENDRIMERS. Michael A. Carnahan and Mark W. Grinstaff, Duke University, Departments of Chemistry and Ophthalmology, Durham, NC.

Dendrimers are highly branched globular macromolecules in which all bonds converge to a focal point or core. Compared with linear polymers, dendrimers have controlled structures with high surface area-to-volume ratios, single molecular weights, low viscosities, and a large number of controllable terminal functionalities. Due to these structural features and unique properties, the application of dendrimers in pharmaceutical and medicinal chemistry is becoming a highly attractive area of dendrimer chemistry. For example, dendrimers have shown significant potential as drug delivery agents by physical entrapment or covalent attachment of drugs. However, commercially available dendrimers such as PAMAM, were not designed as medical polymers. New synthetic procedures and materials are needed to address the lack of biocompatible dendrimers. We are synthesizing hydrolyzable aliphatic poly(ether-ester) and polyester dendrimers with building blocks such as glycerol and either succinic acid or lactic acid, which are known to be biocompatible or degradable to natural metabolites in vivo. This new class of dendrimers is termed "biodendrimers." These biodendrimers contain a tetra-functional core and an AB_2 monomer. To date, G0-G4 biodendrimers composed of glycerol lactic acid and succinic acid have been synthesized in high yield and fully characterized by ^{1}H-NMR, ^{13}C-NMR, IR, MS, and elemental analysis. Preliminary in vitro studies demonstrated that photocrosslinked biodendrimers are suitable materials for sealing corneal wounds. The ability to introduce a wide range of functional groups on biodendrimers will afford polymers which facilitate the design and development of new materials for specific medical and tissue engineering applications.

2:30 PM GG2.4
IN VITRO DEGRADATION OF BIODEGRADABLE POLY-URETHANES FOR TISSUE ENGINEERING. Katarzyna Gorna, Sylwester Gogolewski, Polymer Research, AO/ASIF Research Institute, Davos, SWITZERLAND.

There is an increasing interest in bioresorbable scaffolds for tissue repair and engineering. Ideally, the scaffold should have adequate biological, structural and mechanical properties and would initiate the formation of new tissues and/or organs. In practice, however, scaffolds are seeded with cells to facilitate healing. The mechanical properties of scaffolds depend on the type of tissue to be repaired. Elastomeric biodegradable materials may be preferred for the repair of meniscus, cartilage or cardiovascular tissues. Biodegradable polyurethanes are among the candidates. This study addresses in vitro degradation of biodegradable polyurethanes with varying hydrophilicity designed for tissue-engineered scaffolds and cancellous bone graft substitutes. The polymers were synthesized from aliphatic diisocyanates, diols of poly(ethylene adipate), poly(e-caprolactone), mixtures of pol(e-caprolactone) with poly(ethylene oxide-propylene oxide-ethylene oxide) (Pluronics), and various chain extenders allowing for incorporation of biologically active groups in the polymers. The materials were characterized using 1H-NMR, infrared spectroscopy, viscosity, size exclusion chromatography, contact angle, water uptake, scanning electron microscopy, profilometry, differential scanning calorimetry and tensile testing. Degradation in vitro was carried out in phosphate buffer solution at 37 ± 0.1oC for 48 weeks. The polymers obtained in the study had a molecular weight in the range of 38.000 to 85.000 daltons, a polydispersity index of 1.2 to 3.2, tensile strength, tensile moduli and elongation at break of 11 - 61 MPa, 4.5 - 91 MPa, and 370 - 960%, respectively. The hydrophilic component content was 4 to 7%. Degradation in vitro caused 15 to 20% reduction of molecular weight at 48 weeks for materials containing polycaprolactone, and 70 to 80% for the polymers containing Pluronics in addition to polycaprolactone. The mass loss at this time did not exceed 2%. Three-dimensional porous scaffolds from these polyurethanes implanted in unicortical and tricortical defects in the iliac crest of sheep induced new bone formation. In humans, bone tissue will not form in such defects.

2:45 PM GG2.5
MODELING AND EXPERIMENTAL CHARACTERIZATION OF DEGRADABLE POLY (VINYL ALCOHOL) TISSUE SCAFFOLDS. Penny Martens, Stephanie Bryant, University of Colorado, Dept of Chemical Engineering, Boulder, CO; Troy Holland, BioCure Inc, Norcross, GA; Christopher Bowman, Unviersity of Colorado, Dept of Chemical Engineering, Boulder, CO; Kristi Anseth, Unviersity of Colorado, Dept of Chemical Engineering and the Howard Hughes Medical Institute, Boulder, CO.

Numerous tissue engineering applications can benefit from the use of polymers for the encapsulation of cells (e.g., cartilage tissue engineering). Polymer hydrogels are particularly advantageous because the mild gelation conditions allow encapsulation of cells and the high swelling imparts desirable mechanics and transport properties. However, tuning the degradation behavior of hydrogel cell scaffolds is critically important to match the rate of neo-tissue evolution. In this research, we are using a multifunctional poly (vinyl alcohol) (PVA) that can be photopolymerized to encapsulate cells and also bulk degrades with controllable kinetics. PVA was chosen for the backbone of the macromer because of its history in medical applications and facile modification of its pendant hydroxyl groups. Many properties of the scaffold, e.g., mechanics, swelling and mass loss, need to be characterized as a function of degradation time, as well as the initial network formation and structure. The hydrogel properties were varied by changing parameters related to the macromer structure and polymerization conditions (e.g., molecular weight, functionality, % macromer in solution, and length of the degradable repeat units). By rationally changing these variables, the reaction time, mechanical properties, and total degradation time were readily controlled. Gels with degradation times ranging from 1 to 50 days were synthesized. To further understand what is occurring in the network structure as a function of degradation time, a statistical-kinetic model was developed. This model can be used to predict the erosion profile, volumetric swelling, and mechanics during degradation as a function of the network structure and hydrolysis kinetics. The model predictions have been compared to the experimental results and have shown reasonable correlations.

3:30 PM GG2.6
DECLINE OF POLY (ANHYDRIDE-ESTER) MECHANICAL PROPERTIES AS A FUNCTION OF HYDROLYTIC DEGRADATION. Kenya Whitaker, Rutgers University, Dept of Chemical and Biochemical Engineering, Piscataway, NJ; Kathryn Uhrich, Rutgers University, Dept of Chemistry, Piscataway, NJ.

Poly(Aspirin) is a biocompatible poly(anhydride-ester) which hydrolytically degrades into salicylic acid, an anti-inflammatory analgesic. This polymeric material has a low glass transition (Tg), thus is soft and tacky at room temperature making it difficult to handle. The mechanical properties of poly(Aspirin) were enhanced by copolymerization with para-carboxyphenoxyhexane (p-CPH) monomer. The copolymers have reduced tackiness, but become more brittle with increasing proportions of p-CPH. However, the 50:50 copolymers provided optimal mechanical and handling characteristics. Changes in mechanical properties, Young's modulus (E), water uptake, and Tg were monitored as a function of polymer degradation. These factors were key contributors to the decline of mechanical properties. For hydrolytic degradation to occur, water must first permeate the polymer matrix. Water continues to invade the interior of the polymer matrix via pores that are formed as a result of the degradation occurring at the water/polymer interface. The 50:50 copolymer has been shown by scanning electron microscopy to become more porous upon degradation. Upon drying, the polymer appears to become more brittle as a direct consequence of the increased porosity as evidenced by an increase in Young's modulus. The water within the polymer matrix not only degrades the polymer, but also plasticizes it. Plasticization of the polymer leads to a decrease in Tg, where the rubbery state is mechanically inferior to the glassy state. The Tg is also affected by breakdown of the polymer chains into monomers, and lower molecular weight oligomers.

