Tailoring the properties of PEG-DA hydrogel scaffolds

Brennan Bailey, Raymond Fei, and Melissa Grunlan
Graduate Poster Presentation

Tissue engineering typically relies on a 3-D scaffold to create an environment in which living cells can attach, proliferate, differentiate, and ultimately produce a new extracellular matrix (ECM). It has been identified that in healthy tissues the ECM controls cell behavior, including tissue regeneration, via signaling cascades involving specific binding events *and* non-specific chemical and physical properties. An improved understanding of the correlation between specific scaffold chemical and physical properties and resulting cellular responses would result in the regeneration of tissues with properties more comparable to native tissues. Towards this goal, we describe herein a scaffold system capable of achieving a broad range of tunable chemical (e.g. bioactivity and hydration) and physical properties (e.g. modulus and morphology) as a result of combining inorganic [methacrylated star polydimethylsiloxane] and organic [poly(ethylene glycol) diacrylate] components and different solvents (water and dichloromethane). These scaffolds are expected to be useful in studying cell-material interactions to establish predictive relationships resulting in improved tissue engineering outcomes. Preparation of these scaffolds as continuous gradients will serve as a simple combinatorial method to efficiently prepare a library of compositionally unique hydrogels for evaluation of scaffold properties and the formation of a gradient scaffold to regenerate an osteochondral interface (bone-cartilage interface). The following measurements were made: swelling, dynamic mechanical analysis (DMA) in compression, morphology (SEM and CLSM), PDMS distribution (CLSM) and bioactivity (formation of hydroxyapatite when exposed to simulated body fluid).

Synthesis and Characterization of Composite Hydrogel Particles for Oral Delivery Small Interfering RNA

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Graduate Poster Competition

Introduction: Small interfering RNAs (siRNAs) are short strands of double-stranded RNA that selectively bind and cleave complementary messenger RNA (mRNA), thereby inducing highly potent and specific gene knockdown. The aim of this work was the design and characterization of pH-responsive, degradable hydrogel systems for site-specific delivery of nanoparticle-encapsulated siRNA to the small intestine.

Materials and Methods: Monomers *N*-vinylpyrrolidone, methacrylic acid, and a crosslinking agent were used to synthesize a pH responsive hydrogel. Polycationic nanoparticles (PNPs) were incorporated into the film at 0-5% by weight with respect to monomer content. Swelling experiments were carried out in 0.1 M 3, 3-dimethylglutaric acid buffers from pH 1.2 to 7.2. Cytotoxicity studies were completed with human colon adenocarcinoma (Caco-2) cells.

Results and Discussion: Dynamic swelling experiments from pH 1.2-7.2 showed significant increase in weight swelling ratio above pH 5.2, demonstrating the desired pH response. Incorporation of PNPs had no significant effect on the swelling properties of the film. Cytotoxicity studies demonstrated cell viabilities of greater than 80% of control cells for a concentration range of 0.625-2.5 mg/ml for all formulations tested.

Conclusions: The swelling properties of the materials are suitable for post-synthesis loading of siRNA as well as release at the pH of the small intestine. Preliminary studies indicate low cytotoxicity at relevant concentrations. We are proceeding with loading and release of siRNA, and incorporation of a degradable crosslinking agent.

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Complexation Hydrogels as Oral Delivery Vehicles of Therapeutic Antibodies

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Oral administration of monoclonal antibodies (mAbs) may enable the localized treatment of infections or other conditions in the gastrointestinal tract (GI) or systemic diseases. However, one of the most challenging tasks in antibody therapies if to deal with physical and chemical instabilities of the protein, which invariable lead to loss of biological activity. New families of complexation hydrogels comprised of poly(ethylene glycol) (PEG) chains grafted on poly(methacrylic acid) (PMAA) backbone chain, henceforth designated as P(MAA-*g*-EG), are excellent transmucosal delivery vehicles. This contribution focused on the design and evaluation of hydrogel carriers that will minimize the degradation and maximize the *in vivo* activity of anti-TNF-α, a mAb used for the treatment of inflammatory bowel disease (IBD) in the GI tract and systemically, for the treatment of rheumatoid arthritis. Films of P(MAA-*g*-EG) hydrogels were prepared by UV-initiated free radical solution polymerization and crushed into microparticles. Systems containing various crosslinking agents were evaluated. The length and hydrophilicity of the crosslinking agents affect the hydrogel swelling extent in the aqueous medium. Hydrogel swelling behavior was translated into improved protein release. A detailed evaluation on protein structure and *in vitro* bioactivity was performed in order to determine any instability caused by encapsulation and release from hydrogel microparticles. The capacity of the anti-TNF-α mAb to neutralize TNF-α and protect L929 cellular cultures from the cytotoxic effect of this cytokine was maintained after the mAb was released from P(MAA-*g*-EG) hydrogels. Detailed structural and functional evaluation of released proteins allowed for the rational selection of appropriate polymer composition.

Responsive polycationic nanoparticles for co-delivery of siRNA and chemotherapeutical agents to overcome multidrug resistance in cancer therapy

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There are two main mechanisms by which cells become multidrug resistant (MDR): by increasing drug efflux pumps on the cell membrane and by increasing anti-apoptotic pathways. By encapsulating drugs into nanoparticles that bypass the efflux pumps, drug efflux is reduced, hence increasing the intracellular concentration of the drug. The use of nanocarriers to deliver siRNA, prevents both renal clearance and RNase degradation by protecting siRNA chains, increasing their half-life in blood. It has been suggested that co-delivering drugs and siRNA against anti-apoptotic pathways together in the same delivery system would be more effective in overcoming resistance of cancer cells than co-treatment of cancer cells with delivery systems carrying either siRNA or drugs. Polycationic nanoparticles with a hydrophobic core allow encapsulation of a chemotherapeutic agent and complexation of negatively charged siRNA onto the particles surface. We synthesized polycationic nanoparticles with an hydrophobic core polymerizing the hydrophobic and hydrophilic monomers under a UV source, after emulsion in water. The resulting nanoparticles were pH responsive and have a size that ranges from 140nm, at endosomal pH, to 90nm, at physiological pH. Fluorescein was used as a modal drug due to its similar characteristics in size and hydrophobicity to a wide variety of chemotherapeutic agents. We synthesized biocompatible, highly colloidaly stable, and environmental pH responsive polycationic nanoparticles capable of loading and releasing a model drug in a timely manner.

Electromagnetic field modulation to inhibit biofilm formation on medical device surfaces

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A tridimensional structure of aggregate microorganisms linked to each other while embedded in a self-produced matrix of extracellular polymers, which facilitates adhesion of such a structure on a particular surface, is commonly known as BIOFILM. Biofilms help bacteria adapt to hostile extracellular environments, making it challenging to disrupt them (biofilms) by using antibiotic treatments. Presence of biofilm on medical device surfaces presents a significant health risk for patients and an economic burden for the health care system. The NIH reports that 80% of all chronic infections are attributed to biofilms, and according to CDC data, there are about 1.7 million hospital acquired infections (HAI) in the U.S alone, having direct medical costs ranging between $28.4 to $33.8 billion/yr.

We hypothesize that temperature-controlled electromagnetic fields (EMFs) in synergy with nanostructure coatings provides a platform for disrupting bacterial proliferation. This disruption may generate an internal electrochemical reaction within the bacteria, increasing the concentration of cytotoxic free radicals, altering the pH, killing the bacteria and/or interfering with its growth cycle. The present research aims at developing a biofilm disruption system that can be used in prevention and treatment of HAI, such as ventilator acquired pneumonia (VAP).

Preliminary results using a prototype of an EMF-based *"biofilm disruption system*" showed synergism of temperature-controlled EMF with zinc oxide (ZnO) nanostructure antimicrobial coating that decreased attachment and proliferation of methicillin-resistant *Staphylococcus aureus* (MRSA): ~90% reduction compared with controls (no ZnO or EMF treatment).

A Pluripotent Cell Differentiation System Utilizing a Scalable Cell-Encapsulation Technique and Degradable PEG-Fibrinogen Microspheres

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To satisfy demand for clinically relevant quantities of stem cell-derived therapeutic tissues, techniques for differentiation of pluripotent stem cells must be scalable and yield high concentrations of the desired cell types. Biomaterials can facilitate efforts to achieve both of these goals. In this study, we have demonstrated the efficacy of a highly scalable water-in-oil emulsion method for the generation of polymerized hydrogel microspheres and for the encapsulation and differentiation of mouse embryonic stem cells (mESCs). 10 kDa PEG-diacrylate was conjugated to bovine fibrinogen to produce PEG-fibrinogen hydrogel precursor. Cells were encapsulated at a density of 60 million/mL in PEG-fibrinogen and photo-crosslinked in the presence of both Irgacure 651 and Irgacure 2959. Cell-laden microspheres generated via emulsion had an initial diameter range of 50-250 μm (mean 167 μm). Initial mESC viability was quantified above 50%. Incorporation of fibrinogen within the biomaterial permitted enzymatic degradation of the hydrogel matrix by mESCs. Encapsulated mESCs proliferated within the microspheres to form microsphere-derived embryoid bodies (MdEBs), which further degraded the microsphere matrix over a course of 4 weeks in static suspension culture. Spontaneously contracting MdEBs (5-**%) were observed as early as Day 10 of differentiation and the number of contracting MdEBs increased in response to epinephrine 2.82-fold, on average, following 2 hours of treatment at 200 μM. Gene expression was analyzed on Days 11 and 30 of differentiation. Immunohistochemistry showed that MdEBs produced spontaneously contracting aligned sheets of cardiomyocytes when attached to a gelatin-coated substrate after an initial 2 weeks of suspension culture.

