EITHER

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Influence of CD151 Expression on Prostate Cancer Cell Adhesion to Endothelial Monolayers

Cancer is the second leading cause of death in the United States, with metastatic lesions resulting from circulating tumor cells most often causing the mortality. The basic steps of the metastatic cascade, from initial tumor formation to growth at a secondary site, are a linear sequence of steps composed of a network of mechanisms that are not well understood. One of these steps, the adhesion of cancer cells to endothelial cells during dissemination, is the focus of this research. Specifically, the influence of CD151 expression in prostate cancer cell interactions with endothelial monolayers was quantified. CD151 is a gene that encodes a cell surface glycoprotein that is known to complex with integrins and enhance cancer cell motility, invasion and metastasis. In this study, a parallel plate flow chamber was employed to investigate the role of CD151 in both static and dynamic adhesion of cancer cells to endothelial cells, mimicking the two main theories of tumor cell dissemination. In the first theory, tumor cells are thought to simply lodge in vessels of smaller diameter, allowing for static ligand-receptor interactions. In the second, “docking and locking” as coined by Honn and Tang, tumor cells have been observed to “roll” along the endothelium, breaking and forming weak bonds until firmly adhering. Adhesion assays using the prostate cancer cell line, PC3, and a line of PC3 in which the expression of CD151 was knocked down using shRNA silencing were conducted allowing for both lodging and “docking and locking” for comparison. Preliminary results indicate that a lodging mechanism likely plays a more significant role in metastasis at physiological shear stresses than “docking and locking”. The presence of CD151 has been demonstrated to lead to enhanced initial adhesion to the endothelium, but retention rates were unaffected by CD151 expression once the cells became firmly adhered.

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Drug Delivery from Space-Making Calcium Sulfate/Poly(β-amino ester) Hydrogel Composites

The capacity to quickly augment bone lost as a result of resorption is crucial to ensure suitable application of prosthetics for restoring masticatory function. Calcium sulfate (CS) composites are being developed as 'tenting' barriers to soft tissue infiltration, while allowing delivery of osteogenic agents through direct loading of CS or biodegradable hydrogel (HG) particles during stable CS dissolution to promote vertical bone regeneration. Blank, 1%wt. and 10%wt. HG-particle (150-250µm) CS-composites were fabricated. Destructive mass loss was performed to understand CS dissolution trends. The dissolution rates recorded were consistent to one another via surface erosion, demonstrating the amount of HG-particles loaded did not have a significant effect. Release kinetics and controllability was studied using lysozyme as a model growth-factor directly loaded in CS and in HG-particles embedded in CS. To study composite's potential delivery versatility, a preliminary release study using simvastatin directly loaded in CS was performed. The 10%wt.-HG (286µg lysozyme) and the 1%wt.-HG (30.7µg lysozyme) loaded composites demonstrated a zero-order release, whereas directly loaded protein demonstrated a burst release signifying a near-surface segregation of protein. While hydrogel loading with protein aided in its controlled release, simvastatin demonstrated a controlled release by direct loading alone. CS composites containing biodegradable hydrogel particles for delivering osteogenic biomolecules have potential use as scaffolds for vertical bone augmentation. The hydrogel loading-independence of composite degradation rate may allow for tuning to provide a sustained delivery of drug-loaded particles to stimulate bone regeneration. Composite release studies using lysozyme as a model growth-factor showed promising results for sustained release of protein. Utilizing both hydrogel and direct loadings could allow greater tailoring of the delivery system to optimize bone regeneration potential. The introduction of multiple osteogenic agents could further advance delivery versatility of CS-composites having a potential additive or even a synergistic effect on vertical bone regeneration.

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Characterization of Modular Resilin-based Proteins for Application in Cartilage Engineering

Resilin is an elastomeric protein found in insect cuticles. It has high strain, fatigue lifetime, and resilience. Recombinant resilin-based proteins show similar properties to natural resilin and, based on the mechanical properties, has potential as a cartilage engineering scaffold.

Our laboratory has successfully developed a modular recombinant protein that is composed of: 1) resilin repeats that serve as a mechanical domain, 2) lysine residues that serve as crosslinking sites, and 3) a cell-binding sequence that provides cell adhesion. We have successfully manufactured our resilin proteins in a large-scale system and obtained an average yield of 54.6 mg/L. The identity and purity of our protein were confirmed by SDS-PAGE, Western blot, mass spectrometry, and amino acid analysis. Our results demonstrated that the compressive modulus of the crosslinked resilin protein is 2.4 MPa, which is similar to the compressive modulus of natural cartilage. We also cultured human mesenchymal stem cells (hMSCs) on the resilin-based proteins. Our results showed that the resilin-based proteins are cytocompatible and that the cells can recognize the cell-binding domain in a sequence-specific manner. These results demonstrate that our protein-based biomaterials are promising candidates for regenerative cartilage engineering. Future studies will examine the range of mechanical properties that can be achieved by varying the crosslinking density and protein concentration. We will then explore the effect that these properties have on cartilage differentiation of hMSCs.

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Sequential Drug Delivery System Designed as a Potential Treatment for Periodontitis

The main aim of this research was to develop a multiple drug delivery device that will release four drugs in an appropriate sequence to treat different stages of periodontitis. Drug-loaded and blank cellulose acetate phthalate - Pluronic F-127 (CAPP) films were made using a solvent evaporation technique. The multiple drug delivery device was fabricated by attaching blank and drug-loaded films in the desired order with a backing layer. The drug loaded layers include antibiotic (metronidazole), anti-inflammatory (ketoprofen), anti-resorptive/antibiotic (doxycycline), and osteogenic (simvastatin) drugs. Drug delivery devices were characterized by SEM imaging, *in vitro* drug release, mathematical modeling and bioactivity studies. The *in vitro* studies showed that drug release from the CAPP films occurred in a zero-order (timeindependent) manner. Release of metronidazole, ketoprofen, doxycycline, and simvastatin in the required temporal sequence was achieved. This temporal sequence was designed based on the pathophysiologic stages of periodontitis. The amount of drugs released was comparable to the amount of drug loaded in the CAPP films. The amount of drug loaded/released can be tuned according to a particular drug and its dosage requirement. The bioactivity studies that were conducted on the release supernatants also showed that the drug released from the CAPP device was in bioactive form. The sequential release of multiple drugs can be used to treat the bacterial infection, inflammatory, and bone resorption stages of periodontitis and then subsequently help in regeneration of the alveolar bone. Ongoing work involves implantation of the device in rat model to study the *in vivo* drug release.

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Synthesis of a Novel Injectable, ROS-degradable Tissue-Engineering Scaffold

Injectable, biodegradable poly(ester-urethane) scaffolds possess tunable chemical, mechanical, and biological properties and create porous, cell-inductive tissue scaffolds that are desirable for tissue engineering applications. However, hydrolytic degradation of polyester polymer-based scaffolds generates α-hydroxy acid byproducts that decrease local pH. This triggers an autocatalytic degradation mechanism that can lead to rapid, accelerated scaffold mechanical failure. Here, a novel injectable, reactive oxygen species (ROS)-degradable polyurethane based scaffold has been developed. The scaffold is formed from polythioketal (PTK) prepolymers that are degraded by cell-generated ROS, but not by hydrolysis. Because the injectable PTK-urethane (PTK-UR) formulation selectively degrades by cell-mediated activity, it is predicted to yield better matched rates of cell infiltration and scaffold degradation, which will avoid the sudden, traumatic mode of failure that is common with autocatalytically-degraded polyesters. To optimize scaffold foaming, PTKs with varied levels of reactivity with isocyanates were created by varying the functional end-groups between thiols, hydroxyls, and amines. The reactivities of the different end-functionalized PTK compositions with hexamethylene diisocyanate trimer (HDIt) in the presence of an amine catalyst were quantified using FT-IR. Optimizing the rate of this reaction is key to achieve a desirable working time of the injectable scaffold for potential clinical usage. The thiol- terminated PTK reacted nearly 15 times faster than the hydroxyl-terminated PTK, while the reactivity of the amine-terminated PTK was too rapid to be useful or even be measured by FT-IR. Finally, using a two component mixing process that catalyzes the reaction between the PTK end groups and HDIt, 3D PTK-UR porous scaffolds were created and qualitatively assessed with SEM. These efforts demonstrate this system's potential for creating a new class of biodegradable, injectable scaffolds with properties optimally tuned for regenerative medicine applications.

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Co-delivery of Heat Shock Protein 90 Inhibitors and Composite Iron Oxide Nanoparticle for Dual Cancer Therapy

In this study, core-shell nanoparticles were prepared to concurrently deliver a heat shock protein inhibitor and superparamagentic iron oxide nanoparticles to cancer tumors for chemotherapeutic/hyperthermic dual cancer therapy. The dual cancer therapy involves the use of magnetic nanoparticles (MNPs) raising the temperature of a tumor between 41-45°C through energy dissipation in an alternating magnetic field (AMF), while HSP90 inhibitors down-regulate chaperoning signal transduction for cancer cell survival under heat-mediated stress. We hypothesized that HSP90 inhibition prior to hyperthermia would make cancer cells more susceptible to thermal damage.

