SYMPOSIUM GG
Polymeric Biomaterials for Tissue Engineering

November 26 - 27, 2001

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*Invited paper
9:00 AM #GG1
PROGRAMMING CELL FUNCTION AT THE MICRO-FABRICATED CELL-MATERIAL 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 survival. Using both covalent and non-covalent 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 cell behavior. 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 MOVEMENT. John R. Simon, School of Chemical Engineering, Cornell University, Ithaca, NY.

Controlling cell response to an implantable material is essential to tissue engineering. Fabricating topographical and chemical 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 microstructured on glass surfaces using photoresist photomasks to create transversal behavior of human neutrophils on patterned surfaces. The light microscope was used to observe a light microscope with transmitted light source. The width (2 µm) and length (400 µm) of the ridges were constant. The height (for 3 µm) and the spacing (6 to 14 µm) of the ridges were systematically changed to investigate the effect of micropatterns on neutrophil migration. In addition, the effect of surface chemistry on neutrophil migration was studied by deposition of a thin layer of "inter" biocompatible polymers such as Poly-DL(lysine) and titanium on patterned substrates. More than 95% of neutrophils 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 x 10^-9 cm²/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 suggest that surface chemistry and topography have different effects on neutrophil cell migration. Understanding the behavior of neutrophils formed in binary colloidal structures, which in combination with other proteins and surface energy of the silicon substrate 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. AN ORGANIZATION DIRECTS CELL BEHAVIOR. Eileen M. Higham, Nathaniel J. Glenson, Ilian A. Askoy, Jeffrey D. Carbeck, Princeton Univ, Dept of Chemical Eng, 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 topography 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 the domains 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 produced surfaces that vary from isolated 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 polyelectrolyte glycol. Interfaces produced in this way show that the organization of the cell adhesion 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 two-dimensional cell morphology was coupled with distinct changes in the organization of the cytoskeleton and number of filopodia. For the first time we show that these changes in cell behavior can be directed through protein organization on surfaces, in the past, such effects have only been seen in response to changes in the composition of proteins on surfaces.

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

We studied the physicochemical effects of deep UV irradiation of polystyrene, PMMA, and polycarbonate with respect to cell adhesion and protein immobilization. Polysaccharide moieties immobilized to 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 surface roughening, the clean room compatibility and the small size of achieved structures. It was observed that hepegocytes (HepG2) and fibroblasts (L929) adhered in the presence of serum proteins in the culture medium on the irradiated regions of the substrate and demonstrated normal growth. A spontaneous formation of a patterned protein adsorbed from a multi component mixture like fetal bovine serum, defined adsorbates can be realized by coupling peptides or proteins to the carboxylic groups with carbodiimide activation. We have immobilized horseradish peroxidase, other enzymes and antibodies. Phleomicrocytoma cells (PC12) required a two step adsorption of albumin and collagen or laminin to adhere. Together with supplemented normal growth factor the collagen matrix stimulated the formation of the neurite outgrowth. The described patterning technique may become a useful tool for the study of a variety of defined co-cultures (for example hepegocytes and fibroblasts), neuronal networks, intercellular communication, organogenesis and for applications like biosensors or engineered highly functional tissues and implants as bioartificial organs.

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

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 polymers, depletion of 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, degrades into silicic acid, the degradation product of aspirin. These polymers were designed to influence the tissue response when implanted. Preliminary animal studies indicated that poly[aryl-ether-ester] degradation induced a pronounced, localized response as an inflammation and also for angiogenesis. 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[aryl-ether-ester], several new polymer compositions were synthesized using various other aryl-ether-siloxanes as the polymer backbone. For example, aryl-ether-siloxanes are effective anti-inflammatory agent in treating inflammatory bowel disease. Because current aryl-ether-siloxane delivery systems cause adverse side effects, we prepared several polymers that degrade into aryl-ether-siloxanes including poly[aryl-ether-esters], poly[aryl-methylene]s and poly[aryl-ether-amides]. As a two-proposed approach to drug delivery, antioxidants (e.g. tetrahydroquinine) were physically admixed with the polymers such that two drugs are released - tetrahydroquinine via polymer erosion and salicylate via polymer degradation. The antioxidant-based polyaryl-ether-siloxanes 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.