Ultra Strong, Thermoresponsive Double-Network Hydrogels

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Abstract: An implanted glucose sensor would not only eliminate the inconvenient and painful finger prick test, but would also provide diabetics with more precise control over blood sugar levels thereby improving their quality of life. A sensor membrane that controls the host response (i.e. biofouling) is crucial to the sensor's long-term functionality. We propose a "self-cleaning" membrane based on a thermoresponsive double-network hydrogel.[1, 2] The deswelling of poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogels at temperatures above their volume phase transition temperature (VPTT, ~ 33-35 °C)[3] can be used to release adhered cells and restore glucose diffusion. An electrostatic comonomer (2-acrylamido-2-methyl-propanesulfonic acid, AMPS) and a double network (DN) design was employed to enhance pore size, swelling and stiffness. These properties are attributed to the DN's asymmetrically crosslinked structure[4] and the electrostatic repulsive forces introduced by AMPS[5]. The properties achieved by this membrane design is critical for a self-cleaning glucose biosensor as it is expected to enhance glucose diffusion, self-cleaning and mechanical stability.

Ultra Strong, Thermoresponsive Double-Network Hydrogels

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Objective: An implanted glucose sensor would not only eliminate the inconvenient and painful finger prick test, but would also provide diabetics with more precise control over blood sugar levels thereby improving their quality of life. A sensor membrane that controls the host response (i.e. biofouling) is crucial to the sensor's long-term functionality. We propose a "self-cleaning" membrane based on a thermoresponsive double-network hydrogel.[1, 2] The deswelling of poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogels at temperatures above their volume phase transition temperature (VPTT, ~ 33-35 °C)[3] can be used to release adhered cells and restore glucose diffusion. To improve the mechanical strength and thermosensitivity, an electrostatic comonomer (2-acrylamido-2-methyl-propanesulfonic acid, AMPS) and a double network (DN) design was employed.

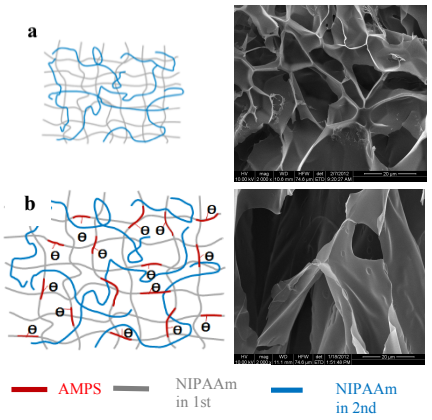

Figure 1. (a) Structure and morphology of PNIPAAm DN (b) Structure and morphology of NIPPAm-*co*-AMPS DN.

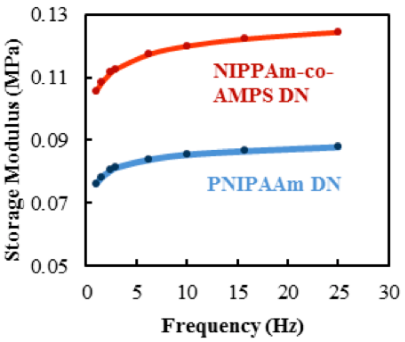

Figure 2. Storage modulus (G') of hydrogels in the compression mode.

Methods: *Preparation of Single Network (SN) Hydrogels*: SN hydrogels were prepared via *in situ* photocure of aqueous precursor solutions. In a 50-mL round bottom flask equipped with a Teflon-covered stir bar, NIPAAm/AMPS (total weight equal to 1.0 g), BIS (0.04 g), and Irgacure (0.08 g) were dissolved in DI water (7.0 g). Different hydrogel compositions were prepared with varying wt% ratios of NIPAAm:AMPS (up to 25:75). A hydrogel sheet was prepared by pipetting the precursor solution into a rectangular mold formed by sandwiching polycarbonate spacers (1.5 mm thick) between two clamped glass microscope slides. The mold was submerged into an ice water bath (~7 °C) and subjected to UV light (UV-Transilluminator, 6 mw/cm^2, 365 nm) for 30 min. After removal from the mold, the hydrogel sheet was rinsed with DI water and then soaked in DI water for 2 days with daily water changes. *Preparation of Double Network (DN) Hydrogels*: The designated SN hydrogel was soaked in a solution of NIPAAm (6.0 g), BIS (0.012 g), Irgacure-2959 (0.24 g), DI water (21.0 g) for 24 hr. The hydrogel sheet was then transferred to a rectangular mold (2.3 mm thick), photocured and purified as above. *Morphology*: Swollen hydrogel specimens were immersed in liquid nitrogen and subsequently freeze-dried in lyophilizer (Labconco Centri Vap Gel Dryer System) overnight. Specimen cross-sections were subjected to Pt-sputter coating and viewed with a field emission scanning electron microscope (FEI Quanta 600 SEM) at accelerated electron energy of 10 keV. *Dynamic Mechanical Analysis (DMA)*: Five discs (13 mm diameter) were prepared as above. DMA of discs was measured in the compression mode with a dynamic mechanical analyzer (TA Instruments Q800) equipped with parallel-plate compression clamp with a diameter of 40 mm (bottom) and 15 mm (top). The swollen disc (13 mm diameter) was blotted with a Kim Wipe, clamped between the parallel plates and silicone oil placed around the exposed hydrogel edge to prevent dehydration. Following equilibration below the VPTT at 25 °C (5 min), the specimens were tested in a multi-frequency-strain mode (1 to 25 Hz).

Results: For the NIPAAm-*co*-AMP DN (i.e. 25:75 NIPAAm:AMPS in the 1st network), SEM revealed larger pores versus the PNIPAAm DN (**Fig. 1**). This agrees with the former's substantially larger swelling upon soaking in DI water. The storage modulus (G', i.e. stiffness) of NIPAAm-*co*-AMP DN was also enhanced (**Fig. 2**).

Conclusions: Thermoresponsive DN hydrogels with enhanced pore size, swelling and stiffness. These properties are attributed to the DN's asymmetrically crosslinked structure[4] and the electrostatic repulsive forces introduced by AMPS[5]. The properties achieved by this membrane design is critical for a self-cleaning glucose biosensor as it is expected to enhance glucose diffusion, self-cleaning and mechanical stability.

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TUNABLE SHAPE MEMORY POLYMER (SMP) FOAMS WITH PDMS SOFT SEGMENTS

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Abstract: Thermoresponsive shape memory polymers (SMPs) are a class of "smart" materials that can be temporarily fixed in a deformed shape and subsequently returned back to their original shape when exposed to heat. Versus non-porous SMPs, porous SMP foams possess unique properties such as low density, high compressibility and enhanced thermal actuating properties. However, most existing SMP foams are based on several commercially available systems and few have offered tunability in foam properties as needed to further expand the applications of SMP foams. In this study, novel SMP foams based on photocrosslinkable macromers containing poly(ε-caprolactone) (PCL) and polydimethylsiloxane (PDMS) segments (AcO-PCL_{40}-*block*-$PDMS_m$-*block*-PCL_{40}-OAc) were prepared via a revised solvent casting/salt leaching (SCSL) method. The obtained foams exhibited high pore interconnectivity and excellent shape memory ability. By changing the PDMS segment lengths (m = 0, 20, 37, 66 and 130), porosity, compressive modulus and degradation rate can be largely varied. Potentially, these SMP foams can serve as scaffolds for an array of biomedical uses.

Biomaterials Day 2012: Graduate Student Poster Abstract Submission

Title: Synthesis and Characterization of Depsipeptide-based Gelation Systems

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We have developed a family of charged depsipeptides based on Fmoc-Lys(Boc)-Lac-OH or Fmoc-Asp(OtBu)-Lac units, using both solution and solid phase peptide methods. Synthesis in solution utilized two protection strategies: activation of the peptide ester with pentafluorophenol or carbobenzyl protection of lactic acid. Purification was performed on silica gel with hexanes:ethyl acetate to yield white crystals (80-95%).

The Fmoc-depsipeptides were used as the growing unit for SPPS, which proceeded with general Fmoc strategies. The cleaving cocktails were investigated to study the stability of the ester bonds under the acidic concentrations needed to remove the Boc or OtBu protecting groups. MADLI of crude samples show little or no hydrolysis of the ester bonds with all cocktails.