3:45 PM GG2.7
BIOERODIBLE POLYPYRROLE. Alexander Zelikin, Venkatram Shastri, David Lynn, Robert Langer, Department of Chemical Engineering and Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA; Ivan Martin, Jian Farhadi, Department of Surgery, Research Division, Kantonsspital Basel, Basel, SWITZERLAND.

Conductive polymers such as polypyrrole (Ppy) have been explored in applications such as microelectronics, analyte sensors, gene chips and substrates for manipulation of cellular functions. Ppy has been explored as guidance channels in sciatic nerve regeneration in rats with good results and shown to possess good biocompatibility. Notwithstanding, the biomedical potential of Ppy has largely been unexplored due to its chemical inertness under physiological conditions. A novel paradigm for the creation of bioerodible conductive materials, wherein the rate of erosion of conductive Ppy

films and pellets is controlled by the hydrolysis of pendant ester groups followed by their ionization is proposed. The premise behind this paradigm is that the hydrolysis of ester pendant groups will result in the formation of water-soluble oligomers of Ppy leading to erosion. We have verified this by demonstrating that the erosion of pellets and films formed from acid functionalized Ppy (Ppy-acid) is a function of the ionization of the acid in that, the rate of erosion is accelerated with increase in pH. We have further demonstrated that the esterification of the acid functionality can be utilized to slow erosion rates thus offering a means for tailoring Ppy with varying erosion profiles. After 60 days of incubation in HEPES buffer (pH 7.2) at 37°C, the mass loss of Ppy-acid pellets was 25%, and that for the ester derivative was 5%. Preliminary studies have shown that these eroding Ppy surfaces are capable of supporting the attachment of primary human bone marrow mesenchymal progenitor cells (MPC). Using real time PCR, it has also been shown that MPC can undergo differentiation into an osteogenic lineage on these erodible Ppy surfaces.

SESSION GG3: IN-ROOM POSTER SESSION
POLYMERIC BIOMATERIALS FOR TISSUE ENGINEERING
Chairs: Kristi S. Anseth and Amy K. Burkoth Poshusta
Monday Afternoon, November 26, 2001
4:00 PM
Hampton (Sheraton)

GG3.1
SYNTHESIS AND PROPERTIES OF NOVEL POLY-ETHYLENEGLYCOL(PEG)-PEPTIDE DIBLOCK COPOLYMERS. Annette Roesler, Harm-Anton Klok, Max-Planck-Institute for Polymer Research, Mainz, GERMANY.

In this contribution we describe the synthesis, purification and structural investigation of novel PEG-b-peptide diblock copolymers, which are of potential interest for applications in drug delivery or tissue-engineering. For these applications it is an advantage if the properties of the materials can be manipulated with external stimuli. The block copolymers described here represent a new approach towards stimuli-sensitive biomaterials. The PEG-block guarantees the compatibility with biological systems. PEG-blocks of different lengths are tested. The block copolymers are prepared by PEGylation of the peptide segment, which is synthesized using Fmoc-solid phase chemistry. Special peptide sequences are used that can undergo reversible pH-dependant structural changes in their secondary structure between β-sheet and α-helices. As a result, the supra-molecular structure of the hydrogels or micelles formed by these diblock copolymers might also be manipulated by changes in pH. CD-, IR- and NMR-spectroscopy are used to observe these structural changes. Main focus of the work is the optimization of the PEGylation reaction of the peptide, the proper selection of the diblock copolymer and the set up of a HPLC method for the separation of byproducts.

GG3.2
AN IN VITRO STUDY OF NANO-FIBER POLYMERS FOR GUIDED REGENERATION. Derick Miller, Karen Haberstroh, Thomas Webster, Purdue University, Department of Biomedical Engineering, West Lafayette, IN.

Biomaterials that successfully integrate into surrounding tissue should match not only the tissue's mechanical properties, but also the dimensions of the associated nano-structured extracellular matrix (ECM) components. The goal of this research was to use these ideals to develop a synthetic, nano-structured, resorbable, polymeric biomaterial that has cytocompatible and mechanical behaviors similar to that of natural vascular tissue. In a novel manner, poly-lactic acid/poly-glycolic acid (PLGA) polymers (50:50 wt.% mix) were synthesized to possess a range of fiber dimensions in the nanometer regime. Preliminary results indicate that decreasing fiber diameter enhances arterial smooth muscle cell adhesion; specifically, arterial smooth muscle cell adhesion increased 15% when polymer fiber diameters decreased from 500 to 50nm. Moreover, ECM proteins (Collagen Type I, III, & IV, fibronectin, elastin, etc.), which will be used to adhere select cell lines to specific regions in the polymer, were analyzed for their ability to selectively enhance arterial smooth muscle cell adhesion after 4 hours. Preliminary results indicate that, compared to other proteins tested, Collagen Type III selectively enhanced arterial smooth muscle adhesion; specifically, compared to albumin, smooth muscle cell adhesion was 60% greater on Collagen Type III. Results of arterial endothelial cell adhesion as a function of nanometer polymer fiber diameter and ECM proteins will also be presented.

GG3.3
FORMATION OF FIBRINOGEN-BASED HYDROGELS USING PHOTOTRIGGERABLE LIPOSOMES. Pochi Shum, David H. Thompson, Junhwa Shin, Zhi-Yi Zhang, Purdue Univ., Dept. of Chemistry, West Lafayette, IN.

Messersmith and coworkers have recently described a technique for producing fibrinogen-based hydrogels via thermally-triggered release of Ca^{2+} from temperature-sensitive liposomes.[2]. We now report an extension of our "cascade" triggering method[3] for producing rapidly gelating fibrinogen-based protein hydrogels using photosensitive calcium-loaded liposomes. Interdigitation-fusion liposomes (IFL), comprised of 38 mol% diplasmenylcholine (DPPlsC) and 57 mol% disteroylphosphatidylcholine (DSPC), were formed in the presence of 10mM $CaCl_2$ and rendered photoactivatable by inclusion of 5 mol% bacteriocholorophyll (Bchl) within the liposomal bilayer membrane. Continuous irradiation (800nm, 800mW/cm^2) of these liposomes under aerobic conditions lead to the photooxidatively-induced leakage of greater than 90% of entrapped Ca^{2+} within 15 minutes. IFL were then used to activate the transglutaminase-catalyzed crosslinking of fibrinogen via Ca^{2+} photorelease. A mixture of Ca^{2+} -loaded IFL, fibrinogen, and a Ca^{2+} -dependent transglutaminase enzyme remained fluid in the dark, but gelled rapidly when irradiated in the presence of air at 800nm and 37°C. SDS-PAGE analysis of the reaction mixture showed that gelation was due to enzymatic crosslinking of the fibrinogen α - and γ -chains. Cryofractured hydrogel samples were analyzed by SEM, revealing a microporous structure with pore diameters ranging between 4-8 μm. This phototriggerable hydrogel system can also be used to readily produce gradient and patterned biomaterial scaffolds. Photoresponsive liposomes based on more readily prepared plasmenylcholine precursors[4] can also be used to activate this system. Potential applications of this phototriggerable hydrogel system in drug delivery will be discussed.