The core-shell nanoparticles were prepared using atomic transfer radical polymerization (ATRP) to coat iron oxide Fe_3O_4 magnetic nanoparticles with a poly(ethylene glycol) (PEG) based polymer shell. The MNP core allows for the remote heating of the particles in an AMF. In our preliminary study, 90 kDa heat shock protein (HSP90) was targeted by using geldanamycin and 17-N-Allylamino-17-demethoxygeldanamycin, chemotherapeutics classified as HSP90 inhibitors. Combinational therapy of Hsp90 inhibitors and hyperthermia on A549 lung carcinoma cells was then investigated to explore potential enhancements in therapeutic effect.

ATRP was successfully utilized to coat iron oxide nanoparticles with a PEG based polymer shell verified through Fourier transform infrared spectroscopy and thermogravimetric analysis. For the time frame of the dual therapy experiments, there was no observable death due to particle toxicity, Hsp90 toxicity, or incubator mediated hyperthermia. No synergistic effect was observed at 125 nM geldanamycin in conjunction with AMF heating to the upper limit of hyperthermia (45 °C) for 30 minutes, suggesting that HSP family members other than HSP90 may retain thermotolerance. Future work involves the refinement of the dual therapy study to demonstrate a synergistic effect of a co-delivery of heat shock protein inhibitors and hyperthermia from magnetic nanoparticles.

ORAL

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Optimization of Cell-Targeted Nanoformulation for Photodynamic Therapy of Head and Neck Cancer

The current clinical mainstays for head and neck cancer treatment, namely, surgical resection, chemotherapy and radiotherapy, can cause significant trauma, systemic toxicity, and functional/cosmetic debilitation of tissue, especially if repetitive treatment becomes necessary due to tumor recurrence. Hence there is significant clinical interest in alternate treatment strategies like photodynamic therapy (PDT) which can effectively and selectively eradicate tumors and can be safely repeated if needed. We have previously demonstrated that the secondgeneration photosensitizer Pc 4 can be formulated within polymeric micelles, and these micelles can be specifically targeted to EGFR-overexpressing cancer cells using GE11 peptide ligands, to enhance cell-specific Pc 4 delivery and internalization. In the current study, we report on the *in vitro* optimization of the EGFR-targeting, Pc 4 loading of the micellar nanoformulation, along with optimization of the corresponding photoirradiation conditions to maximize Pc 4 delivery, internalization and subsequent PDT-induced cytotoxicity in EGFRoverexpressing cells *in vitro*. In our studies, absorption and fluorescence spectroscopy were used to monitor the cell-specific uptake of the GE11-decorated Pc 4-loaded micelles and the cytotoxic singlet oxygen production from the micelle-encapsulated Pc 4, to determine the optimum ligand density and Pc 4 loading. It was found that the micelle formulations bearing 10 mole% of GE11-modified polymer component resulted in the highest cellular uptake in EGFR-overexpressing SCC15 cells within the shortest incubation periods. Also, the loading of ~50 µg Pc 4 per mg of polymer in these micellar formulations resulted in the highest levels of singlet oxygen production. When formulations bearing these optimized parameters were tested *in vitro* on SCC15 cells for PDT effect, a formulation dose containing 400 nM Pc 4 and photoirradiation duration of 400 seconds at a fluence of 200 mJ/cm2 yielded close to 100% cell death.

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Achieving Cancer Immunotherapy Through RNA Interference in Tumor-Associated Macrophages via 'Click', Mannosylated Polymeric Nanoparticles

Macrophages represent an important therapeutic target, because their activity has been implicated in the progression of common, debilitating diseases such as cancer and atherosclerosis. However, macrophage-specific drug delivery within pathologic sites is a significant challenge, as non-specific drug delivery may lead to side effects and undesired interference with molecular mechanisms in healthy tissues. Because CD206 (mannose receptor) is almost exclusively expressed on macrophages and dendritic cells, and upregulated in tumor-associated macrophages, we designed and characterized pH-responsive, mannosylated polymeric micelles in order to achieve CD206-targeted drug delivery. The glycoconjugates improved siRNA delivery into primary murine macrophages by fivefold relative to a non-targeted carrier. The delivered siRNA retained its activity following delivery, resulting in 85±10% knockdown of a model gene within 24h of delivery. Additionally, the glycoconjugates were avidly recognized and internalized by human macrophages, and facilitated the delivery of 13-fold more siRNA into these cells relative to model cancer cell lines. Preliminary results also show that the glycoconjugates co-localize with CD206 in murine breast tumors in vivo, suggesting that these vehicles may become an enabling technology to target pathologic macrophage activity in tumors.

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Heteromultivalent Ligand Modification to Enhance Specific Bioactivity of Vascular Nanomedicine Platforms

Active targeting of nanomedicine vehicles via surface-modification with cell-specific ligands can enhance site-selective delivery of therapeutic cargo. In this context, vehicles for vascular drug delivery have to not only bind to the target site, but also resist dislodgement under blood flow. We postulate that modifying the nanovehicle surface with multiple ligand types (heteromultivalent modification) that simultaneously bind to multiple target epitopes, can enhance the binding specificity, as well as, maintain stable attachment under flow. To this end, platelets provide an excellent biological model, as they render hemostasis by adhering onto vWF via GPIbα and collagen via GPIa/IIa and GPVI, at a vascular injury site, and undergoing subsequent aggregation by binding to fibrinogen via GPIIb-IIIa and to sialoproteins via P-selectin. These same ligand-receptor activities become excessive in vascular diseases like inflammation and thrombosis, and hence mimicking them on a synthetic vehicle can provide effective platforms for vascular drug delivery. Therefore, we have investigated two platelet-mimetic nanomedicine platforms via heteromultivalent modification of liposomes, one mimicking platelet's 'aggregatory' functionalities by simultaneous binding to GPIIb-IIIa and P-selectin, and, the other mimicking platelet's 'adhesive' functionalities by simultaneous binding to vWF and collagen. Using epifluorescence microscopy, we have studied the binding and retention of these vehicles under flow at 0-60 dynes/cm2 on appropriate target surfaces. Our results showed significantly enhanced binding and stable retention of the vehicles on their respective substrates under low-to-high shear. These platforms can be envisioned to carry various therapeutic and imaging agents selectively to vascular disease sites that exhibit high platelet activity.

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Characterization and Optimization of Inhalable PEGylated Phospholipid Microparticles and Nanoparticles Containing Paclitaxel for Targeted Pulmonary Nanomedicine in Lung Cancer

Despite the significant advances in the treatment of lung cancer, it is a disease that still faces poor prognoses and challenges in implementation of treatment. Targeted pulmonary inhalation drug delivery offers many advantages for lung cancer patients in comparison to conventional systemic chemotherapy. In particular, inhalable dry powder formulations of nanoparticles and microparticles containing a chemotherapeutic are advantageous in their ability to deliver drug deep in the lung via optimally sized particles, higher local drug dosage, and long-term storage capability.

In this work, novel advanced spray-dried inhalable PEGylated phospholipid microparticulate/nanoparticulate powders containing paclitaxel were successfully designed and produced via spray drying. Dipalmatoylphosphatidylcholine (DPPC) and dipalmatoylphosphatidylethanolamine poly(ethylene glycol) (DPPE-PEG) at three different PEG chain lengths were mixed with paclitaxel in a dilute methanol solution. SEM images showed spherical particle morphology of the inhalable particles and the diameter range was determined to be 0.6 – 1.2 µm, which is optimal for efficient targeting of the deep lung. DSC and PXRD were performed and allowed for the confirmation of the presence of phospholipid bilayers and/or paclitaxel and their phase transition behavior. The water content of the particles was very low as quantified analytically via Karl Fisher titration (0.44 to 6.48 wt% H2O).

The amount of paclitaxel loaded into the particles was analyzed via UV-Vis spectroscopy resulting in high paclitaxel encapsulation efficiencies (46 - 99 wt%). The dry powder aerosol performance was evaluated using the Next Generation Impactor (NGI) and a dry powder inhaler (DPI) device and the particles demonstrated mass median aerodynamic diameter within the inhalable range (2.7 - 7 µm) as well as high fine particle fractions (43 – 78%) and respirable fractions (44 – 50%). Overall, these results demonstrate this novel therapeutic nanomedicine platform as one capable of effectively delivering paclitaxel directly to the lung for the treatment of lung cancer.

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Biodegradable Elastomeric Substrates with Controllable Stiffness for Regulating Smooth Muscle Cell Behavior

Smooth muscle cells (SMCs) exhibit phenotypic plasticity, which is of critical importance for blood vessel regeneration because synthetic phenotype is needed for cell proliferation and tissue remodeling while contractile phenotype is necessary for forming functional blood vessel. It has been found that substrate stiffness could influence SMC phenotype remodeling and lead to modulation of gene expression. Challenge lies in designing biomaterials that can support fast proliferation of synthetic SMCs and also facilitate the phenotypic conversion into functional contractile SMCs. Photo-crosslinkable poly(□-caprolactone) triacrylates (PCLTAs) recently developed in our group have controllable physical properties after crosslinking for diverse tissue engineering applications. In this study, we have investigated phenotypic modulation of SMCs on elastomeric substrates fabricated by PCLTAs with six molecular weights ranging from 2000 to 20000 g mol-1. Crosslinked PCLTAs were biodegradable through hydrolysis *in vivo* conditions. Crosslinked PCLTA8k, 10k, 20k had melting temperatures (Tm) higher than 37 □C, indicating they were semi-crystalline at body temperature, while crosslinked PCLTA2k, 5k and 7k were amorphous. Through controlling the crosslinking density and crystallinity of the PCLTA networks, we found that the stiffness of these substrates had non-monotonic dependence on the molecular weight of PCLTA. Elastic modulus ranged from 1.6 MPa for amorphous crosslinked PCLTA7k to 194 MPa for semi-crystalline crosslinked PCLTA20k, in which a physical network could be formed by crystallites to enhance the chemical network. Distinct cellular responses to these polymer substrates have been found. Rat SMCs cultured on stiffer crystalline crosslinked PCLTA substrates exhibited stronger stress fibers, larger expanding area, faster growing, and higher expression of contractile phenotype markers. In comparison, the cells cultured on the softer, amorphous substrates had a smaller projecting area and a lower growth rate.

