

Dynamic increase in matrix stiffness promotes invasive tumor phenotype *in vivo*

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Increase in matrix stiffness in soft tissue tumors has been shown to contribute to the development of disease progression *in vivo* and the development of a malignant phenotype *in vitro*. Remodeling and alignment of the extracellular matrix (ECM) is thought to be a prominent driver of this dynamic process. Our group has created an alginate-matrigel hydrogel system capable of dynamic stiffening and weakening upon near infrared irradiation to model this dynamic tumor microenvironment and examine the relationship between dynamic matrix stiffening and tumor metastasis and progression. Gels were mixed with primary tumor cells isolated from transgenic mice where the mouse mammary tumor virus was used to drive expression of the polyoma middle T antigen oncogene and injected into the mammary fat pad of BALB/c mice. Gels were stiffened via transdermal irradiation seven days post injection, a subgroup was stiffened again at 10 days post injection. A subset of each group was given an intraperitoneal injection of NSC23766, a Rac1 inhibitor, after at 2.5 mg/kg/day. Dynamic stiffening resulted in both increased tumor volume and shorter survival time relative to a control unstiffened group. This suggests a role for tissue stiffness in regulation of tumor progression and metastasis. Treatment with NSC23766 led to longer survival times and decreased tumor volume relative to untreated mice, this indicates a role for the Rac1 pathway in tumor sensing and response to changes in mechanical stiffness.

A Versatile, Automated, Optical Cell Counting Approach for the Quantification of Multiple Adherent Cell Types

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Cell counting is a valuable tool used to quantify results in a wide variety of tissue culture experiments. While a number of methods have been devised to accomplish the task of cell counting, existing options are either too noisy, limited in dynamic range, or require cells to be in suspension. In addition, a robust technique for quantitating multiple adherent cell types with high accuracy does not exist. The methodology presented here provides a system for counting one or multiple adherent cell types with high statistical confidence. Using any microscope with a motorized x, y and z axis and the open-source software CellProfiler, a variety of staining methods can be used to quantitate one or multiple cells of interest in most if not all experimental setups. The motorized stage permits in-focus whole well imaging while cellular staining in conjunction with CellProfiler uniquely identifies each individual cell.

Unlike representative snapshots of a well, whole well imaging permits the quantification of all cells in the experimental population providing high statistical accuracy. In addition, single-cell analysis provides a resolution that is superior to bulk ensemble measurements. Together, whole well imaging and single-cell counting permits quantification over a greater dynamic range. While standard cell proliferation assays can typically count from 50 to 25,000 cells, our system can quantify anywhere from 1 to 1 million cells. We foresee applications for our methodology in biomaterials and immunology where quantification of several adherent interacting cell types with high accuracy is a desired but unavailable option.

Mechanical Characterization and DNA Quantification of Porcine SIS-ECM Scaffolds

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Large musculoskeletal defects such as those resulting from trauma often do not heal of their own volition. As such, scaffold material or a graft is required to foster regeneration. An important attribute of scaffolds for biomedical applications is the ability of the scaffolds to retain their mechanical integrity. The objective of this study is to analyze the mechanical properties, and determine the quantity of residual DNA material in four different groups of decellularized porcine SIS-ECM. The four groups in this study were subjected to different methods of decellularization (freeze-thaw, detergent, enzyme and enzyme/detergent), with a non-decellularized group as control. Biaxial testing (Young's modulus using strain energy density models); SEM and FTIR (for structural and protein integrity) and DNA quantification (least residual nuclear material post-decellularization) were conducted to determine which decellularization technique produced SIS-ECM scaffolds with the most favorable properties for applications in tissue regeneration. Data obtained were analyzed using repeated measures ANOVA and Tukey's post-hoc (n=6/group, p<0.05). Results show that the group decellularized with the enzyme (trypsin) displayed superior mechanical strength to the other groups- with a Young's modulus value of 11.7 MPa. Also, decellularization using detergent (TX-100) alone was shown to be most effective in removing nuclear material from the tissue samples; the TX-100 group contained the least residual nuclear material of 47.48 ng/mg of DNA /dry weight of tissue. Results suggest that a combination of these two decellularization techniques may yield optimal mechanical properties and residual nuclear content to support the use of porcine SIS-ECM scaffolds in tissue regeneration applications.

Biodegradable DNA-Enabled Poly(ethylene glycol) Hydrogels Prepared by Copper-Free Click Chemistry

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Significant research has focused on investigating the potential of hydrogels in various applications and, in particular, in medicine. Specifically, hydrogels that are biodegradable lend promise to many therapeutic and biosensing applications. In this work, biodegradable poly(ethylene glycol) hydrogels cross-linked with single stranded DNA are prepared using copper-free click chemistry. Specifically, 4-Arm-PEG-Dibenzocyclooctyne (4-Arm-PEG-DBCO) was prepared by reaction of 4-arm-PEG-NH₂ with paranitrophenyl-DBCO. The 4-Arm-PEG-DBCO was reacted with ssDNA functionalized on both 3' and 5' ends with azide groups in phosphate buffered saline. Hydrogels were then exposed to nuclease solutions and the process of biodegradation was monitored by microscopy. Through the use of this method, biodegradable hydrogels can be formed at room temperature in physiological solutions while avoiding possible harmful effects associated with other polymerization techniques that can be detrimental to cells or other bioactive molecules. Degradation of this hydrogel upon exposure to an endonuclease is demonstrated. This model has the potential to be tailored and expanded upon for use in a variety of applications.

Conductive Polymer-Based Nanoparticles As Photothermal Therapy Agents: Synthesis and Characterization

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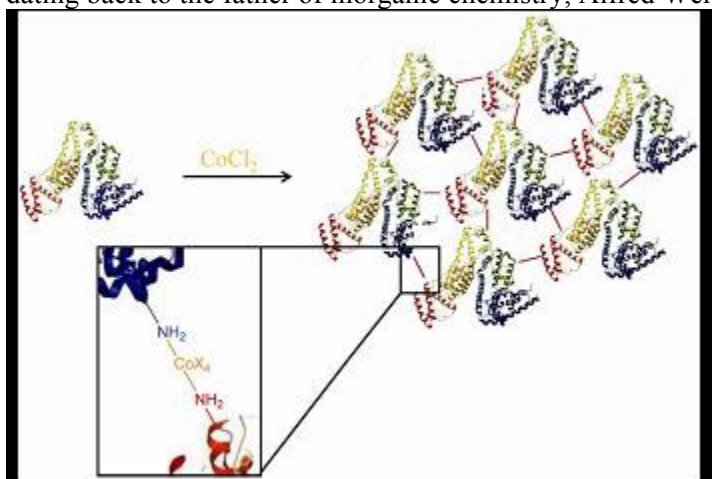
In recent years, photothermal therapy (PTT) has emerged as a viable alternative for cancer treatment. Much effort has been devoted to finding various types of PTT agents with excellent heat generation, photothermal efficiency, biocompatibility, and biodegradability. In this work, polymeric nanoparticles (NPs) composed of poly(1,4-bis(3,4-ethylenedioxythienyl)-2,5-dialkoxybenzenes) (poly(BEDOT-B(OR)₂)) and poly(3,4-ethylenedioxythiophene) (PEDOT) were synthesized using microemulsion polymerization. The NPs were characterized using dynamic light scattering, UV-Vis-NIR spectroscopy, and electron microscopy. The microemulsion polymerization yielded sub-110 nm NPs and the colloidal suspensions exhibited a strong absorbance in the near infrared region. The photothermal transduction and efficiency of these materials were determined and compared to that of commonly used PTT agents. When irradiated with NIR light, the suspensions showed a temperature change of ca. 30 °C with a photothermal efficiency of ca. 43%. *In vitro* cytocompatibility studies were also performed on the conductive polymeric NPs in an effort to determine the concentration limits that could be used without causing toxicity to cells. Cytocompatibility studies for the colloid suspensions were conducted at 24 h and 48 h exposure times, and the NPs were found to be non-toxic at a dose of 0.1 mg/mL. These data suggest that these materials could be good candidates for use as photothermal therapy (PTT) agents.

Synthesis of Cobalt Crosslinked Albumin Nanoparticles and *In Vitro* Evaluation of Macropinocytic Uptake in Gastric Carcinoma Cells

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Abstract

Recent research in the development of bioconjugation has allowed for widespread use in the field of medicinal chemistry research. By conjugating chemical functionality with a bioactive species, acceleration to the drug development process is evident allowing for an increase in the capability to aid in both the treatment and diagnosis of diseases. [1] In this study, albumin nanoparticles (NPs) utilizing a cobalt crosslink to lysine residues on adjacent proteins were synthesized. Our novel strategy can be used to crosslink protein to form nanoparticles ranging from 10 to 500 nm in size. [2] The method utilizes a labile Co^{2+} complex to crosslink lysine residues on adjacent proteins that can then be “locked” into conformation by oxidation to an exchange inert Co^{3+} complex. The coordination chemistry itself has ties dating back to the father of inorganic chemistry, Alfred Werner. [3]



By incorporating a fluorescently labeled marker to our cobalt crosslinked albumin NP (Co-Alb-FITC), we are able to report on *in vitro* characterization as well as preliminary cytotoxicity data. Three key outcomes were demonstrated: first, cancer cells efficiently internalize Co-Alb NPs while displaying high levels of macropinocytic uptake; second, Co-Alb NPs show biocompatibility and potential as a drug delivery vector through demonstrating no toxicity; third, because SNU-5 cells are not known to harbor Ras mutations, this study shows that some non-Ras mutated tumor types also rely on macropinocytosis as a mechanism of cell survival.

References

- [1] Fox, M. E., Szoka, F. C.; Fréchet, J. M. J. Soluble Polymer Carriers for the Treatment of Cancer: The Importance of Molecular Architecture. *Accounts Chem Res* **2009**, 42, 1141-1151.
- [2] Nguyen, D., Cavazos, R.J., Harris, A., Petros, R.A. Utilizing Cobalt Coordination Chemistry for “Traceless” Stimuli-Responsive Bioconjugation Involving Therapeutic Nanoparticles, Protein PEGylation, and Drug-(Bio)Polymer Conjugates. *Comment Inor Chem* **2014**, 34, 59-77.
- [3] Werner, A., A contribution to the constitution of inorganic compounds. *Z Anorg Allg Chem* **1893**, 3, 267.

Development of Chronic Wound Dressing Based on Collagen-Mimetic Proteins

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Objective: Chronic wounds are a growing healthcare issue due increases in elderly population, obesity, and diabetes. Despite the billions of dollars spent on wound care, greater than 30% of chronic wounds fail to close. To address the limitations of standard dressings, we have developed a biodegradable and bioactive hydrogel dressing that incorporates an engineered collagen mimetic protein based on streptococcal collagen-like proteins (Scl2) to guide healing.

