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Biodegradable Polymer-Based Theranostic Nanoparticles: Synthesis, Characterization, and *In Vitro* Studies

Theranostic nanoparticles are a helpful strategy to diagnose, monitor and treat cancer using an all-in-one platform. Elevated tumor proteases can be used as triggers of enzymatically activated nanoparticles (NPs) to develop NIR signal that can provide sufficient contrast between normal and cancerous tissue. In this study, enzymatically activated NPs loaded with the chemotherapeutic drug doxorubicin free base (DOX) were synthesized as contrast and therapeutic agents for optical imaging and treatment of cancer *in vitro*. The core of the NPs was used as a carrier of DOX. The hydrophilic polypeptide poly(L-lysine) placed on the surface of the NPs acts as an anchor for fluorescent molecules and mediates their quenching. Proteolytic enzymes can cleave the polypeptide, providing mobility and space between the fluorophores. As a result of this, cleaved peptide fragments fluoresce. We prepared the NPs from blends of amphiphilic block copolymers by nanoprecipitation. Poly(L-lysine) was used as a model enzymatically cleavable polypeptide which is an anchor for the NIR fluorescent molecule AlexaFluor-750. MDA-MB-231 metastatic breast carcinoma cells which overexpress and secrete high amounts of trypsin were used to monitor fluorescence development by fluorescent microscope and therapeutic efficacy of NPs. The diameter of spherical NPs was in the range of 60–95 nm. The zeta potential of the NPs was -4.27±5.58 mV. Fluorescence activation of the AF750-labeled DOX loaded NPs by treatment with trypsin resulted in the range of a 3- to 23-fold optical signal enhancement within 180 minutes depending on formulation of the NPs. A drug loading of 3 wt% (mg drug/mg NP) DOX in the NPs was achieved. NPs showed fluorescent development of AF750 as well as fluorescence from DOX released into the cells. These results suggest that enzymatically activated doxorubicin loaded NPs are an attractive platform for image-guided cancer therapy.

John R. Clegg, Matthew Harger, Afshan S. Irani, Dr. Pengyu Ren and Dr. Nicholas A. Peppas

The University of Texas at Austin

Toward Protein Recognition with Oligopeptide-Containing Imprinted Hydrogels

Molecularly imprinted polymers (MIPs) have emerged as an active area of research in the biomedical domain because of their superior affordability and environmental stability as compared to natural proteins and antibodies. For applications in medical diagnostics and drug delivery, it is ideal for these materials to recognize single proteins in complex biological fluids. We synthesized MIPs for a series of high-isoelectric point exhibiting model proteins (trypsin, lysozyme, cytochrome c) in order to assess the selectivity MIPs for their template, as opposed to alternative proteins. Trypsin MIPs comprised of poly(methacrylic acid-co-(diethylamino)ethyl methacrylate-co-acrylamide) bound 50% or greater more trypsin, lysozyme and cytochrome c than control non-imprinted polymers (NIPs), while excluding large or low-isoelectric point exhibiting proteins (bovine serum albumin, trypsin inhibitor type II, hemoglobin). We hypothesized that polymer-trypsin specificity amongst high-isoelectric point exhibiting proteins could be achieved by incorporating engineered oligopeptides, screened for trypsin affinity, within MIP networks. Peptide ligands were selected through random generation, followed by a genetic algorithm-like mutation series. Trypsin-oligopeptide affinity was screened using molecular docking simulations in the commercially-available GOLD software. From 7,200 screened peptides, six promising sequences were selected. Selected peptides docked trypsin in high affinity, lacked arginine and lysine residues, and contained cysteine to permit polymer-peptide conjugation via thiol coupling. The affinity of selected peptides for trypsin, as opposed to alternative cationic proteins, was screened experimentally using surface plasmon resonance (SPR) techniques. Peptides selected trypsin with micromolar affinity and were conjugated into MIP and NIP networks at 89% efficiency. We believe these peptide-MIP hybrids pose a promising material strategy for protein recognition in drug delivery and biosensing applications.

3D Printing Vascularized Tissues: Closing the Loop between Computational and Experimental Models

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3D printing offers unique potential to advance the field of tissue engineering by providing a method to establish a vascular space within large 3D tissues. The objective of this work is to close the loop between computational and experimental models involving flow and mass transport in vascularized tissue engineered constructs. By developing reliable computational models for our tissue engineered systems, we can rapidly optimize vascular geometries to maintain cell viability throughout constructs on a physiological size scale.

We first printed branching channel networks with a four-rung ladder design in polyethylene glycol diacrylate hydrogels. We then tracked the flow of fluorescent beads through printed channels at a physiological flow rate of 100 μL/min. After collecting a series of images with frame rates upwards of 100 frames per second, we used the particle image velocimetry package PIVlab to calculate flow rates of beads within the channels. This step provides a basis against which we could compare our computational models. Then, to develop computational models for flow, we first used a Bruker micro-computed tomography scanner to scan the same channels we used in the bead tracking experiments. Using Mimics software, we used these scans to reconstruct 3D meshes for our printed channels. We imported these meshes into the computational fluid dynamics software COMSOL Multiphysics, to predict flow rates and patterns through individual channels. Preliminary evidence has demonstrated a strong agreement between the computational and experimental models. Furthermore, computational models predicted non-obvious flow patterns through the ladder channel networks. Reliable computational models are an invaluable resource in making high throughput optimizations in vessel geometries, flow rates, and cell seeding densities to ensure the viability of cells within a large scale tissue engineered construct.

Using 3D Hydroxyapatite-Collagen Composite Scaffolds and Spatial-Temporal Variation to Promote Vascularized Bone Tissue Regeneration

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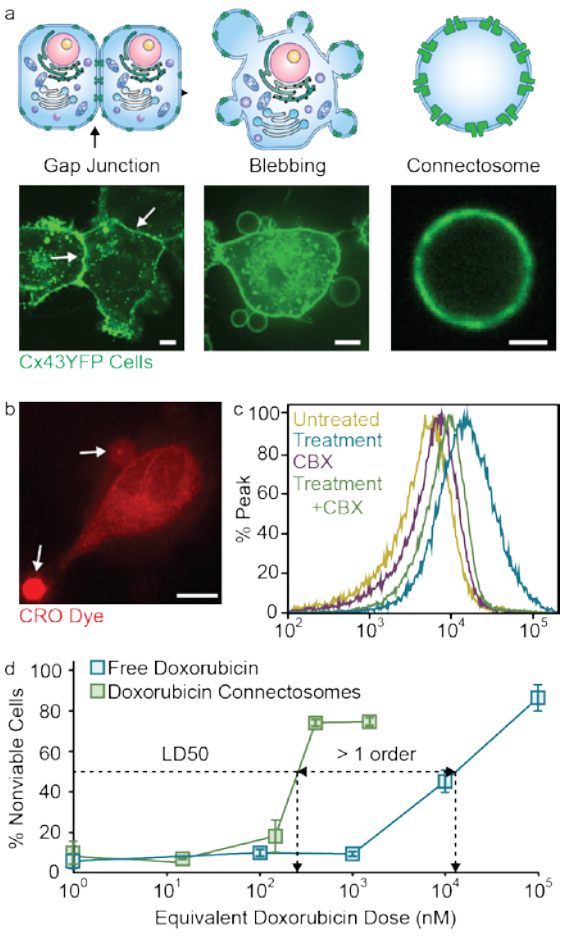
Bone fractures are quite common and while most heal naturally, severe bone injuries such as those caused by trauma often do not heal on their own and require a tissue graft for repair. Many of these grafts fail due to a compromised blood supply. Hence, a means to promote vascularized bone regeneration is needed. As such, spatial-temporal seeding variations of co-culture cells in 3D HA collagen composite scaffolds were evaluated in this study for enhancement of osteogenesis and angiogenesis. Composite scaffolds were prepared by casting 4 mg/ml collagen hydrogels within a porous 3D HA scaffold. Optimized concentrations of human embryonic palatal mesenchymal cells (HEPMs) and human umbilical vein endothelial cells (HUVECs) were seeded with spatial-temporal variation (Group 1: HEPMs seeded 7 days before HUVECs seeding, Groups 2: HEPMs seeded 6 hours before HUVECs seeding, and Group 3: HEPMs and HUVECs cast in the composite scaffolds). Production of vascular markers (VEGF, Ang-1, and angiogenin) and an early osteogenic marker (alkaline phosphatase (ALP)), were measured at regular intervals using ELISA. Groups were compared using 2-way ANOVA across time and Tukey's test ($p<0.05$). Results showed that Groups 1 and 3 had similar trends in ALP throughout the duration of the experiment and vascular markers as well as an initial peak of ALP, which is indicative of osteoblast differentiation. However, Group 2 had reduced and fairly consistent ALP production when compared to Groups 1 and 3, suggesting delayed HEPM attachment on composite scaffolds. All Groups showed an initial early increase in vascular markers Ang-1 (essential for organization, integrity, and maturation of neo-vasculature) and angiogenin (potent inducer of neovascularization *in vitro*). However, vascular marker maturation levels were observed to decrease over time, suggesting the entrapment of vascular proteins in newly formed ECM. Hence, results imply both bone and vascularization are occurring.

Connectosomes for Direct Cytoplasmic Drug Delivery

Avinash K. Gadok, David J. Busch, Brian Li, Silvia Ferrati, Hugh D. C. Smyth, Jeanne C. Stachowiak

Diffusion of drugs across the cell's plasma membrane barrier is an inefficient process. In particular, the requirement for membrane permeability limits drug accumulation in the cytoplasm, undermining therapeutic efficacy and over-constraining drug design. In contrast, gap junctions, transmembrane proteins that connect the cytoplasm of adjacent cells, bypass the plasma membrane, permitting diverse small molecules including chemotherapeutics to move between cells. Our work addresses the challenge of crossing the plasma membrane with a new strategy that uses gap junctions to transport drugs directly into the cytoplasm. Specifically, we developed Connectosomes, cell-derived materials that contain functional gap junction channels and can form gap junctions with cells. Connectosomes are harvested from the plasma membrane of cells that over-express gap junctions (a). These engineered materials transferred dyes and drugs directly to the cellular cytoplasm (c, d). Quantitative analysis revealed that using Connectosomes to deliver doxorubicin reduced the therapeutically effective dose by more than 10-fold (d). This remarkable increase in therapeutic efficacy has the potential to boost the effectiveness of drugs and to address long standing problems with chemotherapy such as toxicity and multidrug resistance. Further, in bypassing the plasma membrane, Connectosomes remove a key constraint on therapeutic design, enabling the delivery of membrane-impermeable drugs.

a, Connectosome formation. b, Connectosomes transported dye to recipient cells. c, Recipient cell fluorescence histograms. Treated cell fluorescence increased 6-fold compared to untreated cells. In the presence of CBX, a gap junction inhibitor, transfer was significantly reduced, illustrating that gap junction-based interaction was responsible for transport. d, Drug-loaded Connectosomes decreased LD50 of doxorubicin by more than 10-fold.