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An in situ forming, mechanically supportive drug delivery system for avascular necrosis of the femoral head

Avascular necrosis of the femoral head (AVNFH) is the death of bone tissue due to an interruption in blood supply and affects over 10,000 new patients each year in the United States. Death of osseous tissue in the femoral prevents bone turnover, resulting in structural collapse and potentially irreversible deformation. In addition to osteoarthritis, pain, and limited mobility, most cases involving collapse will eventually require a hip replacement. An injectable system was developed that combines drug delivery to restore bone turnover with mechanical support to reinforce structurally damaged tissue. The drug release component allows for independent release kinetics of each drug. The scaffold is composed of three phases: a polymer solution that precipitates in vivo, drug-loaded polymer microparticles for prolonged drug release, and biocompatible filler particles for mechanical reinforcement.

Drug release ranging from hours to more than 30 days has been demonstrated from this system, with tunable kinetics based on drug and microparticle chemical properties. N-methyl pyrrolidone, an osteogenic drug as well as an organic solvent, is released within days to allow rapid precipitation of the scaffold and to initiate new bone growth. Clodronate, an anti-resorptive drug, was released within one week to acutely inhibit bone resorption. Simvastatin, and osteogenic agent, was released over 30 days to allow for prolonged new bone formation. Preliminary mechanical data has shown a 68% increase in compressive modulus upon addition of hydroxyapatite microparticles as a mechanical filler, with no effect on release kinetics. This is the first study to combine mechanical support with drug release of an osteogenic and an anti-resorptive agent to treat AVNFH.

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Biomimetic Calcium Carbonate Concentric Microgrooves with Tunable Widths for Promoting MC3T3-E1 Cell Functions

Bone consists of a mineral moiety of hydroxyapatite (HA) and amorphous calcium phosphate deposited on the organic collagen matrix. Osteoblasts adhere to and communicate with each other mainly through adherens junctions, which require calcium-dependent cell-cell adhesionvia cadherins.The interaction between the organic matrix and calcium-containing components is thus critical in bone regeneration and ceramic bone implants have been developed from calcium carbonate (CaCO3), HA, and calcium phosphate.However, long-term observation of these materials showed mismatch of mechanical properties and poor interaction with the surrounding tissue.Facile, biomimetic synthetic approaches are needed in developing biomaterials with improved mechanical properties, biocompatibility, and bioactivity. The components, mechanical properties, and topography of both basement membranes that exist in vertebrate body and bioinspired substrates and scaffolds can modulate cellular functions through activation of transmembrane integrin receptors and the strength of integrin-cytoskeleton links. To mimic these complex topographical features and study their impact on cell functions, micro-patterned surfaces have been fabricated using various biomaterials.Here, we report the fabricationof biomimetic, self-assembled calcium carbonate (CaCO3) concentric microgrooves with groove widths of 5.0 and 10 □m by spontaneous two-step crystal growth on hydrogel matrices made from poly(vinyl alcohol) through simply controlling incubation temperature and their effect on mouse MC3T3-E1 cells. Mouse MC3T3-E1 cells were cultured on flat and microgrooved substrates of CaCO3 and their adhesion, spreading, proliferation, alkaline phosphatase activity, and calcium content were remarkably enhanced by the microgrooves, in particular, the narrower ones. Furthermore, focal adhesions and actin filaments of MC3T3-E1 cells could be aligned on both 5.0-□m and 10-□m-wide CaCO3 grooves. Compared with the original round nuclei on the flat substrates and expanded round nuclei on the narrower microgrooves, the MC3T3-E1 cell nuclei on 10-□m-wide CaCO3 grooves demonstrated preferred entrapment in the grooves and significant alignment with a smaller area after two-day culture.

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Modeling and Experimental Methods to Predict Optimal Oxygen Distribution for Tissue Regeneration in Bone Defects

Connective tissue progenitors (CTPs) are a heterogeneous population of tissue-resident cells that are capable of proliferating and differentiating into one or more connective tissues, including bone forming progenitors or (CTP-Os). CTP-Os are commonly transplanted on allograft and synthetic scaffolds to heal major bone defects. Compromised survival of transplanted progenitors in the center of the scaffold is common due to the absence of efficient mass transfer (i.e. vascularity) primarily due to limited oxygen availability. Oxygen generating biomaterials (OGB) have been developed to improve survival and performance of transplanted cells by providing them with oxygen in the absence of a vasculature. Gas foaming techniques were used to fabricate OGB composed of 50:50 poly(lactic-co-gycolic acid) and sodium percarbonate. CTP oxygen consumption and oxygen release from OGB were measured using closed cell respirometry. To accelerate optimization of OGBs, we have developed a mathematical model that allows us to simulate oxygen distribution in an *in vivo* avascular bone defect. Incorporated into the model are experimental data for the oxygen release rate from a given OGB formulation and the oxygen consumption rate of a given transplanted cell population. With these data, model simulations provide oxygen concentration as a function of space and time, so that the optimal oxygen range can be obtained based on relatively few experiments. This information is essential to minimize the number of experiments in animal models that determine the optimal combinations of scaffold, OGB, and cell concentration. Data collected for model simulations suggest that oxygen consumption of bone marrow-derived nucleated cells is dependent on oxygen concentration. Oxygen release from OGBs is a time-dependent function that must be measured for accurate simulation. Simulations show that the oxygen gradient in an avascular defect has the largest dependence on cell concentration, cell oxygen consumption rate, OGB oxygen generation rate, and OGB geometry.

Andrei Ponta and Younsoo Bae

Tunable Drug Release from Novel Polymer Micelles for Maximized Chemotherapeutic Efficacy

Objective: To increase chemotherapeutic efficacy by utilizing polymeric micelles which can fine-tune drug release.
Methods: Three types of biocompatible block copolymers, poly(ethylene glycol)-poly(aspartate-hydrazide) [HYD], poly(ethylene glycol)-poly(aspartate-aminobenzoate-hydrazide) [ABZ], and poly(ethylene glycol)-poly(aspartate-glycine-hydrazide) [GLY], were synthesized to prepare nanoscale (< 200 nanometers in diameter) polymer micelles. An anticancer drug doxorubicin (DOX) was conjugated to the hydrazide moiety of each block copolymer. Particle size, stability, and other physochemical properties of the drug-loaded micelles were determined. The micelles' drug release patterns were also determined at physiological and intracellular lysosomal conditions (pH 7.4 and 5.0, respectively), confirming the effect of the spacers on tunable drug release capability. In vitro cytotoxicity and intracellular uptake assays were performed comparing micelles with free DOX. In vivo antitumor activity and biodistribution studies were conducted in a subcutaneous xenograft mouse tumor model (NCRNU nu/nu mice, a human cancer A549 cell line).
Results: Polymer micelles from HYD, GLY, and ABZ were successfully prepared, denoted HYD-M, GLY-M and ABZ-M, respectively. In every case, DOX was released at a faster rate in acidic condition (pH 5.0) than in physiological condition (pH 7.4) due to the acid-labile hydrazone bond. Linkers preceding the hydrazone allowed the drug release rates to be fine-tuned by altering the hydrolysis rates of DOX. HYD-M released drug the quickest followed by ABZ-M and GLY-M, respectively. Cytotoxicity assays showed that polymeric micelles were equipotent to free DOX, but their cellular uptake was unexpectedly less than that of free DOX. Interestingly, GLY-M (slowest drug release) led to a greater intracellular DOX concentration than ABZ-M. In vivo studies suggest that tunable drug release would potentially reduce toxicity and improve efficacy.
Conclusion: Drug efficacy was enhanced through the use of polymer micelles which fine-tuned drug release. These promising findings may lead to the development of chemotherapy with enhanced efficacy and reduced toxicity in cancer patients.

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Hemostatic nanoparticles increase survival after liver trauma in rats

Trauma is the leading cause of death for people ages 1-44, with blood loss comprising 60-70% of mortality in the absence of lethal CNS or cardiac injury. Immediate intervention is critical to improving chances of survival. While there are several products to control bleeding for external and compressible wounds including pressure dressings, tourniquets or topical materials (e.g. QuikClot, HemCon), there are no products that can be administered in the field for internal bleeding. We have previously developed hemostatic nanoparticles that reduce bleeding times by ~50% in a rat femoral artery injury model. Here, we investigated their impact on survival following administration in a lethal liver resection injury in rats. Administration of these hemostatic nanoparticles reduced blood loss following the liver injury and dramatically and significantly increased 1-hour survival from 47% in the saline control group to 80%. We further characterized the nanoparticles' effect on clotting time (CT) and maximum clot firmness (MCF) using rotational thromboelastometry (ROTEM), a clinical measurement of whole-blood coagulation. Clotting time is significantly reduced, with no change in MCF. Administration of these hemostatic nanoparticles after massive trauma may help staunch bleeding and improve survival in the critical window following injury.