References
1. The authors would like to acknowledge the support of NIH Grants GM55266 and DE 13030.
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GG3.4
AN INVESTIGATION OF NANO-STRUCTURED CO-POLYMERS FOR USE AS THREE-DIMENSIONAL BLADDER TISSUE CONSTRUCTS. Anil Thapa, Thomas J. Webster, and Karen M. Haberstroh, Purdue Univ, Dept of Biomedical Engineering, West Lafayette, IN.

Conventionally, studies investigating the design of synthetic bladder wall substitutes have involved polymers with micro-dimensional fibers. Since the body is made up of nano-structured components, our focus has been in the use of nano-structured polymers in order to design three-dimensional synthetic bladder constructs that mimic bladder tissue in vivo. Bio-inspired nano-structured copolymers of Poly (lactic acid) and Poly (glycolic acid) have been synthesized by heat-dissolving co-polymer palates (0.5g; Polysciences, Inc.) in chloroform. The copolymers were left partially covered at room temperature and were vacuum dried (at 15 inch gauge pressure) to allow the chloroform to evaporate. Polymer scaffolds (0.5mm x 1mm) were cut from the bulk polymer film and were soaked for various amounts of time (10 min, 30 min and 1 hr) in select concentrations of NaOH (0.1 N, 5 N and 10 N). Scanning electron micrograph images of the control (NaOH untreated) and nano-structured (NaOH untreated) samples provided evidence that treating scaffolds with 10 N NaOH for 1 hr resulted in greatest reduction in polymer fiber diameter. We are presently conducting cell-adhesion experiments using ovine bladder smooth muscle cells and urothelial cells (the two major cell lines that comprise the bladder wall tissue) to investigate the cytocompatibility of these novel nano-fibered polymers compared to conventional, micro-structured polymer. Results of these experiments will be presented.

GG3.5
SURFACE CHARACTERIZATION OF PROTEIN MICRO-PATTERNS FOR DIRECTED CELL GROWTH. Bryan A. Langowski, Gordana Dukovic, Kathryn E. Uhrich, Rutgers University, Dept of Chemistry and Chemical Biology, Piscataway, NJ.

Surface chemical characterization by x-ray photoelectron spectroscopy (XPS) and contact angle was performed on protein-patterned substrates. These substrates are glass micropatterned with proteins such as laminin and bovine serum albumin using photolithography as well as poly(methylmethacrylate) (PMMA) micropatterned with laminin only using either photolithography or microcontact printing. Chemical changes of the substrate surfaces were monitored following each step of the patterning procedure. Samples photolithographically patterned exhibited incomplete protein coverage, and salts from the buffer and developer solutions were detected. PMMA samples prepared by microcontact printing showed increased oxygen content due to oxygen plasma treatment. Surprisingly, silicon was detected on

the PMMA surface, indicating the transfer of matter from the poly(dimethylsiloxane) (PDMS) stamp. The processing factors that affect the transfer of PDMS from the stamp to the PMMA substrate were analyzed by XPS and scanning electron microscopy (SEM).

GG3.6
CELL MICROPATTERNING SUBSTRATES VIA ONE-PHOTON-INDUCED POLYMERIZATION. Elisabeth B. Walsh, Nicole H. Grynaviski, Mark W. Grinstaff, Duke Univ, Dept of Chemistry, Durham, NC.

Cell patterning substrates are of interest for several biological applications. For example, the shape and size of a cell pattern can directly influence cell morphology, adhesion, and motility. Patterning also offers a means to monitor biological processes in a single cell. Further, few options are currently available to create heterogeneous patterns on a single substrate. In this preliminary study, one-photon-induced polymerization is used to create intricate three-dimensional polymeric microstructures for use as cell patterning substrates. This technique is an alternate patterning procedure to microstamping, with the benefits of ease of use and rapidity. Moreover, two- or three-dimensional polymeric islands of different proportions may be placed on a single substrate. Micron-scale resolution affords control of island shape and size, allowing for both single- and multi-cell patterning. Using a continuous wave diode laser as light source (407 nm, 0.4 mW), simple two- and three-dimensional structures were produced on a glass slide from an aqueous solution of PEG diacrylate monomer, eosin Y initiator, and triethanolamine co-initiator. Patterns were viewed in solution by light microscopy, then isolated and characterized by SEM.

GG3.7
RAPID HEPATIC Co-CULTURE SPHERIOD FORMATION ON PLA. Lisa Riccalton-Banks, Robin Quirk, Rena Bhandari, Kevin Shakesheff, University of Nottingham, Pharmaceutical Sciences, Nottingham, UNITED KINGDOM.

The functionality of isolated hepatocyte cultures is generally short lived and is a challenge that must be over come in the endeavour to engineer functional liver tissue. Various methods have been employed in the attempt to maintain differentiated hepatocyte function and several studies have shown the benefits culturing hepatocytes with other cells (Bhandari et al.(2001)). In vivo hepatocytes are organised in a 3-dimensional lobular structure. It has been suggested that the two-dimensional environment in which monolayer cultures are grown, may impede some normal regulatory process. Previous work has shown that hepatocyte cell aggregates can be formed when the cells are cultured on positively charged polystyrene, poly-HEMA or spinner flasks. In these multicellular aggregates the cell-cell contact is maximised and a three-dimensional cyto-architecture, with respect to extracellular components, is attained. Here we describe the rapid self-assembly of multicellular structures when cultured on the non-adhesive substrata of poly (lactic acid) (PLA). Primary rat hepatocytes were co-cultured with hepatic stellate cells on both PLA and tissue culture plastic surfaces. The self-assembly of cell aggregates was observed over time via phase contrast microscopy. The staining of the stellate cells with CellTracker(TM) allowed the identification of theses cells within the cultures. Spheroid formation was found to occur at an earlier time point when the cells were cultured on the PLA surface. SEM images show the round three-dimensional spheroids. The functionality of spheroids formed in this manner was assessed in a number of ways. The activity of cytochrome P-450 enzymes, a classical liver function marker, was positively detected by ethoxyresorufin-o-dealkylase (EROD) assay. The production of albumin, another common indicator of hepatocyte function, was measured via an enzyme linked immunosorbent assay (ELISA). Confocal microscopy of the aggregates incubated with a Live/Dead stain allowed the visualisation of the viability of the cells within the spheroids.
References:
R. Bhandari, L. Riccalton, A. Lewis, J. Fry, S. Tendler, K. Shakesheff (2001) Liver Tissue Engineering: A Role for Co-culture Systems in Modifying Hepatocyte Function and Viability. Tissue Engineering., 7:345-357.

