

Architectural Gradient Scaffolds for Subchondral Restoration

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Considerable interest has been given to various fabrication techniques of scaffolds to seek different means of overcoming bone loss. Studies demonstrate that scaffolds act as temporary matrixes at the site of injury to promote cell proliferation, which in turn promote tissue regeneration. This study focuses on constructing scaffolds that exhibit different gradients of pore architectures and various gradients of collagen coating on their surface in order to mimic physiological structures and compositions and lead to enhanced subchondral bone regeneration. Scaffolds were constructed from porous interconnected templates with a 250 μm , 340 μm or 450 μm pore size formed into spatial gradients in a inferior-superior and external to internal direction of decreasing pore size to mimic subchondral bone. These templates were then coated with a hydroxyapatite (HA) slurry and sintered. Scanning Electron Microscopy was used to identify trabecular thickness and pore size. Compression testing was performed to compare the strength of various scaffolds before and after collagen coating. Finally, three dimensional reconstructions were obtained from the MicroCT which was able to differentiate the different gradient compartments present in the assembly of the scaffold. Scaffolds were coated with various collagen concentrations (0.1%, 0.05%, and 0.25%) to determine the optimal coating concentration. Results show that the trabecular thickness/pore size of each scaffold was not significantly affected after the coating of collagen, indicating that they retained their open porous architecture. Scaffolds coated with 0.05% collagen for 30 minutes showed the strongest trend for an increase in toughness from the uncoated scaffolds ($p=0.082$).

**Select Choroidal Endothelial Cell Functions under
Elevated Pressure and High Glucose Concentrations**

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The present study was motivated by the need to elucidate the effects of diabetic conditions, namely elevated pressure and glucose levels, on constituent cells of the ocular microvasculature.

For this purpose, bovine choroidal microvessel endothelial cells (BCMECs) were cultured under standard cell culture conditions on fibronectin-coated tissue-culture plasticware for 24 hours. These cells were then exposed to either atmospheric, 15, or 25 mm Hg pressure and glucose concentrations ranging from 0 to 30 mM to simulate normal and diabetic conditions, respectively. Controls were cells cultured either under 15 mm Hg in media containing 5 mM glucose or under atmospheric pressure in 0 mM glucose. Viability of BCMECs was monitored after 1, 3 and 5 consecutive days of culture by staining (using the LIVE/DEAD® assay), visualizing, and counting the cells in situ using fluorescence microscopy. BCMEC proliferation was monitored after days 1, 2, 3, and 5 of culture using the CyQuant cell proliferation assay. Cell counts were reported as “cell density”. Experiments were run in duplicates on three separate occasions.

The BCMECs remained viable under all conditions of pressure and glucose concentrations tested. Compared to the respective controls, cell proliferation decreased after 3 and 5 days of exposure to pressure in the absence of glucose. Similar cell proliferation trends were also observed at elevated pressure and in the presence of glucose. The underlying mechanisms of choroidal endothelial cells function under conditions simulating the diabetic eye milieu are subjects of continuing research.

Title: Liquid Crystal Elastomers as Active Substrates for Dynamic Cell Culture

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Abstract

Liquid Crystal Elastomers (LCEs) are materials that show fully reversible shape change in response to external stimuli such as heat, light, electric and magnetic field. LCEs have potential applications in sensors, medicine, artificial muscles, and tissue engineering. There has been growing interest in development of active substrate that can change shape during in vitro cell culture for directing behavior of the cells. LCEs have great potential in this application because they show fast and large amplitude of fully reversible shape change (up to 30% of original shape) and do not degrade easily. We demonstrate use of both thermally responsive LCEs and electrically responsive LCE nanocomposites as active substrates for inducing alignment of cardiomyocytes. Both types of LCEs were coated with thin polystyrene film on the top surfaces to enhance attachment of the cells, and stimulated while immersed in aqueous cell culture medium. The thermally responsive LCEs were put on a top of resistive heater that provided cyclic heating to induce shape change. The LCE nanocomposites, which contain conductive carbon black nanoparticles in elastomer body, were put on top of carbon rods and electric field was applied cyclically. For both types of LCEs, the cells attached well and survived on the LCE surfaces, and local alignment of the cells along the primary direction of strain were observed. The results demonstrated the potential of LCEs as active substrate for dynamic cell culture.

Characteristics and Properties of Silk Scaffolds

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Bombyx Mori silk fibroin has a tunable secondary protein structure allowing changing of material and mechanical properties. This structure has led researchers to create scaffolds for a range of tissues including bones and ligaments. Silk can be molded and shaped to form gels or solid structures.(1) This wide spectrum of possibilities allows mimicking of native tissue.(2) This study analyzed the processing steps in scaffold synthesis to create suitable platforms for pancreatic islet expansion. Briefly, silk cocoons underwent multiple cleansing steps and were lyophilized. The lyophilized scaffolds were treated with methanol. Porous silk scaffolds were developed using hexafluoro-2-propanol (HFIP) to dissolve silk prior to methanol treatment. The silk/HFIP solution was then poured over NaCl, followed by leaching of the NaCl. The scaffolds were characterized using scanning electron microscopy (SEM), Fourier Transform infrared spectroscopy (FTIR) and atomic force microscopy (AFM). Nonporous scaffolds were used as controls. Varying pore sizes (150 to 400 μ m) were observed in this study. FTIR and AFM analyses confirmed structural changes with a change in modulus from 953.3MPa to 4.96GPa, depending on the methanol treatments. The 15% (w/v) HFIP/silk solution was also observed to produce consistent porosity. SEM micrographs confirmed the different pore sizes and consistent porosity throughout the scaffolds. It was concluded that silk scaffolds can be tailored into porous and nonporous scaffolds while maintaining structural properties. Additionally, data observed indicates that silk scaffolds can be tuned to the appropriate properties for mimicking tissue specific applications. Supported in part by funding from the San Antonio Life Sciences Institute.