Multi-Functional Transmembrane Protein Ligands for Cell-Specific Targeting of Plasma Membrane-Derived Vesicles

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Nanoparticle-based drug delivery systems have the potential to target diseased cells on the basis of their receptor expression profiles. Toward this goal, synthetic materials conjugated with antibodies against specific cellular receptors have been shown to concentrate in solid tumors. However, chemical conjugation reactions are difficult to control and frequently compromise the affinity of receptor binding. Further, the difficulty of implementing multiple distinct attachment chemistries effectively limits the number and complexity of targeting ligands that can be used. To overcome these limitations, here we demonstrate that plasma membrane vesicles (PMVs) derived from donor cells can be engineered to express transmembrane protein ligands that precisely target cells on the basis of the expression level of a specific cellular receptor. In particular, PMVs expressing ligands for EGFR bound with high specificity to breast cancer cells expressing distinct levels of this receptor. Furthermore, PMVs can express various other natural and engineered transmembrane proteins to selectively target cell surface proteins. As an example of the generality of this approach we created PMVs expressing a single domain antibody against GFP and showed specific binding to GFP-tagged receptors. Our results demonstrate the versatility of PMVs as targeted drug delivery systems. Further, PMVs provide an approach to insert intact and functional transmembrane proteins into liposomal materials. Moving forward, these capabilities will enable the development of multi-functional biomaterial systems that control the fate of cells and tissues by participating in cellular communication and signal transduction.

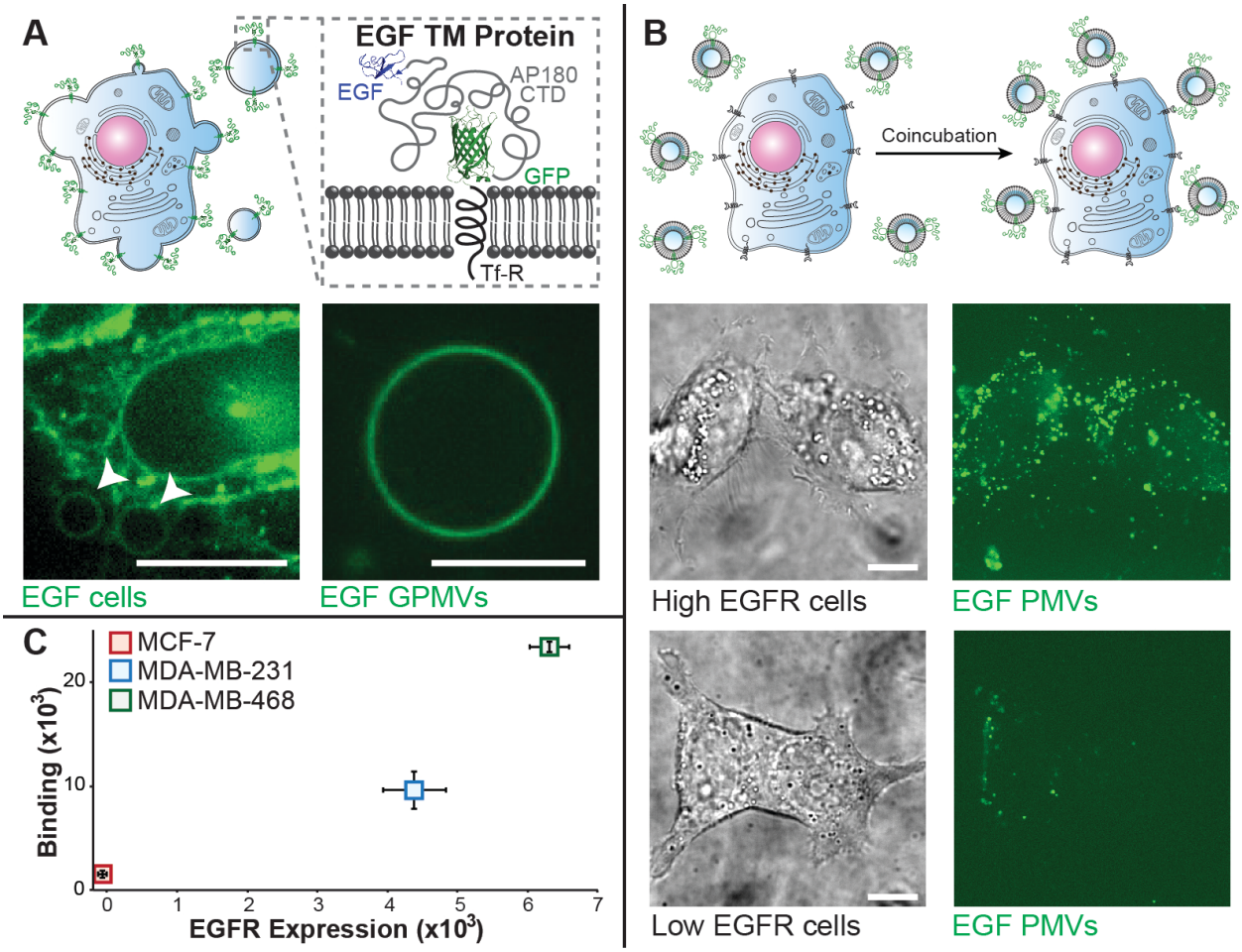

Figure | A) Cellular blebbing **B)** PMVs selectively bind EGFR overexpressing cells **C)** Binding correlates with receptor expression

TETHERED MICROPARTICLES FOR BMP-2 DELIVERY FROM COLLAGEN COATED HYDROXYAPATITE SCAFFOLDS

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There is a strong clinical need to develop synthetic material-based scaffolds [1]. Delivery of biochemical cues to stimulate host's natural healing response may address drawbacks of current approaches for bone regeneration [2]. Bone morphogenetic protein (BMP)-2 is currently the gold standard [3], delivery methods experience initial high-burst release and insufficient local retention [2]. Physical adsorption of BMP-2 to ceramic surfaces is a concern because it results in denaturing of the protein and poor activity [2]. Polymeric drug delivery systems have potential to protect drugs from degradation, and control release at the targeted site [4]. The purpose of this study was to develop a combined platform of biodegradable hydroxyapatite (HA)–collagen scaffold tethered with polymeric microparticles (MPs) which will allow for the sustained slow release of BMP-2. ***Methods:*** HA porous scaffolds were prepared by coating polyurethane sponges in a water–based HA slurry and subsequent sintering. Three BMP-2 release MP systems were prepared using water-in-solvent emulsion evaporation: (a) Poly(lactic acid)(PLA); (b) Poly(DL-lactic-co-glycolic acid)(PLGA)/poly(L-lactic-acid)(PLLA), and (c) PLGA/polyethylenimine(PEI). MPs were tethered to scaffolds by immersing the samples for 30 minutes - 1 hour in 0.05% collagen/MP solution. MP attachment was confirmed by SEM. Scaffold/MPs were immersed in 1XPBS and HCl (24h) and BMP-2 released was measured by ELISA. Biological activity of rhBMP-2 was assessed using human mesenchymal stem cells (MSCs). After 24h of incubation, media was replaced with media supplemented with BMP-2. Alkaline phosphatase activity of the cells in the presence of BMP-2 was determined after 3 days. ***Results:*** SEM pictures confirmed MP attachment to HA/Collagen scaffolds. Encapsulation efficiency of Scaffold/MPs on the scaffolds after 30 min and 1 hour was measured, suggesting burst release of BMP-2 at 24h hours in PBS for PLGA/PEI group. 24h acidic degradation suggests higher loading efficiency for PLA and PLGA/PEI compared to PLGA/PLLA group. Biological activity of BMP-2 was confirmed at day 3 by measuring ALP activity, compared to control. ***Conclusions:*** Polymeric MPs encapsulating rhBMP-2 were prepared using water-in-solvent emulsion evaporation technique and were successfully attached to the HA scaffolds via a Collagen coating for further release of BMP-2. This platform is suitable for prolonging growth factor delivery and maintaining the bioactivity and stability of growth factors for applications in bone tissue engineering based on porous calcium phosphate scaffolds.

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Bioorthogonal Conjugation of Bioactive Proteins to Thiol-Ene Click Microgels

Hydrogel microparticles are attractive drug delivery vehicles for tissue engineering. While covalently tethering growth factors that have short half-lives to microparticles allows for sustained therapeutic presence, a major challenge is maintaining growth factor bioactivity. The use of click chemistry, which offers fast, efficient, specific, and mild reactions, has emerged as a powerful synthetic tool to circumvent this issue. Particularly, the use of bioorthogonal tetrazine-norbornene click chemistry, which proceeds spontaneously without a catalyst and is compatible with biologics, is complementary with thiol-norbornene click chemistry and amenable as a facile tool for bioactive growth factor tethering. Here, we utilize a thiol-ene hydrogel microparticle platform for delivery of bioactive growth factors through bioorthogonal tetrazine conjugation. Briefly, PEG-based hydrogel microparticles were fabricated using thiol-ene click chemistry ([thiol]:[norbornene] ratio of 0.75:1) and an electrospraying water-in-oil emulsion technique. Polymerization was achieved using UV light and an efficient photoinitiator. Thiol-ene click enabled rapid hydrogel photopolymerization and electrospraying resulted in low polydispersity microparticles. Subsequently, a tetrazine modified, protein-of-interest (Tz-protein) was incubated with thiol-ene microparticles to yield protein-functionalized microparticles via tetrazine-norbornene click chemistry. Two model proteins, alkaline phosphatase (ALP) and glucose oxidase (GOx), were explored to demonstrate protein tethering ability and maintenance of bioactivity. Free, untethered Tz-ALP and Tz-GOx were demonstrated to be equally bioactive compared to non-functionalized (NF)-ALP and GOx, respectively. Tz-ALP and Tz-GOx functionalized microparticles showed a dose-dependent bioactivity response compared to NF-ALP and NF-GOx counterparts. Having demonstrated the utility of this approach with model proteins, we are now extending this platform to bone morphogenetic protein-2 delivery, which has relevance for bone tissue engineering. Future studies will investigate the efficacy of this microparticle platform for inducing osteogenic differentiation in vitro and for promoting bone regeneration in vivo.

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Cardiac Differentiation of Reprogramed Amniotic Fluid Derived Stem Cells within a Multifunctional Fibrin/PEG Hydrogel for Congenital Heart Repair

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 can require repeat surgeries due to their inability to grow with the patient. The goal of this study is to create an autologous, implantable cardiac patch that promotes the differentiation of reprogrammed human amniotic fluid derived stem cells (AFSC).