Methods: The Scl2 protein was modified to include integrin-binding motif GFPGER and then functionalized with acrylate-PEG-N-hydroxysuccinimide (Acr-PEG-NHS). Triple helix formation, integrin affinity, and cell adhesion were determined. The biodegradable hydrogel, PEGDTT, was synthesized by adding d,l-dithiothreitol (DTT) and triethylamine (TEA) dropwise to a solution of PEGDA (2 kDa) in dichloromethane (DCM) under a nitrogen blanket. Hydrogels of variable amounts of PEGDTT in PEGDA 6 kDa (100%, 75%, 50%, and 0%) were fabricated to demonstrate a tunable degradation rate. Hydrogel microspheres were fabricated using a Flacktek Speedmixer to create a water in oil emulsion and crosslinked using UV light. Hydrogels were then filtered via vacuum aspiration to remove mineral oil.

Results and Conclusions: A collagen mimetic protein was developed that has improved stability while still maintaining integrin and cell interactions. Hydrogels were synthesized with a tunable degradation rate independent of hydrogel properties and gel microspheres were fabricated that can conform to irregular shaped wound. Overall, this bioactive and biodegradable gel dressing has the potential to improve healing and closure of chronic wounds.

Synthesis and Characterization of Smart Molecularly Imprinted Polymers, Using Structural Analogue Templates, for the Capture and Detection of Biomolecules

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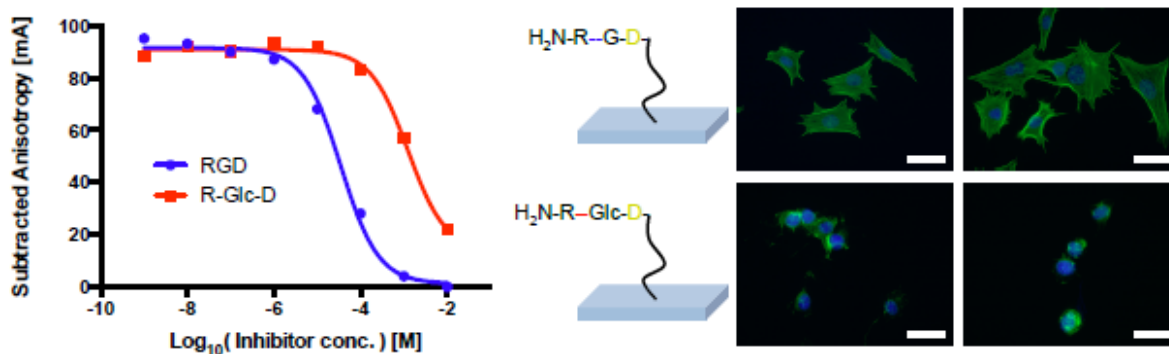
Molecularly imprinted polymers (MIPs), which achieve biomolecule specificity through spatial incorporation of functional monomers, provide an inexpensive and highly stable alternative to biologic molecules used for protein recognition. We present an alternative method for synthesizing MIPs for clinically-relevant biomolecules using protein templates possessing similar molecular weight and isoelectric point. This allows for the detection of many biomarkers, while minimizing production cost. A novel core-shell synthesis method is utilized to generate particles with a hydrophobic core surrounded by a recognitive shell polymerized with acrylate monomers in the presence of template proteins. These MIPs and control non-imprinted particles (NIPs) were subjected to rebinding assays, which assessed their ability to bind template proteins, as well as other proteins possessing similar molecular weight and isoelectric point. Lysozyme (MW=14.3kDa, pI=11.35) and cytochrome c (MW=12kDa, pI=10.2) were used as model protein templates. The imprinting factor, a ratio of MIP to NIP equilibrium binding capacities for a given protein, exhibited by the cytochrome c-templated MIPs was 2.59 for cytochrome c and 1.32 for lysozyme. In contrast, lysozyme-templated MIPs had an imprinting factor of 2.51 for cytochrome c and 2.28 for lysozyme. This suggests that template molecular weight plays a key role in determining the selectivity of MIP pores. Future templates will be rationally selected to possess similar isoelectric point and equal or greater molecular weight to disease biomarkers. Fabrication of MIPs that entrap a fluorophore or therapeutic agent within the hydrophobic core will enable a completely synthetic recognitive nanoparticle for application in medical diagnostics and drug delivery.

Understanding the importance of backbone hydrogen bonding in small peptide self-assembly and the RGD-integrin interaction: consequences for engineering degradable cell-adhesive biomaterials

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Self-assembling peptide-based gel materials are gaining interest for their potential uses in tissue engineering and drug delivery devices. For *in vivo* use, these materials should be degradable by hydrolysis occurring over the time scale of weeks to months. Hydrolytically sensitive ester bonds can be introduced into a peptide backbone by replacing specific amino acid residues with their α -hydroxy acid side chain analogues to form molecules called *depsipeptides*. We have demonstrated the self-assembly, gelation, and slow degradation capability of depsipeptide analogs of short, self-assembling peptides, challenging previous assumptions of the importance of amide-amide hydrogen bonding in stabilizing nanofibrillar assemblies of short, N-conjugated peptides. In this study, we sought to understand the *biological* consequences of ester substitution into the backbone of the cell-adhesive peptide Arginine-Glycine-Aspartic acid (RGD) to assess whether depsipeptide-based materials may be engineered to have cell-signaling capability. Specifically, we aimed to characterize the difference in integrin-binding affinity between the side-chain analogous depsipeptide R-Glc-D (Glc = glycolic acid) and the native RGD. In an assay involving competitive displacement of a FITC-conjugated RGD by soluble RGD or R-Glc-D, we found that RGD had a K_i of 14.1 μM , a 36-fold greater affinity for integrin than R-Glc-D ($K_i = 507.8 \mu\text{M}$). *In vitro* experiments using peptide-functionalized glass surfaces showed that cells cultured on RGD-functionalized surfaces attach and spread to a greater extent than cells on R-Glc-D presenting surfaces. These results suggest that while depsipeptide-protein binding is possible, native backbone bond chemistry and hydrogen-bonding capability may be crucial for functional outcomes of peptide-protein interactions.



Gap Junction Liposomes for Direct Therapeutic Delivery to the Cytoplasm

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Encapsulating chemotherapeutics within liposomes achieves localization to tumors. However, to have their effect, encapsulated drugs must reach the cytoplasm. Most liposomes are endocytosed, giving drugs a narrow time window to escape from endosomes before they are expelled. At present, poor control of endocytosis limits liposomal drug delivery, reducing chemotherapeutic efficiency. *Our work aims to address this problem with a new strategy – a chemotherapeutic delivery approach that uses gap junctions to deliver drugs directly to the tumor cell cytoplasm.* Specifically, we have developed liposomes that contain gap junction channels, and are able to form gap junctions with cells to deliver drugs and small molecules directly to the cytoplasm. To demonstrate the functionality of inserted channels, we opened and closed them by modulating the calcium concentration. Liposomes formed in the presence of fluorescent dye retained the dye in high calcium concentrations, which are known to close channels. However, when calcium was removed, the channels opened and the liposomes released the dye. After determining that the channels could be controlled with calcium, we exposed cells to dye---loaded liposomes and saw that the liposomes were able to form junctions with cells and deliver their dye to the cytoplasm. Addition of carbenoxolone, a gap junction inhibitor, substantially reduced the dye transfer. Finally, liposomes were loaded with doxorubicin, and exposure of tumor cells to doxorubicin---loaded liposomes killed the cells approximately 10 times more efficiently than free, unencapsulated doxorubicin. The ability of gap junction liposomes to transfer drugs rapidly and efficiently across the plasma membrane may provide a mechanism for outpacing drug efflux pumps, a key step toward overcoming multi---drug resistance.

Human pediatric cardiac cells exhibited high viability in 3D culture and limited expression of SSEA-4 and Isl1

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Background: Congenital heart defects (CHD) are the most common birth defects and the leading cause of birth defect-related death in the United States. We have collected right ventricular outflow tract (RVOT) tissue samples from surgical repair of Tetralogy of Fallot, the most common type of cyanotic CHD. Previous studies have shown that implantation of cardiac cells expressing SSEA-4 and Isl1 could lead to improved cardiac tissue regeneration and functional recovery in animal models, and previous experiments in our lab have shown that porous hydrogels composed of a gelatin/chitosan mixture with mechanical properties similar to native cardiac tissue can support high cardiomyocyte attachment and survival. In this study, we isolated human cardiac cells from these pediatric RVOT samples and tested their viability in a gelatin/chitosan hydrogel. We further quantified the expression of SSEA-4 and Isl1 in cultures of these cells. We hypothesize that human pediatric cardiac cells isolated from RVOT have high viability when cultured in a gelatin/chitosan hydrogel, and a subpopulation of these cells expresses SSEA-4 and Isl1.

Methods: RVOT tissue samples were collected during surgical intervention for congenital heart defects from patients between 93 to 242 days in age. Cardiac cells were isolated from RVOT samples through enzymatic digestion using neutral protease and collagenase. Patient samples were collected according to protocols approved by Baylor College of Medicine and Rice University IRBs. Cardiac cells were cultured on 2D surfaces treated with 1% gelatin and in 3D hydrogels composed of a 1:1 blend of low molecular weight chitosan and gelatin (2% w/v). Cultures were seeded at densities between 500 to 1000 cell/cm³ and passaged every 7 to 10 days. The metabolic rate of the cultured cardiac cells were accessed daily with Alamar Blue assay. After 7 to 10 days of culture, the cells were examined with live/dead assay, immunostaining, and flow cytometry.

Results: Human pediatric primary cardiac cells were cultured on gelatin/chitosan hydrogels. The cultures exhibited higher than 80% viability, comparable to 2D cultures, and an approximate 30% increase in metabolic activity was observed over 9 days of culture. A population of the cells stained positive for cTnT and GATA4 in immunostaining. Flow cytometry showed small populations of these cardiac cells exhibited expression of SSEA-4 and Isl1.

Conclusions: Gelatin/chitosan hydrogel is a viable platform for culturing human pediatric cardiac cells. Small populations of these cardiac cells exhibited expression of SSEA-4 and Isl1.