SESSION GG2: DEGRADATION OF BIOMATERIALS

Chairs: Kristi S. Anseth, Amy K. Burkoth Posthuma and David J. Mooney
Monday Afternoon, November 26, 2001
Gardner (Sheraton)

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

Poly(methylene-esters) are biocompatible polymers that predominantly degrade into silicic acid, the degradation product of aspirin. These polymers were designed to influence the tissue response when implanted. Preliminary animal studies indicated that poly(methylene-ester) degradation induced a pronounced, localized response as an inflammation and also for angiogenesis. 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(methylene-esters), several new polymer compositions were synthesized using various other aryl-ether-siloxanes as the polymer backbone. For example, aryl-ether-siloxanes are effective anti-inflammatory agent in treating inflammatory bowel disease. Because current aryl-ether-siloxane delivery systems cause adverse side effects, we prepared several polymers that degrade into aryl-ether-siloxanes including poly(methylene-esters), poly(methylene-esters) and poly(methylene-amides). As a two-proposed approach to drug delivery, antioxidants (e.g. tetrahydroquinine) were physically admixed with the polymers such that two drugs are released - tetrahydroquinine via polymer erosion and salicylate via polymer degradation. The antioxidant-based polyaryl-ether-siloxanes 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
DEGRADABLE POLY (VINYL ALCOHOL) TISSUE SCAFFOLDS. Penny Marrone, Stephanie Bryant, University of Colorado, Dept of Chemical Engineering, Boulder, CO; Troy Holland, Biocure Inc., Norcross, GA; Christopher Bowman, University of Colorado, Dept of Chemical Engineering, Boulder, CO; Kristi Anseth, University 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]. Hydrogels are particularly advantageous because the mild gelation conditions allow encapsulation of cells and the high swelling imparts desirable mechanical and transport properties. However, tuning the degradation behavior of hydrogel cell scaffolds to match the rate of cell growth during tissue evolution is a challenge. In this research, we are using a multifunctional poly (vinyl alcohol) (PVA) that can be photocopolymerized to encapsulate cells and also bulk degrades with controllable kinetics. PVA was chosen for the basis of the macromer because of its history in tissue engineering 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, % macrorner 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 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.

2:30 Pm GG2.5

MODELING AND EXPERIMENTAL CHARACTERIZATION OF DEGRADABLE POLY (ACRYLATE) TISSUE SCAFFOLDS. Penny Marrone, Stephanie Bryant, University of Colorado, Dept of Chemical Engineering, Boulder, CO; Troy Holland, Biocure Inc., Norcross, GA; Christopher Bowman, University of Colorado, Dept of Chemical Engineering, Boulder, CO; Kristi Anseth, University 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]. Hydrogels are particularly advantageous because the mild gelation conditions allow encapsulation of cells and the high swelling imparts desirable mechanical and transport properties. However, tuning the degradation behavior of hydrogel cell scaffolds to match the rate of cell growth during tissue evolution is a challenge. In this research, we are using a multifunctional poly (vinyl alcohol) (PVA) that can be photocopolymerized to encapsulate cells and also bulk degrades with controllable kinetics. PVA was chosen for the basis of the macromer because of its history in tissue engineering 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, % macrorner 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 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.

3:00 Pm GG2.6

DECLINE OF POLY (ANHYDRO-ESTER) MECHANICAL PROPERTIES AS A FUNCTION OF HYDROLYTIC DEGRADATION. Kenya Whittaker, Rutgers University, Dept of Chemical and Biomolecular Engineering, Piscataway, NJ; Kathryn Urich, Rutgers University, Dept of Chemistry, Piscataway, NJ.