Methods: *Cell source:* AFSC were attained from amniotic fluid. Stem cells were isolated and sorted for cKit+ and reprogrammed through mRNA transfection of Yamanaka factors.

Mesoporous Silica Vectors (MSV): MSV (1µm in diameter; 51% of porosity) were loaded with GSK-3 and Wnt inhibitors separately and encapsulated in PLGA microspheres by solid-oil-water (S/O/W) emulsion method. PLGA thickness is altered to tune release mechanics. The release of GSK-3 and Wnt inhibitors from PLGA-MSV, in vitro, was performed in PBS (37°C, under mild agitation).

Fibrin/PEG hydrogel: Fibrin/PEG hydrogels are formed by dissolving fibrinogen was combined at a 1:1 ratio with a bi-functional NHS poly(ethylene glycol) (PEG). After conjugation, reprogrammed AFSC with MSV were mixed into the solution. Thrombin was combined with the cell solution at a 1:4 ratio to initial fibrinogen. Cells within hydrogels were maintained in pluripotent stem cell media for 3 days and assessed for embryonic pluripotency markers. Directed cardiac differentiation of reprogrammed AFSCs was accomplished by MSV release of small molecules inhibiting the GSK3/Wnt signaling pathways.

Results: AFSC 3 days after seeded into fibrin/PEG hydrogels maintain viability and markers of pluripotency Oct4, TRA-1-81(Fig 1). Preliminary release studies of GSK3 containing MSV show delayed release characteristics from different formulations of the hydrogel and vectors (Fig 2). Differentiation studies within fibrin/PEG hydrogels loaded with nanoparticles show an increase in brachyury expression day 1 after the start of differentiation suggesting mesendoderm lineage. With further inhibition of the Wnt signaling pathway, encapsulated cells express early cardiac markers Nkx2.5 and Isl-1.

Conclusions: The current study shows potential for a completely autologous cardiac tissue patch for the repair of congenital heart defects. Successful reprogramming and differentiation of AFSCs proves that functional cardiac cells can result from amniotic fluid. Reprogrammed AFSCs in 3D show that pluripotency is maintained and directed cardiac differentiation can occur by delivery vectors releasing small molecules of the GSK3/Wnt inhibitory molecules.

Novel Nanocomposite to Detect Viability of Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs) are used in angiogenic therapies for regenerating ischemic tissue. It is not fully understood whether the primary role for MSCs in vivo is a paracrine effect or if they are retained and differentiate towards tissue-specific cells. Detecting MSC viability during therapy will provide crucial information in determining their role and developing more effective therapies. Current methods to detect cell viability are limited to fluorescence-based tools, which do not have the requisite penetration for deep tissue in vivo imaging. Poly-D-Lysine (PDL) is well suited as a viability marker because it is readily hydrolyzed by reactive oxygen species (ROS) in apoptotic cells while remaining unperturbed in viable cells.[1] A novel viability probe is being developed which integrates PDL into a gold nanoshell forming sensitive degradable nanocomposites. Upon ROS degradation of PDL, the gold shell loses stability and breaks apart thereby creating a detectable change in absorbance. Degradation of this shell due to ROS species will allow continuous tracking of only viable stem cells. The nanocomposite was found to have maximum absorbance in the 770-950nm range. Degradation of the nanocomposite was tested by addition of hydrogen peroxide over 20 hours and was found to reduce the absorbance by 3.5 fold. This system will be able to accurately detect viability of injected MSCs by measuring changes in spectral absorbance using photoacoustic imaging. Being able to actively detect cell location and viability will provide useful information for developing more effective cell therapies.

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Engineered tissues with perfusable vascular networks created by sacrificial templating of laser sintered carbohydrates

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Engineered tissues densely populated with cells rapidly develop a necrotic core in the absence of convective transport of oxygen and nutrients through vascular networks. Here, we introduce laser sintered carbohydrate materials and their use as biocompatible templates for vascular networks. 3D fluidic networks can be created by encasing sintered carbohydrate templates in diverse hard and soft materials, then dissolving away the templates. This approach provides 3D geometric control, speed, reproducibility, and flexibility with respect to materials.

Based on our experience using carbohydrates as sacrificial vascular templates, we hypothesized that sintered carbohydrates would provide improved control over the architecture of 3D vascular templates. Indeed, we demonstrated that carbohydrate starting materials can be sintered into 3D filament networks (Figure 1). We have demonstrated that carbohydrate materials can be sintered into 3D filament networks, which are self-supporting, water-soluble, and cylindrical and smooth after post-processing. Laser sintered carbohydrate filaments were amenable to sacrificial templating in a variety of bulk matrices to yield open, perfusable channels (Figure 2). Specifically, 3D perfusable networks were formed in cell-laden fibrin, polycaprolactone foams, and PDMS. Thus, this approach is expected to be useful for patterning vascular networks in diverse soft tissues, bone, and microfluidic devices. Already, we have observed that cell activity in thick hydrogels is improved with flow perfusion through sacrificially templated channels.

This novel approach has the potential to meet a major need in the field for reproducible perfusable vasculature within engineered tissue. We expect this technique to be useful for sustaining high cell densities inside large-scale engineered tissues. Independent control of vascular architecture, ECM material, and cell populations also makes this an ideal experimental platform to study angiogenesis, vasculogenesis, interaction between vessels and parenchymal cells, and the effects of flow-perfusion on these phenomena.

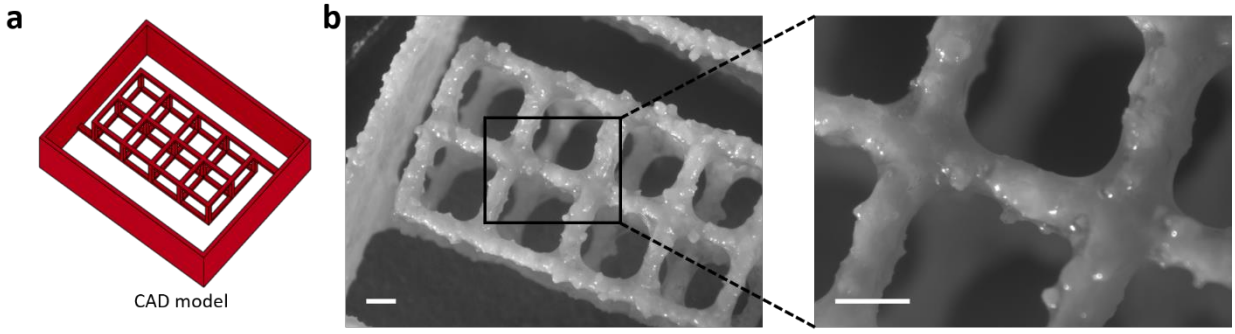
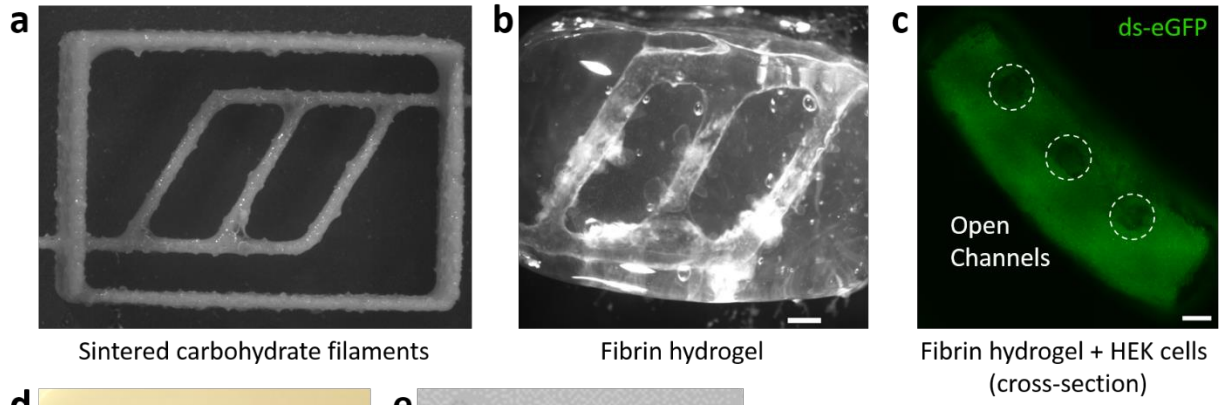


Figure 1. a) A 3D square filament lattice was designed and b) laser sintered from powdered carbohydrates (scale bars = 1mm). Sintered filaments were efficiently fabricated (9 in ~90 mins) with high reproducibility and fidelity on a custom laser sintering platform (github.com/MillerLabFTW/OpenSLS).



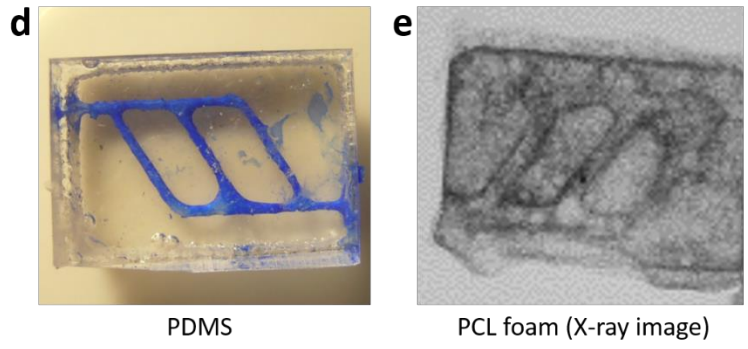


Figure 2. a) A laser sintered carbohydrate ladder network is used as a sacrificial template for open, perfusable channels in diverse materials. Encapsulation in and subsequent dissolution out of b) fibrin hydrogels, c) cell-laden fibrin hydrogels, d) PDMS, and e) PCL foams highlights the flexibility of this approach (scale bars= 1mm).

Time-course of Matrix Stiffening Drives hMSC Differentiation *in vitro*

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Human mesenchymal stem cells provide an exciting and readily available avenue for potential therapies ranging from wound healing and cardiac grafts[1]. However, the multi-lineage potential of hMSCs once transplanted may have an effect on the subsequent population for therapy[2]. Therefore, it is of critical importance to understand the time-course of this differentiation *in vivo*, to do this a dynamic system is needed to mimic the changing graft environment as healing occurs. hMSCs were cultured in an alginate-matrigel hydrogel with the ability to be dynamically stiffened through near-infrared irradiation release of calcium from liposomes[3]. Cells were allowed to culture 7 days in unsupplemented media then either adipogenic or osteogenic induction factors were added. A portion of each group was stiffened at various time-points between 14 and 21 days post gelation to assess the role of stiffening time-course on induction efficiency. hMSCs within gels which have not undergone stiffening exhibited characteristic induction when exposed to the strong factors in the supplemented media. Interestingly, culture in all conditions of the soft group led to positive Oil Red O staining regardless of media composition. Brightfield images additionally show a rounded cell shape typical of adipogenic differentiation. Staining with alizarin red was less conclusive as strong staining only occurred in the group induced towards osteogenesis in the soft condition. Positive Oil Red O staining and rounded, adipogenic shape in all media conditions for soft gels support the conclusion that hMSCs cultured in environments of low mechanical stiffness to be pro-adipogenic. This is additionally supported with reduction in alizarin red staining in the osteogenic non-induced group relative to the osteogenic induced group. Taken together, this reinforces the paradigm that matrix stiffness, and the time-course of stiffness changes, are a strong drivers of hMSC differentiation.