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Hydrogels Containing VEGF Mimetic Peptide for the Neovascularization of Engineered Tissues

The success and enhancement of tissue engineered strategies for the replacement and reconstruction of metabolically demanding tissues is highly dependent on their ability to promote rapid and stable neovascularization within the scaffold. Synthetic hydrogels offer great promise as scaffolds that promote neovascularization and tissue regeneration since they enable controlled study of cell-biomaterial interactions. To this end our lab has shown that matrix metalloproteinase-sensitive (MMP-sensitive) hydrogels of poly(ethylene glycol) with highly controllable biomaterial properties stimulate *in vitro* neovascularization in the absence of growth factor incorporation into the scaffolds. In an effort to increase the rate of neovascularization within tissue engineered PEG scaffolds, we exploited the incorporation of a vascular endothelial growth factor (VEGF) peptide mimetic sequence, QK, developed by D'Andrea et al. which has been previously shown to possess similar bioactivity as compared to that of the full protein. In this study, the QK peptide sequence was modified with a cysteine group to allow its conjugation to a PEG macromer via Michael addition with PEG diacrylate. MMP-sensitive PEG hydrogels containing immobilized RGD adhesion sites and either immobilized QK, soluble QK, or soluble VEGF were crosslinked via free-radical photopolymerization using visible light (λ = 514 nm) in the presence of cellular aggregate co-cultures of endothelial and smooth muscle cells and evaluated for their *in vitro* neovascularization potential. Comparisons of hydrogel groups indicated that gels that included soluble QK and soluble VEGF resulted in an increased area of cell invasion and vascular sprout formation as compared to immobilized QK after a one week culture period ($p < 0.01$). This preliminary data suggests a similar bioactivity between soluble QK and VEGF in the early stages. Future studies will focus on longer *in vitro* cell culture studies that investigate how changes in the concentration of immobilized and soluble QK influence neovascularization.

POSTERS

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Evaluation of Non-functionalized Single Walled Carbon Nanotubes Composites for Bone Tissue Engineering

Introduction: Bone defects and non-unions pose a great challenge in the field of orthopedics. To overcome limitations presented by autografts and allografts, tissue engineering has evolved as a means to develop viable bone grafts. Novel materials of interest in our lab are Single Walled Carbon Nanotubes (SWCNT) which is one atom-thick layers of graphite called graphene, rolled into a cylinder and comprised of carbon with same scale size as of DNA. The overall goal of this project is to develop composites comprised of SWCNT and PLAGA, and to evaluate the interaction of human stem cells (hBMSCs) and osteoblasts (MC3T3 cells) via focusing on cell growth, proliferation, gene expression, extracellular matrix production and mineralization.
Methods: SWCNT composites (PLAGA/SWCNT) and PLAGA scaffolds were fabricated into circular discs using a solvent evaporation technique. For characterization Scanning Electron Microscopy (SEM) and degradation studies were performed. To evaluate biocompatibility MC3T3 cells and characterized hBMSCs were seeded on the scaffolds and cultured over a 7 day period. Adhesion/Proliferation studies using MTS assay and gene expression analysis using real time PCR and western blotting were performed. Morphology studies using Immunofluorescence staining and SEM, and cell survival using Live/Dead assay kit were performed. All experiments were performed at least three times in duplicate and mean ± SEM values along with statistical analysis using ANOVA were performed.
Results: The SEM images for SWCNT composite scaffolds demonstrated uniform incorporation of SWCNT into the PLAGA matrix. Degradation studies over a period of 21 days, demonstrated that PLAGA/SWCNT and PLAGA scaffolds have a similar degradation profile. Immunofluorescence staining and SEM revealed that MC3T3 and hBMSCs cells exhibited normal, non-stressed morphology on the composites and demonstrated focal contact points and normal nuclear formation. Cell proliferation assay revealed that the composite with 10mg SWCNT demonstrated a significantly ($P < 0.05$) higher cell proliferation rate at day 7. Live/Dead assay, demonstrated that cells survived on these SWCNT composites. The real time PCR data demonstrated the expression of osteoblast phenotypic markers (Col I, OPN), mineralization markers (ALP1, OC, BSP) and osteoblast differentiation marker (Runx-2) on these composites. Western blotting demonstrated the expression of OC, OPN, Col I on these composites.
Discussion: We were able to successfully fabricate composites with SWCNT and PLAGA for enhanced mechanical strength. Our results suggested that these tissue engineered SWCNT composites promote cell adhesion, proliferation and gene expression. SWCNT composite with 10mg SWCNT demonstrated the highest rate of cell proliferation and would be the ideal composite concentration. These results demonstrate the potential of SWCNT composites for musculoskeletal regeneration and offer an alternative for bone tissue engineering. Future studies will determine the mechanism of adhesion and optimization of the composites. These are some of the first studies to evaluate SWCNT composites and thus warrant further investigation.

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Pore Size Dependent Protein Capture and Protection from Protealytic Hydrolysis

Active protein capture has great potential in biological catalytic, separation and drug delivery applications. In recent years, significant interest and resources have been placed on the development of materials platforms for the delivery of therapeutic enzymes. Problems have arisen in the development of these materials regarding the implementation of finely tunable and accessible enzyme supports as well as protection of valuable cargo in enzyme hydrolyzing environments. Prevention of protein denaturation and degradation in biological environments is critical to developing bioactive interfaces and devices. Micelle templated porous spherical silica materials with finely tunable pore sizes are synthesized and investigated as a versatile protein capturing support. These materials are prepared by precipitation of silica precursors in the presence of Pluronic (PEO)-(20)-(PPO)-(70)-(PEO)-(20) triblock copolymer surfactant P123 with pore tuning by changing the hydrothermal treatment temperature. Finely tunable pores with sizes ranging in diameter from 3 to 12 nanometer are prepared. The morphology is also tuned to provide large (~15 μm) mostly spherical particles amenable to visualization by scanning confocal microscopy. The sustained activity of the captured proteins in the presence of hydrolyzing proteases will be used to demonstrate the protective capabilities of these materials. As a model system, enhanced green fluorescent protein (EGFP) is used as a representative protein; The activity of EGFP can be compromised by either protealytic degradation or denaturing in solution. Variable pore sizes will be used to demonstrate the effect of pore size on the accessibility and protective capabilities of size selective pockets.

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Design and Evaluation of Human ACL cells on a Novel Tissue Engineered Braided Ligament Construct

INTRODUCTION: The anterior cruciate ligament (ACL) is the most commonly injured ligament of the knee. ACL disruptions are recorded in more than 200,000 patients each year, accounting for more than 150,000 ACL surgeries performed annually(1). The ACL is one of four ligaments that support the knee and the stability is largely dependent on the functionality of the ACL to resist the anterior tibial translational loads (primary) and rotational loads (secondary). Typically, ACL ruptures cannot be repaired naturally because of poor vascularization and an intraarticular environment that is unfavorable for ligament regeneration. Due to its poor healing potential, severe ACL damage requires surgical intervention. Both autologous ligament transplantation and allogenic tissue interventions present concerns for orthopaedic surgeons, including donor site weakness, decreased range of motion, disease transmission, limited availability and unfavorable immunogenic responses.

Investigators have begun to utilize tissue-engineering techniques to create new options for ACL repair, regeneration and replacement. These new options involve artificially fabricated devices that are designed to be mechanically functional tissue scaffolds, and are able to withstand normal mechanical loads. At the same time, these scaffolds must promote ligament development and regrowth in the damaged area. The tissue engineering approach necessitates the implantation of a degradable ACL reconstruction scaffold to temporarily support mechanical loads and promote tissue ingrowth as the scaffold material degrades. This leads to the regeneration and/or restoration of the ACL over time. The goal of this project is to develop and optimize a novel tissue engineered ACL composite ligament that will have both mechanical and biological integrity while promoting healing and regeneration of the ACL. We hypothesize that a novel braided tissue engineered ligament can be designed and support human ACL cellular growth and serve as an alternative option for ligament reconstruction.

RESULTS: Scaffold Construction: The main objective was to create a novel woven ACL ligament using a predetermined turns per inch technique to produce a scaffold with equivalent properties of the native ACL. Through the use of PLAGA fibers, stable ligaments were able to be constructed into 2, 4 and 6 turns per inch orientations.(Fig 1) Characterization studies via SEM demonstrated that both 4 and 6 turn per inch braids produced acceptable pore size and distribution for ACL cellular ingrowth. The secondary structure created through the braiding technique provided braids with qualities of 2, 4, and 6 turns per inch, resulting in three braid orientations with average secondary structure pore sizes of 696µm, 232.25µm, and 25µm, respectively. Cellular studies demonstrated that ACL cells were able to be successfully obtained and initial adhesion to the scaffold over a 7 day period was visualized on the braided ligaments. This was supported by confocal and SEM images, which demonstrated normal morphology of the ACL cells.