SINGLE GROWTH FACTOR RELEASE FROM PLA-BASED MICROPARTICLES FOR RECRUITMENT AND DIFFERENTIATION OF OSTEOPROGENITOR CELLS

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The process of bone repair is orchestrated by multiple signaling growth factors (GFs) (i.e. stromal cell-derived factor-1 (SDF-1 α), tumor necrosis factor alpha (TNF- α), and (transforming growth factor β) TGF- β , etc.)^{1,2}. Microparticles (MPs) have shown promise in delivering molecules to desired sites in the body^{3,4}. The objective of this study was to characterize release profiles of poly(lactic-co-glycolic acid) (PLGA) and polylactic acid (PLA) MPs encapsulating TGF- β and SDF-1 α and TNF- α , respectively. **Methods:** PLA MPs were prepared by dissolving PLA or PLGA in dichloromethane. Each GF (SDF-1, TNF- α , or TGF- β) was added individually to either the PLA or PLGA solution. This solution was added to a poly(vinyl alcohol) (PVA) solution which was then stirred to allow solvent evaporation. MPs were collected by centrifugation, washed and lyophilized, followed by characterization using a scanning electron microscopy. GFs release were measured by adding particles in PBS to concentrator tubes (Vivaspin®6) and kept at 37°C. Media was collected after centrifugation, and MPs were re-suspended in fresh PBS. GFs were quantified using ELISA assays. **Results:** PLGA MPs showed sustained release throughout the 8 weeks, with a maximum release between days 3-5 (46%). Late burst release was observed for PLA MPs between days 5-7 for TNF- α (77%) and 7-9 for SDF-1. **Conclusions:** Formation of drug incorporated MPs by emulsion-evaporation technique allowed for a combined late burst and sustained release profiles over 8 weeks *in vitro*. These MPs systems will be studied in combination with osteoprogenitor cells to promote early recruitment and differentiation, and further bone tissue regeneration.

References: [1] Bai, Y. International Immunopharmacology, 2013. 16: p. 214-223; [2] Lee, K. J. R. Soc. Interface, 2013. 8: p. 153–170; [3] Swami, A. , Springer Science+Business Media. 2012. p. 9-29; [4] Chan, J.M. PNAS, 2010: p. 1-6; [5] Zou, Y. J Biomed Mater Res Part B, 2011. 100B: p. 155-62.

HEALING OSTEOCHONDRAL DEFECTS OF THE KNEE: REGENERATIVE OSTEOCHONDRAL PLUGS

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Tissue engineering may provide an alternative approach to treatment of osteochondral defects (OCDs) - severe damage of articular cartilage resulting in exposure of subchondral bone. Due to the interface from cartilage to bone in OCDs, the ideal materials-based scaffold must spatially direct tissue regeneration as a gradual transition between the two tissue types. Therefore, the scaffold must contain a gradient of chemical and physical properties to act as cues for cell behavior. Previously, we have shown that methacrylated star polydimethylsiloxane (PDMS_{star}-MA) can induce osteoinductivity and bioactivity when incorporated with diacrylate (PEG-DA) in a hydrogel. The chemical and physical properties of planar gels were modified and studied in order to accurately represent the three zones of an osteochondral defect: chondral zone, transition zone, and cancellous bone zone. In this study, we sought to combine these individual layers into a plug-like structure to mimic the autograft plugs currently used in surgery. A salt leaching fabrication technique provides a method to ensure consistent pore size as well as pore interconnectivity throughout the plug. The individual PDMS_{star}-MA/PEG-DA solutions are then added on top of one another to this salt mold. This layer-by-layer design ensures a continuous gradient from one zone to another. The morphology, hydration, and modulus of the osteochondral plug zones were tested to ensure the same properties from the planar scaffold were carried over into the plug. These tests help to ensure the scaffold will cue cells to regenerate into the desired native tissue.

Enhancing Bone Regeneration with Composite Microspheres that Reflect the Osteogenic Niche

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Human mesenchymal stem cells (hMSCs) hold great promise in bone regeneration because of their ability to promote healing of damaged tissues. We have demonstrated that inhibiting peroxisome proliferator-activating receptor gamma with GW9662 reduces negative cross-talk on the cWnt pathway, resulting in a pro-osteogenic hMSC phenotype (OEhMSCs)¹. OEhMSCs secrete an extracellular matrix (hMatrix) that mimics the composition of anabolic bone tissue and strongly enhances hMSC retention and subsequent bone repair *in vivo*¹. Injectable microspheres were developed to co-deliver hMSCs on hMatrix and for controlled release of GW9662. Initial feasibility of this process was demonstrated by incorporating collagen type I, the major component of hMatrix, into polyethylene glycol diacrylate (PEGDA) microspheres. Collagen type I was functionalized with PEG linkers² to enable their stable incorporation into PEGDA spheres, resulting in hMSC adhesion on microsphere surface. The release of GW9662 from poly (lactide-co-glycolide) (PLG) microspheres was measured and quantified. STAR-CCM + was used to model the release of GW9662 from microbeads encapsulated in a hydrogel to compute the non-dimensional concentration profile. This model will be used to simulate different concentrations of PLG microspheres within PEGDA spheres, to evaluate the effect on overall release rates. We believe this systematic characterization of composite microsphere performance will facilitate optimization of bone regeneration and provide insight to identifying key control parameters for microsphere manufacturing.

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References: ¹Zeitouni et al. *Sci Transl Med* 2012; ²Browning et al. *Biomacromolecules* 2013.

Opacification of shape memory polymer foams using tungsten nanoparticles for neurovascular embolic applications

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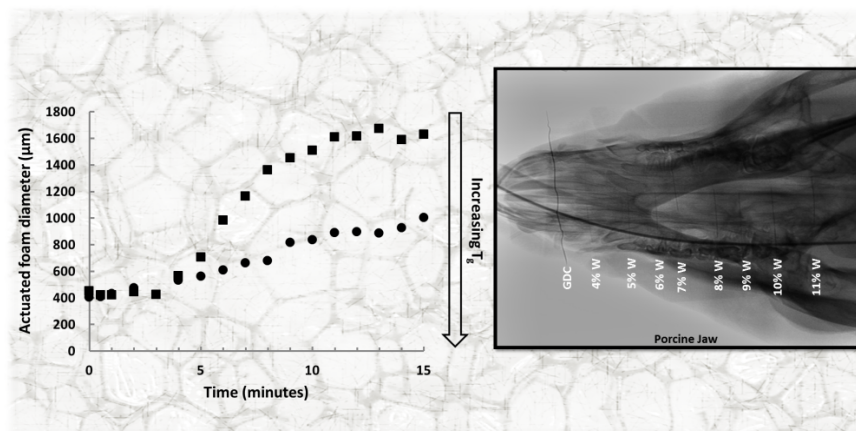
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Shape memory polymer (SMP) foams have been developed for use in neurovascular occlusion applications. These materials are predominantly polyurethanes that are known for their biocompatibility and tunable properties. However, these polymers inherently lack X-ray visibility, which is a significant challenge for their use as implantable materials. Herein, low density, highly porous shape memory polyurethane foams were developed with tungsten nanoparticles dispersed into the foam matrix, at increasing concentrations, to serve as a radiopaque agent. Utilizing X-ray fluoroscopy sufficient visibility of the foams at small geometries was observed. Thermal characterization of the foams indicated altered thermal response and delayed foam actuation with increasing nanoparticle loading (due to restricted network mobility). Mechanical testing indicated decreased toughness and strength for higher loading due to disruption of the SMP matrix. Overall, filler addition imparted x-ray visibility to the SMP foams and allowed for tuned control of the transition temperature and actuation kinetics for the material.



OPTIMIZING MICROCARRIER LOADING FOR ORAL DRUG DELIVERY SYSTEMS

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Current treatments for hemophilia B, a hereditary bleeding disorder characterized by the deficiency of clotting protein, factor IX, rely on injections and infusions that cause pain and discomfort, leading to noncompliance and risk of subsequent bleeding. A non-invasive treatment using an oral delivery system can overcome such issues and increase global access. Anionic complexation hydrogels have been engineered to protect therapeutic agents from the harsh environment of the GI tract and deliver them to the small intestine. We have successfully developed pH-sensitive polymeric systems, notably poly(methacrylic acid) grafted with poly(ethylene glycol) (P(MAA-g-EG)), as vehicles for the delivery of insulin (5.8 kDa), calcitonin (3.4 kDa), and interferon alpha (23 kDa). Here we adopt this method to microcarrier systems for high molecular weight (HMW) drugs, such as human factor IX (hFIX, MW=57 kDa) [1]. Tailoring the polymer network size can improve the loading and release HMW drugs. Based on FITC-dextran loading, increasing the drug molecular weight reduced the loading level. Additionally, the drug size affected the drug distribution within the microparticles, where FITC-dextran (20 kDa) showed uniform distribution throughout and FITC-dextran (50 kDa) shows surface loading. For hFIX loading, decreased crosslinking density increased the loading level, reaching up to 60 µg hFIX/mg particle. Protein release in biorelevant media showed the desired release profile. Protein released in simulated intestinal conditions was at least 80% active. P(MAA-g-EG) systems are viable vehicles for delivering HMW drugs, such as factor IX. Tuning the polymer crosslinking for increased loading capacity can improve the delivery potential.

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References: (1) Peppas NA and Horava SD. Polymers for Delivery of Therapeutic Proteins. U.S. Patent No. UTSB.P1047US.P1, Nov 2014. [Provisional application filed].

Multi-Drug Core-Shell Nanoparticles for Targeted Lung Cancer Dual Therapy

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More than 200,000 new cases have been attributed to lung cancer for 2015, due to poor survival rates (<15%) of lung cancer patients. Thus, a combined therapy incorporating radiation and chemotherapy is proposed where a novel multifunctional dual-drug loaded nanoparticle (MDNP) system consisting of a biodegradable polymer core with radiosensitizer and a pH-sensitive copolymer shell with chemo-drug is used for drug loading and delivery. Furthermore, MDNPs are surface-modified with Folic acid for lung cancer cell targeting. The MDNPs were found to exhibit degradability, excellent stability and both sustained release of the radiosensitizer and pH-dependent release of the chemo-drug. MDNPs were compatible with healthy cells and blood. The MDNPs exhibited a dose-dependent and caveolae mediated uptake by lung cancer cell lines. A reduction in both cancer cell forming colonies *in vitro* and tumor size *in vivo* after treatment with drug loaded MDNPs and radiation indicated the excellent therapeutic efficacy of the designed MDNPs. Thus, a combination of chemotherapy and radiation therapy assisted by the MDNPs represents a potential effective treatment option for lung cancer patients.