GG3.8
ENCAPSULATION OF MAMMALIAN AND BACTERIAL CELLS. Jill A. O'Loughlin, Michael J. Lysaght, Brown University, Dept of Molecular Pharmacology, Physiology and Biotechnology, Providence, RI.

This study compares the microencapsulation in alginate of mammalian and bacterial cells. A vibrating spinneret (frequency at 1000 Hz) was utilized to encapsulate cells in a sterile 1.8% sodium-alginate solution (Inotech). The mammalian cells were primary fibroblasts isolated from sheep heart valve and provided by Dr. Hoffman-Kim at Brown University. The bacterial cells were E. Coli DH5 cells (ATCC). The average measured bead diameter was 1114μm $\pm$ 214 SD as determined using a Coulter particle sizer. All microcapsules were maintained for two weeks in vitro. The encapsulated fibroblasts were kept in a growth medium consisting of Medium 199, 10% Fetal Bovine Serum, and 1% Penicillin-Streptomycin (Gibco). The cells were stored in a humidified 5% CO_2 incubator. The encapsulated bacterial cells were also maintained in a similar incubator with Luria-Bertani medium (10 g/L tryptone, 5 g/L yeast extract (Difco), and 10 g/L sodium chloride (Sigma). We found both the fibroblasts and bacterial cells proliferated in the alginate microcapsules. The E. Coli have a rapid doubling rate, on the order of 15-20 minutes at the optimal growing condition. In contrast, the fibroblasts are slower and take a few days at first and eventually a week or two to double in population. Early pass morphology showed many small cells which divide frequently, and later as the cells age, replication slows down, and they become metabolically quieter and larger as they search to make cell contact. E. Coli cells had a greater growth and viability post encapsulation as observed with digital microscopy, and therefore maintained a higher intracapsular density. Fibroblasts were visualized with trypan blue, which is an indicator of cell membrane integrity. Sterility was a variable in this study, since bacterial may thrive in a non-sterile environment, whereas sterility must be preserved for upkeep of fibroblast cells. We conclude that the fabricated alginate matrix is sufficient to sustain fibroblasts as well as E. Coli cells for up to 2 weeks and that the principle difference between the two cell classes is in proliferation rates.

SESSION GG4: BIOMATERIAL PROCESSING AND NOVEL CHEMISTRIES
Chairs: Kevin Thorne and Kristi S. Anseth
Tuesday Morning, November 27, 2001
Gardner (Sheraton)

9:00 AM GG4.1
ORGANOSILICATE BIOMATERIALS: FROM MONOLITHS TO NANOPARTICLES. Suniti Moudgil and Jackie Y. Ying, MIT, Dept of Chemical Engineering, Cambridge, MA.

Doped silicate materials such as Bioglass have been recognized in orthopedic medicine for their ability to bond quickly and strongly to bone. However, their clinical applications have been limited due to high-temperature processing conditions and low mechanical strength. We have synthesized materials of similar compositions through low-temperature sol-gel techniques for use as a void-filling cement. While the bulk materials increased cell proliferation, these monoliths remained reactive under aqueous conditions, thus limiting their in vivo potential. In order to exploit the advantages of the sol-gel technique while ensuring more complete reaction of the sol-gel monomer at low temperatures, we have minimized the reaction domains by synthesizing nanoparticles with compositions similar to those of Bioglass. Monodisperse organosilicate particles of 10 nm - 1μm have been successfully achieved. With the flexible surface chemistry of silicates, these particles can easily be functionalized with various organic groups. These nanoparticles are being investigated for use as a filler for polymer cements to enhance mechanical strength, and as a colloidal suspension to elicit specific osteoblast behavior. The effects of particle size, concentration and surface chemistry on the mechanical properties of a polymethylmethacrylate (PMMA) matrix have been studied. Low loadings of nanoparticles have been shown to increase both the Vickers hardness and bending strength of PMMA. Particle size and composition have also been shown to affect MC3T3 osteoblast behavior. Cell culture experiments indicated that some organosilicate particles significantly increased osteoblast proliferation. Fluorescent microscopy indicated that these unique particles were ingested by cells and/or adhered strongly to cell surfaces. Subsequent investigation with transmission electron microscopy (TEM) showed that certain nanoparticles entered the cell cytoplasm. The mechanism for nanoparticle uptake and its effect on cell behavior will be discussed in this presentation.

9:15 AM GG4.2
POROUS POLYMER/BIOACTIVE GLASS COMPOSITES FOR SOFT-TO-HARD TISSUE INTERFACES. Kai Zhang, University of Minnesota, Department of Chemical Engineering and Materials Science, Minneapolis, MN; Mary E. Grimm, University of Minnesota, Department of Biomedical Engineering, Minneapolis, MN; Qiwei Lu, University of Minnesota, Department of Chemical Engineering and Materials Science, Minneapolis, MN; Theodore R. Oegema, Jr., University of Minnesota, Departments of Orthopaedic Surgery and Biochemistry, Minneapolis, MN; Lorraine F. Francis, University of Minnesota, Department of Chemical Engineering and Materials Science, Minneapolis, MN.

Bonding of artificial soft tissues, such as cartilage, to hard tissues, such as bone, presents a challenge. Porous polymer/bioactive glass composites are candidate materials for engineering the artificial cartilage/bone interface and possibly other soft/hard tissue

(ligament/bone, tendon/bone) interfaces. A liquid-liquid phase separation technique was used to make porous polymer/bioactive glass composites. Porous polyurethane/bioactive glass composites and polysulfone/bioactive glass composites along with porous pure polymer sheets were prepared. The effects of polymer type, concentration and molecular weight, as well as bioactive glass size and content, on the microstructures of the composites were studied. The composite sheets (thickness: 200-500μm) have asymmetric structures with dense top layers and porous structures beneath. The porous structures consist of large pores (>100μm) in network of smaller (<10μm) interconnected pores. The microstructural features depend most strongly on the type of polymer, the interaction between the polymer and bioactive glass, and the glass content. The dense layers were removed and large pores exposed by: (1) abrasion or; (2) salt leaching from the casting surface. The tissue bonding abilities of the composites were studied in vitro in simulated body fluid (SBF) and in rabbit chondrocyte culture. The growth of hydroxycarbonate apatite (HCA) inside and on the composites after soaking in the SBF for two weeks demonstrates their potential for integration with bone. No apatite was found for the pure porous polymer sheets after soaking in SBF for 2 weeks. Culture studies revealed that composite surfaces were suitable for attachment, spread and proliferation of chondrocytes. The results indicate the potential for the composites to facilitate growth and attachment of artificial cartilage.

9:30 AM GG4.3
IN-SITU MINERALIZATION OF HYDROXYAPATITE FOR A MOLECULAR CONTROL OF MECHANICAL RESPONSES IN HYDROXYAPATITE-POLYMER COMPOSITES FOR BONE REPLACEMENT. Kalpana Katti, Praveen Kumar Gujjula, Arunkumar Ayyarsamy, Timothy Arens, North Dakota State University, Department of Civil Engineering, Fargo, ND.