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Photopolymerization of Microparticles into Porous Scaffolds for Tissue Engineering

Hydrogels are widely used as scaffolds in tissue engineering, because their 3-D structure more closely mimics the natural extracellular matrix compared to 2-D biomaterials. In conventional hydrogels, cell migration and spreading are dictated by the scaffold degradation rate, with lower crosslinking densities being more favorable. However, the coupling of scaffold degradation and cell infiltration conflicts with maintaining suitable mechanical properties desirable for tissue engineering applications. Here, we introduce a porous hydrogel scaffold prepared by photopolymerizing poly(ethylene glycol) (PEG) hydrogel microparticles. The key designs of this scaffold are the porous structure necessary to provide space for cell growth and the non-degradable building blocks that support cells with a constant stiffness. By tuning the microparticle sizes, and thus the hydrogel pore size, as well as microparticle stiffness, we aim to develop cell-instructive scaffolds that enhance cell spreading, migration, and tissue formation. Briefly, microparticles were fabricated using thiol-ene photopolymerization of PEG-tetra norbornene and PEG-dithiol ([thiol]:[norbornene] ratio of 0.75 to 1) via an electrospraying technique. Subsequently, PEG-dithiol was incubated with thiol-ene microparticles and used to crosslink them via photopolymerization to yield porous hydrogel scaffolds. Low polydispersity microparticles with a size range of 100-500 μm were obtained through electrospraying and mechanical properties were manipulated by varying PEG-tetra norbornene molecular weight. *In vitro* studies showed that mouse 3T3 fibroblasts spread well and elongated between microparticles, as evidenced by pseudopodia, after three days culturing on scaffolds. Future studies will investigate the potential for these scaffolds to be used as *in vitro* models to study cell migration and will be cultured with human mesenchymal stem cells to investigate their effects on accelerating tissue repair.

Effects of Antimicrobial Treatment on the Induction of an Osteogenic Membrane in an Infected Bone Defect

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Poly(methyl methacrylate) (PMMA) is a polymer commonly used to induce the formation of an osteogenic membrane for large bone defect reconstruction. This osteogenic membrane is enriched in osteogenic factors, angiogenic factors, and cytokines that contribute to the development of healthy bone. Surgical site infection by *Staphylococus aureus* is a common complication of bone injury and is known to inhibit the healing of bone. Because PMMA can also be leveraged to locally deliver antibiotics, we have developed an antibiotic-releasing porous PMMA construct to simultaneously resolve infection and promote the formation of a pro-osteogenic membrane. We hypothesize that local delivery of clindamycin in an infected segmental rat femoral defect will resolve infection and restore the osteogenic potential of an induced membrane compared to untreated infected defects.

Porous PMMA constructs that release clindamycin over 28 days were fabricated and implanted into an 8 mm rat femoral segmental defect infected with either 0 or 10^3 CFU of *S. aureus*, according to the following study groups: 1) No Infection/No Antibiotic (control), 2) No Infection/Antibiotic, 3) Infection/No Antibiotic, 4) Infection/Antibiotic (n=8/group). After 28 days, defects were cultured and membranes were harvested for evaluation by quantitative polymerase chain reaction (qPCR). No animals in the non-infected groups cultured bacteria; all cultures from animals in the infected untreated groups grew *S. aureus*. One animal in the infected/treated group cultured *S. aureus*. Membranes harvested and evaluated by qPCR demonstrated a significant increase in IL-1β, TNFα , and IL-10 in the infected/untreated group compared to other groups. No significant differences are seen in the levels of IL-6, BMP-2, VEGF, Runx2, or BMP-5.

Preliminary results from this study indicate that while angiogenic and osteogenic growth factor expression were not significantly affected by persistent infection, local antibiotic release can significantly reduce infection and restore the normal inflammatory profile to an induced membrane.

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[1]Biomedical Engineering, Texas A&M University, College Station, Texas
[2]Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, Texas**Bioactive Hydrogel Coatings to Promote Endothelialization of Vascular Grafts**

There is a growing demand for a viable small diameter graft as the number of coronary artery bypass graft (CABG) procedures increases each year. The small diameter graft must resist platelet adhesion to reduce thrombogenicity, must match the compliance of the native tissue to prevent intimal hyperplasia, and must promote endothelialization to ensure incorporation for long term patency of the graft. Previously, we have developed a multi-layer approach to decouple the mechanical requirements from the bioactivity of the graft. The outer layer is an electrospun polyurethane mesh which matches the native properties of the saphenous vein, which is the current gold standard for CABG procedures. The inner layer is based on a poly(ethylene glycol) (PEG) hydrogel, which naturally resists platelet adhesion. To promote endothelial cell (EC) adhesion, the hydrogels have been incorporated with collagen mimetic proteins (Scl2-1). Scl2-1 has a triple helical structure similar to collagen, and has been modified to have selective cell adhesion by incorporating binding sequences for $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins, which promote endothelial cell adhesion. Using this PEG/Scl2-2 platform we will optimize the bioactivity of the intimal layer of the graft to promote endothelialization with appropriate hemostatic regulation. The goal of our current investigation is to understand cell behavior, especially hemostatic regulation, that is influenced by the attachment of integrins to our novel biomaterial. Understanding of this relationship will allow for optimization of our small diameter vascular graft for long-term implantation and efficacy as well as contribute to design optimization for future biomaterials with incorporated Scl2-2 proteins.

Dynamic whole blood study of silicone modified with PEO-silane amphiphiles

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A blood-contacting medical device rapidly adsorbs plasma proteins which initiates clot (i.e. thrombus) formation. Antithrombotic drugs may reduce clotting and associated device dysfunction and ischemia, but put the patient at risk for hemorrhaging. The extreme hydrophobicity of silicone, a common medical blood-contacting device material, makes it highly prone to protein adsorption and clotting. Poly(ethylene oxide) (PEO) is highly protein resistant, but its function depends critically on its presence at the silicone-water interface. To enable rapid and substantial migration of PEO to the silicone surface, we prepared a PEO-silane amphiphile (**Figure 1**) as a surface-modifying additive. Fibrinogen adsorption was substantially reduced, even at low concentrations of the incorporated PEO-silane amphiphile. In order to assess thromboresistance, modified silicones were exposed to whole blood under dynamic conditions using a Chandler Loop. Clotting was evaluated in terms of occlusion time and thrombus generation for silicones modified with varying levels of the PEO-silane amphiphile. Finally, these results related to protein resistance and the ability of modified silicones to undergo water-driven surface-restructuring.

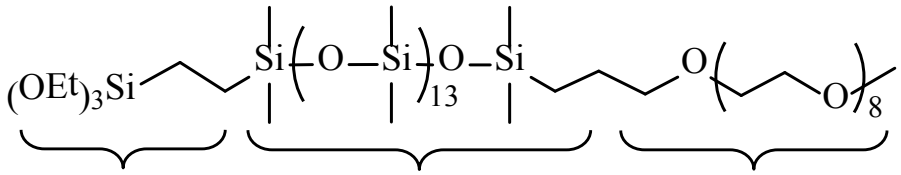

Figure 1: *PEO-silane amphiphile for modification of silicones*

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Co-delivery of multiple therapeutic agents to liver cancer

Off target toxicity and drug resistance are two major factors that currently limit the effectiveness of chemotherapeutic regimens. Drug resistance is particularly prevalent in hepatocellular carcinoma, a disease that currently has a 17% 5 year survival rate, for which death rates have increased over the past 20 years. A promising strategy for overcoming both off target toxicity and drug resistance is through intelligent nanoscale drug delivery systems that co-deliver both a chemotherapeutic and a sensitizing agent, such as short interfering RNA (siRNA). Here, a set of biodegradable tertiary amine methacrylate nanogels with varying hydrophobic comonomer composition were synthesized and investigated as optimal carriers for siRNA and chemotherapeutics.

Nanogels were composed of 2-(diethylamino)ethyl methacrylate and varying ratios of tert-butyl methacrylate and benzyl methacrylate with polyethylene glycol grafts and a degradable disulfide crosslinker. Monomer incorporation was confirmed via FTIR and reaction kinetics were observed in real time using Raman spectroscopy. All formulations exhibited pH dependent swelling and zeta potential with critical swelling transitions at pH 6.5. Loading of siRNA into nanogels was optimized with respect to N/P ratio, buffer ionic strength, and pH. Loading of doxorubicin was optimized and co-loading studies of doxorubicin and siRNA are ongoing. The nanogels were not toxic to human hepatocellular carcinoma (HEPG2) cells after 48 hours at concentrations up to 0.5 mg/mL. Initial transfection studies demonstrated 50% knockdown at N/P ratio of 32. The nanogel framework presented here is a robust intelligent drug delivery system designed to address the major challenges plaguing liver cancer therapy.

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Doxorubicin-Loaded Conductive Polymer Nanoparticles Designed for Laser-Induced Photothermal Therapy and Drug Delivery

Methods for non-invasive, localized cancer treatment are of great importance to theranostic nanomedicine. The successful design of methods for targeting cancer cells while keeping healthy tissue intact encompasses areas of drug delivery, release, and efficacy. Photothermal therapy, a process that uses near infrared (NIR) light and converts it to heat to ablate cells is a widely researched area. The development of nanoparticles made with polymers that exhibit photothermal properties (i.e. that can absorb light in the NIR region and generate heat to ablate cancer cells) and that can act as depots for delivery of anticancer drugs is the main focus of this study. In this work, the chemotherapeutic drug doxorubicin free base (DOX) was encapsulated in nanoparticles prepared using 3,4-ethylenedioxythiophene (EDOT) monomer in a two-surfactant emulsion polymerization process. The DOX loaded PEDOT nanoparticles were characterized using dynamic light scattering (DLS), UV/Vis/NIR absorption and fluorescence spectroscopy, zeta potential analysis, and transmission electron microscopy (TEM). The percent loading of DOX was studied for these nanoparticles at different starting concentrations in order to optimize its encapsulation. Results showed that the nanoparticles have a range of 10-100 nm in size, present strong absorption at 790 nm, and are able to encapsulate DOX at different concentrations up to 1.83% w/w (with respect to polymer). Further studies of these drug-loaded nanoparticles will include drug release and *in vitro* therapeutic efficacy in order to demonstrate their promising applications in light-triggered therapy.