Poly(Acrylic acid) is a biocompatible poly(anhydride-ester) which hydrolytically degrades into acrylic acid, an anti-inflammatory monomer. 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(Acrylic acid) were enhanced by copolymerization with para-carboxyphenoxybenzene (p-CBH) monomer. The copolymers have reduced tackiness, but become more brittle with increasing proportions of p-CBH. 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 poly(CBH) content. 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 and voids. As a result, the degradation occurs at the water-polymer interface. The polymer matrix 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

BIODEGRADABLE POLYPYROMOLECULE. Alexander Zelikin, Venkatesh Shani, 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, Kintzerasal Bazel, 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 for nerve regeneration in rats with good results and shown to possess good biocompatibility. Notwithstanding, the biocompatible potential of PPy has largely been unexplored due to its chemical inertness under physiological conditions. A novel paradigm for the creation of functional biodegradable 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 rate of reaction of the acid group 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 48 h 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 G3: IN-ROOM POSTER SESSION**

**POLYMERIC BIOMATERIALS FOR TISSUE ENGINEERING**

Chair: Kristi S. Anseth and Amy K. Burkot

Posters

**Monday Afternoon, November 26, 2001**

**4:00 PM**

Hampton (Sheraton)

**G3.1** SYNTHESIS AND PROPERTIES OF NOVEL POLYETHYLENEGLYCOL-POLYPETIDE DI.isBlank POLYMER COUMYIERS.

Annette Brandt, Harm-Antos Kocks, Max-Planck Institute for Polymer Research, Mainz, GERMANY

In this contribution we describe the synthesis, purification and structural characterization of novel PEG-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-dependent structural changes in their secondary structure between β-sheet and α-helices. As a result, the supermolecular 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.

**G3.2** AN IN VITRO STUDY OF NANO-FIBER POLYMER JELS FOR GUIDED REGENERATION. Derrick Miller, Karen Haberstroh, Thomas Webber, 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 dimension of the associated nano-structured extracellular matrix (ECM) components. The goal of this research was to use these ideas 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/polyglycolic 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 50 nm. 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 30% 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.

**G3.3** FORMATION OF FIBRINOGEN-BASED HYDROGELS USING PHOTOINITIABLE LIPOSOMES. Poochi Shams, 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 Ca²⁺ from temperature-sensitive liposomes. We now report an extension of our one-pot method to produce rapidly gelating fibrinogen-based protein hydrogels using photosensitive calcium-loaded liposomes. Interdigitated-fusion liposomes (IFL), comprised of 38 mol% dipalmitylphosphatidylcholine (DPPC) and 57 mol% dipalmitoylphosphatidylethanolamine (DPPE) were formed in the presence of 1 mM CaCl₂ and rendered photocleavable by inclusion of 5 mol% bacteriochlorophyll (Bchl) within the liposomal bilayer membrane. Continuous irradiation (800 nm, 800 W/cm²) of these liposomes under aerobic conditions in the photoacid generator led to production of greater than 90% of entrapped Ca⁺⁺ within 15 minutes. IFL were then used to activate the transglutaminase-catalyzed crosslinking of fibrinogen via Ca⁺⁺ photoketone-A mixture of Ca⁺⁺-loaded IFL, fibrinogen, and a Ca⁺⁺ dependent transglutaminase enzyme reneged fluid in the dark, but gelled rapidly when irradiated in the presence of air at 800 nm and 37°C. SANS-PAGE analysis of the reaction mixture showed that gelation was due to enzymatic crosslinking of the fibrinogen α- and γ-chains. Crystallofruated hydrogel samples were analyzed by X-ray, revealing a microporous structure with pore diameters ranging between 4.8 μm. This phototriggerable hydrogel system can also be used to readily produce gradients and patterned biomaterial scaffolds. Photosensitive liposomes based on more readily prepared phycobiliprotein precursors 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 GM56266 and DE 13030.


**G3.4** AN INVESTIGATION OF NANO-STRUCTURED CO-POLYMERS FOR USE AS THREE-DIMENSIONAL BLADDER TISSUE CONSTRUCTS. Anil Thapa, Thomas J. Webber, 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 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 copolymer pellets (0.5g) in chloroform. Incubation steps showed that 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 (0.6 mm x 1 mm) were cut from the bulk polymer film and were soaked for various times of irradiation (0-30 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 greater 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.