DISCUSSION: We have developed an engineered ACL that demonstrates the biocompatibility, structural characteristics and mechanical properties necessary to function as an innovative and unique ACL replacement based on a turns per inch measurement. Additionally, the ligaments supported initial human ACL cell growth. The investigation into the vascularization capabilities, neural system development, and gene expression associated with these scaffolds will further elucidate their qualifications to serve as an ideal structure for ACL reconstruction. Future studies will focus on optimizing the ligament and mechanical strength. These are some of the first studies to evaluate human ACL cells on a ligament matrix designed based on turns per inch in order to maximize strength and cellular ingrowth.

SIGNIFICANCE: To date, there has been no published research investigating the interaction of human ACL cells with the clinically acceptable polymer PLAGA. The creation of a scaffold that is both biocompatible, mechanically sound, and supports native ACL cell growth will allow for improved surgical care and outcomes.

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Effect of Plasticizers and Drug on the Degradation and Release Profiles of Drug Delivery Films

Statement of Purpose

Films composed of 70:30 wt% cellulose acetate phthalate (CAP) and Pluronic F-127 have been proven to be an erosion controlled system, making them an attractive degradable polymer for drug delivery. However, CAP-Pluronic films are typically rigid and unable to conform to varying geometries that may be needed for dental, wound, or other applications. In order to allow for more diverse use of the system, the film needs to be flexible. To impart flexibility, plasticizers were added. The effects of plasticizers on drug release and degradation were studied.

Methods

Films were prepared using solvent evaporation casting. The CAP and Pluronic were combined in a 70:30 weight ratio. Plasticizer [triethyl citrate (TEC) or tributyl citrate (TBC)] was added at a 0, 10, or 20 wt%. Either anti-inflammatory drug, ketoprofen (400 mg), or antioxidant , quercetin (10 mg), were included in the mixture and then all components were dissolved in 8 ml acetone, sonicated, and cast in Teflon dishes. The dishes were left in a 10ºC refrigerator during the evaporation of acetone. Film erosion was measured by incubating samples in phosphate-buffered saline (PBS) on an orbital plate shaker at 37ºC. Aliquots were taken every hour and later analyzed using high performance liquid chromatography (HPLC) to determine drug release profiles.

Results

Plasticizer is added to increase the flexibility of the films resulting in a decreased elastic modulus and ultimate tensile strength and an increase in elongation. However, increasing plasticizer content did not significantly affect the degradation rates of the ketoprofen (Figure 1) or quercetin (not shown) loaded films. Drug release was also not affected by the concentration of plasticizer, TEC or TBC (Figure 1). Whether TBC or TEC was used as plasticizer did not have a noticeable difference in drug release or the degradation of the system. The degradation and drug release had a linear relationship (Figure 2).

Discussion

The drugs were released from the system as the surface of the films eroded, and the amount of plasticizers added did not affect the rate of erosion. This can be seen by the almost linear cumulative release curves for ketoprofen, and also by the degradation versus release for quercetin. Individual drug dosing remains proportional to the surface areaAs the films degraded, the surface area decreased, resulting in a silight decrease in drug release. The two drugs, quercetin and ketoprofen, interact with the CAP-Pluronic system similarly, as they are both small, aromatic, hydrophobic molecules. Ketoprofen loading was 40 times greater than that of quercetin. Interestingly, the loading did not seem to have an effect on the degradation or drug release when comparing these two drugs.

Conclusions

Plasticizers and drug loading do not affect the degradation rates or surface-eroding qualities of CAP-Pluronic films causing them to be an appealing system for flexible drug delivery film.

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Synthesis and Characterization of Peptide-Conjugated Iron Oxide Nanoparticles for Hyperthermia Applications

One of the current challenges of targeted systemic nanoparticle systems utilized in cancer therapy applications is the lack of effective tumor homing. The overall goal of this study is to develop and optimize peptide-conjugated dextran-coated iron oxide nanoparticles for enhanced tumor homing as well as effective hyperthermia treatment. Hyperthermia, the heating of tissue between 42 and 45°C, has been shown to enhance the effects of radiation and chemotherapeutics, but current methods of hyperthermia often result in severe side effects due to lack of localization. The proposed system presents dextran-coated iron oxide nanoparticles functionalized with a tumor homing peptide, CREKA, which has the ability to overcome this limitation by homing to tumor sites while the iron oxide core allows for particle heating upon exposure to an alternating magnetic field. Magnetically-mediated hyperthermia utilizing iron oxide nanoparticles provides the opportunity for localized heating, and this specific particle system would enhance particle accumulation within the tumor vasculature and stroma. The core particle system is synthesized in three steps: iron oxide nanoparticle synthesis and dextran coating, epichlorohydrin crosslinking of the dextran, and amination of the particle coating. CREKA is then conjugated to the particles via a PEG-linker conjugated to the primary amines. The particles have been characterized for size, stability, biocompatibility, and heating capabilities upon exposure to an alternating magnetic field. It has been found that this particle system is stable in PBS and media over at least twelve hours, has a hydrated diameter range of 50-100nm, and can generate enough heat to raise solution temperatures well into the hyperthermia range.

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Delivery of the Photosensitizer Pc 4 in PEG–PCL Micelles for In Vitro PDT Studies

The silicon phthalocyanine Pc 4 is a second-generation photosensitizer that has several properties superior to other photosensitizers currently approved by the FDA, and it has shown significant promise for photodynamic therapy (PDT) in several cancer cells in vitro and model tumor systems in vivo. However, because of the high hydrophobicity of Pc 4, its formulation for in vivo delivery and favorable biodistribution become challenging. To this end, we are studying encapsulation and delivery of Pc 4 in block copolymer micelles. Here, we report the development of biocompatible PEG–PCL micelle nanoparticles, encapsulation of Pc 4 within the micelle core by hydrophobic association with the PCL block, and in vitro PDT studies of the micelle-formulated Pc 4 in MCF-7c3 human breast cancer cells. Our studies demonstrate efficient encapsulation of Pc 4 in the micelles, intracellular uptake of the micelle-formulated Pc 4 in cells, and significant cytotoxic effect of the formulation upon photoirradiation. Quantitative estimation of the extent of Pc 4 loading in the micelles and the photocytotoxicity of the micelle-incorporated Pc 4 demonstrate the promise of our approach to develop a biocompatible nanomedicine platform for tumor-targeted delivery of Pc 4 for site-selective PDT.

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Localized Delivery of Imiquimod to Treat Oral Precancereous Lesions

Objective: Imiquimod, an immune response modifier approved for market (Aldara), has been successfully used to treat acitinic keratosis and superficial basal cell carcinomas. A small number of case studies showed that off-label use of imiquimod was successful in stopping progression of oral dysplasia to oral squamous cell carcinoma. However, use of commercially available cream may be undesirable to use in oral cavity. The objective of this work was to determine the efficiency of a previously designed mucoadhesive system in delivering drug to epithelium and to confirm bioactivity of released imiquimod. Methods: Mucoadhesive films with backing layer were attached to the mucosal surface of 500μm thick porcine buccal tissue contained in a Franz cell. Samples were collected from the receptor compartment at predetermined intervals, and drug quantity was measured using HPLC. Permeability and transport kinetics of imiquimod control solutions and imiquimod-loaded films were compared. Bioactivity of imiquimod released from film supernatants was assessed by measuring TNF-α produced by RAW 264.7 cells via ELISA. Results: Flux rates of imiquimod through buccal mucosal tissue were 1.25μg/cm2/hr and 4.98μg/cm2/hr for mucoadhesive films and control solutions, respectively. This result demonstrates that transport of imiquimod through tissue into simulated saliva was controlled by the films. The mucoadhesive films also decreased permeability of imiquimod through tissue by 50% compared to control solutions, resulting in accumulation of more drug in 500μm thick mucosal tissue. Imiquimod was found to retain bioactivity after undergoing all the processing steps of making films. No significant difference in amounts of TNF-α produced by macrophagic cells was observed using film supernatants and control solutions of same concentration. Conclusion: Mucoadhesive films were able to control drug release and may limit systemic absorption of drug. Decreased permeability of bioactive imiquimod through tissue following release from films may increase localization of drug in cancerous lesions and increase effectiveness of treatment.

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Characterization of Liposomal Nanoparticles for In Vivo Conjugation to Adoptive Transferred T-cells

Adoptive cell transfer (ACT) has proven to be a highly effective method for treating metastatic melanoma, however, the current procedure is expensive, requires expertise, and the adoptively transferred cells do not survive long *in vivo*. 1 In order for the therapy to be effective multiple procedures would be required, further increasing the cost. This project aims to remove the need for repeated injections by arming adoptively transferred T-cells with liposomal nanoparticles. The nanoparticles are decorated with Interleukin-2 (IL-2) on the surface, which aids in targeting the nanoparticles to the adoptively transferred T-cells. Ideally, these nanoparticles will conjugate to the T-cells *in vivo*, since it has been shown that *ex vivo* conjugation has aided proliferation of T-cells and increased treatment efficacy.2The nanoparticles will encapsulate a drug, SHP1/2 inhibitors, which will help prevent down-regulation of the immune response.3 The initial steps in this project include characterization of the nanoparticles, including determining the amount of IL-2 conjugated to the surface of the nanoparticles, the encapsulation efficiency, and the drug time release profile. From there, nanoparticles will be optimized to obtain more desirable encapsulation and drug release profiles.

