(47) Controlled Release of Doxycycline and Metronidazole Using a Complexation Polymer System

Y. Zou and D.A. Puleo

University of Kentucky

Tissue infection is a homeostatic imbalance between tissue and micro-organisms. It is of great importance to deliver antibiotics effectively to kill or inhibit micro-organisms and prevent the formation of infection. Compared to the traditional approach of systemic delivery, localized controlled release of antibiotics can maintain high enough antibiotic concentration over a long duration without causing systemic toxicity. In addition, using two antibiotics can increase the spectrum of antimicrobial coverage and decrease the risk of generating resistant strains of micro-organisms. Therefore, controlled release of two antibiotics can be a viable clinical approach. In this study, the controlled release of two antibiotics [doxycycline hyclate (doxy) and metronidazole (metro)] from six-layer polymer devices was investigated. The release devices were prepared with antibiotic-loaded polymeric microspheres using a pressure-sintering process. The polymer system used here was a blend of cellulose acetate phthalate (CAP) and Pluronic F-127 (PF-127) with a ratio of 7:3. The water-acetone-oil-water (W/A/O/W) triple emulsion process was used to prepare the microspheres. The measurement of antibiotics concentrations released into the supernatant was based on the UV absorbance at two different wavelengths. As doxy and metro both have measurable absorbance at those two peaks, one effective method was established to separate the contribution of two antibiotics at each wavelength and determine their true concentrations. The method's validity was confirmed. In this study, the device continued releasing two antibiotics for seven days, except the first day because the first (top) layer contained only doxy. In addition, a valid method was established that can accurately determine concentrations of two antibiotics simultaneously in release supernatants. Future studies will focus on the system's antibacterial effects.

(46) Fabrication and Characterization of Protein-imprinted Polymers

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University of Kentucky

Molecular imprinting technique is a method to fabricate a polymeric material (molecularly imprinted polymer or MIP) with specific recognition sites on its surface in order to selectively identify specific molecules (template) through preferential binding to these recognition sites. In this work, UV free-radical polymerization was utilized to fabricate MIP. PEG600DMA, the cross-linking monomer, and MAA, the monomer, were mixed with various amounts of lysozyme, used as a model template molecule, along with ethanol and deionized water. DMPA was used to initialize polymerization. Lysozyme was removed in a digestion process by immersing the polymer in protease solution. To evaluate the preferential binding capability of MIP, lysozyme, RNase, or a 50:50 mixture of lysozyme and RNase was added to MIP. Their concentrations were measured in a similar digestion process as used during the MIP fabrication and then compared with one another. It was found that during polymerization, when the amount of lysozyme increased in a certain range, the quantity of binding sites in MIP also increased, as expected. In the evaluation of MIP binding properties, the amount of lysozyme bound to MIP increased as a function of amount of lysozyme in the polymerization solution. Meanwhile, the amount of RNase bound to MIP did not show an observable trend compared with the lysozyme binding. Tests of MIP with the competitive binding mixture of lysozyme and RNase showed less lysozyme than RNase bound when lysozyme concentration was low during MIP fabrication; however, with increasing lysozyme concentration, the MIP bound a greater amount of lysozyme than RNase. Possible explanations could be the different binding mechanisms to MIP between lysozyme and RNase; RNase appears to have non-specifically bound, while lysozyme bound mainly to the specific binding sites in MIP.

(45) Protein Immobilization on Hydroxyapatite-surface Implants to Promote Bone Formation

J. Yewle, Y. Wei, D.A. Puleo, and L. Bachas

University of Kentucky

In bone implant surgery, various materials like titanium, steel, ceramic and hydroxyapatite have been effectively used for years. Hydroxyapatite has a similar composition as that of bone, therefore it has been also applied as a coating biomaterials. However natural bone growth on the hydroxyapatite surface is difficult. In this presentation, we will discuss how to make the implants prone to bone growth. Hydrazine bisphosphonates are used as bifunctional chelates to immobilized protein on hydroxyapatite without change in protein conformation. Proteins are selectively attached to the bisphosphonates through a hydrazone linkage. Effective protein immobilization on hydroxyapatite was demonstrated using enhanced green fluorescent protein as a model protein and the immobilization was determined with fluorescence spectroscopy.