**G3.5** SURFACE CHARACTERIZATION OF PROTEIN MICRO-PATTERNS FOR DIRECTED CELL GROWTH. Bryan A. Longawa, Gordana Dukovic, Kathryn E. Uhric, 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 micro-patterned with proteins such as laminin and bovine serum albumin via photolithography as well as poly(methyl methacrylate) (PMMA) micro-patterned with laminin only using either photolithography or microcontact printing. Chemical changes of the substrates were monitored following each step of the patterning procedure. Samples photolithographically patterned exhibited incomplete protein coverage, and solids from the buffer and developer solutions were detected. PMMA samples prepared by microcontact printing showed increased oxygen content prepared by 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**

Elisha B. Walsh, Nicole H. Gryczkowski, Mark W. Ginsberff, 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 allows the designator biological microstructures in a controlled manner. 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 large threedimensional microstructures on a single-cell patterning substrate. 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. Micro-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 dimethacrylate monomer, eosin Y initiator, and triethylamine co-initiator. Patterns were viewed in solution by light microscopy, then isolated and characterized by SEM.

**GG3.7**

**RAPID HEPATIC CO-CULTURE SPHEROID FORMATION ON PLA**

Lisa R. Rizzicato-Bekal, Robin Quirk, Reza 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 overcome in the endeavor to engineer functional liver tissue. Various methods have been employed in the attempt to maintain differentiated hepatocyte function and several studies have shown the benefit of 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 influence the expression of regulatory molecules. Previous work has shown that hepatocyte cell aggregates can be formed when the cells are cultured on positively charged polystyrene, poly-HEMA or spinin films. In these multicellular aggregates the cell-cell contact is maximised and a three-dimensional cytoarchitecture, with respect to extracellular components, is attained. Here we describe the rapid self-assembly of multicellular structures when cultured on the non-adhesive substrates 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 these 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) many. The production of albumin, another common indicator of hepatocyte function, was measured using 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:


**GG3.8**

**ENCAPSULATION OF MAMMALIAN AND BACTERIAL CELLS**

Jill A. O'Loughlin, Michael J. Lyngard, 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 1000 Hz) was utilized to encapsulate cells in a sodium alginate/sodium calcium alginate (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 ± 214 SD μm 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 15% Fetal Bovine Serum, and 1% Penicillin-Streptomycin (Gibco). The cells were stored in a humidified 5% CO2 incubator. The encapsulated bacterial cells were also maintained in a similar incubator with Luria-Bertani medium (10 g/L trygon, 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 had a rapid doubling rate, on the order of 15-20 minutes at the optimal growth 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 fewer cells become maturely greater 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 intracapsule density. Fibroblasts were visualized with trypan blue, which is an eosinophil marker of cell metabolism. 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 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**

**ORGANIC/SILICATE BIOMATERIALS: FROM MONOLOLITHS TO NANOPARTICLES**

Sumit Mudgal and Jackie Y. Ying, MIT, Dept of Chemical Engineering, Cambridge, MA.

Doped silica 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 vitreous form of Bioglass. 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 10-1 μm 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 deliver specific osteoinductive behavior. The effects of particle size, concentration and surface chemistry on the mechanical properties of a polymethylacrylate (PMMA) matrix have been studied. Low loadings of nanoparticles have been shown to improve both the Viadon hardness and bending modulus of PMMA. Particle size and composition have also been shown to affect MC3T3 osteoblast behavior. Cell culture experiments indicated that these organosilicate nanoparticles significantly increased osteoblast proliferation.

Fluorescent microscopy indicated that these unique particles were ingested by cells and 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 effect on cell behavior will be discussed in this presentation.

9:15 AM **GG4.2**

**POROUS POLYMER/BIACORE 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. Ogemar, Jr., University of Minnesota, Department of Orthopedic Surgery and Biochemistry, Minneapolis, MN; Lorraine P. Francis, University of Minnesota, Department of Chemical Engineering and Materials Science, Minneapolis, MN.