Comparing Mechanical Properties of Silk-Coated and Collagen-Coated Hydroxyapatite Scaffolds for Subchondral Regeneration

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Extensive interest has been given to various fabrication techniques of scaffolds to seek different means of restoring volumetric bone loss. Studies demonstrate that scaffolds act as temporary matrices at the site of injury to promote cell proliferation, which in turn promotes tissue regeneration [1]. Various coatings, architectural factors, biomechanics and porosity are taken into account when enhancing the biomaterial design. Scaffolds with pore size of 100μm-300μm wide were found to be best suited for cell migration, bone regeneration and capillary formation [2]. Collagen has been used to coat scaffolds, as it is the main protein component in bone and reveals exceptional biocompatibility [3]. Silk films are also used as coatings, as they have superior mechanical properties [4]. This study focuses on constructing porous, structural hydroxyapatite scaffolds with different coatings to identify samples with optimal mechanical properties to mimic trabecular bone like structures for subchondral regeneration. Scaffolds were constructed by sintering porous cylindrical polyurethane templates coated with a hydroxyapatite (HA) slurry. Two scaffolds groups were formed: one group consisted of collagen type I coated scaffolds of three concentrations (0.05%, 0.1%, and 0.25%) another group was composed of silk coated scaffolds of three concentrations (3%, 5%, and 7%). Two techniques were used: dipping the scaffold into the coating solution 6 times at 5 minute intervals, or soaking the scaffold into the solution for 30 minutes. Scaffolds coated with 0.05% collagen for 30 minutes showed the strongest trend for an increase in toughness compared to uncoated scaffolds ($p=0.082$). Scaffolds coated with 7% silk for 30 minutes had an increase in ultimate stress ($p=0.001$) and toughness ($p<0.001$) compared to the uncoated control group. It was identified that the trabecular thickness and pore size of each scaffold was not significantly affected after collagen or silk coatings, and they retained their open porous architecture after each coating ($p>>0.05$). *References:* [1] Dhandayuthapani, B et al. Polymer Scien 2011; Hindawi Publishing Corp, [2] Karageorgiou, V et al. Biomat 2005; Elsevier, [3] Glowacki, J et al. Biopolymers 2008; Wiley Periodicals, Inc, [4] Wang, Y et al. Biomaterials 2006; Elsevier.

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Differential Protein Sensing Using Ionic Polymer Coated Gold Nanoshells

Synthetic polymers can be designed to differentially bind proteins by incorporating monomers that form non-covalent interactions with specific residues on protein surfaces. We have found that complementary charge-charge interactions between ionic monomers and proteins have a greater effect on differential binding than hydrophobic interactions or hydrogen binding. Such ionic polymers can be synthesized on the surface of nanomaterials to develop receptors with built-in signal transduction. For example, the localized surface plasmon resonance (LSPR) of noble metal nanomaterials is sensitive to changes in local refractive index and can be exploited to detect molecular binding events near the nanomaterial surfaces. In particular, nanomaterials with high aspect ratios, such as gold nanoshells (AuNSs), have enhanced LSPR sensitivity compared to colloidal gold. In the current work, we synthesize ionic polymers on AuNSs and take advantage of their enhanced LSPR sensitivity to differentiate among proteins with varying isoelectric points.

Hydrophobically modified AuNSs were encapsulated in poly(maleic anhydride-*alt*-1-octadecene)-grafted-poly(ethylene glycol) methacrylate (PMAO-PEGMA) via solvent displacement. PMAO-PEGMA coated AuNSs were combined with N-isopropylacrylamide (NIPAM), methacrylic acid (MAA), and N,N'-methylene bisacrylamide and purged with nitrogen while heating to 70°C before initiating polymerization. Poly(NIPAM-co-MAA) coated AuNSs were incubated with two high isoelectric point (pI) proteins, lysozyme and cytochrome c, and one low pI protein, bovine serum albumin (BSA). Absorbance spectra were measured to detect LSPR response to increasing protein concentrations.

Successful growth of poly(NIPAM-co-MAA) on AuNSs was characterized by dynamic light scattering and transmission electron microscopy. Bare AuNSs and PMAO-PEGMA coated AuNSs showed negligible LSPR shifts upon incubation with any protein. However, poly(NIPAM-co-MAA) coated AuNSs exhibited concentration dependent shifts in LSPR wavelength upon incubation with high pI proteins (lysozyme and cytochrome c) for concentrations down to 175 nM, but no shifts were observed for the low pI protein, BSA, demonstrating the importance of charge-charge interactions in differential protein sensing.

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A Gold Nanoparticle-Based Transfection Agent to Direct Macrophage Polarization

Macrophages of the innate immune system dramatically influence muscle recovery after injury [1]. The relationship between pro-inflammatory, phagocytic M1 macrophages, pro-regenerative M2 macrophages, and muscle recovery is highly dependent on the time of pro- to anti-inflammatory macrophage phenotype shift [2]. Supplementing ischemic injury sites with polarized macrophages can improve muscle functionality; delayed treatment with M2-polarized macrophages significantly improves muscle recovery following ischemic injury and reperfusion [2]. Macrophage polarization is characterized by the metabolism of arginine: M1 cells will metabolize arginine into inducible nitric oxide synthase (iNOS), while M2 cells will synthesize arginase [3]. We have developed a gold nanoparticle-based silencer RNA (siRNA) transfection agent to knock down iNOS expression and promote the pro-regenerative M2 phenotype during muscle repair. Using a gold nanoparticle-based agent may promote endosomal escape within the macrophage via laser irradiation at the gold nanorod peak absorbance wavelength [4]. Therefore, this transfection agent can be used to temporally regulate injected and host macrophage polarization upon lasing.

Gold nanorods (NRs) were synthesized to have an LSPR wavelength of 808 nanometers with a 20 nanometer silica coating. NRs were coated in a layer of 25 kD linear polyethylenimine (PEI), to positively charge the particle and help transfection efficiency via the proton sponge effect. siRNA was electrostatically bound to the PEI intermediate layer. PEI and siRNA coating of the particle was confirmed with zeta potential measurements and gel electrophoresis. *In vitro* evaluation of transfection efficiency of the NR agent with and without irradiation was compared to the commercially available transfection agent Lipofectamine. The widely adaptable nature of this particle makes it an effective tool to both improve macrophage-based therapies and to elucidate the role of key inflammatory markers in muscle degradation and recovery.

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Ultrahigh-throughput generation and characterization of cellular aggregates in laser-ablated microwells of poly(dimethylsiloxane)

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Aggregates of cells, also known as multicellular aggregates (MCAs), have been used as microscale tissues in the fields of cancer biology, regenerative medicine, and developmental biology for many decades. However, small MCAs (fewer than 100 cells per aggregate) have remained challenging to manufacture in large quantities at high uniformity. Forced aggregation into microwells forms uniform aggregates, but commercial sources of microwells are expensive, complicated to manufacture, or lack the surface packing densities that would significantly improve MCA production. To address these concerns, we custom-modified a commercial CO_2 laser cutter to provide complete control over laser ablation and directly generate microwells in a poly(dimethylsiloxane) (PDMS) substrate. We overrode native laser cutter microcontrollers to implement an open-source 3D printing microcontroller workflow, a z-axis stage, and a vacuum to prevent ablation debris accumulation. We achieved ultra-rapid microwell production speeds (>50,000 microwells/hr) at high areal packing densities (1,800 microwells/cm^2) over large surface areas for cell culture (60 cm^2). PDMS substrate distance from the laser focal plane during ablation generated microwells with a variety of sizes, contours, and aspect ratios. MCAs of hMSCs, murine 344SQ metastatic adenocarcinoma cells, and human C4-2 prostate cancer cells were generated in our microwell system with high uniformity within 24 hours. For 344SQ cells, we generated more than 100,000 MCAs with low diameter polydispersity (62.0 ± 10.8 μm diameter) when seeding at 25 cells/microwell cell density. Moreover, MCAs formed in our microwell system maintained invasive capabilities in 3D migration assays. 344SQ MCAs demonstrated epithelial lumen formation on Matrigel®, and underwent EMT and invasion in the presence of TGF-β. Given the applicability to multiple cell lines, we expect our technique for high-throughput fabrication of customized microwell structures will find broad utility in the generation and cultivation of multicellular aggregates for use in regenerative medicine and tumor engineering applications.

Clicking Polymers Together:
Assembly of Complex, Biologically Useful Polymer Structures from Efficient Chemistries

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A new paradigm encompassing several distinct chemical reactions and, more importantly, a generalized approach to molecular design and synthesis has been rapidly adopted in the fields of chemical synthesis, biotechnology, materials science, drug discovery, surface science, and polymer synthesis and modification. The *Click Chemistry* paradigm focuses on implementation of highly efficient reactions that achieve quantitative conversion under mild conditions. As such, these reactions represent ideal candidates for further development, understanding and implementation. In particular, the synergistic combination of these click chemistries with photochemical initiation and polymer formation has been used to afford 4D control of polymer formation, structure and patterned assembly, particularly as useful in the devleopment of biomaterials by design. Here, we will focus on three distinct vignettes related to our implementation of photoclickable polymer systems. The first of these areas focuses on the combination of chemistry and biospecificity to develop polymerization-based biodetection approaches that enable ultrahigh sensitive detection of target compounds. The second focuses on the development of smart, responsive materials based on covalent adaptable networks (CANs) where the ability to controllably alter the network structure is used to alter topography and other material properties. This behavior is achieved by forming materials which can be switched reversibly from elastic to plastic simply by exposure to light. Finally, the development and implementation of click nucleic acids (CNAs) based on the thiol-ene click reaction will be presented. This distinct class of oligonucelotides combines the vast advantages of synthetic oligonucleotides such as peptide nucleic acids with the power of click reaction chemistry to form materials that hybridize with both natural and synthetic olignonucleoitides via Watson-Crick base pairing while being simple to produce in large scales appropriate for directed assembly and other high value materials applications.