Insitu mineralization of hydroxyapatite (HAP) and role of organics in initial nucleation and growth of HAP is critical for the resulting nano and microstructure of HAP. Insitu mineralization of hydroxyapatite (HAP) in the presence of Ca binding polymers such polyacrylic acid has shown some promise towards improvement of mechanical response of uniaxial compressed HAP/polymer composites to loading. This work represents fundamental studies on the nature of insitu HAP precipitation on resulting microstructure of the composite and bulk mechanical properties. Specifically, an experimental study, evaluating role of initial stage mineralization of HAP on bulk mechanical responses is conducted. Fourier transform infrared (FT-IR) spectroscopic (with micro attenuated total reflectance) techniques are utilized to evaluate the association of polymer (polyacrylic acid) with HAP during mineralization of HAP. Insitu HAP exhibits a faster mineralization as compared to the exsitu mineralization samples, This improved kinetics is responsible for altering the resulting micro and nanostructure of the HAP/polymer composite. Small spectral changes are detected in the absorbance spectra of insitu HAP as compared to exsitu samples. Changes in mechanical response to loading included improvement in strain-to-failure and resulting toughness characteristics of the insitu composite. The control and development of molecular-level associations of polymer with HAP is suggested to be critical for the resulting macro properties. Our results may have significant implications in design of nanocomposites for biomedical applications.

9:45 AM GG4.4
CHARACTERIZATION OF TISSUE DEVELOPMENT IN CO-EXTRUDED POLYMERIC SCAFFOLDS. Newell R. Washburn, Alamgir Karim, Eric J. Amis, Polymers Division, NIST, Gaithersburg, MD; Kimbleree Potter, AFIP, Rockville, MD.

An investigation of osteoblastic cell proliferation in co-extruded polymer scaffolds is presented. Poly(ethyl methacrylate) scaffolds having continuous porous networks with characteristic length scales ranging from 20-100 micrometers were prepared. Cells seeded on the scaffolds were cultured in a perfusion bioreactor. Matrix deposition was monitored in situ with magnetic resonance imaging and, after 6 weeks, tissue development was assessed using histological techniques. The influence of scaffold pore size and geometry on cell activity will be discussed.

10:30 AM GG4.5
BIOABSORBABLE MEMBRANE AS TISSUE SURROGATES FOR BONE CELLS. Kwangsok Kim, Wanda Chen, Meiki Yu, Steven Zhong, Dufei Fang, Benjamin Hsiao, Benjamin Chu, SUNY at Stony Brook, Dept of Chemistry, Stony Brook, NY; Michael Hadjiargyrou, SUNY at Stony Brook, Dept of Biomedical Engineering, Stony Brook, NY.

Poly(lactide) (PLA) has been widely studied for medical applications because of its biodegradability as well as biocompatibility. However, PLA generally possesses slow biodegradation rate, and its formation in film is usually too stiff for many applications. In this study, we will demonstrate a unique fabrication technique using electrospinning to produce a nanostructured flexible membrane, which was found to be an ideal vehicle for storage and delivery of tissue cells. The membrane possesses randomly interconnected webs of submicron size fibers, which biodegradation properties can be controlled by processing parameters and material compositions. We will also demonstrate the incorporation of bone cells in these membranes. We found that the cell release efficiency can be adjusted by the membrane morphology, and the cell survival rate is very high.

10:45 AM GG4.6
ANHYDROUS SCAFFOLD FABRICATION FROM SENSITIVE BIODEGRADABLE POLYMERS. Michael Hacker, Joerg K. Tessmar, Markus Neubauer, Esther Lieb, Torsten Blunk, Achim Goepferich, Michaela B. Schulz, Dept of Pharmaceutical Technology, University of Regensburg, Regensburg, GERMANY.

The need for biodegradable polymers that allow to control cell-biomaterial interactions for tissue engineering applications is constantly rising. One strategy to satisfy this demand focuses on the synthesis of polymers that allow to covalently bind bioactive proteins and peptides to the polymer surface. In many cases these materials contain functional groups which are prone to hydrolysis. To process such polymers into macroporous scaffolds an anhydrous small scale fabrication technique is required. Furthermore, the generated pore microstructure should be highly interconnected to ensure an even distribution of seeded cells and their sufficient nutrient supply. A recently published processing technique is promising to fulfill these needs [1]. With paraffin microparticles dispersed in solutions of polymer in halogenated solvents, polymer foams were obtained after hexane leaching. Although this technique avoids the use of water, problems evolve from the high extraction temperature, the low biocompatibility of paraffin and solvent. The goal of our study was to overcome these problems by using triglyceride microparticles as porogen and non-halogenated solvents. With poly(D,L-lactic acid)-block-poly(ethylene glycol)-monomethyl ether copolymers (Me.PEG-PLA) as model polymers, we identified the decisive process parameters for scaffold fabrication and tested scaffold functionality using rat marrow stromal cells. Extraction temperature and time, as well as polymer to porogen ratio and amount of polymer solvent were found to be key parameters. Especially the extraction temperature had to be carefully adapted to glass transition temperature of the polymer to avoid detrimental effects on the scaffold structure. Concomitantly, the melting point of the lipid particles had to be adjusted precisely to the extraction temperature to obtain an interconnected pore structure. The correlation of process parameters with polymer properties allowed us to use this technique for processing different polymer types, such as poly(D,L-lactic-co-glycolic acid) and amine reactive PEG-PLA derivatives, without the need of laborious experiments.
[1] V.P. Shastri et al., PNAS, Vol. 97 (2000), No. 5, 1970-1975.

11:00 AM GG4.7
IMPROVED SYNTHESIS OF SALICYLATE-BASED POLY-ANHYDRIDES. R.C. Schmeltzer, Theodore J. Anastasiou, Kathryn E. Uhrich, Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ.

We developed an improved synthetic procedure for polymeric prodrugs that hydrolytically degrade into pharmacologically active components. These poly(anhydride-esters) are composed of sebacic acid linked to either salicylic acid, and 4- or 5-aminosalicylic acid. Salicylic acid is an antipyretic, anti-inflammatory analgesic used for a variety of medical applications. The aminosalicylates are currently used for treating similar conditions such as tuberculosis and inflammatory bowel disease. By incorporating these compounds into the polymer backbone, the mechanism and kinetics of drug release is controlled as a function of the polymer chemistry. We previously reported one coupling step in the prepolymer synthesis that involved protection of the reactive carboxylic acid group of the salicylates, followed by deprotection once the coupling was complete. Herein, we report a modified synthetic route that eliminates the need for the carboxylic protection/deprotection steps. The current method yields prepolymer in quantitative conversion in fewer steps, thus increasing the overall yields. We also report a modification of the "traditional" melt-condensation polymerization that results in materials with higher molecular weights. Previous melt-condensation polymerizations of the poly(anhydride-esters) were done according to methods established by Conix in the 1950's, with molecular weights ranging from 2500 to 6000. Our modified method yielded molecular weights of the same polymers up to 45000. These improvements and novel apparatus for melt-condensation will be discussed.