Bending of articular soft tissues, such as cartilage, to hard tissues, such as bone, presents a challenge. Porous polymer/biacore glass composites are candidate materials for engineering the articular cartilage/bone interface and possibly other soft/tissue
(ligament/hone, tendon/hone) interfaces. A liquid-liquid phase separation technique was used to make porous polymer/biocomposite glass composites. Polyurethane foams and polysulfone/biocomposite glass composites along with porous pure polymer sheets were prepared. The effects of polymer type, concentration and molecular weight, as well as biocomposite glass size and content, on the microstructures of the composites were studied. The composite sheets (thickness: 201±50 μm) have asymmetric structures with dense top layers and porous structures beneath. The porous structures consist of large pores (> 100 μm) in network of smaller (10 μm) interconnecting pores. The mechanical strengths depend most strongly on the type of polymer, the interaction between the polymer and biocomposite 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 cultures. The growth of hydroxyapatite on the HCA side and on the composites after soaking in the SBF for two weeks demonstrated an increase in potential for interaction with bone. HAp 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.

10:30 AM GC6.4 CHARACTERIZATION OF TISSUE DEVELOPMENT IN COLLAGEN-FORMING POLYMERIC SCAFFOLDS: Newt W. Bashil, Abhikar Karim, Eric J. Amis, Polymers Division, NSF, Fairbanks, AK, Mimekimee Potter, AFIP, Rockville, MD.

An investigation of osteoblast cell proliferation to coextruded polymer scaffolds is presented. Poly(ethylene methacrylate) scaffolds having continuous porous networks with characteristics scale ranging from 211 to 110 μm are prepared. Cells seeded on the scaffolds were exposed to a perfusion culture. 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 porosity size and geometry on cell activity will be discussed.

40 AM GG4.5 BAHAiORAL MEMBRANE AS TISSUE SURROGATES FOR BONE CELLS: Kwang-Suck Kim, Wenda Chen, Melik Yu, Steven Zhong, Dufei Fang, Benjamin Heino, Benjamin Chu, SUNY at Stony Brook, Dept of Chemistry, Stony Brook, NY, Michael Hvidjorg, SUNY at Stony Brook, Dept of Biomedical Engineering, Stony Brook, NY.

Poly(lactic) [PLA] has been widely studied for medical applications because of its biodegradability as well as biocompatibility. However, PLA generally has high biodegradation rate, and its formation in vivo 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 delivery for tissue cell. The bone cell 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 these membranes. We found that the cell release efficiency can be adjusted by the membrane morphology, and the cell survival rate is very high.

9:30 AM GC6.3 IN SITU MINERALIZATION OF HYDROXYAPATITE FOR A MOLECULAR CONTROL OF MECHANICAL RESPONSES IN HYDROXYAPATITE-POLYMER COMPOSITES FOR BONE REPAIR.

PLACED AT: Menasha, PK, Parkland, VC, Long, Ann, Arukumke Ayers, Timothy Arvey, 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. In situ mineralization of hydroxyapatite [HAP] in the presence of Ca binding polymers such as polyacrylic acid has shown some promise towards improved 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 basic 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 [poly(acrylic acid) with HAP during mineralization of HAP. In situ HAP exhibits a faster mineralization as compared to the ex situ 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 ex situ samples. Changes in mechanical response to loading included improvement in strain-to-failure and resulting toughness for the composite. The control and development of molecular level associations of polymer with HAP is suggested to be critical for the resulting micro properties. Our results may have significant implications in design of nanocomposites for biomedical applications.

11:00 AM GC6.7 IMPROVED SYNTHESIS OF SALICYLATE-BASED POLY-ANHYDRIDES R.C. Schmeltzer, Theodore J. Anastasio, Kathryn E. Ulrich, Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ.

We developed an improved synthetic procedure for polymeric produgs that hydrolytically degrade into pharmacologically active components. These poly(anhydride-esters) are composed of sebacic acid linked to either salicylic acid or 4-methylsalicylic acid. Salicylic acid is an anti-inflammatory, anti-inflammatory analgesic used for a variety of medical applications. The salicylates 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 polymer synthesis that involved protection of the reactive carboxylic group of the salicylic acid, followed by deprotection once the coupling was complete. Herein, we report a modified synthetic route that eliminates the need for the coupling protection/deprotection step. The protected coupling pre polymer in quantitative conversion in a few steps, thus increasing the overall yield. 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 these polymers up to 45000. These improvements and novel approach for melt-condensation will be discussed.