Skeletal Fracture Risk Following Local Bone Injury

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While it has been previously observed that an injury to the skeleton results in metabolic changes in intact bones distant to the site of the injury, calcium loss at such distant skeletal sites following a local bone injury has never been specifically investigated. In order to determine skeletal effects following local injury, a standardized critical sized defect in the rat femur was treated with either fully demineralized, partially demineralized or freeze dried allograft bone and observed after 4, 8 and 16 weeks (n=4/group). In this study we analyzed the calcium loss at different distant skeletal locations including contralateral femurs, radius and ulna, iliac crest, lumbar vertebrae and calvaria following surgical bone restoration. The primary objective of this study was to determine whether an injury resulting in the loss of bone tissue (locally) causes loss of calcium from the skeleton at a distant site from the site of injury. We performed micro-computed tomography in order to analyze the variations in bone quality using bone mineral density, bone volume to tissue volume ratio, bone surface density, cortical thickness and trabecular architectural parameters across the different groups. The differences between groups were analyzed using two way ANOVA followed by Tukey's post hoc test ($p < 0.05$). While significant architectural differences were not observed in the lumbar vertebrae, transient changes in density were seen at other sites. These findings will guide the practice of clinical care in treating bone injuries including potential calcium supplementation regimen to mitigate future fracture risk in the patient population.

Multi-functional Meshes to Prevent Intestinal Anastomotic Leakage and Surgical Adhesions

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More than 600,000 surgeries are performed each year to treat colon diseases, a majority of which require an intestinal anastomosis. Poor healing of these anastomoses after digestive surgery can lead to severe postoperative complications due to anastomotic leaks as well as obstructions and chronic pain due to surgical adhesions to surrounding tissue. To improve outcomes, we have developed methodology to generate bilayer meshes that have a gelatin layer to promote healing at the anastomoses and a bioinert, biodegradable hydrogel layer to prevent intra-abdominal adhesions. If successful, these studies will provide a single approach to improve two prevalent and severe complications of gastrointestinal surgical procedures.

METHODS: The biodegradable hydrogel was synthesized by adding d,l-dithiothreitol and triethylamine dropwise to PEG(2k)DA in dichloromethane. In situ crosslinked gelatin was electrospun using double-barrel syringes loaded with gelatin/2,2-trifluoroethanol(TFE) and 1,4-diazabicyclo[2,2,2]octane in one barrel and hexamethylene diisocyanate/TFE in the other. The solution was dispensed at 1mL/hr and 10kV was applied to the needle tip. Hydrogel degradation was conducted in PBS, while mesh degradation was performed in collagenase solution. Composites were fabricated by dip coating the gelatin meshes with hydrogel precursor solution and photocuring. Human dermal fibroblast adhesion as a function of hydrogel degradation was evaluated 3 hours post seeding.

RESULTS: Tuning of degradation rates of hydrogel and gelatin mesh was demonstrated by changing the concentration of PEGDTT and crosslinker ratio, respectively. In addition, selective cell adhesion was observed with high adhesion and spreading on the gelatin layer with minimal adhesion on the hydrogel layer.

Overall, this work highlights the strong potential of a bilayer mesh with tunable cellular adhesion and degradation rate to promote healing of anastomoses while reducing surgical adhesions.

**for the graduate poster competition*

Evaluation Of Polyelectrolyte Hydrogels Incorporating Poly(L-Lysine) As A Stimulant Of Chondrogenic Differentiation For Cartilage Tissue Engineering

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Objectives: This study focuses on the use of poly(L-lysine) (PLL), which has been shown to up-regulate mesenchymal condensation during developmental skeletogenesis *in vitro*, as an early chondrogenic stimulant of mesenchymal stem cells (MSCs). We characterized the effect of PLL incorporation on the swelling and degradation of oligo(poly(ethylene) glycol) fumarate) (OPF)-based hydrogels as functions of PLL size and dosage. Furthermore, we investigated the effect of PLL incorporation on the chondrogenesis of hydrogelencapsulated MSCs.

Methods: The swelling ratio and mass loss of PLL-laden hydrogels were determined (n=4) at days 1, 7, 14, and 28. For the cell study, MSCs were encapsulated (10 million cells/mL) into PLL-laden hydrogels, cultured *in vitro*, and retrieved for biochemical and gene expression analyses at various time points over 28 days.

Results: The swelling ratio of OPF hydrogels was affected by the incorporation of PLL during fabrication, with PLL-containing groups exhibiting decreased swelling compared to blank hydrogels by day 28. When MSCs were encapsulated, the incorporation of PLL of 225 kDa resulted in early (day 7) enhancements of type II collagen gene expression and type II/type I collagen expression ratios when compared to negative controls.

Conclusions: PLL can function as an inductive factor that primes the cellular microenvironment for early chondrogenic differentiation but may require additional biochemical factors for the generation of fully functional chondrocytes. Further, the incorporation of PLL into OPF hydrogels influences their swelling behavior, which may be leveraged for the development of constructs with desirable swelling properties for cartilage repair.

Acknowledgements: This study was supported by the National Institutes of Health (R01 AR048756)

Osteochondral tissue repair using a bilayered hydrogel composite delivering spatially-guided dual growth factors

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The present work investigated the use of biodegradable hydrogel composite scaffolds, based on the macromer oligo(poly(ethylene glycol) fumarate) (OPF), to deliver growth factors for the repair of osteochondral tissue in a rabbit model. Bilayered OPF composites were used to mimic the structural layers of the osteochondral unit, and insulin-like growth factor-1 (IGF-1) and bone morphogenetic protein-2 (BMP-2) were loaded into gelatin microparticles and embedded within the OPF hydrogel matrix in a spatially controlled manner. Three different scaffold formations were implanted: 1) IGF-1 in the chondral layer, 2) BMP-2 in the subchondral layer, and 3) IGF-1 and BMP-2 in their respective separate layers.

The quantity and quality of osteochondral repair was evaluated at 6 and 12 weeks with histological scoring and micro-computed tomography (micro-CT). While histological scoring results at 6 weeks showed no differences between experimental groups, micro-CT analysis revealed that the delivery of BMP-2 alone increased the number of bony trabecular islets formed over that of IGF-1 delivery alone. At 12 weeks post-implantation, minimal differences were detected between groups for cartilage repair. However, the dual delivery of IGF-1 and BMP-2 had a higher proportion of subchondral bone repair, greater bone growth at the defect margins, and lower bone specific surface than the single delivery of IGF-1. These results suggest that the delivery of BMP-2 enhances subchondral bone formation and that, while the dual delivery of IGF-1 and BMP-2 in separate layers does not improve cartilage repair under the conditions studied, they may synergistically enhance the degree of subchondral bone formation.

Poster Group: Graduate Poster Competition

SELF-CLEANING, MECHANICALLY ROBUST MEMBRANES FOR IMPLANTED GLUCOSE BIOSENSORS

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A subcutaneously implanted glucose biosensor would provide diabetics with continuous monitoring of blood sugar levels, thereby reducing short- and long-term complications. Biofouling of the biosensor membrane (e.g. the attachment and accumulation of proteins and cells) represents a major obstacle to achieving long-term functionality as this quickly compromises glucose diffusion. In this work, we have prepared “self-cleaning” membranes with thermoresponsive hydrogels based on crosslinked poly(N-isopropylacrylamide) (PNIPAAm). PNIPAAm hydrogels reversibly switch between a water-swollen state to a deswollen state when heated above the volume phase transition temperature (VPTT, ~35°C). This process can be utilized to cause cellular detachment (i.e. self-cleaning). Unfortunately, crosslinked PNIPAAm hydrogels exhibit poor thermosensitivity (i.e. rate and extent of deswelling/reswelling) as well as poor mechanical properties which can contribute to reduced self-cleaning and fracture, respectively. To overcome this, we have prepared double-network (DN) PNIPAAm hydrogels in which an electrostatic comonomer, 2-acrylamido-2-methylpropane sulfonic acid (AMPS), was introduced into the first network at varying levels. These DN membranes containing varying % AMPS essentially maintained the convenient VPTT of PNIPAAm hydrogels. With increased % AMPS levels, reswelling kinetics increased which may be attributed to the greater pore size as observed in SEM. For the membrane prepared with the highest level of AMPS (75%) in the 1st network, exceptional strength was noted (~17.2 MPa). Glucose diffusion rates of a membrane containing 50% AMPS in the 1st network were similar to a PEG membrane control. The PNIPAAm-co-AMPS DN hydrogels demonstrate exciting potential towards achieving a self-cleaning membrane with excellent mechanical properties.

Hydroxyapatite-Carbon nanotube (HA-CNT) composite scaffolds for bone tissue engineering

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Biomaterial scaffolds have been extensively investigated to function as synthetic graft substitutes and meet a growing need to regenerate bone defect sites caused by traumatic injury, cancerous resection or congenital defects. It has been shown that synthetic HA has good regenerative properties as a bone graft substitute due to its strong osteoconductive nature. Carbon nanotubes (CNTs) have also been previously shown to function mechanically as matrix reinforcing fillers and biologically to promote bone growth in composites. The primary objective of this study was to determine whether CNT incorporation into porous interconnected HA scaffolds provided a biomechanical benefit in terms of increased strength, toughness and/or fluid permeability. HA scaffolds were prepared using a previously described template coating process, CNT (Molecular Rebar Design Austin, TX) were added to make three different groups with 0%, 1% or 5% CNT concentration per unit mass of HA. Scaffolds were then sintered under one of four gas treatments: no flow, or a steady flow of air, nitrogen or argon. Characterization methods included evaluation of porosity and architecture by pycnometry and microCT analysis, mechanical characterization by pure compression and permeability by a custom flow apparatus. No significant differences were found between the groups for porosity, or density. The mechanical characterization showed that the samples sintered under air flow exhibited significant differences ($p < 0.05$) reduction in strength from 1% to 5% CNT. Permeability testing showed that the 1% and 5% CNT sintered under air flow exhibited significantly higher permeability compared to the rest of the groups ($p < 0.05$).

SELF-FITTING SHAPE MEMORY POLYMER SCAFFOLDS FOR BONE DEFECT REPAIR

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Cranio-maxillofacial (CMF) bone defects can result from trauma, infection, congenital deformities or tumor removal. Currently considered the gold standard, transplantation of harvested autologous grafts is limited by complex grafting procedures, donor site morbidity and limited availability. A particular difficulty is shaping and fixing the rigid autograft tightly into the defect in order to obtain osseointegration and to prevent graft resorption. Tissue engineering has been explored as an alternative strategy for the treatment of CMF bone defects. Thus, to overcome the limitations of autografting, we have prepared a “self-fitting” shape memory polymer (SMP) tissue engineering scaffold that could conformally fit into an irregular CMF defect with the mere application of warm saline (~60 °C). Thermoresponsive SMPs are capable of being fixed into a temporary shape and subsequently returned to the original shape when exposed to heat. Therefore, in this study, inorganic-organic scaffolds were prepared from diacrylated macromers comprised of inorganic polydimethylsiloxane (PDMS) and organic poly(ϵ -caprolactone) (PCL) segments, AcO-PCL_n-*block*-PDMS_m-*block*-PCL_n-OAc. High pore interconnectivity was achieved via a revised solvent-casting/particulate-leaching (SCPL) method. By specifically varying macromer composition (i.e. PDMS segment length), the impact on pore interconnectivity, self-fitting and shape memory behaviors, mechanical properties and degradation was systematically studied. The SMP foams were found to have favorable properties for use as self-fitting bone defect scaffolds.