Following purification with HPLC, our goal is to investigate the assembly process of Fmoc-oligodepsipeptide gels. Our control studies were conducted with oligopeptides Fmoc-(Lys-Ala)$_4$-OH and Fmoc-(Asp-Ala)$_4$-OH. The controls were synthesized under standard Fmoc-SPPS methods and purified with HPLC. The equivalence points for Fmoc-(Lys-Ala)$_4$-OH) and Fmoc-(Asp-Ala)$_4$-OH were found to be pH 5.17 and pH 6.47 respectively via titration tests. Fmoc-(Lys-Ala)$_4$-OH (20.60-5.2 mg/ml) and Fmoc-(Asp-Ala)$_4$-OH (1.6 mg/ml) were dissolved in water and mixed. Gel formation was determined with the upside-down vial method, and our results yield gels with total peptide concentrations of 3.75-11.10 mg/ml. Our initial studies suggest that Fmoc-oligodepsispetide gels may form under similar conditions.

THE INFLUENCE OF ENHANCED CORE HYDROPHOBICITY ON MEMBRANE DISRUPTIVE PROPERTIES OF POLYCATIONIC NANOSCALE HYDROGELS CONTAINING 2-(DIETHYLAMINOETHYL) METHACRYLATE

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Abstract

Many advances in drug delivery are now predicated upon the rational design of polymers tailored for specific cargo and engineered to exert distinct biological functions. In particular, hydrogels have been instrumental in the development of polymeric systems for release of therapeutic agents. These materials are attractive for transmucosal and intracellular drug delivery because of their facile synthesis, inherent biocompatibility, tunable physicochemical properties, and capacity to respond to physiological stimuli. Our current work aims to develop a responsive hydrogel platform for delivery of small interfering RNA to disease targets along the gastrointestinal tract. Dynamic light scattering, zeta potential measurements, hemolysis assays, and cytotoxicity assays were employed to understand the influence of polymer composition on pH-dependent swelling, effective surface charge, membrane disruption, and cytocompatibility with model cell lines. The addition of *tert*-butyl methacrylate (*t*-BMA) lowers the onset of pH-dependent swelling from ~ pH 7.8 to pH 7.0 and the networks with 30 mol% t-BMA (PDETB30) reach maximum volume swelling near pH 6.0, a value characteristic of the early endosomes. Additionally, pyrene fluorescence spectroscopy demonstrated that greater ionization (i.e. lower pH) is required to induce a hydrophobic-hydrophilic phase transition in PDETB30 networks as compared to those without t-BMA. The addition of t-BMA markedly expands both the pH and concentration range at which these networks effectively disrupt erythrocyte membranes, suggesting the increase in network hydrophobicity has significant impact in the membrane disruptive properties of these nanoscale hydrogels.

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ENHANCING THE ANTI-FOULING PROPERTIES OF SILICONES

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Adsorption of blood proteins by blood-contacting materials induces thrombosis which compromises device success. Silicones have been used in many biomedical applications because of their excellent bulk properties including thermal and oxidative stability, gas permeability, low modulus, flexibility, and biocompatibility. However, silicones generally exhibit poor resistance to plasma proteins because of their extreme hydrophobicity. To reduce protein adsorption, silicone surfaces have been hydrophilized by various approaches involving physical and chemical treatments. In contrast, poly(ethylene oxide) (PEO; or poly(ethylene glycol) PEG) is a neutral, hydrophilic polymer which exhibits high protein resistance due to its hydrophilicity and configurational mobility. Thus, incorporation of PEO into silicones may improve the latter's protein resistance. In this study, PEO-silane amphiphiles containing a flexible, hydrophobic siloxane tether were used to prepare PEO-modified silicones (with and without silica filler), surface-grafted silicone coatings (with silica filler) and surface-grafted coatings on a model substrate (silicon wafer). PEO-silane amphiphiles with variable siloxane tether length (n = 0, 4, 13) were prepared with the basic formula: α-$(EtO)_3Si(CH_2)_2$-oligodimethylsiloxane$_n$-block-[PEO_8-OCH_3]. Silicones (without silica filler) were prepared by the condensation cure of a stoichiometric balanced ratio of each PEG-silane amphiphile (n = 0, 4, 13) and α,ω-bis(Si-OH)PDMS (M_n = 3000 g/mol). Silica-filled silicones were prepared by crosslinking varying concentrations of PEG-silane amphiphile (n = 13) with an acetoxy cure RTV silicone (20% silica). In general, we observed that protein adsorption was decreased as the siloxane tether length was increased. Thus, amphiphilic PEG-silanes are useful to prepare anti-fouling silicone coatings.

Submission for undergraduate poster presentation

Two-part Oral siRNA Delivery Systems: Polycationic Hydrogel Nanoparticles and Alginate Matrices

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Despite the promise of using siRNA to treat disease, there are many challenges associated with its oral administration and the development of oral controlled release formulations. Effective oral delivery systems must protect the therapeutic agent through the GI tract, allow for cellular uptake, and then promote endosomal escape and release of siRNA into the cytosol. In this work, we propose polycationic hydrogel nanoparticles protected by alginate matrices which may be used as carriers to overcome these challenges.

Polymer nanoparticles composed of 2-(diethylamino) ethyl methacrylate, poly(ethylene glycol) methyl ether methacrylate, tetraethylene glycol dimethacrylate, and hydrophobic methacrylate monomers were synthesized using Activators ReGenerated by Electron Transfer Atom-Transfer Radical Polymerization (ARGET ATRP). Following synthesis and purification, the nanoparticles were characterized with dynamic light scattering and hemolysis. Using dynamic light scattering, nanoparticles were observed to swell at low pH; incorporating a hydrophobic monomer decreased the volume swelling ratio. Hemolysis of red blood cells was used as an indicator of endosomal membrane disruption by the nanoparticles; adding a hydrophobic component to the polycationic nanoparticles resulted in strong membrane disruption at acidic pH 6.5 (endosomal pH) but not at pH 7.4 (extracellular pH).

The nanoparticles were incorporated into an alginate matrix in order to investigate a method of protecting the carrier (and siRNA cargo) during GI transit. Alginate beads encapsulating the nanoparticles were synthesized by dropping nanoparticle/alginate dispersions into a calcium chloride solution. The fluorescently labeled nanoparticles enable characterization of the nanoparticle loading and release from the alginate particles to study the system feasibility.

This work was supported by the U.S. National Science Foundation (CBET 10-33746) and a National Science Foundation Graduate Research Fellowship to DCF (DGE-1110007).

In vitro Characterization of Tissue Engineered Cardiac Patch using Gelatin-Chitosan Composite Hydrogel.

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A 3-D scaffold comprised of self-assembled polycaprolactone (PCL) sandwiched in a gelatin-chitosan (GC) hydrogel was developed as a biodegradable patch for surgical reconstruction of congenital heart defects (CHD). These multilayered scaffolds have suturability and high tensile strength provided by the PCL core, along with cardiomyocyte binding sites, and control of the degradation rate provided by the GC hydrogel. Blended or pure MW PCL scaffolds were formed by self-assembly in an aqueous environment, then assessed for pore structure, elastic modulus, tensile strength and degradation time. SEM analysis showed that scaffolds containing lower MW PCL have larger pore sizes. These samples have ~1.8 MPa of elastic modulus. The degradation rate increased in scaffolds containing 10 kDa PCL in physiologic conditions. PCL scaffolds were coated with a GC mixture to facilitate cell attachment and form 3-D tissues. These coated scaffolds showed no significant alteration in tensile stress, strain and tensile modulus. However the compressive modulus of the composite tissue was in a range more similar to native tissue (~15 kPa for 1:1=G:C). A 3-D hive like porous structure was formed with a mean pore diameter ~80μm, allowing for invasion of neonatal rat ventricular myocytes (NRVM). NRVM were viable for 14 days, and formed spontaneously beating engineered tissues. In summary, we were able to form a multilayered scaffold containing NRVM with sufficient tensile strength for use in CHD repair.

Modulation of Activity of Valvular Interstitial Cells by In-vitro Culture with Biomimetic Poly(ethylene glycol) Diacrylate Hydrogels

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Activation of valvular interstitial cells (VICs) is crucially important for the extracellular matrix (ECM) synthesis, which plays a critical role to maintain the homeostasis of the resident cells, the ECM structures, and the mechanical property of the leaflets. However, the relationship between VICs activation and ECM is not well understood. In this study, poly(ethylene glycol) diacrylate (PEGDA) hydrogel was used as a scaffold ("blank slate") that can be grafted with a variety of bioactive peptides to modify its biological function, and thus provides an ideal model to study VICs activation *in vitro*. In two-dimensional culture, various amount of cell-adhesive peptide RGDS (0.5-5 mM) were incorporated into the hydrogel network. We found that time for initial cell adhesion and elongation is RGDS concentration dependent. By increasing the RGDS concentration in the hydrogel, cells adhered on the surface more quickly, while no cell adhesion was found on the surface of pure PEGDA hydrogel. Moreover, more cells elongated and showed strong smooth muscle alpha actin staining (activated) in a short period when higher concentration of RGDS was applied. In three-dimensional culture (encapsulation of cells in the hydrogel), matrix metalloproteinase-sensitive peptide (GGGPQGIWGQGK, abbreviated PQ) and RGDS, were grafted to the hydrogel network. Activation of VICs and secretion of MMP2 (for degradation of the scaffold) was found when cultured in 5% (w/v) PEG-PQ hydrogel with 5 mM RGDS. In summary, PEGDA hydrogel is an ideal template to study activation of VICs *in vitro*, which will shed light on valvular biology and heart valve tissue engineering.