11:15 AM GC6.8 NEW SUBSTITUTED POLYLACTIDES AS CANDIDATE MATERIALS FOR TISSUE SCAFFOLDS: Gregory L. Baker, Milton R. Smith III, Mao Yin, Tianqi Liu, Chun Wang, Department of
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(b lactate), 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 biodegradable polyesters is through the synthesis of novel polyesters from monomers that are known to be either natural metabolites or biodegradable and thus termed biodendrimers. Specifically, our dendrimer monomer units are glycolic acid, glycidyl ether, and serine. We have constructed biodendrimers through condensation reactions to form ester linkages between branching units and multifunctional cores. Conversion of tri-o-hydroxypropylglycerol (THPG) to a bifunctional monomer (G1P) by reactions with 4-pentenoic acid and 4-pentenoic acid chloride (G1) was achieved. The glycol ether linkage of the dendrimer core is then linked to another dendrimer core to form the dendrimer branching unit (DIBU). GLGASE-2 is a dendrimer branching unit with two G1P units and GLGASE-4 is a dendrimer branching unit with four G1P units. Further generations were formed by the condensation reaction of GLGASE-2, branching units to the dendrimer core followed by subsequent addition of further dendrimer branching units via a catalytic hydrogenation reaction. The physical characteristics of these biodendrimers, including their ability to degrade to natural metabolites and the number of functionalizable end groups, suggest that biodendrimers are ideal for use in several important biomedical engineering applications.
degradation, be osteoconductive (promote bone cell attachment), and be formed in situ to eliminate or reduce implant fabrication and allow for facile filling of surgically created 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 acylated or methacrylated. These oligomers resulted in a high density 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 the incorporation of other oligomers (i.e., other 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. Additional hydrophilic and functional (e.g., of the phosphate activity and mineralization) of primary rat calvarial osteoblasts attached to network surfaces are dependent on the oligomer chemistry. One specific oligomer, 2EO11LAA (dihydroxy ethylene glycol core with 10 lactic acid units on either side), formed networks with osteocartilaginous properties similar to that of tissue culture polystyrene and 50:50 poly(lactic-co-glycolic acid). 3-D polymer scaffolds with a controlled macroscopic architecture were readily fabricated from this material by mixing a porogen with the macromer, subsequently photopolymerizing the material, and then leaching the porogen 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 POLYHACIDRIDE ORAL PRODRUGS
Theodore J. Anastassiadis and Kychrin E. Ulrich, Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, N.J.
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 poly(hydroxides containing 4- and 5-aminosalicylic acid backbones linked through either ester, amide, or carbamate bonds were synthesized. In these materials, polymer degradation and the drug release via the catalyzed hydrolysis (pH or enzyme) of the hydroxide bond as well as the 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 0.01 to 100 kDa. Thermal characterization was performed by thermogrammetric analysis (TGA) (TD) and differential scanning calorimetry (DSC) (TG, Tm). TDs were all above 350°C, with a notable absence of Tg’s, likely due to the high density of the materials as well as inter- and intramolecular hydrogen bonding. The relationship between aminosalicylic acid structure (4- or 5-substitution) and backbone length (amide, amide, or ester) on polymer degradation rate and drug release were investigated. The degradation of these materials was carried out at acid, neutral and basic pH’s, ranging from 3 to 8, in absence of enzymes, and at neutral pH in the presence of extremes, amylase, and an acid reflux. Polymers were solvent-cast into films, then degraded by placing the buffer at the appropriate pH. At defined time points the spent buffer was 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) COMPLEXES
Xiaolan Liu, Zhihong Han, Jacob Schwartz, Tong Cui, and Jie Lin, Unversity of North Texas, Dept. of Physics and Materials Science, Denton, TX.
Hydroxypropylcellulose (HPC) and polyacrylic acid (PAA) complexes were studied using turbidometry measurements and laser light scattering. The phase transition temperature of the complexes proved to depend on PAA concentration, HPC content, and pH. The transition temperature of the complexes increased 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 a higher temperature with an increase in pH. The application for this complex would be used for controlled drug release and biodegradable delivery systems based on environmental stimuli such as pH or temperature.