Title: Synthesis and Applications of Antioxidant Carbon Nanomaterials

Authors: Lizanne G. Nilewski, William K. A. Sikkema, Dr. James M. Tour*

Abstract:

Carbon nanomaterials represent an expanding field with broad applications to many disciplines of chemistry and biology, and have been applied as drug delivery vehicles, biosensors, imaging agents, tissue scaffolds, and therapeutics. This work covers the use of highly oxidized carbon nanomaterials called PEG-HCCs (PEGylated hydrophilic carbon clusters) as drug delivery vehicles and as antioxidants. PEG-HCCs catalytically convert superoxide to oxygen and hydrogen peroxide at a rate faster than most single-active-site enzymes, and they also quench hydroxyl radicals, making them potent antioxidants. PEG-HCCs have been shown to carry out therapeutic functions that have been unattainable from enzymes or small molecule antioxidant treatments; they have been studied in vitro and in vivo and were successfully applied to treat models of traumatic brain injury, stroke, cancer, rheumatoid arthritis, and multiple sclerosis. This work covers the synthesis, characterization, and antioxidant mechanisms of PEG-HCCs as well as their biomedical applications.

Nanoparticles for gene therapy: an alternative treatment for hindlimb ischemia

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Introduction: Peripheral arterial disease (PAD) is a severe impairment of arterial vessels resulting in obstruction of normal blood flow. This decreased supply results in acute hindlimb ischemia, a disease with high morbidity and mortality rates. Common treatments for hindlimb ischemia include thrombolytic and anticoagulant therapy, surgical revascularization, and amputation; however, these therapy modalities have limitations such as lack of re-endothelialization and high rates of restenosis. To overcome the shortcomings associated with current treatments, the goal of this research project is, therefore, to develop and formulate nanoparticles (NPs) loaded with human DNA plasmids Erythropoietin receptor (EPOR) to enhance angiogenesis, and/or restore vessel functions, while preventing further tissue necrosis at ischemic sites for use as an alternative PAD treatment. EPO receptor signaling is selected for this research due to its role in both angiogenesis and protection of endothelial cells under hypoxic conditions, especially that of the ischemic hindlimb.

Materials and Methods: Poly(lactic-co-glycolic acid) (PLGA) nanoparticles encapsulating the human EPO receptor DNA plasmid were fabricated using a standard double emulsion technique. PLGA NP surfaces were coated with polyethyleneimine (PEI) via adsorption. Glucose is also used with the plasmids to maintain its bioactivity and stability during particle formation. Characterization of these NPs such as particle size, surface charge, and stability was determined using the dynamic light scattering (DLS) technique. Releases of pDNA from these NPs was investigated by incubating them in buffer solutions up to a period of 28 days. Blood clotting time and hemolysis analysis were carried to evaluate hemocompatibility properties of these NPs while *in vitro* studies to assess for cytocompatibility and therapeutic activities of fabricated NPs were performed using human aortic endothelial cells (HAECs). Current testing on the efficacy of these NPs as protective tools to cells in hypoxic environments is being studied.

Results and Discussion: The fabricated NPs showed positive surface charges with average diameters of around 200 nm (Figure). Release studies demonstrated a sustained release of the payload, EPOR DNA plasmid from the NPs. Moreover, these NPs demonstrated their stability in different aqueous solutions, including serum. They also showed that they were hemocompatible via acceptable whole blood clotting time and very low levels of hemolysis. In addition, these nanoparticles were cyto-compatible with HAECs, and the cellular uptake of these NPs by HAECs was dose dependent.

Conclusions: PEI-PLGA NPs could be used to load plasmid DNA as an alternative therapy to treat PAD. Our results show that these NPs are not only stable, cyto- and hemo-compatible, but also provide sustained releases of the payload. These results provide the green light for further analysis of the ultimate effects on these nanoparticles in restoring vessel function at ischemic sites. Future work includes more detailed *in vitro* and *in vivo* studies of EPOR DNA plasmids-loaded PLGA NPs for the treatment of PAD.

Acknowledgements: We would like to acknowledge funding support from the National Institutes of Health (NIH) U01 HL111146 and R01 HL118498 grants, and American Heart Association (AHA) Grant-in-Aid award.

Polymeric Nanoparticle-Based Enzymatically Activatable Near-Infrared Nanoprobes for Optical Detection of Cancer

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Statement of Purpose: Near infrared (NIR) fluorescent nanoprobe approaches are emerging as an alternative for the detection and image guided resection of tumors. Tumor lysosomal proteases can be used as triggers of enzymatically activated nanoprobes (EANPs) to develop NIR signal that can provide sufficient contrast between normal and cancerous tissue, thereby enabling specific imaging of tumors. In this study, EANPs were synthesized as cancer-specific contrast agents for optical imaging and characterized *in vitro*. Polymeric Nanoparticle-Based Enzymatically Activatable Near-Infrared

Results: Size of spherical EANPs were in the range of 70–150 nm. Fluorescence activation of the AF750-EANPs demonstrated with up to 15-fold optical signal enhancement within 120 minutes. The health of MDA-MB-231 breast cancer cells was confirmed during and after exposure to EANP suspensions via microscopy and MTT assay, even at high concentrations (1 mg/mL). Enhanced fluorescence was observed on the MDA-MB-231 cells exposed to EANPs (Figure). Fluorescence decreased with increasing concentrations of TLCK protease inhibitor. Dilute suspensions of the EANPs imaged at depths of up to 4 mm, as well as up to a 13-fold signal-to-background ratio in tissue phantoms.

Conclusions: Nanoprecipitation of copolymer blends containing poly(L-lysine) was utilized as a method for preparation of highly functional nanoprobes with high potential as contrast agents for fluorescence based imaging of cancer.

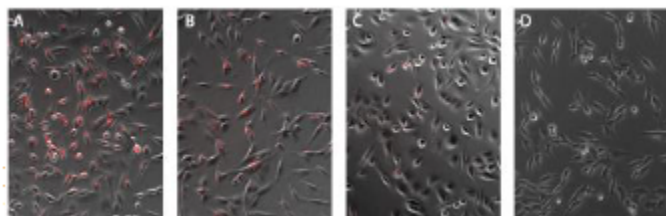


Figure. Overlay images of AF750 fluorescence in MDA-MB-231 cells exposed to 0.5 mg/mL AF750-labelled EANPs in the presence of increasing concentrations of TLCK protease inhibitor.

(A) 0 μ M TLCK, (B) 31 μ M TLCK, (C) 250 μ M TLCK, (D) No EANPs and 0 μ M TLCK.

Thermo-responsive, multimodal imaging enabled nanoparticles towards cancer therapy.

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Theranostic drug delivery systems (TDDS) have been shown to effectively treat various cancers as they can be used to detect the tumor region and deliver drugs locally for an effective killing of cancer cells. Our objective in this project is to develop new biodegradable thermo-responsive fluorescent polymer-coated magnetic nanoparticles (TFP-MNPs) to function as effective TDDS for cancer therapy.

The proposed TFP-MNPs were formulated and characterized for their physical, fluorescent, magnetic, and drug release properties. *In vitro* cytotoxicity and cellular uptake were also assessed using normal cells (human dermal fibroblasts and prostate epithelial cells) and prostate cancer cell lines (PC3 and LNCaP cells), respectively. *In vitro* and *in vivo* detection of TFP-MNPs by magnetic resonance imaging (MRI) and fluorescence imaging were performed using agarose phantoms and tumor bearing mice. *In vivo* therapeutic capacity of drug-loaded TFP-MNPs is currently being evaluated in animal models.

TFP-MNPs had average diameters of 135 nm, surface charge of -31 mV, LCST of 39°C and, a temperature-dependent drug release profile. They were also cytocompatible and demonstrated a dose-dependent uptake in prostate cancer cell lines. *In vivo* fluorescence imaging of animals administrated with TFP-MNPs showed the localization of these nanoparticles at the tumor sites.

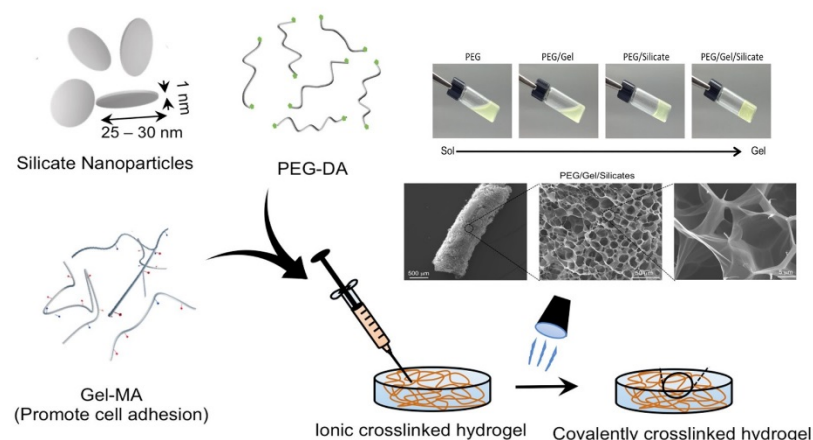
TFP-MNPs demonstrated cytocompatibility, degradability, and temperature responsive drug release and possessed fluorescent and MRI imaging capabilities, thus indicating a potential to serve as efficient TDDS for cancer therapy. Future work will involve *in vivo* studies to determine their therapeutic efficacy to treat prostate and skin cancers.

Microfiber Fabrication from Nanoparticle Polymeric Solutions for Cellular Encapsulation

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INTRODUCTION: Here we present a solution for constructing microfibers using poly (ethylene glycol) (PEG) diacrylate, silicates, and methacrylated gelatin (Gel) via ionic and covalent crosslinking. By controlling the interactions between nanoparticles and polymers tunable properties are achieved.



EXPERIMENTAL: PEG (mw. 10kDa) hydrogels (5% wt/vol) were synthesized from PEG-diacrylate prepolymer. Silicate nanoparticles (1 mm thick, 20-30 mm diameter) were incorporated at 4% wt/vol. Gel was incorporated at 1% to aid in cell attachment to the scaffold.