BUILDING AN IMPLANTABLE GLUCOSE BIOSENSOR

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Recently, we have proposed a "self-cleaning membrane" to reduce biofouling of implanted biosensors and thereby extend its lifetime and efficacy. This membrane is based on a thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAAm) which undergoes reversible deswelling/reswelling when heated and cooled, respectively, above and below its volume phase transition temperature (VPTT, ~ 35 °C). Thermal modulation leads to release of adhered protein and cells (i.e. self-cleaning). Coté and co-workers have reported a fluorescent glucose-responsive assay based on the reversible de-aggregation of a protein (Alexa 647-concanavalin A, "ConA") with a glycodendrimer (Alexa 594-glycodendrimer,"GD") when glucose levels rise causing an increase in ConA's fluorescence. We foresee that a functional biosensor could be created by formation of the assay-containing membrane with a cylindrical geometry (1.2 mm x 5 mm) which may be inserted via needle. Towards the goal of ultimately creating this biosensor, we have explored several strategies for assay incorporation into the membrane. Because aggregation/de-aggregation is required for assay functionality, we hypothesized that it must be provided enough space to permit this process. Also, the assay must not significantly leach from the membrane nor should glucose diffusion through the membrane be compromised. We evaluate three distinct strategies. First, direct incorporation of the assay during membrane formation. Second, introduction via assay-loaded calcium carbonate ($CaCO_3$) sphere carriers during membrane formation and the sequential dissolution of spheres to produce assay-loaded pores. Third, formation of a cylindrical membrane with a hollow cavity which could subsequently be filled with the assay.

Myocardial Remodeling of ECM Patch in an Ovine Model

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Treatment of congenital cardiovascular defects has been limited to synthetic or xenogenic materials susceptible to significant inflammatory response, infection, and thrombosis. This has generated interest in using biological implant materials that can overcome these limitations, as well as grow with the patient. Small intestinal submucosa-derived extracellular matrix (SIS-ECM) has demonstrated remodeling in bladder reconstruction, and may be a potential patch material for heart defects. This study investigated the bioelectrical, mechanical, and histological properties of an ECM patch implanted in the right-ventricular outflow tracts (RVOT) of juvenile ovine (n=4) at 5 and 8 months. After the 5 and 8 month implantation period, the RVOT and native tissue were characterized. *In vivo* electrical mapping confirmed that the SIS-ECM patch conducted an organized electrical signal. No significant difference was measured in stiffness between the native and SIS-ECM patch at 8 months (0.976±0.3 vs. 0.614±0.2 MPa, p-value=0.333). At 5 and 8 months, the ECM had undergone significant ECM remodeling, and neovascularization was evident more at 5 months compared to 8 months (3.08±1.2 vessels/mm^2 vs. 0.88±0.3 vessels/mm^2, p-value=0.004). Immunohistochemistry showed that the implant site was populated with locally aligned muscle cells positive for sarcomeric α-actinin, CD45, and troponin I and T at 5 and 8 months. This study demonstrated that the SIS-ECM patch was capable of remodeling to behave as native, functional ventricular tissue in ovine. Further studies including a greater number of animals and advanced imaging will be performed to validate the functionality of the patch over time.

INORGANIC-ORGANIC HYDROGEL SCAFFOLDS FABRICATED AS CONTINUOUS GRADIENTS

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It has been shown that cells actively respond to the chemical and physical properties of their environment, whether this environment is the natural extracellular matrix (ECM) or a synthetic, three-dimensional (3D) tissue engineering scaffold. Therefore, optimizing and understanding the interactions between cells and a scaffold's material is essential for the regeneration of native-like tissues. Here, we describe a method for altering, and potentially enhancing, the properties of organic, 3D poly(ethylene glycol) diacrylate (PEG-DA) hydrogel scaffolds via the incorporation of inorganic methacrylated star polydimethylsiloxane ($PDMS_{star}$-MA). $PDMS_{star}$-MA is known to be bioactive and has been shown to induce bone-like differentiation in mesenchymal stem cells (MSCs). Because of this, $PDMS_{star}$-MA has been incorporated into a PEG-DA hydrogel as a "gradient." Ideally, this scaffold containing a continuous gradient of $PDMS_{star}$-MA will initiate MSC differentiation towards a gradual transition from mineralized bone- to cartilage-like tissue. Furthermore, the hydrogels were fabricated in both water and solvent, which altered the distribution of $PDMS_{star}$-MA and the scaffold's porosity due to the differing polarities of the solutions. Hydration and mechanical testing were used to study the effects of both the $PDMS_{star}$-MA concentration gradient and the choice of fabrication solution. This combinatorial screening method will be used to rapidly assess cell-material interactions towards the regeneration of a more native-like osteochondral interface.

Microwave Absorption Property of Nanoparticles for Biomedicine

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Keywords: thermoacoustic tomography (TAT), photoacoustic tomography (PAT), super paramagnetic iron oxide nanoparticle (SPIONs), gold nanoparticles (AuNPs), gold nanoclusters (AuNCs)

Abstract

This research focuses on the microwave properties of nanoparticles for use as contrast and hyperthermic agents. Currently, visible light is used for irradiation of nanoparticle hyperthermia agents1,2. Additionally, visible/Near-infrared light is used for photoacoustic tomography (PAT) imaging3.Compared to optical wavelengths, frequencies between 10 MHz to 3 GHz transmit through tissue with high penetration depth4. Thus, deep cancerous cells and malignant tissue may be treated and imaged. These nanoparticles could enable the use of a hybrid microwave/acoustic technique known as thermoacoustic tomography. Here, quantitative measurements of the heat generation in super paramagnetic iron oxide nanoparticle (SPIONs) , gold nanoparticles (AuNPs), and gold nanoclusters (AuNCs) induced by microwave energy at 3 GHz, are presented and compared.

SPIONs are the most investigated and efficient nanoparticles for microwave heating5,6. However, high concentrations are still required. AuNPs, which support plasmon resonances, should not provide absorption in the radio frequency and microwave regions. However, there have recently been conflicting reports about AuNPs and the conversion microwave energy into heat7,8. AuNCs are a new form of ultra-small (<2.5 nm) AuNPs9,10 which do not support plasmonic resonances but may provide microwave absorption due to subconduction band transitions. However, these nanoparticles have not yet been studied in this frequency region.

Microwave testing of the nanoparticles reveals that SPIONs are the most efficient absorber of microwave energy in this frequency region. As expected, AuNPs were found to have negligible microwave absorption. Interestingly, the study indicates that AuNCs do possess absorption in this frequency region and may provide an alternative to SPIONs.

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CHARACTERIZING THE INJECTABILITY OF A HIGHLY POROUS BONE GRAFT

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Undergraduate Poster Competition

Objective: A clinical need exists for a bone graft that can fill irregularly shaped defects encouraging integration with host tissue. We utilized emulsion templating to develop a high internal phase emulsion (HIPE) to provide a high porosity, injectable bone graft that can withstand physiological loads. In this work we characterized the injectability of our system in terms of work and set time and component cytocompatibility.

Methods: Work and set time were quantified with a dynamic mechanical analyzer and the tack-free time test, respectively. The effect of injection needle diameter on scaffold pore size was analyzed via scanning electron microscopy (SEM). The cytocompatibility of HIPE components was tested using LIVE/DEAD® assays.

Results: Preliminary injection results indicated a decrease in average pore size with an increase in needle gauge. Work and set time were quantified at 55 minutes and 1.5 hours, respectively using the aqueous phase soluble initiator ammonium persulfate. All HIPE components were cytocompatible except for the initiator. To address this, an organically-soluble initiator was used to improve cytocompatibility. This also provided an interconnected pore system.

Conclusions: The polyHIPE system can be used as an injectable graft as indicated by our work and set times and measured cytocompatibility. Current work is focused on tuning the interconnectivity of the polyHIPE system using the organic initiator. The work and set time will then be characterized for the new system. Future work will determine the feasibility of encapsulating human mesenchymal stem cells in the polyHIPE for cell delivery to the defect site.

Effects of Varying Stiffness of Hyaluronic Acid Hydrogels on Morphology and Viability of Prostate Cancer Cells

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Traditional 2D systems for cell culture in which cells are grown as a monolayer have been well-characterized, but are not sufficiently representative of the natural cellular environment. These 2D systems limit the possible architectures in which cells can be grown and often change gene expression, limiting the ability of these systems to be predictive of in vivo outcomes. Three-Dimensional cell culture systems in which cells are grown suspended in natural or synthetic polymeric hydrogels offer a more biomimetic environment.