(44) Lipid-Pluronic Ultrasound Contrast Agents

R.E. Wilson and T.M. Krupka

Case Western Reserve University

The advent of microbubble contrast agents has enhanced the capabilities of ultrasound as a medical imaging modality and stimulated innovative strategies for ultrasound-mediated drug and gene delivery. While the utilization of microbubbles as carrier vehicles has shown encouraging results in cancer therapy, their applicability has been limited by a large size which typically confines them to the vasculature. To enhance their multifunctional contrast and delivery capacity, it is critical to reduce bubble size to the nanometer range without reducing echogenicity. In this work, we present a novel strategy for formulation of nano-sized, echogenic lipid bubbles by incorporating the surfactant Pluronic, a triblock copolymer of ethylene oxide co-propylene oxide co-ethylene oxide into the formulation. Five Pluronics (L31, L61, L81, L64 and P85) with a range of molecular weights (Mw: 1100 to 4600 Da) were incorporated into the lipid shell either before or after lipid film hydration and before addition of perfluorocarbon gas. Results demonstrate that Pluronic-lipid interactions lead to a significantly reduced bubble size. Among the tested formulations, bubbles made with Pluronic L61 were the smallest with a mean hydrodynamic diameter of 207.9 ± 74.7 nm compared to the 880.9 ± 127.6 nm control bubbles. Pluronic L81 also significantly reduced bubble size to 406.8 ± 21.0 nm. We conclude that Pluronic is effective in lipid bubble size control, and Pluronic Mw, hydrophilic-lipophilic balance (HLB), and Pluronic / lipid ratio are critical determinants of the bubble size. Most importantly, our results have shown that although the bubbles are nano-sized, their stability, and in vitro and in vivo echogenicity are not compromised. The resulting nanobubbles may be better suited for contrast enhanced tumor imaging and subsequent therapeutic delivery.

ABSTRACT PRESENTATIONS

Development of Biomimetic Hydrogels for Guided Endothelial Cell Morphogenesis, Organization and Vessel Formation

A.P., Y. Yuan, C. Klinge, and A.S. Gobin

University of Louisville

A major obstacle to the application of therapeutic angiogenesis is the inability to assess them *a priori* in a controlled *in vitro* environment. *In vivo* neovascularization, through both vasculogenic and angiogenic processes, involves the complex integration of biochemical signaling, microenvironment degradation and remodeling, and cellular communication. In addition, both a spatial and temporal orchestration of signals and processes have been found to exist between the cellular component and the soluble factors and the extracellular matrix (ECM) components. However, the exact mechanisms involved in these processes are currently not well understood primarily due to the lack of well controlled models. Thus, the goal of this research is to develop a biomimetic material composed of poly (ethylene glycol) (PEG) and biochemical signals to examine in a controlled environment the spatial organization and temporal sequence of angiogenesis during wound healing. In order to accomplish this goal, both vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) are being presented in a PEG hydrogel to investigate the influences on vascular cell behavior.

Endothelial cells on the surface of hydrogels with grafted VEGF (50ng/ml) or FGF (50ng/ml) were assessed for mitogenic, motogenic and apoptosis survival responses as compared to no growth factor present. It was observed that there was a significant increase in cell number over a 4 day period, a robust random migration response, and a strong apoptosis survival rate. Our results indicate that biochemical signals can be incorporated into hydrogel system to initiate vascular cell morphogenesis. Both VEGF and FGF can be covalently grafted into the hydrogel without significant reduction in its bioactivity and stability. The long term presentation of these biosignals allows us then to study angiogenesis in a controlled and tailorable 3D environment.

(43) Antioxidant Activity of Therapeutic Degradable Polymer Poly(trolox ester) to Suppress Oxidative Stress Injury in the Cells

P. Wattamwar, Y. Mo, R. Wan, R. Palli, Q. Zhang, and T. Dziubla

University of Kentucky

Oxidative stress is a pathological condition that has been implicated as a central player in a variety of diseases, including vascular and neurodegenerative diseases. More recently, oxidative stress has also been shown to be involved in the biological incompatibility of many materials, especially at the nanoscale. As such, there is a critical need for new biomaterials that can inhibit this response, improving the compatibility of medical devices. In this work, we polymerized trolox, a synthetic antioxidant and water soluble analogue of Vitamin E, to form an oxidation active polymer as a new class of biomaterial. Synthesized poly(trolox ester) polymers were formulated into nanoparticles using a single emulsion technique and their size was controlled by changing the polymer concentration in the organic solvent. Nanoparticle cytotoxicity, protective effects against cellular oxidative stress and degradation kinetics were all evaluated. Poly(trolox ester) nanoparticles were found to have little to no cytotoxicity and were capable of suppressing cellular oxidative stress induced by cobalt nanoparticles. In vitro degradation studies of poly(trolox ester) nanoparticles indicate that the antioxidant activity of nanoparticles was derived from its enzymatic degradation to release active antioxidants.