11:15 AM GG4.8
NEW SUBSTITUTED POLYLACTIDES AS CANDIDATE MATERIALS FOR TISSUE SCAFFOLDS. Gregory L. Baker, Milton R. Smith III, Mao Yin, Tianqi Liu, Chun Wang, Department of

Chemistry and Center for Fundamental Materials Research, Michigan State University, East Lansing, MI.

Two critical physical properties of polymers used for tissue scaffolds are their glass transition temperature and their hydrolytic degradation rate under physiological conditions. Most scaffolds are based on poly(lactide), poly(glycolide) and glycolide/lactide copolymers, and thus the range of properties that can be extracted from these two polymer systems is limited. One strategy for expanding the range of properties available from lactic acid polymers is through the use of substituted lactic acids as the starting materials for degradable polymers. We have found that elaborating the methyl group of lactic acid leads to high molecular weight degradable polymers with glass transitions ranging from -35 C to 100 C. In addition, copolymers prepared from these new monomers provide predictable control over the glass transition and degradation rates.

11:30 AM GG4.9
SYNTHESIS AND CHARACTERIZATION OF DENDRIMERS FROM α-HYDROXY AND AMINO ACIDS. Meredith T. Morgan and Mark W. Grinstaff, Duke University, Depts of Chemistry and Ophthalmology, Durham, NC.

Dendrimers are three-dimensional polymers formed through iterative additions of branching units to a multi-functional core. Characteristics of dendrimers include a globular structure, high surface area to volume ratio, high density, low viscosity, and a large number of end units which can be functionalized. The generation number of the dendrimer increases with each successive coupling, doubling the number of end units and increasing the molecular weight exponentially. We are synthesizing novel dendrimers from monomers that are known to be either natural metabolites or biocompatible and thus termed biodendrimers. Specifically, our dendrimer monomer units are glycerol, glycolic acid, and serine. We have constructed biodendrimers through condensation reactions to form ester linkages between branching units and multifunctional cores.
Cis-1,3-O-benzylidine-(2-O-acetic acid)-glycerol (BG-GA) was synthesized by reacting cis-1,3-benzylidine glycerol (BG) with chloroacetic acid in the presence of NaH in dioxane at 45°C for 24 hours. DCC chemistry was used to couple BG-GA with serine ethyl ester HCl in the presence of DCC, HOBt, TEA to yield the serine derivitized building block (GLGASER). The primary alcohol on the alpha carbon of serine was then protected with a silyl group to yield the dendrimer branching unit GLGASER-Si. GLGASER-Si was then coupled with BG in a DCC reaction and deprotected via catalytic hydrogenolysis to afford the dendrimer core G0-P(GLGASER-Si)-OH. Further generations were formed by DCC coupling reactions of GLGASER-Si branching units to the dendrimer core followed by subsequent activation/deprotection of the branching units via catalytic hydrogenolysis. The physical characteristics of these biodendrimers, including their ability to degrade to natural metabolites and the large number of functionalizable end groups, suggest that biodendrimers are ideal for use in several important biomedical engineering applications.

11:45 AM GG4.10
IN VITRO SYNTHESIS OF POLYHYDROXYALKANOATE BY IMMOBILIZED PHA SYNTHASE. Young-Rok Kim, Hyun-Jong Paik, Carl Batt, Cornell Univ, Nanobiotechnology Center, Ithaca, NY; Geoffrey Coates, Cornell Univ, Dept of Chemistry and Chemical Biology; Christopher Ober, Cornell Univ, Dept of Materials Science and Engineering.

A system is currently under development to fabricate microscale systems for biopolymer production in an attempt to generate material with enhanced functionality. Polyhydroxyalkanote (PHA) is an aliphatic polyester produced in nature. PHA has received significant interest from industry and academia because it is a biocompatible and biodegradable thermoplastic with potential applications in consumer and medical products. Currently, PHA is largely produced using microorganisms, although nascent efforts to express it in plants are being made. One promising research area is in vitro synthesis of PHA, which will potentially permit the formation of unique crystalline forms of the polymer. For the first time, we have developed an immobilized in vitro system for the production of PHA. PHA synthase genetically engineered with a His-Tag peptide was immobilized on various supports, including agarose, gold, glass and anodic alumina, through a Ni-NTA linker. PHA synthase retained the ability to synthesize PHA in its immobilized form. The morphologies and properties of synthesized biopolymer documented.

SESSION GG5: *IN-SITU* FORMING BIOMATERIALS AND BIOMATERIALS WITH CONTROLLED STRUCTURE
Chairs: Kevin Thorne and Jennifer L. West
Tuesday Afternoon, November 27, 2001
Gardner (Sheraton)

1:30 PM *GG5.1
TUNING THE PROPERTIES OF ARTIFICIAL EXTRACELLULAR MATRICIES. Kevin E. Healy, Depts. of MS&E and Bioengineering, Univ. of California at Berkeley, Berkeley CA; Ranee A. Stile, Northwestern University, Evanston, IL.

We have embarked on a long-term project to create artificial polymeric extracellular matrices (ECMs), or scaffolds, that are environmentally responsive and tunable with respect to mechanical properties (e.g., G*), incorporation of biological ligands, and degradation by proteases. In general, polymer scaffolds do not integrate well with either cells or tissues, which decreases the likelihood that regenerated or repaired tissue will fully integrate with the native existing tissue. One approach to circumvent this problem is to use peptide sequences as ligands to promote bimolecular engagement between either receptors on the surfaces of cells or proteins of the native ECM. To achieve this goal, we have exploited the phase behavior of poly(N-isopropylacrylamide) [p(NIPAAm)] in aqueous media to synthesize injectable hydrogels containing NIPAAm and acrylic acid (AAc) monomers. When heated from 22°C to body temperature (i.e., 37°C), the p(NIPAAm-co-AAc) hydrogels demonstrated a significant increase in G* (i.e., rigidity) without exhibiting a significant change in either volume or water content. To induce direct interaction between cells and the p(NIPAAm-co-AAc) hydrogels, we functionalized the AAc groups in the hydrogels with peptides containing relevant sequences found in ECM macromolecules. The chemical modification of the hydrogels has been verified via solid-state 1H magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectroscopic, lower critical solution temperature (LCST), and volume change studies. The peptide-modified P(NIPAAm-co-AAc) hydrogels were viscous at 22°C and could be injected through a small-diameter aperture. Rat calvarial osteoblasts (RCO) seeded into these peptide-modified hydrogels were viable for at least 21 days of in vitro culture and demonstrated significantly greater proliferation when cultured within the peptide-modified p(NIPAAm-co-AAc) hydrogels, as compared to control hydrogels. Environmentally-responsive hydrogels can be tuned to incorporate native functions of the ECM into the polymer and serve as ideal materials to test hypotheses addressing regulation of tissue regeneration.