RESULTS/ DISCUSSION:

Using a simple mixture of PEG,

Gel, and Silicates micron-scale fiber structures are formed with robust mechanical properties (Figure 1). Addition of Silicates and Gel to PEG increases the viscosity. With regard to the compressive moduli, we observed a significant ($p < 0.05$) difference between samples containing and not containing both Gel and Silicate, indicating that the unique combination of PEG/Gel/Silicates is mechanically robust. As proof of concept, RFP MOSJ cells were encapsulated with the microfibers and GFP-labeled MC3T3s to the surface. 24-hr cell cycle analysis indicate that cells are entering a phase of growth, suggesting Gel/Silicate enables cell spreading which could be attributed to the presence of magnesium in Silicates.

CONCLUSIONS: We have demonstrated the fabrication of nanocomposite microfibers through the use of a simple system. The addition of Gel/Silicates to PEG has strong potential for easy cellular incorporation. Long term use of this hydrogel can be for specific geometries or to use as a printing ink for 3D printers that enable precise models of tissues.

**Torsional Evaluation of Collagen Coated Hydroxyapatite with Varying rhBMP-2 Dosages
in an *In Vivo* Critical Sized Rabbit Radius Model**

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Recombinant human bone morphogenetic protein-2 (rhBMP-2) is a growth factor utilized to encourage bone regeneration. Increased use of rhBMP-2 at high dosages for bone regeneration has led to many concerns including swelling found in spinal fusions. This study uses varying rhBMP-2 dosages with hydroxyapatite (HA) scaffolds (15mm length, 3x5mm oval cross section, 80% porosity) that were coated with collagen and paired with a collagenous periosteal membrane. The scaffolds were implanted in a critical sized (15mm) diaphyseal radial defect in New Zealand white rabbits. The groups examined were ACS+76µg rhBMP2 (clinically used INFUSE dosage), HA+76µg rhBMP2, HA+15µg rhBMP2, HA/Col+15µ rhBMP2 and HA/Col+15µg rhBMP2+BMSCs. The *in vivo* study lasted 8 weeks with radius and ulna extracted post-euthanasia. Before torsional evaluation (1°/sec), the distal ends were embedded in epoxy and the ulna removed from load transfer. The stiffness, torsional toughness, maximum angle and maximum torque in all groups were not significantly different ($p < 0.05$) from the nonsurgical controls. The stiffness was observed to increase with reduced rhBMP2 dose and was greatest in the HA/Col+15µ rhBMP2 group. The HA+15µg rhBMP2 and HA/Col+15µ rhBMP2 groups showed comparable maximum torque (1.014 ± 0.222 Nm and 0.987 ± 0.424 Nm respectively) to the nonsurgical controls (1.013 ± 0.261 Nm). The ACS+76µg rhBMP2 (INFUSE), HA+76µg rhBMP2, and HA/Col+15µg rhBMP2+BMSCs groups had maximum torque values of 0.861 ± 0.22 Nm, 0.866 ± 0.204 Nm, and 0.861 ± 0.317 Nm respectively. These results confirm that scaffolds with reduced dosages of rhBMP-2 can produce torsional strength similar to nonsurgical controls and current clinical dosages. This study was supported in part by the Orthopaedic Extremity Trauma Research Program USAMRMC # W81XWH-08-1-0393.

Surface Hydrolysis Mediated PEGylation of PNIPAAm Nanogels

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Thermoresponsive nanogels, such as those composed of N-Isopropylacrylamide (NIPAAm) have been studied for the use in nanocomposites for the externally triggered delivery of chemotherapeutics for years. One issue that they encounter is the body's immune response leading to local temperature increases that prematurely trigger the release of any loaded payload. In order to overcome this a stealth coating of polyethylene glycol (PEG) is necessary in order to prevent the opsonization and removal by the reticular endothelial system. In the past, the methods for coating these systems have deleterious effects on the temperature response of the nanogels. In order to avoid this we have developed a hydrolysis aided mechanism to surface coat the particles using biocompatible EDC/NHS chemistry. This mechanism converts relatively unreactive acrylamide units to acrylic acid near the surface providing functional groups for the attachment of amine terminated PEG chains. This method has no impact on the swelling response of the nanogels while providing substantial coating to prevent the protein adsorption that leads to opsonization. The extent of hydrolysis is measured with potentiometric titrations and nuclear magnetic resonance. These systems also demonstrate resistance to uptake by model immune cells in the form of RAW 264.7 compared to both unmodified PNIPAAm and P(NIPAAm-co-Acrylic Acid) systems. Furthermore, this new method of surface modification offers a robust reaction mechanism to modify relatively unreactive nanogels without affecting their innate properties.

Development of Suturable and Bioactive Hydrogels to Promote Endothelialization of Vascular Grafts

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There is a growing clinical need for a synthetic vascular graft that can remain patent in small diameter applications. To be successful, the vascular graft must be thromboresistant, match the compliance of the native tissue to prevent intimal hyperplasia, and promote endothelialization. We propose a multi-layer approach to decouple the mechanical requirements of the graft from the requisite cell-material interactions. The outer layer is an electrospun polyurethane mesh which matches the native properties of the saphenous vein, the current gold standard for CABG procedures. The electrospun mesh is then coated with a poly(ethylene glycol) (PEG) hydrogel that is resistant to platelet adhesion. To promote endothelial cell (EC) adhesion, a collagen mimetic protein (Scl2) is incorporated into the gel. The protein has been engineered to have selective cell adhesion by incorporation of binding sequences for $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins (Scl2-2). We have previously demonstrated that we can modulate endothelial cell adhesion and migration using this bioactive hydrogel platform. However, when implanted in porcine carotid arteries, suturing of the vessel to the graft caused particles to dislodge with caused damage downstream in smaller vessels. In order to enhance the suturability of the inner layer, we have incorporated N-vinylpyrrolidone (NVP), which prevents damage to the hydrogel during suturing. In summary, we have demonstrated that the proposed multilayer design has the potential to promote endothelialization of the vascular graft and is robust enough to resist damage during implantation.

Hyaluronan Hydrogels as Biomimetic Spongiosa Layer for Tissue Engineered Heart Valves

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The only treatment for severe aortic valve disease is valve replacement surgery, but current replacement options of glutaraldehyde-fixed bioprosthetic tissue or mechanical valves have shortcomings. A tissue engineered heart valve would provide autologous living tissue capable of somatic growth. Most previously studied tissue engineered grafts are homogenous makeup, which does not recapitulate the physiological structure or function of the valve. These types of valves have not had much success in animal trials or clinically, but it is hypothesized that a valve which more closely resembles the structure of the native valve will improve on these previous results. The aortic valve has a tri-layered structure, with each layer having distinct ECM makeup to provide unique mechanical function. This structure will be recapitulated in a synthetic polymer scaffold, with each layer being independently tuned before being laminated together to form a tri-layered tissue engineered valve. The middle spongiosa layer is made up of mostly proteoglycans and glycosaminoglycans and serves the mechanical functions of shock absorption, lubrication between outer layers, and leaflet motion dampening. Hyaluronan is a natural glycosaminoglycan which makes up 60% of the GAGs in valve tissue. Crosslinked hyaluronan hydrogels can support valve cell growth and 3D and serve the biochemical and biomechanical functions of the spongiosa layer of the valve. The research presented compares the use of crosslinked hyaluronan hydrogels to PEGDA based hydrogels in the context of a biomimetic spongiosa later in a tissue engineered heart valve.

PEO-silane amphiphiles to prevent protein adsorption on silicone: Dependence on PEO-segment length and concentration

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Poly(ethylene oxide) (PEO) has been incorporated into biomaterials (*e.g.* silicones) to prevent protein adsorption and subsequent thrombosis, but its performance *in vivo* is limited. Previously, we demonstrated that PEO-silanes modified to be amphiphilic with a hydrophobic siloxane tether exhibit improved protein resistance versus conventional, non-amphiphilic PEO-silane when incorporated into silicone. This effect is attributed to the siloxane tether enhancing restructuring of PEO to the silicone surface in response to water exposure. When PEO-silane amphiphiles of different PEO-segment lengths ($n = 3, 8, \& 16$) were incorporated into silicones at a single molar concentration (50 μmol per 1 g silicone), $n = 8$ exhibited the greatest rate and extent of water-driven surface restructuring. In the present study, the concentration of each PEO-silane amphiphile was systematically varied to determine the minimum concentration required to confer substantial surface restructuring and protein resistance. For each PEO-segment length, medical-grade silicone was bulk-modified with 5 different concentrations of the corresponding PEO-silane amphiphile (5, 10, 25, 50 & 100 μmol per 1.0 g silicone). The $n = 8$ PEO-silane amphiphile proved to be the most efficient in improving silicone surface wettability, with concentrations as low as 10 μmol (< 2 wt%) achieving significant surface restructuring and protein resistance. The results indicated that restructuring behavior is dependent more so on the PEO-segment length than PEO concentration.

Effect of Flow Conditions in a 3D Tumor Model Generated Using a Flow Perfusion Bioreactor

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Abstract

Most preclinical drug screening systems fail to accurately drug efficacy, as cells are plated on culture dishes devoid of signaling cues normally present *in vivo*. In order to overcome these issues, several three-dimensional (3D) tumor models have been proposed, with partial recapitulation of tumor-stroma and tumor-extracellular matrix interactions. Along this direction of research, this study investigated the use of flow perfusion bioreactor as a bone tumor model for Ewing Sarcoma (ES).

We hypothesized that flow perfusion bioreactor would mimic those mechanical stimuli experienced by ES cells in the native bone tumor niche, improve cell culture conditions via promotion of nutrient supply, and ultimately lead to a more physiologically relevant cell phenotype and drug response than samples cultured in static conditions.

Significantly, flow-derived shear stress promoted autocrine IGF-1 ligand production by ES cells and elicited a super-additive release of IGF-1 in the presence of exogenous ligand. In a rate dependent manner, flow perfusion enhanced the sensitivity of ES to anti-IGF-1R signaling agent, which is an effect not observed with conventional chemotherapeutic doxorubicin. Beyond the direct effect upon cell phenotype, flow perfusion increased mass transport throughout the biomimetic 3D scaffold, which ultimately enriched ES culture over static conditions.

Collectively, our tissue-engineered system provides an improved method to study cancer cells *ex vivo*, and offers to narrow the gap between preclinical and clinical drug activity.