(42) Material Design Strategies for Bone and Nerve Regeneration: Controlled Physical Properties and Regulated Cell Responses

L. Cai and S. Wang

University of Tennessee

In an effort to develop suitable biomaterials with controllable physical properties for different tissue-engineering applications such as bone and nerve regeneration, we present a facile synthetic method to achieve self-crosslinkable poly(ε-caprolactone) diacrylates (PCLDAs) and triacrylates (PCLTAs). This novel method uses potassium carbonate (K_2CO_3) as the proton scavenger other than triethylamine in the literature to avoid side reactions and make purification significantly easier. Furthermore, we employ a material design strategy of combining a crystallite-based physical network and a crosslink-based chemical network together to modulate material properties and cell responses. PCLDAs with different molecular weights are blended with another crosslinkable biomaterials, poly(propylene fumarate) (PPF), to regulate the physical properties and photocrosslinking characteristics. Since different PCLDAs have different crystallinities and melting points while PPF is amorphous with a higher density of crosslinkable segments, the mechanical properties of photo-crosslinked blends can be modulated efficiently while distinctively by varying both crosslinking density and crystallinity with the PPF composition in the blends. Thermal properties such as glass transition temperature (Tg), melting temperature (Tm), and the heat of fusion (dHm) have been measured and correlated with their mechanical and rheological properties. Surface characteristics such as surface morphology, hydrophilicity and the capability of adsorbing serum protein from cell culture medium have also been examined for the crosslinked polymer disks. MC3T3 cells and Schwann precursor cell line (SPL201) have been applied to evaluate the in vitro biocompatibility of this series of polymeric networks and the roles of surface chemistry, crystallinity, and stiffness in regulating cell responses.

Protein-Based Scaffolds in Tissue Engineering Applications

J. C. Liu

Purdue University

Recombinant proteins have been explored for a number of tissue engineering applications. In particular, artificial proteins based on structural repeats of elastin and silk have been investigated for use in soft tissue and orthopedic applications. Compared to synthetic or natural materials, recombinant proteins have a number of advantages that include: exquisite control over sequence and composition; a precise molecular weight; and the modular nature, which allows for the composition of domains to be easily altered. We are developing a family of artificial extracellular matrix (aECM) proteins for tissue engineering applications such as cartilage engineering and vascularization. These proteins consist of i) structural repeats derived from elastin, resilin, or abductin to confer mechanical properties; ii) bioactive sequences to elicit desired cellular responses; and iii) lysine residues or the non-natural amino acid para-azidophenylalanine to crosslink proteins into three-dimensional scaffolds. In this work, we show that cell responses can be modulated by ligand identity, density, and context. We are currently exploring the use of these materials for stem-cell based therapies.

Polymeric Platform for Affinity-Based Drug Delivery

H. von Recum

Case Western Reserve University

While many research groups have explored the use of polymers for the controlled release of therapeutic agents, this release is typically dependent strictly on diffusion. Our research group has been exploring the use of molecular interactions between drug and polymer to change the rate of release to be substantially longer and more linear than that capable by diffusion-based drug delivery. In our research novel hydrogels made from polymers of cyclodextrin were fabricated and explored for the controlled release of antibiotics, hormones, chemotherapeutics, anti-angiogenic compounds and other small molecule drugs. This presentation will focus on recent work on the antibiotic delivery platform where beta-cyclodextrin (affinity-based) polymers were compared to control dextran polymers (whose release relies solely on diffusion mechanisms). Release from the cyclodextrin hydrogels was found to be capable of killing bacteria in vitro beyond 30 days where dextran hydrogels was unable to kill bacteria (both Gram positive and negative) beyond 15 days. This release was additional dependent on drug type (moderately hydrophilic vs. hydrophobic) and conventional parameters such as crossliniking density. These observations translated to therapeutic application in a device infection model where controlled release implants were capable of eradicating an infection of Staphylococcus aureus within 2 weeks and maintain an uninfected state beyond 4 weeks. The current clinical option, a one-time wound flush at the time of implantation, was found to show similar levels of infection to untreated controls as both 2 and 4 weeks. The result of this work is a versatile platform that can be tuned to deliver a range of therapeutics from minutes to months, and can be applied to a host of biomedical applications including hernia repair meshes, orthopedic implants, cardiovascular devices, and cancer therapies.