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"Study of characteristics of Fmoc-FRGD peptide hydrogel and its depsipeptide derivatives"

Short peptide-based self-assembling materials show potential for biomedical application by their ability to form self-supporting hydrogels, micro- and nanoparticles. However, few studies have been done to analyze the molecular interaction between peptides that results in self-assembly, long-term biocompatibility and biodegradability all of which are important for practical application. Recent work by our group and others proposes that depsipeptides, in which an amide bond in the peptide backbone has been converted to an ester bond, can interact specifically with cells in a way that is dependent on the side chains, yet has distinct gelation and degradation behavior. These results suggest that depsipeptides can be an appropriate molecule for analyzing the molecular interactions for self-assembly as well as being a platform for supporting 3D cell culture. To investigate the capability of depsipeptides, we synthesized Fmoc-FRGD, which can form a hydrogel and promote cell attachment, and its depsipeptide derivatives, Fmoc-FRGlcD and Fmoc-FGlcRGD (where 'Glc' is glycolic acid), and analyzed their gelation and degradation characteristics. Results from the gelation study demonstrated that Fmoc-FRGD and Fmoc-FRGlcD formed gels using either a pH or solubility change when the concentration was above 3mg/mL. However, Fmoc-FGlcRGD only formed a gel by solubility change when it the concentration was above 10mg/mL. For the degradation study, two depsipeptides demonstrated a slow and highly linear degradation profile, but Fmoc-FRGD did not show any degradation. Conclusively, we show that the ester bonds in depsipeptides can enhance biodegradabilityof the formed hydrogel, and gelation and degradation may be regulated by controlling the length of molecule and location of ester bond in short peptide structure.

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Ultra-Strong, Thermoresponsive Double Network Membranes for Implanted Glucose Biosensors

Ultra-strong, thermoresponsive hydrogels have the potential to increase the lifetime of implantable glucose biosensors by improving durability and reducing membrane biofouling. Membrane biofouling – the adsorption and accumulation of proteins and cells - limits glucose diffusion thereby disabling the sensor in typically less than one week. In this work, we evaluated the performance of ultra-strong, thermoresponsive double network (DN) hydrogels based on poly(*N*-isopropylacrylamide) (PNIPAAm) and electrostatic comonomer, 2-acrylamido-2-ethylpropane sulfonic acid (AMPS) as implantable glucose biosensor membranes. The hydrogels were comprised of a tightly crosslinked, negatively charged first network [P(NIPAAm-*co*-AMPS)] with varying levels of AMPS (100:0 to 25:75 wt% ratio of NIPAAm:AMPS; i.e. "0%" to "75%" AMPS) and a loosely crosslinked, neutral second network comprised of PNIPAAm. For such PNIPAAm-based membranes, "self-cleaning" may be achieved by cycling the membrane above and below the volume phase transition temperature (VPTT, ~35 °C). The incorporation of the AMPS comonomer led to a dramatic enhancement in both swelling kinetics and ultimate compressive strength (~17.2 MPa for 75% AMPS). Compared to a PEG control, the DN hydrogels were confirmed non-cytotoxic using LDH assays and were permeable to glucose at similar diffusion rates to permit rapid sensing. To examine the "self-cleaning" functionality, *in vitro* cell adhesion and release were studied. Cell adhesion was most prevalent on the 50% AMPS hydrogel due to cells exhibiting a greater affinity to negatively charged surfaces. However, the 75% AMPS hydrogel showed poor cell adhesion due to the increased surface hydrophilicity seen with greater AMPS content. Moreover, the most cell-adhesive composition (50% AMPS) demonstrated cell-release after two thermal cycles, confirming the membranes' ability to "self-clean" and potentially extend biosensor lifetime.

Mechanical Stimulation of Collagen-Fibrin Hydrogels for Skeletal Muscle Regeneration

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Introduction: 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. We then tested the following collagen:fibrin ratios: 100:0, 75:25, 50:50, 25:75, and 0:100.

Methods: Characterization methods included material stability evaluation over 14 days with and without cells (rat skeletal myoblasts L6), uniaxial tensile testing, rheology and *in vitro* myogenesis. Combining the information from the cell study with the mechanical studies, the 25:75 group was chosen to continue into dynamic studies. The 75:25 group was also evaluated in the current study in order to better elucidate the roles of both collagen and fibrin and their degradation on the myogenic process during dynamic stimulation. The two groups (75:25 and 25:75) were evaluated under both static and dynamic conditioning. For dynamic conditioning, samples were stimulated for 2 hrs daily at 1 Hz with 10% longitudinal cyclic stretch using a uniaxial tensile bioreactor for 7 days. This study evaluated myogenic potential via dsDNA quantification; staining with MF20 for muscle fibers; and RNA expression of Myogenin, MyoD, and Myosin Heavy Chain (MHC) using RT-qPCR. Data collected from these studies were analyzed using ANOVA and Tukey's pot hoc test.

Results: The study indicated an increase in concentration of dsDNA in the dynamic groups vs the static groups. Staining showed increased cell alignment with the direction of strain and the presence of MF20 (DSHBY, University of Iowa) for muscle fibers.

Conclusion: The dynamic stimulation allowed myoblast proliferation and differentiation over 7 days to generate an organized biomimetic skeletal muscle like extracellular matrix in collagen:fibrin hydrogel blends.

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Ligand Size Regulates Membrane Receptor Uptake within Clathrin-Coated Pits

Clathrin-mediated endocytosis (CME) is a dominant process of internalization of membrane receptor proteins which enables cells to control receptor levels on the membrane. Understanding this pathway is key to manipulating the link between extracellular stimuli and intracellular signaling. Because these receptors share conserved adaptor motifs facilitating entry into clathrin-coated pits (CCPs), targeting these adaptor motifs to control receptor membrane levels is inherently non-specific. Recent findings from our lab and others, however, indicate that steric bulk can exclude specific receptors from CME with greater specificity. Here we show that a large, size-tunable PEG polymer can cause a similar effect when conjugated to a protein ligand. Fluorescently tagging clathrin pits, a small model receptor, and a protein ligand reveals decreased incorporation of ligand and receptor into pits with increasing bound ligand size. This decreased CCP incorporation increases receptor levels on the membrane, demonstrating that large, semi-synthetic ligands can be tuned to specifically target a receptor for membrane retention. Further, a linear relationship between the number of bound ligands on the membrane and receptor incorporation into CCPs was revealed. Fitting a statistical lattice model to the experimental trend reveals that loading of the model receptor into CCPs is non-cooperative. For receptors known to require dimerization or other cooperativity in order to be effectively endocytosed, we expect that this platform will enable further probing of CME loading dynamics. Moreover, the ability to retain receptors at the membrane opens avenues to modulate specific signaling pathways *in vitro*. This precise control over receptor signaling is useful in elucidating receptor signaling dynamics and developing novel therapeutics for diseases caused by reduced receptor signaling.

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Protein Resistance of Silicones Modified with PEO-Silane Amphiphiles: The Role of PEO-Segment Length and Concentration

Silicone is commonly used for blood-contacting medical devices, but its high surface hydrophobicity makes it susceptible to protein adsorption and subsequent thrombosis. To improve protein resistance, poly(ethylene oxide) (PEO) can be incorporated into the silicone; however, this requires the PEO chains to be able to migrate to the surface-biological interface where protein adsorption occurs. We have recently shown that PEO-silanes are unable to serve as efficient "surface modifying additives" for silicones. In contrast, PEO-silane amphiphiles developed in our lab show a high capacity to, under aqueous exposure, undergo water-driven surface restructuring to present PEO to the silicone surface and confer protein resistance. The amphiphile is comprised of a hydrophilic PEO-segment, a flexible, hydrophobic siloxane tether, and a reactive trialkyloxysilane group: α-$(EtO)_3Si(CH_2)_2$-oligodimethylsiloxane$_m$-*block*-[PEO_n-OCH_3]. When amphiphiles of varying PEO-segment lengths (n = 3, 8, & 16) were incorporated into silicones at a single concentration, n = 8 showed the greatest rate and extent of water-driven surface restructuring. In this study, we varied the concentration of the PEO-silane amphiphile to determine the minimum concentration needed for enhanced surface restructuring and protein resistance. The amphiphile was incorporated into a medical-grade silicone (MED-1137) at concentrations ranging from *5-100 µmol/g MED-1137*. Concentrations as low as *10 µmol/g MED-1137* achieved significant surface restructuring and protein resistance. The results indicated that amphiphile restructuring behavior, depends more on PEO-segment length than concentration. Future work will focus on determining the stability of these modified silicone surfaces under prolonged exposure to an aqueous environment.

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Tunable, "Self-fitting" Shape Memory Polymer Scaffolds for Cranial Bone Defect Repair

Currently considered the gold standard in cranial defect treatment, the transplantation of harvested autologous bone tissue is limited by graft availability, donor site morbidity and complex grafting procedures. A particular difficulty is shaping and fixing the rigid autograft tightly within the defect in order to promote osseointegration and lower the risk of graft resorption. Tissue engineering has been explored as an alternative strategy, although a scaffold for cranial bone tissue engineering must still be able to conformally "fit" within the defect. Thus, to overcome current treatment limitations, we have developed a "self-fitting," porous shape memory polymer (SMP) scaffold able to be "fitted" into a confined, cranial defect upon the mere application of warm saline. Previously, we have reported SMP scaffolds prepared of a polycaprolactone diacrylate (PCL-DA) SMP network that exhibited high, interconnected porosity, strength, and degradability. Developing upon this work, in this study, we have prepared semi-interpenetrating networks (semi-IPNs) of PCL-DA with rigid, biodegradable poly(L-lactic acid) (PLLA) seeking to enhance tunability of scaffold mechanical properties and degradation rate. Porous scaffolds, via a revised solvent-casting particulate-leaching (SCPL) method, and the corresponding solid films were prepared, and, by varying PCL:PLLA ratio and PCL molecular weight, the impact on thermal properties (i.e. crystallinity and T_m), mechanical properties (i.e. stiffness and strength) and the degradation profile was systematically studied. Notably, solid films exhibited highly tunable mechanical properties and degradation rate, and shape memory behavior was maintained. Initially, porous scaffolds have shown high pore interconnectivity of controlled pore size. In future work, porous semi-IPNs will be further investigated as "self-fitting" scaffolds for cranial bone defect repair.

The 2 for 1 Silk Enthesis

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Introduction: Recreating the native transition regions between tissues, such as bone to ligament (the enthesis), has been a complex task for biomaterial researchers. Most approaches seek to take two separate materials and combine them, such as self-assembled monolayers for joining layers. Overall these approaches have not provided a stronger enthesis than the gold standard autograft anchors. The drawbacks of autologous medial patella tendon for anterior cruciate ligament reconstruction, include donor site morbidity and a 20% incidence of secondary surgery in the first 6 years (1). Our chosen material, silk, has been utilized for bone and ligament grafts with varying treatments, leveraging its tunable mechanical properties. Using this information, we seek to create a 2 for 1 transition, matching the properties of 2 tissues with a continuous graft of 1 material. This initial study provides information on the treatment timeframes needed to create mechanical properties similar to the bone-ligament enthesis.