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Solid Freeform Fabrication of High Porosity Foams

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Introduction:

Tissue engineering has emerged as a promising solution to the limitations of current bone grafting procedures. However, it remains challenging to produce scaffold architectures that approach the complexity and function of native tissues. Emulsion templating is capable of producing porous scaffolds through polymerization of high internal phase emulsions (HIPEs) to form porous foams (polyHIPEs). We have previously demonstrated the potential of this system to generate injectable bone grafts with mechanical properties comparable to cancellous bone¹. We have expanded upon this platform to fabricate grafts with hierarchical architectures by 3D printing the polyHIPE into predefined geometries.

Methods:

Biodegradable, hydrophobic, acrylate-functional macromers are combined with surfactant and photoinitiator, emulsified with water to form a viscous paste, and deposited layer-by-layer into geometric shapes using a HYREL 3D printer equipped with an EMO-25 paste extruder. Emulsions were cured via UV light (365 nm) in a printing process we term cure-on-dispense (COD) to form strong, cohesive shapes.

Results and Conclusion:

Recently we have been able to optimize the rheological properties to prevent slumping and improve scaffold fidelity. A sufficiently high zero-shear viscosity (1500 Pa*s at 0.001 1/s) and low viscosity under typical extrusion (10 Pa*s at 50 1/s) has improved deposition precision to allow lattice structures and complex features within 3D printed models. In addition, dual printing of a poly(lactic acid) shell with the COD polyHIPE infill was utilized to significantly increase the compressive modulus and strength of composite scaffolds.

References: 1. Moglia, R.S., et al., Injectable polyHIPEs as high-porosity bone grafts. *Biomacromolecules*, 2011. 12(10): p. 3621-3628.

Cure-On-Dispense Printing of High Porosity Foams using Redox Initiation

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Introduction: Tissue engineering has emerged as a promising solution to meet the limitations of current bone grafting procedures by harnessing the body's ability to heal itself. Emulsion templating is capable of producing porous scaffolds through polymerization of high internal phase emulsions (HIPEs) to form porous foams (polyHIPEs). Previous work from our laboratory has created injectable bone grafts with mechanical properties comparable to cancellous bone utilizing redox initiation^[1]. Herein, we demonstrate an extension to this work to fabricate grafts with hierarchical architectures via 3D printing of polyHIPEs using a redox-based system to enable cure-on-dispense printing.

Methods: A biodegradable, hydrophobic, acrylate-functional macromer was combined with surfactant and initiator, emulsified with water to form a viscous paste, and deposited into geometric shapes using a Prusa i3 3D printer. Two separate HIPEs were created, containing either an oxidizing agent (benzoyl peroxide, BPO) or reducing agent (trimethylaniline, TMA). These components were forced through a mixing head, initiating a redox reaction, and deposited to create the desired construct.

Results and Conclusion: We have designed a system to enable printable redox-based polyHIPE with rapid curing after dispensing at low temperatures. This development coupled with the emergence of affordable, open source, 3D printing technologies has paved the way for the fabrication and processing of complex 3D scaffold designs which could serve for future high-fidelity bone grafts.

[1] Moglia RS, Whitely M, Dhavalikar P, Robinson J, Pearce H, Brooks M, et al. Injectable Polymerized High Internal Phase Emulsions with Rapid in Situ Curing. *Biomacromolecules*. 2014;15:2870-8.

Clindamycin-releasing Porous Poly(methylmethacrylate) space maintainers for Prevention of Mandibular Infection

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Infection is a common and devastating consequence of injuries to the craniomaxillofacial region. In order to address this difficulty, antibiotic-releasing porous space maintainers have been developed to address infection prevention and maintenance of the defect architecture.

The objective of this study is to evaluate the effects of antibiotic release kinetics and dose on soft tissue healing over a porous poly(methylmethacrylate) space maintainer implanted in critical size infected rabbit mandibular defect.

Three groups of clindamycin-releasing porous space maintainers were fabricated: Burst Release, High Dose Sustained Release and Low Dose Sustained Release. The space maintainers were implanted into an infected 10 mm bicortical rabbit mandibular defect ($n = 10/\text{group}$). After 12 weeks, mandibles were evaluated by gross observation, culture, microcomputed tomography, and histology.

Release of clindamycin from the Burst group occurred over 7 days, after which no further release was noted; release of clindamycin from the sustained release groups occurred over 28 days. The inoculated bacteria was not recovered from any animal. Gross healing over the defect was similar between all groups ($p > 0.05$). However, the PLGA High groups exhibited significantly less dehiscence than either the Burst or PLGA Low groups ($p < 0.05$).

While it appears that the release kinetics have no effect on the healing of soft tissue over the space maintainers, the results from this study indicate that a high dose extended release of antibiotic may be useful in preventing dehiscence of healed mucosa.

Encapsulation of Polyanhydride Nanoadjuvants in Biodegradable Microgels for Oral Delivery

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The Global Vaccine Action Plan (GVAP), published in 2012, designated non-syringe delivery mechanisms as a research priority for development of next-generation vaccines (1). Oral vaccination has potential to stimulate both systemic and mucosal immune responses while improving ease of distribution, administration, and safety compared to injection-based administration. However, there are several challenges to overcome to effectively induce immunity, including maintaining antigen stability in the harsh conditions of the gastrointestinal tract (e.g. acidic pH, enzymatic digestion), to reach the antigen-sampling cells of the small intestine. Our proposed solution is a Polyanhydride-Releasing Oral Microparticle Technology (PROMPT) which exploits the unique properties of two biomaterial systems. Polyanhydride nanoparticles (PNPs) function as an antigen carrier and vaccine adjuvant to confer protection. Microencapsulation within pH-responsive hydrogels enable safe transport of the antigenic payload through the stomach and delivery into the small intestine.

In this work, pH-responsive hydrogels were adapted to incorporate biodegradable crosslinking strategies and optimized for delivery of nanoparticles. Hydrogels composed of poly(methacrylic acid) with either grafted PEG tethers, denoted P(MAA-g-EG), or co-monomer N-vinyl pyrrolidone, (P(MAA-co-NVP)), were modified to incorporate the pH-responsive chemical crosslinker dimethacryloyl hydroxylamine or an oligopeptide for selective degradation in intestinal conditions. Degradation studies indicate therapeutic release in physiologically relevant conditions, and *in vitro* assessment indicates no cytotoxicity of microgels or degradation byproducts with intestinal epithelial and macrophage cell lines. Additionally, PNPs are readily internalized by cultured macrophages. These results indicate PROMPT has potential as an adaptable platform for oral vaccine administration.

1. *Global Vaccine Action Plan Strategic Objectives*. 2013, World Health Organization.

Synthesis and Characterization of Cationic Nanogels for Enhanced Cancer Therapy

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Cancer cells can be intrinsically drug resistant or can become drug resistant when exposed to chemotherapeutics. Specifically, cancer cells can develop resistance through up-regulation of efflux pumps or anti-apoptotic pathways. In addition, chemotherapeutic regimens can essentially select for a cancer cell subpopulation resistant to the chosen therapy. As such, methods to improve chemotherapeutic regimens to combat drug resistant cancer are needed.

Here, cationic nanogels were synthesized with either randomly incorporated or localized pH responsive and hydrophobic functional groups. The presence of localized functional groups are hypothesized to enhance pH responsive properties as well as drug loading to reduce off target effects associated with premature release of therapeutic agents.

The swelling properties of cationic nanogels were observed with dynamic light scattering from pH 4-9. The onset of the pH dependent swelling was modulated by varying monomer feed ratios. Typical nanogels exhibited hydrodynamic diameters of 80-100nm in collapsed state to 120-140nm swollen in 1x PBS at 25C. Similarly, zeta potential was used to track apparent surface charge on the nanogels through the same pH range. Positive zeta potential on the order of 15mV was observed for swollen gels with almost neutral zeta potential observed at pH 7.5.

Initial screening of the randomly polymerized nanogels exhibited minimal cytotoxicity when incubated with RAW 264.7 murine macrophages at concentrations up to 0.1mg/mL for 24 hours.

This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program.

Interpenetrating Collagen-Fibrin Hydrogels for Skeletal Muscle Regeneration

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Biomimetic hydrogel scaffolds have been used extensively for *in vitro* investigation and to create synthetic grafts for wound healing applications such as skeletal muscle. Previously, we performed an *in-vitro* screening of natural hydrogels, evaluating collagen I, agarose, alginate, fibrin and collagen-chitosan. The results indicated that collagen and fibrin were best suited as myogenic scaffolds compared to the other groups tested. For the current study, we used the following collagen:fibrin ratios: 100:0, 75:25, 50:50, 25:75, and 0:100. Characterization methods included evaluation of material stability over 14 days with and without cells (rat skeletal myoblasts L6), uniaxial tensile testing, rheology and *in vitro* myogenesis. Statistical differences were determined using a two-way ANOVA with Tukey's post hoc test (n=6, p<0.05). The stability test indicates that the groups have an increasing level of degradation with increasing fibrin content. The addition of cells increased degradation in the case of the 50:50 blend, but extracellular matrix (ECM) deposition caused slower material degradation in all other hydrogel blends. This allows us to potentially tune the rate of scaffold degradation to match the rate of ECM synthesis by skeletal myoblasts. The rheological data shows that all groups have predominantly elastic behavior rather than viscous. Furthermore, the elastic moduli was similar between all groups and comparable to skeletal muscle myoblasts. *In vitro* testing of the gels using L6 cells over 14 days indicated that the blended hydrogels led to greater myogenesis based off immunofluorescent staining for myosin heavy chain compared to the pure collagen or fibrin groups.

Design and development of pH-responsive hydrogel systems for the oral delivery of therapeutic proteins

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Protein therapeutics have vast utility in the treatment of a variety of debilitating disorders such as diabetes and growth hormone deficiency. However, due to their large size and fragile structure, their administration is limited to painful injections. While oral delivery is preferred, the harsh, degradative nature of the gastrointestinal tract, the short residence time in the absorptive small intestine, and a restrictive epithelial barrier, are all challenges that must be overcome by an oral delivery system.

This work proposes an oral delivery carrier composed of a pH-responsive hydrogel capable of remaining collapsed at low pH (such as in the stomach) and only swelling to release the protein at the neutral pH of the small intestine. This hydrogel system, designated as P((MAA-co-NVP)-g-EG), contains methacrylic acid to impart pH-responsiveness, N-vinyl pyrrolidone for increased hydrophilicity, and poly(ethylene glycol) to provide mucoadhesion for increased residence in the small intestine. Formulations of P((MAA-co-NVP)-g-EG) have been synthesized by UV-initiated free-radical polymerization and have shown the desired swelling profiles, remaining collapsed at low pH and swelling only at pH > 5. Various proteins, including insulin, growth hormone and ovalbumin, have been successfully loaded into the hydrogel carrier, at 40-80% loading efficiencies. These hydrogels are also capable of preferentially releasing the proteins at neutral pH, while limiting release at low pH. *In vitro* cytotoxicity studies indicate minimal-to-no cytotoxicity of the hydrogels to intestinal cell models. *In vivo* bioavailability studies in Sprague Dawley rats show that growth hormone loaded hydrogels can successfully deliver the protein in therapeutically relevant doses.