(41) Electrochemically Aligned Collagen Scaffolds Induce Tendon Hypertrophy In Vivo

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Purdue University

Introduction: Collagen type I has been extensively studied as a promising biomaterial for tendon tissue engineering applications. However, weak mechanical strength of the collagen constructs is a major limitation. We have previously developed and reported a novel methodology to form densely packed electrochemically aligned collagen (ELAC) threads with mechanical properties converging upon the natural tendon. In this study, we assessed the in vivo response of rabbit patellar tendon (PT) to braided ELAC bioscaffolds. Methods: ELAC was synthesized by loading dialyzed monomeric collagen solution between two electrodes. On application of an electric current for 12 hours, the collagen molecules align and form highly oriented collagen threads. Nine individual genipin-crosslinked collagen threads were braided together to form the ELAC bioscaffold. Each PT was longitudinally incised bilaterally (N = 4, New Zealand female white rabbits) from the patellar to tibial enthesis over the central axis. One limb served as the sham-operated control and the other received the ELAC bioscaffold, which was inlaid along the length of the tendon. Rabbits were euthanized four months postoperatively, the PTs were harvested and analyzed by histology. Results and Discussion: The ELAC bioscaffold did not degrade notably and exhibited limited integration with the surrounding tissue. A low-grade granulomatous inflammation was evident around the perimeter of the implant. Quantitative histology revealed that the cross-sectional areas of PTs with the implant were up to 40% larger compared to the sham-operated control. Since the implant lacked any bioinductive factors, we believe that the hypertrophic effect on the surrounding tissue was via the cytokines released by the cells present in the granulomatous core around the implant. Current work is focused on assessing the quality of the hypertrophic tissue by mechanical testing and electron microscopy. We conclude that ELAC has the potential to regenerate damaged tendons by inducing a prolonged low-grade granulomatous inflammation.

(40) Probing Protein Conformational Changes with Atomic Force Microscopy (AFM)

S. Trajkovic, S. Daunert, and Y. Cai

University of Kentucky

Conformational changes are very important for understanding various biomolecular interactions in proteins. They result from the intrinsic flexibility of protein structure. Immobilization of proteins on a surface, while still maintaining biological functionality is invaluable tool in array-based screening techniques. However, immobilized proteins often behave differently from their counterparts in bulk solution. Therefore, the development of a biosensor that is based upon conformational change in immobilized protein would provide a precious insight for a variety of applications. This work explores the conformational change of apocalmodulin (apo-CaM) immobilized on a chemically patterned surface. Calmodulin (CaM) is a small (148 amino acid residues) calcium binding protein involved in the regulation of a number of cellular signaling events. Prior to calcium binding, apo-CaM adopts a globular shape with an approximate size of 5 nm in which the helices in both EF hands are packed together. Upon calcium binding however, CaM adopts a more open conformation with the two symmetrical globular domains separated by a flexible "hinge" region that extends the protein to approximately 7 nm. Genetically engineered apo-CaM with a cysteine on the C-terminus was immobilized on the mercapto (-SH) terminated surface pattern through the cysteine-Hg-mercapto coupling. The average height of the immobilized apo-CaM was measured as 4.8 nm using AFM. After the apo-CaM pattern was incubated in calcium solution, the average height of the protein increased to 7.0 nm, indicating a conformational change of apo-CaM to CaM. The result shows that immobilization of apo-CaM on solid support does not interfere with the ability of the protein to bind calcium. This result paves the way towards the development of wide array of biosensors.

(1) Oligomer Content as an Important Design Parameter for Controlling Collagen Polymerization Kinetics and Matrix Microstructure-mechanical Properties

J. Bailey and S.L. Voytik-Harbin

Purdue University

The search is on for next generation engineered biomaterials that recreate biological and physical signaling capacity inherent to the extracellular matrix (ECM). Collagen, a biopolymer native to the ECM, represents the primary determinant of the physical features of the cellular microenvironment, making collagen biomaterials ideal candidates for biomedical applications. However, routinely used collagen formulations, which are primarily monomeric, are limited by their highly variable polymerization kinetics and low mechanical integrity. Collagen has a relatively untapped element that contributes significantly to its in vivo microstructural-mechanical properties, native covalent crosslinks between collagen molecules (i.e. monomers). Crosslinks formed in vivo serve to increase ECM mechanical stiffness and strength. In the present study, oligomer-rich and monomer-rich collagen formulations were isolated from porcine skin. Spectrophotometric-based turbidity studies showed that formulations with increased oligomeric content resulted in drastically decreased polymerization times. In addition, the resultant polymerized 3D matrices showed distinct fibril microstructures with increased mechanical stiffness and strength when tested in shear and compression formats. These findings demonstrate that oligomer content is an important design parameter that can effectively be manipulated to precision tune polymerization potential of engineered collagen constructs allowing for the optimization of polymerization rate and mechanical signals while maintaining biological signaling.