2:00 PM GG5.2
IN SITU FORMING HYDROGELS USING SELF-ASSEMBLY OF FLUOROALKYL-ENDED POLY(ETHYLENE GLYCOL) FOR SUSTAINED RELEASE OF THERAPEUTIC PROTEIN. Giyoong Tae, Julia A. Kornfield, California Institute of Technology, Dept of Chemical Engineering, Pasadena, CA; Jeffrey A. Hubbell, Inst for Biomedical Engineering and Dept of Materials, ETH-Zurich and University of Zurich, Zurich, SWITZERLAND.

A new class of materials and method to induce in situ transitions from an injectable state to a hydrogel using self-assembly of associating polymers is described for applications that include tissue engineering and controlled release. Poly(ethylene glycol)s modified with fluorocarbon end groups form hydrogels by the hydrophobic interaction of the ends. The physical properties of the gel can be systematically tuned over a broad range by choice of PEG and fluoroalkyl lengths. The hydrogel state can be transformed into an injectable state by the addition of a bio-tolerable organic solvent, such as N-methyl pyrrolidone (NMP), and this solution can be restored to a gel state quickly after injection by removal of the organic solvent by diffusion. In vitro characterization of sustained release of human growth hormone (hGH) using this injectable depot shows that hGH remains stable inside the hydrogel, and is released over a prolonged period (2-3 weeks) without irreversible aggregation or an initial burst. The rate of release appears to be controlled by diffusion of hGH through the hydrogel. Preliminary in vivo experiments show that the material is very well tolerated subcutaneously in rats.

2:15 PM GG5.3
DEVELOPMENT OF *IN SITU* FORMING 3-D LACTIC ACID BASED POLYMER SCAFFOLDS FOR BONE TISSUE ENGINEERING. Jason A. Burdick, Univ of Colorado, Dept of Chemical Engineering, Boulder, CO; Kristi S. Anseth, Univ of Colorado, Howard Hughes Medical Institute, Dept of Chemical Engineering, Boulder, CO.

Although bone has the ability to heal itself, there are many instances (e.g., significant bone loss due to extreme trauma or tumor removal) when biomaterials may accelerate the regeneration process. For such applications, an ideal orthopaedic biomaterial should have controllable

degradation, be osteoconductive (promote bone cell attachment), and be formed *in situ* to eliminate *ex vivo* implant fabrication and allow for facile filling of irregular shaped bone defects. With these criteria in mind, tetrafunctional lactic acid oligomers were synthesized by a ring opening polymerization of lactide on low molecular weight ethylene glycol cores that were subsequently acrylated or methacrylated. These oligomers react via a photoinitiated polymerization to form highly crosslinked degradable networks with high conversions reached on a clinical time scale (<5 minutes). The hydrophobicity and, consequently, the degradation rate of these networks are readily altered through changes in the oligomer chemistry (i.e., number of ethylene glycol and lactic acid repeat units). Networks have been synthesized with degradation ranging from ~20 to 55% mass loss after eight weeks by simply changing the oligomer composition. Additionally, the viability and function (e.g., alkaline phosphatase activity and mineralization) of primary rat calvarial osteoblasts attached to network surfaces are dependent on the oligomer chemistry. One specific oligomer, 2EG10LA (diethylene glycol core with 10 lactic acid units on either side), formed networks with osteoconductive properties similar to that of tissue culture polystyrene and 50:50 poly(lactic acid-co-glycolic acid). 3-D polymer scaffolds with a controlled macroscopic architecture were readily fabricated from this material by mixing a poragen with the macromer, subsequently photopolymerizing the material, and then leaching the poragen from the scaffold. These scaffolds were seeded with osteoblasts and cultured in a spinner flask for periods up to eight weeks. Preliminary results indicate that osteoblasts attached throughout these scaffolds proliferate and produce collagen networks with culture time.

2:30 PM GG5.4
CHARACTERIZATION AND DEGRADATION OF AMINO-SALICYLATE POLYANHYDRIDE ORAL PRODRUGS.
Theodore J. Anastasiou and Kathryn E. Uhrich, Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ.

Aminosalicylic acids are currently used for treating a variety of conditions such as tuberculosis and inflammatory bowel disease. However, side-effects, serum half-lives, and the need for targeted delivery limit their usefulness. To overcome these problems, a series of polyanhydrides containing 4- and 5- aminosalicylic acid backbones linked through either ester, amide, or azo bonds were synthesized. In these materials, polymer degradation and thus drug release occurs via the catalyzed hydrolysis (pH or enzyme) of the anhydride bond as well as the azo, amide, or ester bonds. Because of this enzyme and/or pH dependence, polymer degradation, and hence drug release can be targeted to the gastrointestinal system where the pH ranges from approximately 1 through 8. The chemical structures of these novel materials were confirmed by NMR and IR spectroscopy. Molecular weights were determined by gel permeation chromatography (GPC) and/or viscosity measurements, and were approximately 9,000 amu. Thermal characterization was performed by thermogravimetric analysis (TGA) (Td) and differential scanning calorimetry (DSC) (Tg, Tm). Td's were all above 350°C, with a notable absence of Tg's, likely due to the rigid nature of the materials as well as inter- and intra-molecular hydrogen bonding. The relationship between aminosalicylic acid structure (4- or 5- substitution) and backbone linkage (amide, azo, ester) on polymer degradation rate and drug release were investigated. The degradation of these materials was carried out at acidic, neutral and basic pH's, ranging from 3 to 8, in absence of enzymes, and at neutral pH in the presence of esterases, amidases, and azo reductases. Polymers were solvent-cast into films, then degraded in phosphate buffer at the appropriate pH's at 37°C. At defined time points the spent buffer was removed, replaced with fresh solution, and analyzed by HPLC to monitor the drug release.

2:45 PM GG5.5
TEMPERATURE-INDUCED PHASE TRANSITION OF HYDROXYPROPYL CELLULOSE (HPC) AND POLY(ACRYLIC ACID) (PAA) COMPLEX.
Xihua Lu, Zhibing Hu, Jacob Schwartz, Tong Cai, and Jie Lin, Univ of North Texas, Dept of Physics and Materials Science, Denton, TX.

Hydroxylpropyl cellulose (HPC) and polyacrylic acid (PAA) complexes were studied using turbidity measurements and laser light scattering. The phase transition temperature of the complexes proved to depend dramatically on PAA concentration, HPC concentration, and pH. The driving force for this phenomenon seems to be due to the hydrogen bonding and hydrophobic interaction of the macromolecules. The phase transition temperature was also found to be dependent on the molecular weight of two polymers. The phase transition temperature of the complexes decreased with the increase of the molecular weight due to stronger interaction in higher molecular weight. When PAA and HPC concentrations are closer, the complexes are more stable above the phase transition temperature. The phase transition of the complexes shifts to higher temperature with increase in pH. The application for this complexes would be used for controlled drug release and bioadhesive drug delivery systems based on environmental stimuli such as pH or temperature.

3:30 PM GG5.6
POLYELECTROLYTE MULTILAYERS: A NANOSCALE APPROACH FOR CREATING CELL-INTERACTIVE SURFACES.
Jonas D. Mendelsohn, Sung Yun Yang, Allon Hochbaum, Michael F. Rubner, Massachusetts Institute of Technology, Dept of Materials Science and Engineering, Cambridge, MA.