Materials and Methods: *Bombyx mori* silk cocoons were processed to remove sericin leaving only fibroin. Then, the silk was either dissolved in hexafluoro-2-propanol for porous scaffolds through salt leaching or lyophilized in cylindrical molds for non-porous scaffolds. The silk was treated with methanol (2, 4, 8, and 12 hours) to show the range of mechanical properties that could be obtained. The samples were then fully characterized using various techniques including mechanical testing and scanning electron microscopy.

Results: The stiffness values ranged from 9.79±3.12 to 18.12±5.40 N/mm for the 2 and 12 hour treatments respectively, with the 4 and 8 hr treatments having intermediate properties.

Conclusion: By varying the methanol treatment times of the silk scaffolds, we created different mechanical properties, by leveraging the transition to a β-sheet dominant protein conformation. This serves as a proof of concept towards creating a single material with an elasticity gradient to mimic the bone-ligament enthesis.

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Development of a Tissue Engineered Periosteum for Bone Regeneration

Nearly 2 million patients undergo bone repair annually to treat critical-sized defects caused by trauma or infections. Unfortunately, current bone grafting procedures often fail to achieve natural healing stimulated by the periosteum such as controlled, temporal delivery of multiple growth factors resulting in insufficient regeneration. Therefore, we propose to develop a tissue engineered periosteum wrap that can deliver multiple growth factors with independent release kinetics to be used in combination with bone grafts. Various in-situ crosslinking of electrospun gelatin will be used to modulate release kinetics. In-situ crosslinking of electrospun gelatin was performed using a double barrel syringe (1:1 barrel ratio). One barrel was loaded with a 10 wt% solution of bovine-derived gelatin in 2,2,2-trifluoroethanol (TFE), and a 1,4-diazabicyclo[2,2,2]octane catalyst at 5 wt% of solids. The second barrel was loaded with a solution of hexamethylene diisocyanate crosslinker in TFE at a concentration equal to 5X ratio of isocyanate/amine. The solutions were dispensed through the mixing head at a rate of 1 mL/hr with 10 kV being applied to an 18 gauge needle placed 12 cm away from the collector. Electrospinning uncrosslinked gelatin was performed similarly using a single barrel syringe. Release kinetic studies were conducted by incorporating a model protein, FITC-albumin, into both gelatin solutions prior to loading the solutions into the syringes. The electrospun grafts were then soaked in PBS for up to one week with release kinetics analyzed up to 7 days. Electrospun 5X in-situ crosslinked gelatin grafts demonstrated sustained release kinetics up to 7 days compared to electrospun uncrosslinked gelatin grafts. This research underscores the potential of a tissue engineered periosteum wrap to enhance the regenerative potential and bridging of bone grafts.

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3D Printing of Emulsion Inks with Thiol-ene Chemistry

Tissue engineering has emerged as a promising solution to limitations in the current treatment options for bone injuries. Emulsion templating is a fabrication technique used to produce porous scaffolds via polymerization of high internal phase emulsions (HIPEs). Our lab has adapted this platform to 3D printing of emulsion inks and demonstrated fabrication of grafts with predefined geometries and hierarchical porosities. Extruded HIPEs are polymerized via exposure of specific photoinitiator to UV. While this process is relatively quick, reaction of radicals with molecular oxygen can result in formation of unreactive peroxy radicals which can prematurely terminate polymerization. Uncured material may cause increased line spreading, decreasing the fidelity of the primary layers of printed constructs. In this study, we present a recent development to our material, incorporation of a multifunctional thiol-ene molecule, to increase material resistance to oxygen inhibition. HIPEs were fabricated using specific macromers including propylene fumarate dimethacrylate, pentaerythritol tetrakis (3-mercaptopropionate), and pentaerythritol tetraacrylate mixed with surfactant and photoinitiator. The homogenized mixture was emulsified with water to form a viscous paste. To determine low shear viscosity, rheological studies were performed on all samples. We were able to demonstrate thiol-based HIPEs matched the appropriate viscosity needed for extrusion from 3D printer. Future work will involve printing single lines to look at differences in line spreading, gel fraction, effects of tetrathiol on cytocompatibility, and characterizing mechanical properties of printed constructs. Thiol-base chemistry has the potential to improve resistance against oxygen inhibition in our emulsion inks. By limiting premature termination of polymerization, prints will cure rapidly allowing for instantaneous retaining of shape, translating to increased print fidelity and construct shape integrity.

3D Printed Haversian Scaffolds for Critical Bone Trauma

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Introduction: When an autograft is used to fill a bone defect, patients more often complain about the second surgery site than the initial injury. The secondary surgery also introduces chances for complication or infection. Bioengineered bone grafts, or scaffolds, have be developed in order to remedy this problem. Successful bridging has been observed in scaffolds made from hydroxyapatite (HA), a mineral native to bone, by way of polymer casting. The architecture of these scaffolds, however, resembles trabecular bone and therefore do not have the sufficient mechanical properties to support bone mechanical loading. Although compact, or cortical, bone has higher mechanical properties, the architecture is difficult to replicate as the interconnected Haversian canals are on the order of micrometers.

Methods and Materials: By using three dimensional printing, the negative space within cortical bone can be made into a cast. This cast will then be filled with an HA slurry and heat sintered. The HA will bind to the cast as the sintering occurs, leaving an HA scaffold with microporous Haversian structure. These scaffolds will then undergo compression and flexure testing to determine the mechanical properties of the material and architecture. The diameter of the Haversian canals will be variable, testing for the relationship between interconnectivity, volume of canals, and the mechanical properties.

Anticipated Results: The erosion/dilation of the Haversian canals is expected to have a significant effect on the mechanical properties of the scaffold. As the canal size decreases it is expected that the printer accuracy will also decrease.

Conclusion: An ideal printable structure for bone grafts allows for effective customization through the assisted use of medical imaging technology. Bone grafts that can support more stress may lead to better stabilizing techniques. These findings will help develop a standardized cortical bone scaffold to improve healing of bone defects post trauma or surgery.

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Influence of Molecular Architecture in the Design and Development of a pH-Responsive Nanoscale Hydrogel Platform for Tumor-Targeted Drug Delivery

The lack of specificity in traditional chemotherapeutic administration leads to significant dose-limiting toxicities and requires patients to wait for long periods between treatments. During this time, cancer cells have an opportunity to recover and develop drug resistance[1]. To improve treatment and reduce toxicity to healthy tissues, we have developed a novel, intelligent nanoscale hydrogel carrier (nanogel) for targeted delivery of multiple chemotherapeutic agents.

Here we show that the nanogel molecular architecture can be tailored to carry a variety of cargos with widely varying physicochemical properties, and release the cargo only in response to an acidic intracellular environment. Nanoparticle-mediated combination therapy offers many advantages including the ability to signal different pathways in cancer cells, maximize therapeutic efficacy against specific targets, target different phases of the cell cycle, and overcome efflux-driven mechanisms of resistance[2]. Further, it allows PK/PD to be dictated by the *in vivo* distribution of the nanogels rather than the physicochemical properties of the free drugs, ensuring optimal ratios are delivered to the cytosol.

The nanogels are comprised of: 1) hydrophilic, cationic monomer 2-(diethylamino)ethyl-methacrylate, 2) tetraethylene-glycol-dimethacrylate crosslinker, and 3) surface-grafted poly(ethylene-glycol)-methacrylate. To improve drug-polymer interaction, we investigated the impact of hydrophobic alkyl-methacrylate monomer inclusion through systematic variation of monomer chain length and steric bulk. The physical properties of the resulting nanogels were compared using dynamic light scattering, zeta potential, titration, pyrene fluorescence, and red blood cell hemolysis to elicit the influence of polymer composition on swelling ratio, surface charge, pKa, hydrophile-hydrophobe phase transition, and erythrocyte membrane disruption capability. The therapeutic delivery potential was analyzed using model hydrophobic and hydrophilic agents.

The nanogels resulted in well-defined and controllable particle size, morphology, and composition. Varying both the chain length and steric bulk allowed for precise control over the thermodynamic response (swelling ratio), dynamic behavior (pKa and membrane disruption potential), and drug-polymer interaction (therapeutic delivery potential). Nanogels synthesized with cyclohexyl methacrylate demonstrated both an improved ability to mediate endosomal rupture and enhanced ability to co-load and deliver multiple drugs.

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Optimizing the Surface Properties of Hydrogel Nanoparticles to Enable the Delivery of Therapeutic Agents for the Treatment of Ovarian Cancer

Current ovarian cancer treatment plans typically include chemotherapy with radiation or surgical removal of the tumors. However, traditional chemotherapy does not distinguish between healthy and cancerous cells and typically leads to significant side effects. To improve treatment, we aimed to develop long-circulating, biocompatible nanogels that respond to the acidic intracellular environment for controlled delivery of chemotherapeutics. There are three major requirements for nanoparticles to deliver their payload to the tumor. They need to: 1) be stable in the circulation without releasing drug prematurely, 2) evade opsonization and accumulate in the tumor efficiently, and 3) intelligently release drug inside the tumor cells. This means the nanogel must have sufficient time in blood circulation to reach intended sites of the body and accumulate in and around the tumors via leaky vasculature.

The Peppas lab previously developed intelligent, nanoscale hydrogels (nanogels) and demonstrated success in loading and releasing chemotherapeutic agents. In this work, we optimized the nanogel surface properties to avoid the body's natural clearance mechanisms and increase circulation time, while still maintaining the necessary characteristics to promote cellular uptake. The nanogels are synthesized using a UV-initiated free radical polymerization and are comprised of 2-(diethylamino)-ethyl-methacrylate (DEAEMA) and tetra(ethylene-glycol)-dimethacrylate (TEGDMA). DEAEMA is a hydrophilic monomer with ionizable amine pendant groups that enable the pH-activated swelling, and TEGDMA is a hydrophilic crosslinking agent that forms 3-dimensional polymer networks. To optimize a stealth coating, we synthesized a series of nanogels with varying levels of surface-grafted poly(ethylene-glycol)-methacrylate (PEGMA) and analyzed the impact to the nanogel physicochemical properties. PEGMA is a hydrophilic monomer that is used to provide stability during circulation in the bloodstream. We demonstrated that the zeta potential changes drastically as a function of PEGMA coverage on the surface of the nanogel, while the isoelectric point remains constant. The volume swelling ratio (VSR) of the nanogels was significantly influenced by the amount of PEGMA, with an increased percentage relating to a decreased VSR. Ultimately, we found that adjusting the PEGMA leads to varying nanogel surface characteristics that may be exploited to enable long-circulation and effective transport of the nanogels to the tumor site.