3:30 PM GG5.6
POLYIONELECTROLYTE MULTILAYERS: A NANO SCALE APPROACH FOR CREATING CELL-INTERACTIVE SURFACES
James D. Mendelsohn, Haoyun Yang, Allen Hochstein, 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 repetitively 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 nanoscale control of surface topography, composition, and surface properties. Applications ranging from opto-electronic devices to biocatalysts to tailored 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 govern the resulting multilayer thin film architecture, it is possible to create either bio-interfaces to which cells from a model marine fibroblast cell line readily adhere or, on the contrary, surfaces that are essentially bio-inert and prevent all observable cell attachment. The ability to micro-pattern 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, wetting measurements, and chemical characterization will be used to help explain the cell resistance or responsiveness 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 devices 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.
Hyun-Joon Kong, David J. Mooney, Unversity of Michigan, Departments of BioLogie & Materials Sciences, Chemical Engineering, and Biomedical Engineering, Ann Arbor, MI.
Alginates have been extensively studied as synthetic extracellular matrices for soft tissue engineering, due to their bio-compatibility and simplicity in gelation. To avoid her molecule function as extracellular matrices, the physical properties (e.g. mechanical and degradation behaviors) of the gel 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 modulus and the strains of hydrogels are independently controlled by varying the concentration of calcium ions, are controlled by altering the molecular size of alginate chains via gelation, and by altering the solids concentration. In particular, mixing alginate chains of high molecular weight (Mw ~ 250,000) and alginate chains of low molecular weight (Mw ~ 50,000) facilitates the formation of gels with broad ranges of mechanical properties, at a constant solids concentration. In addition, these binary gels do not show any deterioration in their mechanical properties over a month during incubation in vitro. To induce the degradation of the binary hydrogel in a controlled manner, while maintaining comparable mechanical properties for the desired period, the number ratios of β-D-mannuronic acid to α-L-guluronic acid (N Ma /N Ga ) in the non-irradiated alginate chains are varied to tune the associative forces between cross-linking molecules. The incorporation of alginate chains with higher N Ma /N Ga enhances the degradation rates, as indicated by changes in elastic modules 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 CHONDRONETIC TO MODIFIED ALGINATES: THE ROLE OF CROSSLINK DENSITY AND SUBSTRATE STIFFNESS.
Nicholas Genis, Frank C. Neffe, Ander S. Niel, and Steven W. Frey, University of Massachusetts Medical School, Worcester, MA.
Alginates is a co-polymer of mannuronic 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 differentiation 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-mediated 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 well coated with 2% control and RGD-alginate crosslinked with Ca²⁺ or Ba²⁺, 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 Ca²⁺ 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 Ba²⁺ surfaces had similar moduli to those surfaces crosslinked with high density Ca²⁺. Equilibrium attachment for low density Ba²⁺ surfaces was similarly consistent with levels for high density Ca²⁺. 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
Padminy Rajagopalan, William Mergiotti, Michal Dembo, and Joyce Y. Wang, 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 substrate technique. GRGDS peptide or fibronectin are covalently bound to N-hydroxy succinimide ester, which is incorporated in the polyacrylamide gels during polymerization. Fibroblast spreading on GRGDS modified gels differs significantly in comparison to fibronectin modified gels. The average area for fibroblast cells on GRGDS is ca. 621 ± 272 micron² and on fibronectin is ca. 1000 ± 500 micron². Traction stresses exerted by fibroblast cells on polyacrylamide gels (estimated Young’s Modulus 30,000 N/m²) modified with GRGDS peptide was calculated to be 4.75 kdynes/cm². Traction stresses for fibroblast cells on gels (estimated Young’s Modulus 3400 N/m²) modified with fibronectin was 17.5 kdynes/cm².