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An Improved in vitro Model for the Study of Endothelial Cells Using Micropatterned Surfaces

Sickle cell anemia, malaria, and cancer are a few of the deadly diseases that utilize blood vessels as a means of migration throughout the body. Adhesion of harmful cells to the endothelial lining of the circulatory system is an integral step in the metastasis of blood borne diseases. As a result of shear stress produced by blood flow through veins and arteries, the endothelium undergoes a distinct morphological change resulting in a more elongated and unidirectional morphology. It has recently been suggested that such changes in cell morphology can affect surface expression profiles, which in turn affects cell-cell binding and interaction to the endothelial wall. Currently, most researchers are using *in vitro* flow models or static well-plates to culture endothelial cells. However, traditional *in vitro* flow systems take approximately 24 hours to obtain a valid morphology, and static well plate studies result in cobblestone morphology more random in orientation than *in vivo* endothelial cells. In this study, we are investigating the use of micropatterned glass surfaces to statically culture human vein endothelial cells (HUVECs) in the desired elongated and unidirectional morphology and this system's effects on surface chemistry of the HUVECs. Microscopy and flow cytometry were used to compare the morphology and surface expression of HUVECs grown on control blank slides, on micropatterned grooves, and under flow conditions. HUVECs cultured on micropatterned grooves demonstrated the desired elongated and unidirectional morphology. Morphology analysis showed that HUVECs cultured on micropatterned grooves were statistically more elongated and unidirectional than HUVECs cultured on control blank slides. Preliminary data on flow adaption and surface expression profiles will also be presented.

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Biocompatible multilayer co-fired ceramic for biomedical lab on chip applications

Low temperature co-fired ceramic (LTCC) electronic packaging materials are applied for their ease of fabrication, three dimensional features and integration of multifunctional component, such as optical and electrical functions. For these reasons LTCC is attractive for biomedical microfluidics and Lab-on-a-Chip systems. However, commercial LTCC system, optimized for microelectrics applications, have unknown cytocompatibility, and lack optical transparency, and not designed for biomedical applications. In the current work, both LTCC and transparent high temperature co-fired ceramic (HTCC) will be developed starting with materials of known composition and biocompatibility. The LTCC material is a glass-ceramic fabricated from a lime silicate glass (Schott B270) and pure alumina (HP DBM, Baikowski Malakoff Inc.). The transparent HTCC has been fabricated from ultrapure alumina, widely utilized for its bio-inert properties. In-vitro biocompatibility of both materials has been evaluated using human umbilical vein endothelial cells (HUVEC). The HUVECs attached and spread on the surface of both the LTCC and MLCC substrates, and also in the leachate obtained by soaking both materials in cell media at least for five days. The cell density and percentage of live cells in MLCC leaching media were comparative with those of control. The live cell percent in LTCC leaching media was the same as that of control. However, the cell density was less than control, which may be due to slight pH change during the incubation. Those results indicate developed LTCC and transparent HLCC are biocompatible for biomedical applications.

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Hemostatic Nanoparticles to Halt Bleeding

Blood loss is the primary cause of death in both battlefield and civilian trauma. There are currently many available treatments for external hemorrhage; however, there are few options to address internal bleeding. We address this issue with polymer nanoparticles designed to augment hemostasis after intravenous delivery. These particles can also be used as a drug delivery platform to aid in tissue repair. Nanoparticles are synthesized from a block copolymer consisting of poly(lactic-co-glycolic acid), ε-poly-Llysine, and poly(ethylene glycol). The resulting nanospheres average 200-300nm measured by DLS and SEM. The targeting peptide RGD is bound to the end of the PEG to allow the particles to bind to activated endogenous platelets. These particles were tested in rat injury models. Initial work in a rat injury model shows that these particles are effective in reducing bleeding time and improving survival.

Amanda Clark

Effect of Processing Temperature on Poly(lactic-co-glycolic acid) Scaffold Properties and Bioactivity of Insulin-like Growth Factor

Poly(lactic-co-glycolic acid) (PLGA) is commonly used as a scaffold because it exhibits biodegradable and biocompatible properties. PLGA allows for the encapsulation of growth factors, such as insulin-like growth factor I (IGF-I), which has been shown to stimulate the synthesis of proteoglycan and type-II collagen while enhancing chondrocyte matrix synthesis. The objective was to determine if there is an effect of temperature on IGF-I after being encapsulated in a PGLA scaffold and it's ability to increase cell proliferation.

In this study an IGF solution was incubated at various temperatures to order to find the range at which IGF can withstand. To evaluate the effectiveness of IGF, this solution was be added onto cells and monitored for DNA production in comparison to the control. It was found that IGF could withstand up to 50°C and still be maintain bioactivity. Afterwards, the IGF was encapsulated into PLGA microspheres and fabricated into scaffolds, from which the release profile was measured. The released supernatant was also added onto cells and the results to verify that the IGF bioactivity was not lost due to scaffold fabrication. As a final evaluation cells were seeded onto the IGF encapsulated scaffolds to evaluate the effect of the added IGF, which showed an increase in GAG and DNA content at 3 and 4 weeks as compared to the blank scaffolds.

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Mimicking the extracellular matrix via metal-assembled collagen mimetic peptides.

The extracellular matrix (ECM) is a complex mixture of proteins, polysaccharides and growth factors. Altogether, the components of the ECM offer a number of physical and biochemical cues for cells to attach, grow, proliferate, migrate and differentiate. Threedimensional (3D) scaffolds, either natural or synthetic, that mimi such features offer a great advantage in regenerative medicine. They can be used as temporary carriers of cells and growth factors (GFs) for implantation into damage tissue. We have previously reported the design of a collagen mimetic peptide (CMP) that assembles into a mesh-like 3D structure upon the addition of metal ions and its potential for the culture of human cells [1]. Here, we report the design of CMPs that can be functionalized with His-tagged cargo, within the 3D scaffold, via metal coordination. We show that the addition of GFPHis8 and human epidermal growth factor (hEGF-His6) has minimal effect in the assembly process. Additionally, we show that the bound hEGF-His6 can be released gradually *in vitro* for 5 days and induces cell proliferation in an EGF-dependent cell line. Furthermore we functionalized the CMPs with the cell adhesion sequence (RGDS) to promote cell adhesion and differentiation of various human cell lines, such as human umbilical vein endothelial cells (HUVEC), human Mesenchymal stem cells (hMSC) and the human breast epithelial cells lines, MCF10A and 3522-S1.

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Synthesis and Characterization of a Bioactive Polymeric Prodrug for Bone Regeneration

Biodegradable polymers used in bone regenerative applications include poly (lactic-co-glycolic acid) (PLGA) and poly (lactide) due to biocompatibility, adjustable degradation kinetics, and mechanical strength. However, they carry a limited amount of active agents, and residual particles still induce some degree of inflammation. Developing a bioactive poly (simvastain) diblock copolymer has been proposed for bone tissue healing.
An oligomeric poly (simvastatin) block was synthesized by ring opening polymerization. Characterization was done using Fourier Transform Infrared Spectroscopy (FTIR), gel permeation chromatography (GPC), nuclear magnetic resonance (NMR), and diffraction light scattering (DLS). Blended controls were made using both components used to synthesize the copolymer in comparable molar ratios to compare results against the synthesized copolymer. The IR spectrum of the diblock copolymer show characteristics from both poly (ethylene glycol) methyl ether (mPEG) and simvastatin monomer components, an increased alkyl to carbonyl stretch ratio representing monomer addition to mPEG, and a slight carbonyl shift indicative of new ester bond formation. Controls of both components blended in comparable molar ratios to the copolymer showed spectra more reflective of simvastatin. GPC revealed MWs of the crude copolymer ranging from 10,000 to 18,500 Da, with an mPEG block of 5000 Da. NMR integration showed supporting evidence of the formation of an oligomeric simvastatin block.
Characterization methods have shown evidence that that oligomeric blocks of poly (simvastatin) were synthesized. Simvastatin is a well known hypolipidemic prodrug, but the purposes of the present copolymer utilize other desirable properties of the monomer in its active form. These properties include being osteogenic by up-regulating bone morphogenic protein -2 and possessing anti-inflammatory and angiogenic properties. Together, these attributes make poly (simvastatin) desirable as a bioactive micellar and/or polymeric drug delivery system for therapeutic applications in bone regeneration.