Mechanically Stiff Nanocomposite Hydrogels at Ultralow Nanoparticle Content

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Although hydrogels are able to mimic native tissue microenvironments, their utility for biomedical applications is severely hampered due to limited mechanical stiffness and low toughness. Despite recent progress in designing stiff and tough hydrogels, it is still challenging to achieve a cell friendly, high modulus construct. Here, we report a highly efficient method to reinforce collagen-based hydrogels using extremely low concentrations of a nanoparticulate-reinforcing agent that acts as a cross-link epicenter (Figure 1). Extraordinarily, the addition of these nanoparticles at a 10,000-fold lower concentration relative to polymer resulted in a more than 10-fold increase in mechanical stiffness and a 20-fold increase in toughness. We attribute the high stiffness of the nanocomposite network to the chemical functionality of the nanoparticles, which enabled the cross-linking of multiple polymeric chains to the nanoparticle surface. The mechanical stiffness of the nanoengineered hydrogel can be tailored between 0.2 and 200 kPa simply by manipulating the size of the nanoparticles (4, 8, and 12 nm), as well as the concentrations of the nanoparticles and polymer. Moreover, cells can be easily encapsulated within the nanoparticulate-reinforced hydrogel network, showing high viability. In addition, encapsulated cells were able to sense and respond to matrix stiffness. Overall, these results demonstrate a facile approach to modulate the mechanical stiffness of collagen-based hydrogels and may have broad utility for various biomedical applications, including use as tissue-engineered scaffolds and cell/protein delivery vehicles.

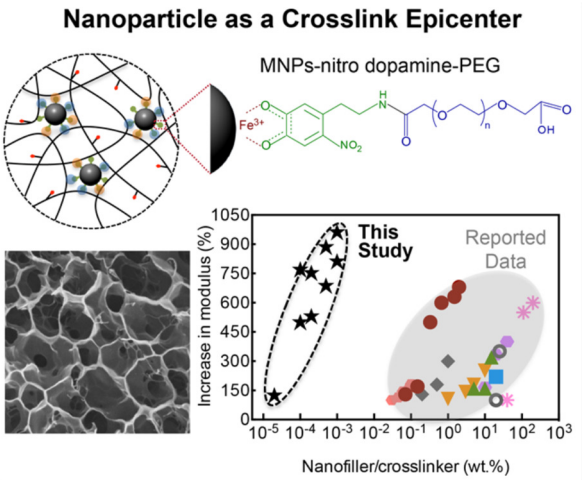

Figure 1. Schematic showing nanoparticle conjugated with multiple polymeric chains, thus serving as crosslink epicenter. Hydrogel's microporous structure via SEM and a comparative plot of increase in modulus using various crosslinkers.

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Multicomponent Nanocomposite-Interpenetrating Network Hydrogel Bio-inks for 3D Bio-printing

Advanced bioinks for 3D printing are rationally designed materials intended to improve the functionality of printed scaffolds outside the traditional paradigm of the ‘‘biofabrication window’’. While the biofabrication window paradigm necessitates compromise between suitability for fabrication and ability to accommodate encapsulated cells, recent developments in advanced bioinks have resulted in improved designs for a range of biofabrication platforms without this tradeoff. In this paper, we describe a new multiple-component bioresorbable hydrogel bioink for low-cost extrusion bioprinting of freestanding 3D structures (>3cm tall) while retaining high encapsulated cell viability. This bioink uses ionic-covalent entanglement interpenetrating networks composed of gelatin methacrylate and kappa carrageenan to strengthen the gel and stabilize printed structures. The laponite nanosilicates imbue the bioink with shear thinning properties that reduce shear stress on cells during printing, improve the mechanical stability of printed structures, and help direct cell fate. 3D bioprinting is an increasingly popular strategy in tissue engineering because of its potential to replicate the precise microarchitectures and cell distributions of human tissue. However, progress is being hamstrung by a lack of suitable bioinks. To our knowledge, this is the first bioresorbable hydrogel bioink that can be used to print freestanding human-scale 3D structures while maintaining high cell viability and biocompatibility.

Keywords: Nanocomposite, Bioink, 3D Printing, 3D Bioprinting, Nanosilicate, Laponite, Interpenetrating Network, Ionic-Covalent Entanglement, Hydrogel, Bioactive

Gelation of Bio-polymers by Active-Center Rich Hierarchical Nano-assemblies (ACRiHN) of 2D-Transition Metal Dichalcogenides

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Two dimensional (2D)-Transition metal dichalocogenides (TMDs e.g. MoS_2 and WS_2) have gained unprecedented attention in recent due to their unique physical and chemical properties associated with atomic arrangements and well-defined layered structures. Nanosized 2D flakes offer exceptionally large aspect ratio, higher electron density and easy access to lattice atomic defects for further manipulation. All these fascinating properties by 2D TMD flakes, significantly divergent from their bulk counterpart, have laid down the platform for researchers to explore, understand and realize their wide-variety of translational research. The defect sites on lattice plane act as active center for chemical conjugation to polymer and induce gelation without any catalyzing agent. However due to lack of enough active centers, conjugation and thus gel formation with singular sheets cannot simply be realized. To overcome this issue we propose active center-rich hierarchical nanoassembly (ACRiHN) of TMDs as an alternative whereby due to presence of umpteen number of active centers thiolated polymers can be easily conjugated and hence can form hydrogel (Figure 1). In the present work we show the optimization of ACRiHN synthesis for gelation of multi-arm thiolated polyethylene glycol (PEGSH) and their properties studied for mechanical and rheological robustness. Microstructural analysis of transverse section of hydrogels by SEM confirms the regular pore formation of the size of approx. 10 µm. Compressive modulus studies revealed that the stiffness of the gels can be altered by manipulating TMD concentrations, thus TMD not only does induce gelation but help for stiff network as well. Further cellular behavior studied by using hMSC (human mesenchymal stem cell) confirmed the gels are compatible and sense the mechanical cues from the surface stiffness.

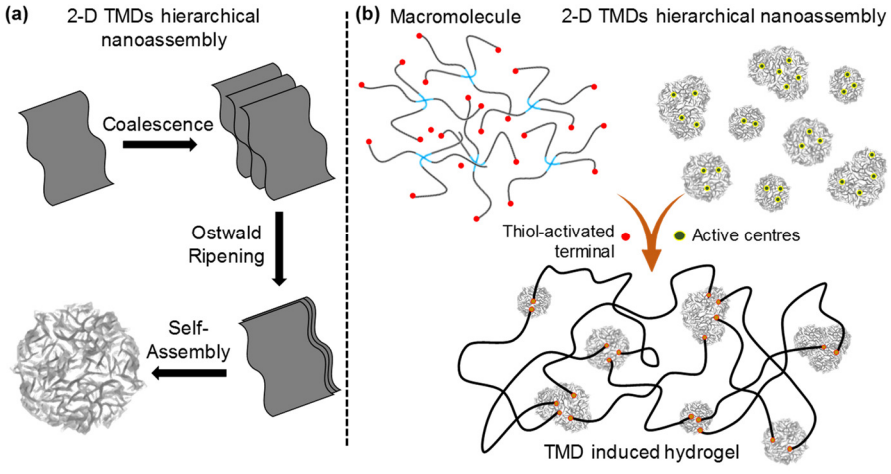

Figure 1: (a) Active center-rich hierarchical nanoassembly (ACRiHN) of TMD flakes (b) These active centers offer conjugation centers for thiol terminated polymer for chemical crosslinking, thus induce gel formation. These TMDs induced hydrogels can be used for stem cell differentiation to generate soft tissues like cartilage.

In vivo regenerative response enhanced in critical size bone defects using High Performance Micro Environments

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Introduction: Bone marrow mesenchymal stem cells (BM-MSCs) are a common treatment of non-union fractures due to their ability to immunomodulate the microenvironment as well as enhancing osteogenesis. To improve the functionality of recruited BM-MSCs in a wound healing environment *in vivo*, a cell culture substratum referred to as High Performance Micro Environment (HPME) was generated using bone marrow stromal cells. Previous studies indicated that HPME is primarily composed of extracellular matrix proteins such as collagen types I, VI, XII, fibronectin, tenascin and transforming growth factor beta inducible protein. HPME significantly increased the yield of BM-MSCs, the expression of IL-10, and the expression of stage specific embryonic antigen (SSEA)-4 when compared to tissue culture plastic (all $p<0.05$).

Materials and Methods: In the current study femoral segmental critical sized defects were created in Sprague Dawley rats. Groups analyzed were: A) Defect filled with Medtronic™ Mastergraft® (HA+TCP-ß) granules B) Defect filled with Mastegraft and HPME. Each group also had a Cytoplast collagen wrap around the defect site. Both groups were allowed to heal for 4 and 8 weeks (n=7) and analyzed by μCT (9μm resolution). Data was analyzed using ANOVA followed by Tukey's test for post hoc analysis of significance.

Results: Functional regeneration was measured by the polar moment of inertia (PMI) from μCT analysis, and it was found that both groups exhibited significant increases in their PMI ($p<0.01$). There was significant increase in bone regeneration from 4 to 8 weeks in the presence of HPME ($p=0.017$).

Discussion: Results suggest that the while the bone growth trends are similar in the first four weeks, continued regenerative activity is maintained in critical sized defects in the presence of HPME.

Acknowledgments: This study was performed at the University of Texas at San Antonio and supported by a grant from StemBioSys, Inc.

Polypyrrole coated Polyvinylidene fluoride aligned electrospun fibers for biomedical applications

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Introduction: Polyvinylidene fluoride (PVDF) is an electroactive polymer with excellent piezoelectric properties and biocompatibility; however, drawbacks for regenerative medicine applications include hydrophobicity and poor drug release capability. These limit its applications for direct use as a substrate for electrically stimulated tissue engineering applications. Various modifications have been attempted to improve its biological properties including the preparation of composites and surface modification of PVDF. Poly-pyrrole (PPy), a bio-compatible polymer is an ideal candidate to elicit such responses because of its conductivity and electroactivity for the regeneration of multiple tissues including bone, nerve, skeletal muscle, and cardiac muscle.

Materials and Methods: In this study, PVDF electrospun microfibers have been prepared through conventional electrospinning, coated with PPy and characterized using Scanning Electron Microscopy (SEM), attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and Sessile Drop contact angle measurement. All experiments were performed on a minimum of 6 replicates to ensure repeatability.

Results: SEM images exhibit *in situ* polymerization of PPy on PVDF fibers. In addition, fibers are aligned after the polymerization. ATR-FTIR results show that β ratio is 72% and also confirm the polymerization of PPy on PVDF, reaching optimal coating polymerization after 18 hours. Advancing contact angle results revealed an increase in contact angle after the polymerization during first 12 hours; however, it has decreased and a decrease with continued polymerization after 12 hours from 120 ^{o} to 110^{o}.

Conclusions: We demonstrate the synthesis of a PPy-PVDF system with uniform coating and demonstrable electroactivity. These results can serve as a basis for manufacturing synthetic grafts for encouraging cell migration and differentiation using electrical stimulation.