It has been shown that stiffness of the 2D substrate on which cells are grown has a strong effect on many cell processes, including morphology, migratory behavior and viability. Three-dimensional systems also offer tunable stiffness by changing crosslinking density. In these studies we examined how changes in the stiffness of a three-dimensional hyaluronic acid matrix affect the morphology and viability of multiple prostate cancer cell lines. These 3D systems potentially can be employed in high-throughput screening applications for testing new anti-cancer compounds.

Development of a Model Surface for Investigation of Glutaraldehyde-Fixed Heart Valve Surface Modification

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Introduction: Surface modification has successfully improved the hemodynamic properties and immunogenicity of vascular grafts, and could be leveraged to improve the performance of glutaraldehyde-fixed (GA-fixed) heart valve replacements as well. However, valves have a complex geometry and greater thickness that impedes analysis of the surface modifications. To address this problem, we created a model GA-fixed surface to enable facile analysis of surface modification techniques.

Methods: To create our model surface, glass coverslips were coated with 1.35% collagen I solution at 37°C for 30 min and then GA-fixed in 0.2% glutaraldehyde for 3 days. Evenness of the coated surface and glutaraldehyde incorporation were analyzed by XPS. Acute toxicity of the GA-fixed surface was determined by culturing human dermal fibroblasts for 24 hours on collagen and GA-fixed coverslips, and comparing to fibroblasts cultured in a transwell plate with GA-fixed porcine tissue.

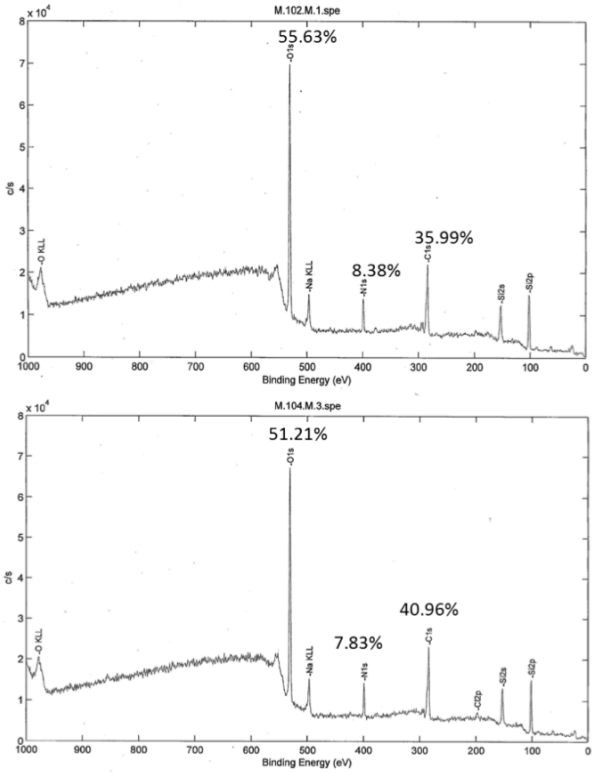

Figure 1. XPS analysis of collagen-coated (top) and glutaraldehyde-fixed (bottom) coverslips. Percentages reflect the atomic concentration of each element, indicating an increase in collagen after the fixation process.

Results: XPS spectrum readings for carbon and nitrogen confirm collagen coating on the coverslip surface. The atomic concentration of carbon increased from 35.99% to 40.96% between the collagen-coated and GA-fixed groups, indicating incorporation of glutaraldehyde molecules into the surface coating. Fibroblasts cultured on both surfaces exhibited normal spindle-shaped morphology, whereas cells cultured with GA-fixed tissue were rounded and unhealthy.

Conclusion: We created a model GA-fixed surface for testing of various surface modification methods. This surface, however, does not mimic the toxicity exhibited by glutaraldehyde-fixed heart valves. Further work is needed to create a model surface mimicking cytotoxic effects to predict the response of cells to the modified surface *in vivo*.

Intelligent Polymeric Biomaterials for the Oral Delivery of Hydrophobic Therapeutic Agents for Cancer Treatment

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We have designed nanoscale drug carriers that can help achieve oral delivery of hydrophobic cancer drugs, thereby minimizing side effects associated with intravenous delivery and improving overall patient compliance. Hydrogel nanoparticles comprising of methacrylic acid co-polymerized with hydrophobic monomer tert-butyl methacrylate, and grafted with poly(ethylene glycol) tethers, were synthesized using an UV-initiated emulsion polymerization technique. Incorporation of methacrylic acid allows the hydrogel to be responsive to changes in the pH, as it passes from the stomach (low pH) to the upper small intestine (high pH), upon oral administration. Doxorubicin was chosen to be a model hydrophobic chemotherapeutic to evaluate the loading and release capabilities of these nanoparticles. The collapsed diameter (at pH 2) of the particles was found to be in the range of 80-100 nm, while the swollen diameter (at pH 7) of the particles ranged from 120-140 nm, as obtained by dynamic light scattering. Nanoparticle formulations with varying crosslinking density and core hydrophobicity were able to load doxorubicin with efficiencies ranging from 36-55% within 2 hours of incubation with the drug solution. As the percentage of crosslinking density of the nanoparticles was increased, their ability to swell as well as their therapeutic agent loading and release capability, decreased. The physically encapsulated doxorubicin was completely released within a period of 4-6 hours, at pH 7.4, while still being retained at pH values representative of conditions in the stomach.

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EFFECTS OF PROCESSING AND STORAGE CONDITIONS ON PEG HYDROGEL PROPERTIES

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Objective: Poly(ethylene glycol) (PEG) hydrogels are widely used as biomaterial scaffolds due to their highly tunable properties and established biocompatibility. In this study, PEG hydrogels have been chosen as the intimal layer of an off-the-shelf vascular graft. Previous research has suggested that processing and long-term storage of hydrogels could alter scaffold properties. The aim of these studies was to determine the potential effects of varied processing and storage conditions on PEG hydrogel mechanical properties, swelling ratio, and bioactivity to assess their potential use in off-the-shelf vascular grafts.

Methods: Mechanical properties and swelling ratio of swollen PEG hydrogels (10, 20 and 30 wt%, MW 3.4 and 6 kDa) were measured after different processing treatments (swelling, swelling then vacuum drying, vacuum drying, lyophilization). Acrylated collagen was crosslinked into 10 wt% PEG (3.4k) diacrylamide hydrogels at 4mg/mL, and the resulting gels were either lyophilized or swollen and stored at -20°C or 25°C. After up to six weeks of storage, endothelial cell adhesion and spreading on resulting samples were assessed.

Results: PEG hydrogel swelling ratio and mechanical properties were not significantly affected by processing conditions for any composition. These results suggest that the grafts can be dried without significant effects on intimal layer properties. In the near future, effects of storage conditions on bioactivity of PEG-collagen hydrogels will be examined by measuring EC adhesion and spreading. The ability to dry and store these scaffolds for long periods without detriment to material properties or bioactivity could significantly improve the utility of this vascular graft design.

Title: Microwave-assisted Synthesis of Carbon Dots

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Abstract:

Carbon Dots (Cdots) are a new type of luminescent nanomaterial which have strong potential in biological and biomedical applications, and have been the subject of intense research in recent years. Cdots are nanostructures consisting primarily of carbon atoms and can have similar optical performance as quantum dots under UV excitation. Moreover, properties including good bio-compatibility, water solubility, and a non-toxic response show that Cdots are safe for the body or the environment [1-2]. Thus, they are good candidates for both *in vivo* and *in vitro* applications. These advantages give Cdots strong potential in bio-labeling or bio-imaging.

Several synthesis routes have been explored including laser ablation [3], electrochemical [4], and acid dehydration of carbohydrates [5]. However, these methods either involve high cost or complex reactions. Here, we explore an innovative and simple microwave-assisted synthesis toward the production of Cdots from glucose. Photoluminescence properties are studied and discussed through emission and absorption spectrum. The components and structure are investigated by transmission electron microscopy (TEM), Atomic force microscopy (AFM) and mass spectrum. The results show the Cdots exhibit globular structure and the average size is 2.7 nm in diameter. The emission curves of Cdots are broad, extending across the visible spectrum, and exhibit a shift with excitation wavelength.

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MANIPULATION OF PROTEIN FUNCTIONALIZATION INCREASES CELL INTERACTIONS WITH BIOACTIVE HYDROGELS

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Objective: Poly(ethylene glycol) (PEG)-based hydrogels are widely used as biomaterial scaffolds due to their "blank slate" properties that allow for controlled introduction of bioactivity. We have fabricated PEG hydrogels with a modified collagen-mimetic protein derived from group A Streptococcus, Scl2.28 (Scl2-2) for use in vascular grafts. These gels induce endothelial cell (EC)-specific adhesion but at relatively low levels. We propose that modifying Scl2-2 functionalization could enhance these interactions. The aim of these studies is to determine the effects of functionalization density on PEG-Scl2-2 hydrogel initial and sustained bioactivity.