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Achieving Tunable Degradation of PolyHIPE Bone Grafts

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Introduction: Injectable bone grafts serve as a space-filling substrate for bone tissue engineering. Previously, our lab has reported the development of highly porous, interconnected, injectable bone grafts (polyHIPEs) achieved utilizing emulsion templating and redox initiation. These polyHIPEs are characterized with compressive properties comparable to cancellous bone but lack degradation profiles that match rates of *in vivo* tissue regeneration. This work focuses on the development of a thiol-containing macromer, diethylethylene glycol dimethacrylate thiol (DEGMA-T), to increase the hydrolytic degradation rate of polyHIPE bone grafts.

Materials and Methods: Macromer phases for the polyHIPE bone grafts consisted of 0:1, 1:1, and 1:3 wt% ratios of DEGMA-T: 1,4, butanediol methacrylate (BDMA). Each macromer phase composition was separated into two equal amounts and 10 wt% surfactant (PGPR) was dissolved in each. The water soluble redox initiators, 15 wt% ammonium persulfate and iron gluconate were dissolved in the aqueous phases for each HIPE composition before added to their respective organic phase. HIPE emulsification was achieved using a Flacktek Speedmixer 150 FVZ-K, and both reducing and oxidizing portions of each polyHIPE were then combined to initiate crosslinking. *In vitro* hydrolytic degradation of the three polyHIPE compositions was assessed over a 12 week period in PBS and 0.1 M NaOH. Compressive stress and modulus, and pore architecture were investigated to characterize the polyHIPEs.

Results and Conclusions: Incorporation of a thiol-based macromer, DEGMA-T, increased the hydrolytic degradation of the polyHIPE bone grafts. SEM analysis indicated minimal changes in pore architecture and interconnectivity after incorporation of DEGMA-T. Compressive strength and modulus values decreased but remained within the range of cancellous bone. This work demonstrated that the incorporation of a thiol-incorporated methacrylated macromer can be used to increase the hydrolytic degradation of polyHIPE bone grafts to better match rates of *in vivo* tissue regeneration.

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Electrochemically-Pre-adsorbed Collagen Promotes Adult Human Mesenchymal Stem Cell Adhesion on Optically Transparent Nanostructured Carbon Substrates

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The present study was motivated by need to understand and control protein adsorption on material surfaces prior to subsequent cell interactions for the success of tissue engineering and regeneration applications and was inspired by recent findings that applied electrical potential results in increased protein adsorption onto nanostructured carbon films.

For this purpose, rat-tail, Type I Collagen was adsorbed electrochemically on the surface of optically-transparent carbon (OTC) under 0.4, 0.8 or 1.5 volts, at room temperature, for 3 hours. Adult, human, mesenchymal stem cells (hMSCs) in medium (without serum) were allowed to adhere on the surface of each substrate under standard cell culture conditions for 2 hours. The hMSC were then stained, visualized, and manually counted in situ. The data of hMSC adhesion were averaged, reported as “cell density” (cells/cm²), and compared to the respective controls. Controls were hMSCs seeded in parallel on either (1) tissue culture polystyrene (non-conductive substrate), (2) OTC without exposure to the electrical potential, or (3) pre-adsorbed protein on OTC without exposure to the electrical potential. The controls were maintained under similar conditions and analyzed using the aforementioned techniques.

hMSC adhesion was highest ($p < 0.001$) when Type I Collagen was pre-adsorbed on OTC substrates under 0.8 volts. hMSC adhesion was similar on all OTC controls tested and on substrates with pre-adsorbed collagen under 0.4 volts. The mechanisms underlying the electrochemical adsorption of proteins which modulate subsequent cell adhesion on material surfaces need elucidation and are the subject of continuing research.

Sequential Click Reactions for the Polymerization and Functionalization of Poly(ethylene glycol) Based Hydrogel Microparticles

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Abstract

Hydrogels have been extensively used in tissue engineering and regenerative medicine because of their ability to mimic key aspects of the extracellular matrix. Hydrogel microparticles have been of particular interest for delivering therapeutics and cells, and the delivery of bioactive cues from hydrogel microparticles has been demonstrated both in vitro and in vivo. While a number of strategies for hydrogel microparticle preparation and functionalization have been described, emerging tools that fall under the paradigm of click chemistry may provide some important advantages owing to the specificity and efficiency of these reactions. Here, we describe a novel and effective method for producing bioactive, protein-functionalized poly (ethylene glycol) (PEG)-based hydrogel microparticles using sequential thiol-ene and tetrazine click reactions. Briefly, a tetra-functional PEG-norbornene macromer (7.5-15wt%; 20kDa) and dithiothreitol crosslinker were mixed off-stoichiometry ([SH]:[norbornene] = 0.75:1), emulsified with an aqueous dextran solution (40 wt%; 40kDa), and then rapidly photopolymerized using lithium acylphosphinate (2mM) and 365nm UV light (10mW/cm²; 5min). Characterization of the resulting hydrogel microparticles indicated that microparticle diameter increased with PEG concentration. Subsequently, protein conjugation to the microparticles was achieved via tetrazine click chemistry to unreacted norbornene groups in the particles. Protein conjugation was demonstrated using a model fluorescent protein. Finally, microparticles were functionalized with alkaline phosphatase, and enzyme activity was shown by conversion of *p*-nitrophenylphosphate phosphate. Collectively, these results demonstrate that sequential click reactions can be employed to control microparticle synthesis and functionalization. Future studies will investigate potential cellular applications in vitro and in vivo.

Keywords

hydrogels, microparticles, suspension polymerization, click chemistry, protein functionalization