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Honeycomb-patterned Polymer Substrates for Regulating Cell behavior

Many methods such as phase separation, colloidal templating, and lithography have been used to fabricate porous topography with ordered structures. Among these methods, the breath-figure method is very simple and economical, in which polymer is dissolved in a volatile and water-immiscible solvent and then the solution evaporates quickly in a humid environment. The sharp drop of the temperature in polymer solution upon rapid solvent evaporation causes condensation of water vapor in air. Subsequently polymer precipitates and stabilizes water droplets with identical diameters orderly packed in a hexagonal array on the solution/air interface as the result of capillary force and convection currents. Then honeycomb polymer films can be obtained after complete drying. We present honeycomb-patterned poly(□-caprolactone) (PCL), crosslinked poly(□-caprolactone) triacrylate (PCLTA), and comb-dendritic tri-block copolymer (PLLA-PEG-PLLA) films directly fabricated using the breath-figure method without assistance of a surfactant in a relatively non-toxic, water-miscible solvent, tetrahydrofuran. Pore diameters of 10, 6, and 3.5 □m for PCL films and 3.0 and 5.6 □m for crosslinked PCLTA were achieved by controlling the airflow rate. Pore diameters of 0.95, 1.92, and 3.1 □m for the triblock copolymers were achieved by modulating the polymer solution volume coated on glass substrates. Mouse pre-osteoblastic MC3T3-E1 cell adhesion, spreading, proliferation, mineralization, and expression of integrin subunits of □1, □2, and □1 were evaluated on honeycomb-patterned PCL and crosslinked PCLTA films. Mouse neuronal progenitor cell (NPC) attachment and proliferation on honeycomb-patterned triblock copolymer films were also studied. Honeycomb-patterned PCL and crosslinked PCLTA films could remarkably promote MC3T3-E1 cell adhesion, spreading, proliferation compared with the flat films. This effect was more prominent when pore size was smaller. On the contrary, NPC attachment and proliferation could be suppressed and they were more difficult to form neurospheres on the honeycomb-patterned films of the triblock copolymer compared with the flat ones.

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Protein-based biomaterials for bone regeneration

Tissue engineering of bone is a promising approach to regenerate large bone defects that do not self-heal. Protein-based biomaterials have been studied as potential scaffolds for bone regeneration. We are developing an artificial protein containing bioactive cues to promote stem cells to differentiate and produce a bone matrix.

DNA encoding the desired protein sequences was cloned. The resilin-based protein containing bioactive cues was expressed in *E.coli* by IPTG induction at 37°C and purified by a salting out and heating method. Osteogenic differentiation of human mesenchymal stem cells on adsorbed protein was characterized by Alizarin red S staining.

DNA encoding resilin repeats from *Anopheles gambiae* and a peptide derived from bone morphogenetic protein-2 (BMP-2) was successfully cloned. Resilin-based proteins were successfully expressed and purified, as confirmed by SDS-PAGE analysis. Human mesenchymal stem cells (hMSCs) were viable on adsorbed proteins. hMSCs grown on the protein containing the BMP-2 peptide accelerated bone formation, as assessed by Alizarin red S staining at 11 days of culture. This accelerated bone formation was sequence specific; cells grown on a protein containing a scrambled version of the BMP-2 peptide did not show a similar acceleration.

We developed a resilin-based artificial protein containing a BMP-2 derived peptide and investigated the effect of the protein on osteogenic differentiation. The inclusion of the BMP-2 peptide in the modular protein did not disrupt the bioactivity of the peptide. Accelerated osteogenic differentiation of hMSCs was observed on the protein containing the BMP-2 peptide and the observed effect was sequence specific. The ability of the resilin-based protein to promote osteogenic differentiation will be further characterized by evaluating alkaline phosphatase (AP) activity and the relative gene expression level of osteogenic markers to assess its potential as a tissue engineering matrix.

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Nanotopographical Control of Surface Morphology Through Ion Beam Irradiation for Improved Cell Response

Pseudoaneurysms are common in soldiers who suffer from high-velocity gunshot wounds/penetrating blast injuries (PBI) to the head and neck. Treatment in theater is limited to coil embolization or surgical clipping. There is a need for a minimally invasive procedure that will induce closure of the aneurysm neck orifice due to the prevalence of this type of injury in military personnel. This work focuses on nanopatterning a biocompatible stent coating through ion beam irradiation that will induce growth of the absent tunica media across the pseudoaneurysm neck. Nanoscale surface morphology has been shown to play an important role in cell regulation, differentiation, adhesion and proliferation. In order to control these features, irradiation utilizes a number of parameters, including ion species, beam energy, and angle of incidence. A variety of surfaces can be patterned using ion beam irradiation, especially metals and semiconductors. In this study, gold, palladium, and silicon surfaces were patterned through ion beam irradiation using argon and xenon. Human umbilical vein endothelial cells (HUVEC) were then cultured on the surfaces of the samples and the DNA damage was assessed and compared to that caused by unpatterned samples of the same materials using the Comet Assay. The surface morphology of the samples was examined using atomic force microscopy (AFM) and scanning electron microscopy (SEM), while surface chemistry was examined through x-ray photoelectron spectroscopy (XPS) and surface wettability measurements.

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Synthesis and Characterization of Quercetin based Poly (β-amino ester) particles to suppress Cellular Oxidative Stress

Oxidative stress is a pathophysiological condition defined by an increased production of reactive oxygen species (ROS, e.g., , singlet oxygen (1O2), superoxide radicals O2._,nitric oxide (NO.)), which can result in the temporary growth arrest of cells at lower concentration levels to cell disintegration or necrosis at very high ROS levels[1]. A number of antioxidants (e.g. curcumin, quercetin) are capable of directly scavenging ROS or chelating transition metals, which catalyze ROS generation, short-circuiting the self-propagating oxidative stress state and control the cell death.

However, very poor solubility of these molecules in aqueous medium along with relatively short activity life poses a barrier to their use as a drug to suppress oxidative stress efficiently. To overcome this limitation, quercetin was conjugated as poly (β-amino ester) (PBAE) cross-linked gel microparticles and nanoparticles. PBAEs are hydrolytically degradable polymers with relatively low cytotoxicity and have been frequently used for targeted drug delivery of hydrophobic drugs, gene delivery etc. Phenolic bonds of quercetin were replaced with acrylate groups for further reaction. Acrylated quercetin based gels were synthesized by bulk reaction of quercetin with PEG(400)DA and primary diamine, later cryo-milled into microparticles while nanoparticles of about 250nm size were synthesized by suspension polymerization in heptane-acetonitrile system. Time dependent degradation studies of different quercetin-PEG(400)DA ratio gel particles in PBS helped in tuning the controlled release of quercetin and maintaining the prolonged activity of quercetin during incubation as compared to pure quercetin.

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Tandem Drug Delivery from Hydrogels for Antibiotic Resistance Emergence Inhibition

Due to the emergence of antibiotic resistant strains of bacteria, the medical community faces a major challenge with a decreasing number of useful antibiotics available worldwide. To pawn this troubling trend, new approaches must be advanced to actively impede bacterial antibiotic resistance emergence in wounds. One approach is the co-delivery of antibiotics with agents that directly inhibit evolutionary adaptive mechanisms, e.g., reactive oxygen species (ROS) mediated antibiotic resistance emergence. It has been shown that the endogenous production of ROS in bacteria after a sub-lethal antibiotic threat can result in increased bacterial diversity, and thereby potential resistance via recA mediated SOS response DNA break/repair malfunction. In this work, a biodegradable hydrogel with a small molecule antibiotic, vancomycin, and large molecule antioxidant payload, catalase, was developed to achieve sustained co-release with the intention of interfering with the ability of *Staphylococcus aureus* to develop antibiotic resistance under sub-lethal antibiotic insult. While free-drug loading of both vancomycin and catalase have shown extended release previously, integration of vancomycin into the poly(β-amino ester) hydrogel is being developed further to tune the vancomycin release rate to the degradation rate of the hydrogel. Further, an antibiotic resistance based assay has been formed to help determine the proper reagents to be added alongside vancomycin to try and cut into the effect of bacterial resistance emergence against vancomycin, and determine whether or not ROS are a major route of antibiotic resistance formation.

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Tuning Nerve Cell Functions on Distinct Polymer Networks with Controllable Mechanical Properties

Mechanical, chemical, and topological properties are three major factors that influence cell behavior on the substrates. In this study, we used two types of biodegradable and photocrosslinkable polymers, poly(ε-caprolactone) triacrylate (PCLTA) and poly(ethylene glycol) diacrylate (PEGDA), with different molecular weights to demonstrate the roles of mechanical and chemical factors in regulating nerve cell behavior. Crosslinked PCLTAs were hydrophobic while crosslinked PEGDAs were hydrophilic and formed hydrogels. All the networks were amorphous at 37 °C to ensure good controllability of mechanical properties by well-defined crosslinking density. Rat Schwann cell precursor line (SpL201) cells were used to evaluate their performance in terms of proliferation and differentiation. We found that SpL201 cells could proliferate more and differentiate better on stiffer substrates of both networks but differences existed between these two networks because of surface chemistry. The mechanisms how cell functions were tuned by surface properties have been investigated by blocking of β1 integrin prior to cell attachment. We found that SpL201 cells no longer showed dependence on mechanical properties if β1 integrins were blocked. The present results provide guidance for designing optimal nerve conduits made by crosslinked PCLTA and filled with PEGDA hydrogels for peripheral nerve regeneration. Besides the effect of substrate stiffness, we also present in the poster our recent findings on the roles of microgroove dimensions and grafted polyethylene glycol or poly(L-lysine) chains in regulating nerve cells on PCL networks.