Methods: Scl2-2 and collagen were functionalized with acrylate-PEG-N-hydroxysuccinimide (Acr-PEG-NHS) at varied ratios of Acr-PEG-NHS:NH_2 to produce proteins with varied PEG linker densities (1X, 0.5X, 0.1X). EC adhesion and spreading on PEG-protein gels was evaluated after swelling for up to six weeks. Protein crosslinking efficiency and retention was measured with a protein quantitation assay.

Results: EC adhesion and spreading was significantly increased on PEG-protein hydrogels with decreased functionalization density. However, decreasing functionalization density reduced crosslinking efficiency for PEG-collagen and PEG-Scl2-2 hydrogels (~80% for 1X to ~60% for 0.1X). Additionally, gels swollen for up to 6 weeks were found to have steadily decreasing levels of crosslinked protein over time.

Conclusions: EC adhesion and spreading data suggests that decreasing functionalization density could be utilized to provide enhanced cell interactions with bioactive PEG-based hydrogels. However, it also lowers crosslinking efficiency and could result in greater loss of protein over time. Currently, the effect of PEG matrix biostability on protein retention and EC interactions is being investigated.

Externally-Triggered Thermally-Responsive Nanocomposites for Advanced Therapeutic Delivery

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The localized and targeted concentration of drug agents using nanocarriers with external spatiotemporal control confers significant improvements over traditional drug distribution methods that result in systemic bioavailability. With chemotherapeutics utilized in anticancer regimens, adverse side effects and improved clinical outcomes would be concurrently expected if drug levels are maintained in high concentrations exclusively in the intended tissues. Toward this objective, responsive nanocomposites based on hydrogel nanoparticles and encapsulated iron oxide cores, rely on external triggering by AC induction heating to conduct thermal energy from the inorganic nanoparticle core to the surrounding temperature-responsive polymer. Heating from within releases encapsulated therapeutics by a combination of convective and diffusional processes induced by the expanding polymer network in the physiological environment. The surface of the nanocarrier is modified for improved in vivo utility by the attachment of a poly(ethylene glycol) brush and antibodies for active targeting.

Interpenetrating polymer network (IPN) hydrogel nanocomposites have been synthesized for this application, where a poly(acrylamide)-poly(acrylic acid) IPN experiences an upper-critical solution temperature (UCST) thermal swelling response during heating. The particles are synthesized by a reverse-emulsion process, and are purified by a series of strategies, including sedimentation, extraction, dialysis, and tangential flow filtration. Furthermore, the processes that underscore the reverse-emulsion polymerization have been examined with dynamic light scattering and electron microscopy to determine which variables are the most significant in regard to engineering particle morphology, as well as swelling responses critical to achieving therapeutic utility and desired pharmacokinetics.

Environmentally Responsive Polymeric Carrier Systems for Oral Delivery of Protein-Based Chemotherapeutic Drugs

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Statement of Purpose: A major area of interest in developing more effective cancer treatments is the potential to orally administer therapeutic agents selectively to targeted sites in the gastrointestinal tract, liver, colorectal, and pancreas. Our work has been to develop pH-responsive carriers using poly(methacrylic acid grafted poly ethylene glycol) (P(MAA-g-EG)) nanoparticles with the hydrophobic polymer *tert*-butyl methacrylate (*t*BMA) incorporated into the network. The protein-based chemotherapeutic agent Interferon Alpha (IFNα) is being investigated for the treatment of liver, colorectal, and pancreatic cancers using our nanoparticles as carriers.

Methods: P(MAA-g-EG-co-*t*BMA) nanoparticles were synthesized using photo-emulsion polymerization method previously reported by our group [1]. Dynamic light scattering (DLS) was used to determine the size of the particles as pH increased. Additionally cytocompatibility, bioavailability, and transport studies were performed using the human colon adenocarcinomal (Caco-2) cell line.

Results: The DLS data showed that particles underwent a 3-fold increase in volume when exposed to pH above 4.8. This swelling transition point indicates that drug release would occur at the upper small intestine [1]. Cytocompatibility studies showed that cell viability did not significantly decrease when incubated with the nanoparticles. The transport studies showed that when using the P(MAA-g-EG-co-*t*BMA) nanocarriers, IFNα was transported across the cell monolayer much more efficiently compared to free IFNα.

Conclusions: The P(MAA-g-EG-co-*t*BMA) nanocarriers show promise for the oral delivery of toxic chemotherapeutic agents.

This work was supported in part by NIH grant U54-143837-02.

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Submission for graduate student poster presentation

COMPARING ARGET ATRP WITH TRADITIONAL FREE RADICAL POLYMERIZATION FOR VERSATILE POLYCATIONIC HYDROGEL NANOPARTICLES

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Advances in controlled radical polymerization technology create opportunities for new – and possibly improved – biomaterials for drug delivery. Activators regenerated by electron transfer (ARGET) ATRP is a simple, robust ATRP technique that can occur in the presence of limited air with relatively low concentrations of copper catalyst. ARGET ATRP avoids UV-initiation, which confers advantages including ease of conducting multiple simultaneous reactions, synthesis protocols that can be scaled without consideration of the UV light source intensity and duration, as well as the ability to use photosensitive components such as dyes or biologically derived components in the polymerization reaction. This work converts a traditional free radical photoemulsion nanoparticle synthesis to an ARGET ATRP-based scheme and compares the resulting polycationic hydrogel nanoparticles. Polycationic hydrogel nanoparticles may be used for the delivery of oligonucleotides such as siRNA as well as co-delivery of siRNA with small molecule drugs.

The polycationic hydrogel nanoparticles are composed of 2-(diethylamino) ethyl methacrylate (DEAEMA) for pH-responsiveness, poly(ethylene glycol) methyl ether methacrylate (PEGMA) for enhanced colloidal stability and biocompatibility for drug delivery, a crosslinking agent for enhanced nanoparticle stability, and hydrophobic methacrylate monomers to impart core hydrophobicity. The nanoparticles swell from ~100nm at low pH to ~150 nm at high pH, according to dynamic light scattering. Characterization *in vitro* suggests the cytotoxicity of the new ARGET ATRP-based nanoparticles is comparable to that of particles synthesized using the traditional free radical polymerization method, and that reduced toxicity can be achieved by increasing the amount of hydrophobic monomer.

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Long-term Culture of Human Cardiac Cells

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Abstract

Primary human cell lines could provide insight for understanding cardiac tissue functions and devising regenerative therapies. In this study, we investigated the isolation and long-term culturing of cardiac cells from human right ventricular outflow tract (RVOT) samples obtained from surgeries for congenital heart defect repair. Cells were isolated by enzymatic digestion. Isolated cells were then subjected to serum supplemented Medium 199 based media, and allowed to attach to attachment factor treated surface for 12 hours. The adhered cells were cultured for at least 7 days at 37^{o}C and 5% CO_2 and examined using light microscopy during various stages of culturing. The cultured cells were found to be proliferating and exhibited fibroblast-like morphology. These cells lacked the characteristic striation of cardiomyocytes and did not spontaneously contract. After the culturing period, the cells were subjected to immunostaining and quantitative real time polymerase chain reaction (qRT-PCR) assays. Compared to amniotic fluid-derived stem cells (AFSC,) a non-cardiac cell control, the cultured cells stained positively for cardiac-specific troponin T (cTnT) and GATA-4, protein markers for cardiac lineage. In agreement with this result, the cultured cells were found to have expressed significantly ($p<0.05$) higher levels of mRNAs encoding cTnT and GATA-4 than AFSCs through qRT-PCR. These results indicated that a population of proliferating cells of cardiac lineage was able to be isolated from RVOT samples and maintained in long-term cultures. These cells could be used in future investigations of cardiac cell differentiation.

Synthetic Strategies to Improve Functionalization of PEG-Diamine

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Objective: Injured anterior cruciate ligaments (ACL) have poor healing capacity due to inadequate vasculature and cell proliferation. Our goal is to develop a tissue engineered ACL that provides adequate mechanical properties and graded load transfer to new tissue. Highly reactive amine-functionalized poly(ethylene glycol) (PEG-NH_2) will be used to synthesize the soft segment of an enzymatically degradable polyurea-based ACL scaffold. This study focuses on improving the functionalization and lowering the coupling of PEG-NH_2 during synthesis. Incomplete functionalization of PEG-intermediate (PEG-I) allows nucleophilic substitution from the unreacted hydroxyl groups which, in turn, couples PEG chains via ether formation. Additionally, leaving group reactivity influences PEG-I and PEG-NH_2 functionalization by affecting the reaction kinetics of PEG-I and PEG-NH_2 synthesis. The effects of various leaving groups on functionalization of PEG-I and PEG-NH_2 were investigated to provide improved reproducibility of soft-segment synthesis for ACL grafts.

Methods: Azeotropically dried PEG-OH (6000 kDa) was reacted with triethylamine and a leaving group (4-toluenesulfonyl chloride (TS), methanesulfonyl chloride (MS), or 4-fluorobenzenesulfonyl chloride (FS)) in dichloromethane at 0°C. Resulting PEG-I was reacted with ammonium hydroxide to yield PEG-NH_2. Percent functionalization of PEG-I and PEG-NH_2 was measured with NMR and coupling was characterized with gel permeation chromatography.