The layer-by-layer (LbL) processing of polyelectrolytes, whereby oppositely charged polymers are repeatedly and sequentially adsorbed onto a substrate to yield multilayer ultrathin films one molecular layer at a time, has emerged in recent years as a promising approach for creating polymer films with nanoscale control of structure, composition, and surface properties. Applications ranging from opto-electronic devices to biosensors to tailorable surface coatings all have been exploited using this simple yet highly versatile technique. Recently, we have explored the use of thin film multilayer assemblies composed of alternating layers of the weak polyelectrolytes poly(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH) and the hydrogen bond-forming polyacrylamide (PAAm) to create cell-interactive surfaces. With subtle changes in the polymer pH assembly conditions, which thus govern the resulting multilayer thin film architecture, it is possible to create either bio-interfaces to which cells from a model murine fibroblast cell line readily adhere or, on the contrary, surfaces that are essentially bio-inert and prevent all observable cell attachment. The ability to micropattern cell adhesion molecules (e.g., fibronectin) onto an otherwise bio-inert polyelectrolyte multilayer surface will be demonstrated as a powerful way to direct desired cell adhesion and eliminate non-specific attachment. Furthermore, protein adsorption studies, wettability measurements, and chemical characterization will be used to help explain the cell resistance or adhesiveness of these multilayers. With the ability to create nanostructured conformal coatings with controllable cell behavior onto substrates of any size or shape, including complex medical implants or tissue engineering scaffolds, polyelectrolyte multilayers may introduce a robust new method for effectively engineering biomaterial-tissue interactions.

3:45 PM GG5.7
MICROSTRUCTURAL CONTROL IN DESIGN OF ALGINATE HYDROGELS AS EXTRACELLULAR MATRIX MATERIALS.
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Alginate hydrogels have been extensively studied as synthetic extracellular matrices for soft tissue engineering, due to their biocompatibility and simplicity in gelation. To satisfactorily function as extracellular matrices, the physical properties (e.g. mechanical and degradation behaviors) of the gels should be readily controlled. These properties are key factors for the interaction with cells, and thus the formation of new tissues. We propose to tailor the microstructure of alginate gels by adjusting the size distributions of alginate chains, the composition of the alginate, and the resulting intermolecular interactions in a constitutive manner. The compressive elastic moduli and yield strains of hydrogels, which are ionically cross-linked with calcium ions, are controlled by altering the molecular size of alginate chains via g-irradiation, and by altering the solids concentration. In particular, mixing alginate chains of high molecular weight (M_w ~250,000) with irradiated alginate chains of low molecular weight (M_w ~ 50,000) facilitates the formation of gels with broad ranges of mechanical properties, even at a constant solids concentration. In addition, these binary gels do not show any deterioration in their mechanical properties over a month during incubation *invitro*. To induce the degradation of the binary hydrogels in a controlled manner, while maintaining comparable mechanical properties for a desired period, the number ratios of β-D-mannuronic acid to α-L-guluronic acid ($N_{M/G}$) in the non-irradiated alginate chains are varied to tune the associating forces between cross-linking molecules. The incorporation of alginate chains with higher $N_{M/G}$ enhances the degradation rates, as indicated by changes in elastic moduli and swelling ratios over time. Alginate gels with well-controlled properties will likely be useful for the engineering of a wide variety of tissues, and the approach to microstructural control may be widely applicable to other polysaccharide systems.

4:00 PM GG5.8
ADHESION OF CHONDROCYTES TO MODIFIED ALGINATES: THE ROLE OF CROSSLINK DENSITY AND SUBSTRATE STIFFNESS.
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Alginate is a copolymer of manuronic and guluronic acid that gels in the presence of divalent cations. The degree of crosslinking can be controlled by the concentration and identity of the crosslinker.

Alginate is used for the 3D culture of chondrocytes because it keeps the cells in a spherical conformation, promoting the differentiated phenotype. The grafting of RGD adhesion ligands to alginate has been shown to enable chondrocytes to form integrin-mediated cytoskeletal attachments to alginate and that cell-mediate crosslinking of the material alters the mechanical properties of the gel. The factors regulating this attachment have not been characterized, though the adhesion of other cell types has been shown to be regulated by substrate mechanics. Chondrocytes were isolated from calf articular cartilage by collagenase digestion and cultured in T-175 flasks with F-12 and 10% FBS for one week. Cells were resuspended at 50,000 cells / mL in serum-free media and transferred to wells coated with 2% control and RGD-alginate crosslinked with Ca^{2+} or Ba^{2+}, ranging from 5.0 to 62.5 mmol / g alginate. Chondrocyte attachment to RGD-alginate, as measured by phase contrast microscopy, fit first-order binding kinetics models. The equilibrium attachment level increased, and the characteristic time constant of attachment decreased, as Ca^{2+} crosslinker density rose. The stiffness of RGD-alginate surfaces was calculated by measuring the resultant forces after applied uniaxial confined compression. At low crosslinker densities, samples from Ba^{2+} surfaces had similar moduli to those surfaces crosslinked with high density Ca^{2+}. Equilibrium attachment for low density Ba^{2+} surfaces was similarly consistent with levels for high denity Ca^{2+}. Increasing substrate stiffness increased the magnitude and rate of chondrocyte adhesion, independent of the chemical identity of the crosslinking agent.

4:15 PM GG5.9
CELL MORPHOLOGY AND TRACTION FOR FIBROBLAST CELLS ON FIBRONECTIN VERSUS RGD MODIFIED HYDROGELS. Padmavathy Rajagopalan, William Marganski, Micah Dembo, and Joyce Y. Wong, Department of Biomedical Engineering, Boston University, MA.

Surfaces of biomaterials that can exhibit controlled cell response are extremely important for the development of future medical implant devices. Immobilizing extracellular matrix proteins or short peptide sequences on the surface of a biomaterial leads to enhanced cell attachment as well as specific cell interactions. Obtaining quantitative information on cell-substrate interactions is vital to applications such as tissue engineering, and wound healing. In particular, determining the effect that chemical and mechanical properties of the biomaterial substrate exert upon cell migration, morphology, and cellular traction will lead to the development of implants with improved biocompatibility. Here we present preliminary data on cell morphology and cellular traction stresses for NIH 3T3 fibroblast cells on thin polyacrylamide gels using the elastic substratum technique. GRGDSP peptide or fibronectin are covalently bound to N-hydroxy succinimide ester, which is incorporated in the polyacrylamide gels during polymerization. Fibroblast spreading on GRGDSP modified gels differs significantly in comparison to fibronectin modified gels. The average area for fibroblast cells on GRGDSP is ca. 621 $\pm$ 272 $micron^2$ and on fibronectin is ca. 1000 $\pm$ 500 $micron^2$. Traction stresses exerted by fibroblast cells on polyacrylamide gels (estimated Young's Modulus 30,000 N/m^2) modified with GRGDSP peptide was calculated to be 4.75 $kdynes/cm^2$. Traction stresses for fibroblast cells on gels (estimated Young's Modulus 3400 N/m^2) modified with fibronectin was 17.5 $kdynes/cm^2$.