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A novel pH-responsive mixed micelle for siRNA delivery

Small interfering RNA (siRNA) has potential to be applied therapeutically to treat cancer or other pathologies whose etiology is related to aberrant gene overexpression. However, because of its size and charge, siRNA requires a carrier for effective intracellular delivery. Previous studies have established the efficacy of a pH-responsive diblock copolymer-based micelle for the delivery of siRNA. The polymer consists of a pH-responsive, endosomolytic block composed of butyl methacrylate (BMA), propyl acrylic acid (PAA), and dimethylaminoethyl methacrylate (DMAEMA) that is hydrophobic and drives micelle self-assembly. The second polymer block consists of a corona-forming poly(DMAEMA) block, which is positively charged at pH 7.4 and condenses anionic siRNA. However, nanocarriers with positive surface charge often have poor hemocompatibilty and poor blood circulation times, making them impractical for clinical translation. In order overcome this limitation, we have designed a mixed micelle approach that leverages the same endosomolytic terpolymer core but has a mixed corona containing varying levels of PEG. Micelles containing 0%, 25%, 50% and 75% PEG on the corona are consistently ~ 35 nm in diameter as determined by DLS. The PEG shows effective charge shielding, as the zeta potential of the micelles decreases from +17.2 mV to -0.540 mV with the increasing percentage of PEG. The micelles fully complex siRNA and demonstrate pH-responsive behavior finelytuned for endosomal escape. Studies also indicate that with the addition of PEG, fewer micelles nonspecifically interact with red blood cells, indicating the addition of PEG will increase hemocompatibilty and circulation time in vivo. Studies are currently being performed to evaluate the cellular uptake and gene silencing of these micelles in cancer cell lines. Based on the current data, these novel, pH-responsive mixed micelles show promise as safe, effective siRNA carriers.

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Smooth Muscle Cell Behavior on Poly(-caprolactone) Triacrylate Networks Grafted with Poly(ethylene glycol)

Poly(caprolactone) triacrylate (PCLTA), a photo-crosslinkable polymer synthesized in our lab via a facile method, is a promising injectable biomaterial because crosslinked PCLTA has excellent biocompatibility, biodegradability, and well-controlled physical properties. However, the high hydrophobicity of semi-crystalline PCLTA networks may limit their potential tissue engineering applications. We found that the hydrophobicity of these PCLTA networks can be greatly relieved when PCLTA is photo-crosslinked together with hydrophilic methoxy poly(ethylene glycol) monoacrylate (mPEGA). To further clarify the role of mPEGA in modification of PCLTA networks and regulation of rat smooth muscle cell (SMC) behavior, we prepared a series of photo-crosslinked mPEGA/PCLTA with various mPEGA compositions (m) of 0-50% and different nominal molecular weights of 350, 2000, and 10000 g mol-1 for mPEGA. The bulk and surface properties of these polymer networks were characterized and SMC behavior on these polymer substrates was investigated. PEG grafted chains on the surface of PCLTA networks significantly reduced surface hydrophobicity, frictional coefficient, and serum protein adsorption. Longer mPEGA chains had a stronger effect in modulating these material properties. SMC adhesion and proliferation exhibited parabolic dependence on the composition of mPEGA350 and maximized at m of ~5%. For mPEGA10000 with the longest chain length, a sharp, monotonic decrease was found in both SMC adhesion and proliferation. This study clearly revealed that grafting a small fraction of short PEG pendant chains to PCLTA networks could enhance cell attachment and proliferation on the polymer substrates by reducing surface hydrophobicity. In contrast, a high fraction of grafted PEG chains or long PEG chains could significantly prohibit SMC adhesion and proliferation via strong repulsion to both proteins and cells. The expression levels of three contractile gene markers for SMCs cultured on crosslinked mPEGA350/PCLTA substrates also demonstrated a similar non-monotonic trend by having maxima at m of 5%.

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Evaluation of Human Umbilical Vein Endothelial Cells and Low Temperature Co-fired Ceramic Materials for Fluidic Trans-endothelial Electrical Resistance Applications

The expansion of low temperature co-fired ceramic (LTCC) materials into microfluidic systems technology has the ability to enable many new applications due to their ability to combine complex three dimensional structures with optical, fluidic, electrical functions. It is vital to evaluate the biocompatibility of LTCC materials before expanding into biomedical research. The few biocompatibility studies on low temperature co-fired ceramics generally show an adverse response of cells exposed to thick film pastes used in generating the electronic circuitry patterns. In this study, biocompatibility of Human Umbilical Vein Endothelial Cells (HUVECs) was examined on HL2000 LTCC tape and two of their conductive pastes. The biocompatibility was assessed by monitoring cellular attachment and viability of HUVECs up to three days. This study examines the idea of harmful components leaching out of the LTCC materials being used. Results indicate difficulty in initial attachment of Human Umbilical Vein Endothelial Cells to sintered HL2000 tapes, but no hindrance of cellular attachment and growth onto the two conductive pastes. Outcomes also refute the idea of harmful leachates from low temperature co-fired ceramic materials by displaying cellular attachment and growth for up to three days of cell culturing. Lastly, this study examines the possibility of low temperature co-fired ceramics as a Trans-endothelial Electrical Resistance (TEER) device. Results show the LTCC device's signal to noise performance better than the simple chopsticks method but worse than the Endohm12 cup method. Findings also display an increase in resistance due to endothelial cellular growth within the channel of the device. These results provide a basis for biological devices using low temperature co-fired ceramic materials.

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Effectiveness of anti-biofilm agents against *Staphylococcus aureus* biofilms

The goal of this work was to determine which anti-biofilm agent was the most effective at disrupting and inhibiting biofilm growth in a well plate.
An established anti-biofilm plate assay was used in order to test the effectiveness of several different anti-biofilm agents against colonies of *Staphylococcus aureus*. The drugs investigated were d-amino acids (d-proline, d-phenylalanine, and d-tyrosine), xylitol, lactoferrin, lysostaphin, vancomycin, and gentamicin.
The d-amino acids, xylitol, and lactoferrin were not shown to inhibit or disrupt biofilms in the plate assay compared to the negative control. The broad spectrum antibiotics, vancomycin and gentamicin, were only effective at inhibiting the biofilm growth, not disrupting existing films. The enzyme lysostaphin proved to be the most effective treatment for inhibiting biofilm growth at concentrations as low as 10 µg/ml but were also able to disrupt existing biofilms at higher concentrations of 100 µg/ml.
Lysostaphin has shown great potential as an anti-biofilm agent capable of disrupting an existing biofilm and inhibiting biofilm formation in the preliminary *in vitro* tests. This dual action of lysostaphin makes it a promising candidate for future anti-biofilm biomaterials which could eliminate an existing biofilm and prevent it from re-growing. The success of these initial trials warrants further investigation into the ability of lysostaphin to be loaded into a delivery vehicle and incorporated into a device.

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NANOTOPOGRAPHICAL CONTROL OF SURFACE MORPHOLOGY THROUGH ION BEAM IRRADIATION FOR IMPROVED CELL RESPONSE

Pseudoaneurysms are common in soldiers who suffer from high-velocity gunshot wounds/penetrating blast injuries (PBI) to the head and neck. Treatment in theater is limited to coil embolization or surgical clipping. There is a need for a minimally invasive procedure that will induce closure of the aneurysm neck orifice due to the prevalence of this type of injury in military personnel. This work focuses on nanopatterning a biocompatible stent coating through ion beam irradiation that will induce growth of the absent tunica media across the pseudoaneurysm neck. Nanoscale surface morphology has been shown to play an important role in cell regulation, differentiation, adhesion and proliferation. In order to control these features, irradiation utilizes a number of parameters, including ion species, beam energy, and angle of incidence. A variety of surfaces can be patterned using DIS, especially metals and semiconductors. In this study, gold, palladium, and silicon surfaces were patterned through ion beam irradiation using argon and xenon. Human umbilical vein endothelial cells (HUVEC) were then cultured on the surfaces of the samples and the DNA damage was assessed and compared to that caused by unpatterned samples of the same materials using the Comet Assay. The surface morphology of the samples was examined using atomic force microscopy (AFM) and scanning electron microscopy (SEM), while surface chemistry was examined through x-ray photoelectron spectroscopy (XPS) and surface wettability measurements.

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Multiple Macromer Hydrogels for Multiphase Drug Release

Biodegradable hydrogels are of interest for drug delivery applications due to their resemblance to biological tissue and their ability to absorb large amounts of biological fluids. Here, hydrogels were synthesized from multiple macromers to demonstrate step-wise degradation and multiphase drug release profiles. Control over the degradation and release profiles of multiple macromer hydrogels has potential applications in implantable, extended release drug delivery devices in which removal would not be needed after administration. Herein, macromers were synthesized from diethylene glycol diacrylate (A), poly(ethylene glycol) diacrylate (n=400) (H), and isobutylamine (6) in 1.2:1 molar ratios of total diacrylate to amine with diacrylate ratios of A:H (0:1), (1:1), and (2:1). Multiple macromer hydrogels were synthesized via UV photo polymerization with a 365nm UV flood source and an intensity of 8-10mW/cm2. Degradation and swelling studies were conducted gravimetrically, and fluorescence correlation spectroscopy was used to track diffusion coefficients at different stages of degradation. Fluorescently tagged lysozyme, trypsin, and bovine serum albumin were loaded into the multiple macromer hydrogels and release was tracked using fluorescence spectroscopy.