Title: Elastomer/Gelatin Composite Membranes for Treatment of Cutaneous Mold Infection

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Abstract (Limit 250 Words):

Necrotizing invasive mold infections in soft tissues present a clinical challenge to treat and result in significant morbidity and mortality in an increasing population of patients with severe immunosuppression or trauma. Systemic antifungal therapy is often limited by fungal destruction of the local vasculature, preventing delivery of therapeutics to the site of infection. We have developed an injectable, biodegradable membrane to serve as both a local antifungal delivery vehicle as well as scaffold for regenerating cutaneous tissues. Previously, we had demonstrated that poly(glycerol sebacate) acrylate (PGSA) can be used for the controlled delivery of antimicrobial compounds. However, by introducing gelatin microparticles (GMPs), hybrid natural/synthetic material composite membranes can be synthesized that allow for pore formation upon GMP degradation. Uncrosslinked PGSA was loaded with the antifungal medication voriconazole (VRC) and mixed with gelatin microparticles (GMPs) to produce a liquid composite. This injectable solution was crosslinked in situ by exposure to blue light from a conventional dental lightemitting diode (LED) system. The physicochemical properties, degradation rate, VRC release rate, and its bioactivity against a pathogenic fungal isolate (*Aspergillus fumigatus* strain Af293) were evaluated. Pores within PGSA/GMP composite membranes were formed and increased in diameter over time after incubation in collagenase-containing media. VRC was successfully released at physiologically-relevant concentrations over 15 days, a clinically-relevant time span. The released VRC was capable of mitigating the growth of *A. fumigatus*. As an injectable membrane capable of the local release of bioactive antifungals, this therapy represents a paradigm shift in the treatment of cutaneous fungal disease.

Synthesis and Characterization of Biodegradable Polyurethane-ureas as Biomaterials for Soft Tissue Applications

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The development of biomaterials that match the mechanical properties of native tissue has been subject to extensive investigation in tissue engineering.¹ A mismatch in mechanical properties can result in early device failure or stress shielding. Much of the interest in biodegradable synthetic materials has focused on polyesters that display high tensile strength and modulus with low elongation.¹ However, these materials are subject to creep and do not recapitulate the dynamic range in properties of soft tissues. Elastomeric polyurethanes can provide the tunability and mechanical properties needed to address the current limitations. In this work, we have developed a library of biodegradable poly(ether ester urethane-urea)s based on variations in the hard segment content and chemistry with a broad range of mechanical properties. Mechanical testing of the B-PURs displayed a moduli ranging from 18 MPa to 233 MPa and ultimate tensile strength from 17 to 33 MPa). This library allows for appropriate selection of material by correlating tensile properties with reported tissue values. Specifically, by varying properties, these B-PURs can be used as biomaterial scaffolds for many musculoskeletal applications such as rotator cuff repair. To achieve the gradient of mechanical properties typical of native rotator cuff tissues, we developed a co-electrospinning technique to produce scaffolds that mimics the tendon to bone interface.

References: 1. Ma et al. *Biomarcomolecules*, 2011.

Reprogramming and Cardiac Differentiation of Amniotic Fluid Derived Stem Cells for the Repair of Congenital Heart Defects

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Statement of Purpose: Congenital heart defects are the most common type of birth defect and the leading cause of infant death. Current repair strategies involve surgery using inactive repair materials, which are unable to grow with the patient. Defects can be detected as early as the first trimester where most severe defects require immediate surgical intervention at birth. This time between diagnosis and surgery can be used to engineer functioning cardiac tissue. The goal of this study is to create an autologous, implantable cardiac patch system consisting of a PEG-fibrin hydrogel and signaling inhibitors released at specified time points promoting cardiac differentiation.

Methods: Cell source: By adapting previous cardiac differentiation of iPSC protocols, reprogrammed AFSCs were first cultured in 2D to determine appropriate growing conditions for cardiac differentiation.

Hydrogel: Cell viability in a PEG-fibrin hydrogels was assessed using reprogrammed AFSCs. The cells were exposed to GSK3/Wnt inhibitors at days 0 and 3, respectively. The cells were analyzed for early and late stage cardiac markers.

Results: 2D studies showed that induced amniotic fluid derived stem cells were capable of differentiating into beating cardiac cells 15-21 days from the start of differentiation. When encapsulated within PEG-fibrin hydrogels, reprogrammed AFSCs show high viability and exhibit cardiac differentiation potential.

Conclusions: The current study shows the potential for a completely autologous cardiac tissue patch for the treatment of congenital heart defects. AFSCs can be readily isolated in utero at the time of diagnosis, then induced and differentiated into a beating cardiac lineage. This construct can be grown into functional, beating cardiac tissue which can be implanted directly into the patient at the time of surgical intervention.

In Vitro and In Vivo Mineralization and Osteogenesis of Injectable Stem Cell Laden Hydrogels

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Reconstruction of craniofacial bone defects remains a clinical challenge due to limitations of current treatments, such as allografts and autografts, and associated risks. A minimally invasive tissue engineering alternative is a thermoresponsive hydrogel system that undergoes a sol-gel phase transition at body temperature, allowing for injection in and contouring to a defect site, as well as localized delivery of stem cells or growth factors. The aim of this study was to first, investigate the osteogenic capacity of injectable, dual-gelling hydrogels composed of poly(*N*-isopropylacrylamide) (PNiPAAm)-based thermogelling macromers (TGM) and polyamidoamine (PAMAM) crosslinkers, with and without the incorporation of gelatin microparticles (GMP) as sites for cell attachment, to support the osteogenesis of primary rat mesenchymal stem cells (MSC) *in vitro*, and second, to evaluate the regenerative potential of the hydrogel-cell constructs for bone formation *in vivo*. 20 wt % hydrogels with loaded GMPs significantly supported encapsulated MSC viability and promoted osteogenic differentiation over 28 days *in vitro* as demonstrated by Live/Dead confocal microscopy, histological staining, and biochemical assays. This was reflected in the *in vivo* results, in which joint incorporation of GMPs and MSCs within the hydrogel system led to significant hydrogel mineralization, bone formation and bone tissue ingrowth in an 8 mm rat critical size cranial defect. The results indicate that injectable, dual crosslinking hydrogels can be successfully created to support cell viability and direct osteogenesis both *in vitro* and *in vivo*. These self-mineralizing injectable hydrogels show promise as a minimally invasive strategy for stem cell delivery in craniofacial tissue engineering.

Intravascular Canine Patent Ductus Arteriosus Closure Device

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Patent ductus arteriosus (PDA) is a congenital cardiovascular disease in which a connection between the aorta and pulmonary artery does not close shortly after birth. If this defect is not closed, it can lead to serious complications and even death. A prototype device, the nitinol foam cage (NFC), has been developed in an attempt to address the shortcomings of the current treatment methods. The NFC utilizes a nitinol frame and a shape memory polymer foam to promote embolization and tissue healing.

The NFC's mechanical properties were evaluated and compared to the Amplatz Canine Ductal Occluder's (ACDO) in multiple mechanical and *in vitro* experiments. The NFC exerted similar radial pressures to those of the ACDO for all but the two largest vessel diameters tested, but it required a much lower force to dislodge the device from its ideal position compared to the ACDO. The NFC exhibited minimal device migration, remained in the desired location in the *in vitro* models, and received positive clinician feedback, including that it offered less resistance and was easier to deliver in the same sized sheath as the ACDO. While the ACDO exhibited superior mechanical properties, the NFC performed well in the *in vitro* experiments, warranting further development of this design as an alternative method to treat PDA.

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Nanoparticle Delivery via Angioplasty Balloons for Treatment of Atherosclerosis

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American Heart Association credits cardiovascular diseases with over 1.73 million deaths per year. Current treatment strategies like bare or drug eluting stenting and angioplasty suffer from restenosis, late-stent thrombosis, and reduced drug exposure at the atherosclerotic site. Here we have designed an alternative nanoparticle (NP) delivery strategy by coating drug loaded biodegradable Urethane-doped polyester (UPE) NPs directly onto angioplasty balloons. Two different coating methods, A) Layer-by-Layer (LbL) electrostatic coating and B) Acrylic acid hydrogel (AAH) coating, are investigated. Based on the electron microscopy (SEM and TEM) and dynamic light scattering (DLS), UPE NPs had an average size of about 300 nm. The NPs also showed a sustained release of the model drug over 21 days, and they were stable and degradable. The loading efficiencies of drug-loaded UPE NPs were about 80% and 65% for AAH and LbL coatings, respectively. In addition, NPs were compatible with human aortic endothelial cells by MTS assays. *Ex Vivo* UPE NP transfer efficiency studies also showed that, a larger number of NPs being transferred to the rat arterial wall by the AAH coating method. Thus, UPE NPs with the AAH coating technique potentially can be used to obtain high drug-loaded NP delivery to the atherosclerotic regions.

Long-Term Safety and Stability of Carbon Nanotube Fiber as Active Interfaces with Excitable Tissues

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Implanted active interfaces can be used to treat neurologic disorders as well as to restore, replace, or supplement body functions. Examples of currently used active interfaces include the leads for pace-makers, deep brain stimulation (DBS) electrodes for Parkinson's disease, and electrodes for neural prostheses. However, state-of-the-art metal electrodes are inadequate for long-term neuronal stimulation and recording due to their high stiffness, sub-optimal electrochemical properties, proneness to bending fatigue failure, and in some cases, chemical and mechanical instability in biological environment. Carbon nanotube (CNT) fiber, with its softness and remarkably low contact impedance, represents an attractive alternative for applications involving an implanted active interface. Our previous studies have shown that CNT fiber electrodes perform just as well or better compared to metal electrodes through in vitro electrochemical characterization, and in vivo neural recording and stimulation in rodent models with Parkinson's disease. CNT fiber microelectrodes were also found to elicit less brain-specific inflammatory response than commercial metal electrodes. In order for CNT fiber to have wide applications as biomaterial for active interfaces, long-term safety and stability in physiological on other than the brain must be demonstrated. In this work, we assess the long-term stability of CNT fiber and compare it to that of metals in vitro in presence of oxidative species, representative of the biological condition created under inflammatory response. Furthermore, we evaluate biocompatibility of CNT fiber through sub-chronic and chronic in vivo studies.