(2) Development of Antioxidant Polymeric Nanoparticles for the Suppression of Doxorubicin mediated Vascular Chemotoxicity

K. Baldridge, P. Wattamwar, K. Anderson, and T. Dziubla

University of Kentucky

The therapeutic window of chemotherapeutic agents is limited by the toxic side effects caused by the non-specific accumulation of drug in non-cancerous tissue. While active targeting can reduce this effect, even the best targeting systems still have >80% of the delivered drug distribute to healthy tissue. As such, concomitant suppression of toxicity in healthy tissue with active targeting of chemotherapeutics may provide a means of improving treatment outcomes by allowing greater doses of chemotherapeutic to be used. As such, a means of selectively targeting healthy tissue is needed; nanoparticles provide a way of achieving this control. Doxorubicin (DOX) is one non-specific chemotherapy agent. It is cytotoxic via three mechanisms:1) intercalation into a cellâ€™s DNA, 2) interference with Topoisomerase II, causing double strand breaks in the DNA, and 3) generation of reactive oxygen and nitrogen species (ROS and RNS) that damage the cell. By nature, cancer cells reproduce more rapidly and are thus more susceptible to mechanisms 1 and 2. Non-cancerous cells, however, are affected mostly by the third mechanism. The hypothesis of this study is that antioxidant polymeric nanoparticles (AoP NPs) delivered concurrently with DOX will provide protective effects effect against DOX toxicity in non-cancerous cells. To evaluate this hypothesis, a series of antioxidants are evaluated as chemoprotectants against DOX cytotoxicity in Human Umbilical Vein Endothelial Cells (HUVEC). In addition, the effects of free (non-polymeric) antioxidants delivered with DOX were studied: Trolox (a water-soluble form of vitamin E), eugenol (an antioxidant naturally occurring in cloves) and liposomal vitamin E (vitamin E loaded into phospholipid micelles).

(39) IGF-I Releasing PLGA Scaffolds for Growth Plate Regeneration

S.k. C. Sundararaj, R.D. Cieply, T.A. Milbrandt, and D.A. Puleo

University of Kentucky

Growth plate is highly organized cartilaginous tissue found at the end of long bones and is responsible for longitudinal growth of the bones. Growth plate fracture is one of the major types of bone injuries occurring in children. Growth plate fracture leads to retarded growth and unequal limb length, which might have a lifelong effect on a person’s physical stature. The methods that are being currently used to treat growth plate fractures do not regenerate the injured growth plate or its functions. This research is a tissue engineering approach for the treatment of growth plate injury. Low molecular weight, acid terminated poly-(lactic-co-glycolic acid) in the form of biodegradable porous scaffolds was used as drug delivery vehicles in this study. Insulin-like growth factor I (IGF-I), which can stimulate cartilage formation was encapsulated within PLGA microspheres by double emulsion method. IGF-I microspheres were mixed with salt and made in the form of porous scaffolds by pressure sintering and leaching. The release profile of the IGF-I from the PLGA scaffold showed a biphasic release pattern. In vitro studies were done by seeding rat bone marrow cells (BMCs) on the top of IGF-I encapsulated PLGA scaffold and the effects of released IGF-I was studied. The final in vivo studies were conducted by creating growth plate injury and implanting scaffolds in the anterior side of the tibia of the New-Zealand white rabbits. Following radiographic imaging, bone angle measurements were made to observe the changes during the course of the study. At the end of the study, the animals were euthanized, and bones with implant were harvested for histology. The tissue sections obtained showed regeneration of cartilage, albeit with disorganized structure, at the site of implantation. This work will be a significant step towards tissue engineering of growth plate cartilage.