Results & Conclusions: PEG-MS yielded the lowest coupling of PEG-NH_2 with high percent amination. Low reactivity of PEG-MS prevented reversion to PEG-OH and reduced coupling of PEG-NH_2. Alternatively, PEG-FS caused the highest percent coupling of PEG-NH_2 due to the highly reactive nature of the leaving group. Thus, decreasing leaving group reactivity of PEG-I improved functionalization and reduced the degree of coupling in PEG-NH_2. The resulting PEG-NH_2 can be used to synthesize the soft segment of a polyurea ACL graft designed for cell-mediated degradation. Current work is focused on using lower molecular weight polyol and alternative methods of amination to synthesize PEG-NH_2.

DETERMINATION OF THE OSTEOCONDUCTIVITY OF HIGHLY POROUS, INJECTABLE POLYHIPES FOR BONE GRAFTS

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Objective: Orthopedic surgeons have long been interested in a synthetic material that fully integrates with host tissue to improve healing and remodels to resemble native bone. We have successfully utilized an emulsion templating technique to fabricate a polymerized high internal phase emulsion (polyHIPE) with compressive strength and modulus comparable to cancellous bone. Previously, we determined key variables for modulating pore architecture which influences mechanical properties, mesenchymal stem cell phenotype, and nutrient/waste transfer. We have expanded on this work by examining the cytocompatibility of compositional elements and determining methods to produce an osteoconductive, injectable polyHIPE for bone grafts.

Methods: Propylene fumarate dimethacrylate (PFDMA) purification techniques were altered to ensure complete removal of methacrylic acid. A LIVE/DEAD® assay was utilized to determine the cytocompatibility of the PFDMA, surfactant, radical initiator, and electrolyte added during HIPE fabrication. hMSC adhesion and spreading were monitored on polyHIPEs and polymer films utilizing cells stained with CellTracker™.

Results: The aqueous-phase soluble initiator ammonium persulfate (APS) exhibited poor cytocompatibility while 2,2′-azobis(2-methylpropionitrile) (AIBN), an organic-phase soluble initiator, was cytocompatible at concentrations required for polyHIPE formation. Additionally, AIBN produced polyHIPEs with interconnected pores. Adhesion data comparing films with and without the surfactant polyglycerol polyricinoleate (PGPR) indicated that PGPR has an effect on protein adsorption and/or conformation resulting in poor cell adhesion.

Conclusions: Recent work has indicated the cytocompatibility of PFDMA polyHIPE components. Currently, we are pursuing the incorporation of acrylated PEG-RGD into polyHIPEs to introduce cell binding sites. Future work will focus on determining graft osteoconductivity as well as increasing osteoinductivity through incorporation of hydroxyapatite nanoparticles.

pH-Responsive Hydrogels for Oral Delivery of Therapeutic Proteins

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Traditional delivery of therapeutic proteins by intravenous injection is an invasive method which results in low patient compliance with doctor-recommended treatment. Oral delivery is strongly preferred to increase patient compliance and quality of life. We have previously developed pH-responsive hydrogel vehicles, based on poly(methacrylic acid) grafted with poly(ethylene glycol), or P(MAA-*g*-EG), for the oral delivery of insulin. Our current work is focused on expanding this technology for other proteins, specifically salmon calcitonin which treats osteoporosis, Paget's disease, and hypercalcemia. Calcitonin, like approximately half of all bodily proteins, displays a high isoelectric point that results in different charge properties compared to insulin. As a result, different hydrogel delivery systems are required for effective release.

We have synthesized pH-responsive hydrogels of itaconic acid (IA) and N-vinyl pyrrolidone (NVP), designated P(IA-*co*-NVP), by UV-initiated free radical polymerization using tetra(ethylene glycol) dimethacrylate (TEGDMA) crosslinker. In equilibrium and dynamic-pH swelling studies, these carriers displayed favorable swelling characteristics for delivery in the small intestine, remaining collapsed in acidic conditions and swelling to weight ratios of 15-20 at neutral conditions. Formulations with higher itaconic acid content displayed greater swelling. All carriers displayed greater swelling in a dynamic-pH study than previous formulations of P(MAA-*g*-EG) or P(MAA-*co*-NVP), indicating the potential for greater release of protein in the small intestine. Bovine serum albumin was loaded into and released from P(IA-co-NVP) microparticles to evaluate the copolymer's ability to deliver large molecular weight proteins. The carriers all demonstrated delivery capability, although P(MAA-co-NVP) microparticles were more effective at loading and releasing large doses.

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ANTI-THROMBOGENIC HEMODIALYSIS CATHETER COATINGS BASED ON PEO-SILANE AMPHIPHILES

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Hemodialysis catheters are increasingly being used to treat patients with end-stage renal disease (ESRD). Such catheters are often prepared polycarbonate-polyurethane copolymers (PCUs) due to their superior mechanical properties versus silicones. Unfortunately, PCU catheters are unable to adequately control surface-induced thrombosis (i.e. clotting). Thus, heparin-locks are used following dialysis but have limited efficacy, do not treat extraluminal thrombosis, are expensive and pose serious health risks. Heparin-coated PCU catheters are commercially available but are expensive and their efficacy has not been conclusively established. Thus, a heparin-free surface coating that could be grafted to the intra- and extraluminal surfaces of PCU catheters presents an attractive alternative for controlling thrombosis. We have developed a coating that prevents the non-specific adsorption of plasma proteins that initiates the coagulation cascade. Although poly(ethylene oxide) (PEO; or poly(ethylene glycol) PEG) exhibits exceptional protein resistance *in vitro*, its success *in vivo* is limited. In this study, PEO-silane amphiphiles with variable PEO lengths (m = 3, 8, 16) were prepared with the basic formula: α-$(EtO)_3Si(CH_2)_2$-oligo-dimethylsiloxane$_{13}$-block-[PEO_m-OCH_3]. The flexible, hydrophobic siloxane tether will introduce amphiphilicity and molecular mobility to the surface so as to reduce protein adsorption. The PEO-silane amphiphiles were covalently grafted onto a model substrate (silicon wafer) and their surface properties evaluated. In addition, we have evaluated surface-grafting as well as blending strategies to modify carbothane surface properties.

FABRICATION OF INJECTABLE AND HIGH POROSITY POLYMIPE SCAFFOLDS FOR SOFT TISSUE REGENERATION

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Motivation:
Soft tissue repair requires elastomeric, permeable scaffolds that conform and change with healing tissue. Current soft tissue scaffolds have adequate mechanical properties but are either non-injectable or non-porous. Injectable scaffolds that cure *in situ* have the potential to improve tissue integration and healing while eliminating the need for costly molds. Additionally, highly porous scaffolds increase nutrient/waste transport, necessary for neotissue viability. In this study, polymerized medium internal phase emulsions (polyMIPEs) were investigated as tunable scaffolds that could be both injectable and highly porous.

Methods and Results:
PCL diol and triol were end capped in bulk with an excess of hexamethylene diisocyanate at 75 °C under nitrogen until complete, as determined with FTIR spectroscopy. An overhead stirrer was used to emulsify the PCL-diisocyanate and triisocyanate (PCL-DI, –TI) with 20 wt% polyglycerol polyricinoleate (PGPR 4125) and an aqueous solution of 1% $CaCl_2$ added at 1 mL/min. Prior to polymerization, all MIPEs had a mayonnaise-like consistency capable of flowing through a syringe and then cured in 5 hours at 37 °C. Scanning electron microscopy showed that pores were interconnected and up to 1mm in diameter, appropriate for cell migration and nutrient/waste transport. Scaffold mechanical properties and pore structures were tuned by varying polyMIPE composition to manipulate crosslink density and emulsion stability.

Summary/Conclusion:
Injectable, elastomeric scaffolds have been fabricated with tunable pore architectures and mechanical properties suitable for soft tissue applications. These scaffolds form to any defect geometry and their open pores facilitate mass transport and cellular infiltration without sacrificing elastomeric behavior.

EFFECTS OF HUMIDITY AND SOLUTION VISCOSITY ON ELECTROSPUN FIBER MORPHOLOGY

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Objective: Electrospinning is advantageous for many tissue engineering applications because it produces polymeric nanofiber meshes that can direct and support cell growth while having tunable degradation rates and mechanical properties. These properties can be modulated by controlling fiber microarchitecture, such as fiber orientation and diameter. However, current efforts at controlling fiber microarchitecture are hampered by poor understanding of the role of processing variables (e.g. humidity). We aim to gain a better understanding of the process of fiber formation under various electrospinning conditions in order to improve control of fiber morphology. In this study, we investigate the effect of humidity and solution viscosity on fiber morphology.

Methods: Carbothane®, a poly(carbonate urethane), poly(ethylene glycol) (PEG), and polycaprolactone (PCL) were electrospun using the appropriate parameters for each to achieve uniform fibers. Humidity was altered from 25 to 75% using a humidifier with a feedback controller and a dehumidifier. The Carbothane® solution viscosity was varied by altering solution concentration from 15 to 20 wt% and was measured with a rheometer. Resultant fiber morphologies were analyzed using scanning electron microscopy (SEM).