(38) Modulation of Hydroxyapatite Nanocrystal Growth and Morphology by Polyelectrolytic Peptides and Its Potential In Vivo Applications

J. Diegmueller, X. Sun, X. Cheng, and O. Akkus

Purdue University

Interactions of synthetic peptides containing charged amino acids with hydroxyapatite (HAP) have been used as models of noncollagenous proteinsâ€™ interaction with apatite crystals in bone. While there is some knowledge on the affinities of these peptides with HAP, the effects of the type of charged peptides, the influence of their molecular weight and concentration on the crystal has not been systematically investigated. In this study, four types of charged peptides, poly-arginine (Arg), poly-aspartate (Asp), poly-glutamate (Glu), and poly-lysine (Lys), were evaluated on their affinity to HAP as well as their effects on mineral nucleation/growth kinetics and the resulting crystal size and shape. Negatively charged peptides (poly-Asp, poly-Glu) were shown to have greater affinity for HAP than positively charged peptides (poly-Arg, poly-Lys), and higher molecular weight (HMW) of peptide further increased such affinity. Negatively charged peptides at low concentrations also promoted higher yield of crystals with greater crystallinity, but such effects were inhibited at high concentrations. Lastly, the addition of different types of peptide all decreased the dimensions of the HAP crystals formed compared to control. Poly-Asp, poly-Glu, and poly-Lys were also found to significantly affect the crystal morphology by increasing the aspect ratio of resulting crystals. Thus, these results suggest that polyelectrolytic peptides can be used for surface coating of orthopaedic implants to promote their anchorage to the mineral phase of neighboring bone tissue. Moreover, combined with bone apatite crystal isolation and characterization techniques, these polyelectrolytic peptides might be shown in following animal studies to be promising candidates for regulation of growth and morphology of apatite nanocrystals in vivo to improve the mechanical properties of bone, which can serves as a potential treatment to certain bone-weakening diseases, such as osteoporosis.

(3) Peptide Mediated Synthesis of Catalytically Active Pd Nanowire Networks

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Peptides are known to mediate the production of an array of inorganic materials on the nanoscale; however, the final materials are typically limited to spherical nanoparticles ranging from 2 - 150 nm. Altering the shape of the nanostructure can fundamentally change both the physical and electronic properties of the material to impart a higher level of functionality, especially for catalytic purposes. Using environmentally friendly reaction conditions, we have been able to produce Pd nanowire networks (NWNs) in the presence of the R5 peptide, which is able to self-assemble and act as a template for shaped nanomaterials synthesis. Pd^{2+} ions are able to bind to the lysines of the peptide, which sequester the metal ions within the biological framework. Upon reduction with $NaBH_4$, production of the linearly branched Pd NWNs is achieved and directed by the sterics of the system. The metallic materials were characterized by UV-vis spectroscopy, high-resolution transmission electron microscopy, and diffraction methods. The NWNs were exceedingly stable in the ambient environment and did not precipitate even after two months. The Pd NWNs demonstrated a high degree of catalytic reactivity for C-coupling reactions via Stille coupling. Using 0.50 mol% Pd, quantitative product yields were achieved in 24.0 h with a turnover frequency of 430.0 ± 9.8 mol BPCA (mol Pd.h)$^{-1}$. Such studies demonstrate the possible capabilities achieved by using peptide-mediated materials syntheses where final functional products are achieved that operate under bio and eco friendly conditions.

(4) Poly(ethylene glycol)-grafted Poly(propylene fumarate) Networks for Regulating Surface Physicochemical Characteristics and MC3T3 Cell Behavior

L. Cai and S. Wang

University of Tennessee

Injectable material, poly(propylene fumarate) (PPF), was modified by photo-crosslinking with methoxy poly(ethylene glycol) monoacrylate (mPEGA) at seven mPEGA compositions of 0% to 30%. We examined the bulk and surface physicochemical properties and their roles in influencing mouse MC3T3 cell adhesion, spreading, and proliferation. Unlike PPF crosslinked with PEG dimethacrylate, PEG dangling chains on the surface of crosslinked PPF did not influence the swelling ratio in water significantly while increased surface hydrophilicity greatly. As expected, surface friction coefficient and the capability of adsorbing proteins from cell culture medium were found to decrease continuously with increasing the mPEGA composition. However, MC3T3 cell responses to the modified surfaces did not follow the trend when the mPEGA composition was less than 7%. We found that MC3T3 cell attachment, spreading, and proliferation reached maximum at the mPEGA composition of 5-7%. Bulk properties such as thermal and rheological properties of uncrosslinked PPF/mPEGA blends and mechanical properties of photo-crosslinked PPF/mPEGA blends were also investigated and correlated with surface characteristics to elaborate on modulation of cell responses through controlled material properties. Besides revealing that PEG dangling chains may enhance cell responses by increasing hydrophilicity when their fraction on the hydrophobic surface is small, the present study also offers a new method of improving the wettability and performance of the scaffolds made from this widely used bone-tissue-engineering material, PPF.

(37) Use of Diagnostic Ultrasound to Noninvasively Characterize In Situ Forming Polymer Implants

L. Solorio, B.M. Babin, R.B. Patel, J. Mach, and A.A. Exner

Case Western Reserve University

In situ forming drug delivery systems provide a means by which a controlled release depot can be physically inserted into a target site without the use of surgery. The release rate of drugs from these systems is often related to the rate of implant formation. Currently, only a limited number of techniques are available to monitor phase inversion, and none of these methods can be used to visualize the process noninvasively. In this study, diagnostic ultrasound was used to visualize and quantify the process of implant formation in a phase inversion based system. Implants comprised of three different molecular weight poly(lactic-co-glycolic acid) (PLGA) polymers dissolved in 1-methyl-2-pyrrolidinone (NMP) were studied. The implants were encapsulated in a 1% agarose tissue phantom for five days. Quantitative measurements of the gray-scale value, swelling, and precipitation were evaluated using image analysis techniques, showing that polymer molecular weight has a considerable effect on the swelling and formation of the in situ drug delivery depots. This study demonstrates, for the first time, that ultrasound can be used to noninvasively and non-destructively monitor and evaluate the phase inversion process of in situ forming drug delivery implants.

(36) PEG-Fe_3O_4 Hydrogel Nanocomposites for Combined Chemotherapy and Hyperthermia Treatment of Cancer

J. Shapiro, S. Meenach, J.Z. Hilt, and K. Anderson

University of Kentucky

Cancer is a leading cause of death worldwide. Chemotherapy and radiation are conventional cancer treatments that are still limiting for some types of cancer despite their widespread use. Hyperthermia, the heating of cancerous tissues to 40-45°C, has shown to increase the efficacy of both chemotherapy and radiation. For example, hyperthermia treatment has been shown to increase the efficacy of paclitaxel, a chemotherapeutic that disrupts mitosis. We hypothesize that iron oxide hydrogel nanocomposites can be used for a dual-therapy application to treat cancer. Hydrogels composed of the macromer poly(ethylene glycol) (PEG) methyl methacrylate and crosslinker poly(ethylene glycol) dimethacrylate were fabricated with iron oxide nanoparticles incorporated in the hydrogel matrix. These nanoparticles can be remotely heated by an alternating magnetic field (AMF), thus producing the hyperthermia effect from the hydrogel nanocomposite. When imbibed with paclitaxel, the hydrogels have the potential to provide synergistic heating and chemotherapy in a local area. Due to the temperature-responsive nature of PEG, swelling analysis indicated an inverse relationship between temperature and volume swelling ratio (Q), and between crosslinking density and Q. AMF heating of the hydrogel nanocomposites indicate higher crosslinked hydrogels exhibit a greater DT, supporting previous studies that show gels with lower Q values heat higher due to higher iron oxide mass to gel volume ratio. Concerning paclitaxel release studies, hydrogels with lower crosslinking densities exhibited an overall faster release due to increased drug effective diffusivity. It was also shown that hyperthermia treatment increases cytotoxicity for M059K glioblastoma. At the analyzed concentrations, paclitaxel did not significantly decrease cell viability, however, changes in cell morphology indicated cytotoxic potential. At this time, studies to prove the hypothesis that increased cytotoxicity will result from combined treatment are in progress. In conclusion, PEG-iron oxide hydrogel nanocomposites have the ability to remotely deliver hyperthermia treatments, allowing potential use in combination therapies.

(5) Phase Inversion of Spherical PLGA Implants

A. Carlson

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Poly Lactic-co-Glycolic Acid (PLGA) is a biocompatible degradable polymer that can be used as an in situ forming drug delivery vehicle. PLGA has both hydrophobic and hydrophilic sections and when placed in water, it forms a spherical implant which contains the deliverable drug inside the center, to be dispersed as the implant degrades. This polymer combined with fluoroscein which is used as a “mock drug”, NMP (N-Methyl-2-pyrrolidone) and pluronic P85 make up the implant solution used for the following phase-inversion experiments.