Results and Conclusions: Fiber morphology was modulated from beaded fibers to beads-on-strings to uniform fibers by increasing relative humidity or solution viscosity. We hypothesize that humidity alters fiber morphology by changing the amount of charge dissipation from the fibers. Additionally, preliminary data suggests that higher viscosity increases polymer chain entanglements and prevents fiber breakage into beads. This understanding of electrospun fiber formation provides a systematic method for modulating fiber microarchitecture to improve the properties of tissue engineering scaffolds.

Quantifying the interaction between adeno-associated virus vectors and human fibronectin for substrate-mediated gene delivery

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Substrate-mediated gene delivery (SMGD) combines tissue engineering (TE) with gene delivery (GD), providing unique opportunities for genetic modulation of target cells associated with tissue regeneration. SMGD involves immobilization of GD vectors on or within a biomaterial. This approach results in high concentrations of transgenes within the biomaterial microenvironment, leading to increased GD to cells that are seeded or migrate into the biomaterial. Adeno-associated virus (AAV) is gaining momentum as a delivery vector due to its high efficiency, long-term transgene expression, and relatively low pathogenicity. We previously showed AAV2 binds to the ECM protein fibronectin (HFN); but to date AAV2 binding strength has not been quantified to a substrate other than its primary cellular receptor, heparan sulfate (HS). Since biomaterials used in TE frequently include ECM components, inherent AAV2-ECM interactions may be leveraged to immobilize AAV2 onto a biomaterial, circumventing the need to chemically conjugate gene vectors to substrates. Here we utilized Quartz Crystal Microbalance with Dissipation to quantify AAV2-HFN binding affinity. We observed AAV2 follows Langmuir binding kinetics, quickly forming a monolayer over HFN. We derived an apparent AAV2-HFN K_D of 16nM, indicating an 8-fold increase in binding strength over the AAV2-HS interaction. This result suggests AAV2 can be immobilized onto HFN via inherently strong interactions. However, SMGD from HFN still allows for high transduction efficiency with AAV2, indicating the overall dynamics in the scaffold microenvironment allow for appropriate virus-cell interactions that lead to successful GD. Continued investigation of AAV-ECM interactions should aid in the development of new SMGD approaches.

Design of pH-Responsive Carriers for the Oral Delivery of High Molecular Weight Protein Therapeutics

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Protein therapeutics have traditionally been delivered by intravenous or subcutaneous injections, which can cause discomfort and lack of patient compliance. An oral delivery scheme is preferred, although it is limited by the harsh conditions present in the GI tract that degrades any unprotected protein. We have focused on the synthesis of polymer networks, such as poly(methacrylic acid) grafted with poly(ethylene glycol) chains, or P(MAA-g-EG), capable of protecting the protein and releasing it in the upper small intestine. In our current work we examine additional proteins such as growth hormone (GH). This particular therapeutic agent is used for the treatment of growth hormone deficiency, Turner's Syndrome, and Prader-Willi Syndrome. Its large size, 22 kDa and about 4 times larger than insulin, presents unique loading and delivery challenges.

The carrier for the delivery of growth hormone is a family of hydrogel networks based on methacrylic acid, which imparts the pH-responsive behavior, N-vinyl pyrrolidone, a highly hydrophilic monomer, and poly(ethylene glycol), providing mucoadhesive properties. The hydrogels, designated as P((MAA-co-NVP)-g-EG), were synthesized using UV-initiated free-radical polymerization and a tetra(ethylene glycol) dimethacrylate (TEGDMA) or a poly(ethylene glycol) dimethacrylate (PEGDMA) crosslinker. Both of these networks showed favorable swelling characteristics, remaining collapsed at low pH and volume swelling ratios up to 20-25 at neutral pH. The polymer pores were approximately 5 times larger than the hydrodynamic radius of GH. Additionally, dynamic swelling studies were completed at varying pH-values and showed desired swelling profiles and ratios. These studies indicate that P((MAA-co-NVP)-g-EG) is an excellent candidate for GH release.

Presence of IHH in "Growth Plates" of Cartilage Nodules Grown in a Rotating Bioreactor. T.C. Woernle, P.J. Duke, A.Derese, A. Menchaca, Dept. of Orthodontics, School of Dentistry, UTHSC, Houston, TX

Previous studies have found that cartilage nodules grown in a bioreactor contain regions resembling epiphyseal growth plates, which mineralize and secrete the angiogenic factor VEG-F. To further characterize these growth plates, the current study compared the location of the signaling factor Indian hedgehog (IHH) in cartilage nodules grown in a bioreactor from embryonic limb buds to its location in growth plates of adult mice. Methods: Fore and hind limbs removed from C57/Bl/6 embryos on day 13th of development were used to form a single cell solution which was aggregated on a rotating platform in an incubator overnight. Nodules were then placed in a rotating bioreactor (Synthecon, Inc.) for 2 weeks with ½ the medium changed every other day. Nodules were fixed, sectioned, and stained with anti-IHH antibody (Millipore). The harvested growth plate of an eight week old C57/Bl/6 mouse was stained as a control. Results: The location of IHH staining in nodules grown in a bioreactor closely resembles that found in the growth plates of a C57/Bl/6 mouse. The IHH is located primarily in the prehypertrophic region and in the various perichondria of both tissues. Conclusion: This experiment provides further evidence that the cartilage nodules grown in a bioreactor contain functioning growth plates. Besides having zones, these nodules secrete the same molecules as does the growth plate itself, thus possibly serving as models of normal and abnormal cartilage formation in the early steps of endochondral ossification.

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This will be in the graduate competition as Tim is a 3rd year dental student.

Tiffany Vo
07/8/12
TX Biomaterials Day Abstract

Physicochemical characterization of dual gelling injectable scaffolds for craniofacial tissue engineering

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Injectable, *in situ* forming hydrogel scaffolds capable of delivering bioactive molecules offer promise in craniofacial tissue engineering as minimally invasive strategies for regenerating complex orthopedic defects. In particular, thermosensitive materials are attractive scaffold candidates since they can undergo a reversible phase transition from a soluble to insoluble state at a tunable lower critical solution temperature. However, the main challenge with such hydrogels is that they are not degradable and undergo syneresis, limiting their application for effective bone repair. A novel two-component system involving poly(*N*-isopropylacrylamide)-based macromers and polyamidoamine crosslinkers was designed that imparts non-shrinking and degradable properties. Incorporation of the ringed monomer, dimethyl-*γ*-butryolactone acrylate, provides hydrolysis-dependent modulation of the lower critical solution temperature over time for the creation of soluble byproducts. Various formulations of the thermogelling macromer and polyamidoamine crosslinker were synthesized via free radical polymerization and assessed using proton nuclear magnetic spectroscopy and time-of-flight electrospray ionization spectroscopy. Full factorial swelling and degradation studies demonstrated that the diamine functionalized crosslinkers increased the equilibrium degree of swelling through epoxy-based reactions with glycidyl methacrylate moieties of the base polymer to create non-shrinking, degradable scaffolds. The hydrogels demonstrate tunable mechanical properties based on the polymer content and degree of crosslinking. In vitro cytocompatibility studies of the leachables and degradation products also showed that the hydrogels were non-cytotoxic to cells, indicating that stem cell delivery via these constructs may be favorable. The injectable two-component hydrogel is a promising tissue engineering scaffold for minimally invasive cell-based craniofacial therapies.

Investigating Electrospinning as a Method to Introduce Anisotropic Mechanical Behavior in Hydrogels used for Heart Valve Tissue Engineering

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Abstract

A tissue-engineered heart valve uses cells, growth factors and a scaffold to regenerate native tissue and mimic its functions. Native aortic heart valves have a high level of anisotropy to withstand circumferential stress and extend radially to close the valve; thus, the designed scaffold must have similar mechanical properties. Hydrogels, like poly(ethylene glycol) diacrylate (PEGDA), are widely used in tissue engineering applications; however, their isotropic nature presents a major challenge in their functionality. This study hypothesizes that hydrogels used as scaffolds for heart valve tissue engineering can be made anisotropic by fiber reinforcement via electrospinning.

The electrospinning technique produces highly porous, three-dimensional nanofibrous structures. A high voltage source is used to create an electric field between a droplet of polymer solution at the tip of a needle and a collector plate. The morphology of the fibers can be controlled with solution properties and other variables, such as the flow rate and the voltage. In order to produce anisotropic scaffolds, a rotating mandrel is used to collect the polymer of choice: polycaprolactone (PCL). The resulting scaffolds are treated with ammonium persulfate (APS), acrylated, and bound to PEGDA. Scanning electron microscopy and mechanical testing are used to characterize the scaffolds. Elastic modulus data indicate that anisotropy is observed in the electrospun PCL. APS treatment and acrylation did not affect the fiber alignment and anisotropy; however, the elastic modulus in the parallel direction decreased. In conclusion, electrospun anisotropic PCL maintained its mechanical properties after its modification and binding to PEGDA.