With phase inversion, it is necessary to quantify the amount of water that is needed for the implant to phase invert fully so as to know what environments the drug delivery implant can be used in within the body. The implant solution was injected into vials containing various concentrations of water and NMP ranging from 0% to 100% water. The implants were allowed to form at 37C, then freeze dried in order to quantify the amount of actual implant that had phase-inverted without the added mass of the NMP/water solution. The effects of varying PLGA molecular weight as well as varying pluronic P85 amount in the implant solution on phase inversion were studied. The implant solutions with the lower molecular weight PLGA polymers phase-inverted the fastest, while implant solutions with higher molecular weight polymers never reached 100% phase-inversion. Pluronic was varied from 0% to 5% and the results showed that the implant solutions with the smaller amounts of pluronic phase-inverted more quickly than the solutions with higher pluronic concentrations. These results will aid others in their choices for proper concentrations in drug delivery implant solutions.

(6) PC-μCP Synthesized Environmentally Responsive Hydrogel Nanocomposites over Biomedical Devices

H.D. Chirra

University of Kentucky

The ability to incorporate responsive hydrogels at the micro- and nanoscale has resulted in a wide variety of applications in the biomedical field. Hydrogel nanocomposites can be tailored to exhibit unique properties due to the combination of the nanoparticle system and the response property of the hydrogel, thereby expanding their potential applications in the diagnostic and therapeutic field. Herein, polymerization controlled by micro contact printing (PC-μCP) was applied to synthesize micro- and nanostructured environmentally responsive hydrogel composites (e.g. crosslinked N-isopropyl acrylamide matrix with gold nanoparticles). XY control of the hydrogels was achieved using microcontact printing, and the Z/thickness control was achieved using a variety of polymerization techniques such as UV photopolymerization and atom transfer radical polymerization (ATRP). The responsive behavior of the hydrogels to external stimuli was characterized using optical microscopy, UV-vis spectroscopy, atomic force microscopy (AFM), and scanning electron microscopy (SEM). Using PC-μCP based techniques, it is possible to synthesize controlled responsive hydrogel nanocomposites for potential applications in the biomedical field.

(35) A Portable, Reagentless Potassium Sensor Capable of Real Time, in Vivo Detection and Monitoring.

D. Scott, L. Moschou, and S. Daunert

University of Kentucky

Potassium plays a critical role in many physiological processes, thus the ability to monitor the potassium levels of biological fluids in real time would greatly enhance diagnostic and therapeutic capabilities in many clinical settings. To that end a reagentless, portable, fiber optic detection system has been developed. The sensor utilizes a potassium ionophore and a proton chromoionophore contained in a lipophilic optode membrane to monitor changes in potassium concentration. The sensing membrane has been integrated with an optical fiber by depositing the precursor solution on the tip of the fiber, allowing the sensing system to be incorporated with a catheter to allow for in vivo monitoring at the point of interest. The sensor responds to changes in potassium concentration selectively, reproducibly and reversibly with a fast response time of one minute. The sensor would be suitable for a variety of clinical applications desiring the detection and monitoring of in vivo potassium.

(34) Controlled Cell Adhesion Properties of Silicate Cross-linked PEO Nanocomposites

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Purdue University

We investigate the structural, mechanical and biological properties of nanocomposite films made from poly(ethylene oxide) (PEO), which is physically cross-linked with silicate nanoparticles. We present tunable mechanical properties ranging from extensible tough to brittle and find that the mechanical strength of the nanocomposites is proportional to the silicate concentration. Crystallization of PEO is suppressed with increased silicate concentration due to a decrease in the number of free PEO chains capable of rearranging to form crystallites. Polymer crystallization can be related to the adhesion of NIH 3T3 fibroblast cells. Moreover, adhesion of fibroblasts strongly depends upon the silicate concentration, as cells do not adhere to unmodified PEO surfaces. Crystalline regions within films, which contain areas of high PEO concentration, become amorphous upon immersion in cell culture media and further prevent cellular adhesion. Cell viability remains unchanged, regardless of the nanocomposite composition. The properties of these nanocomposites are suitable for applications that require controlled cell adhesion and the bioglass characteristics added by the silicate nanoparticles can be used for the repair of bone defects.

(7) Effects of Pore Size on Degradation Rate and Modulus of Poly(lactic-co-glycolic acid) Scaffolds Before and During Degradation

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Poly(lactic-co-glycolic acid) (PLGA) has been commonly used as a scaffold for tissue engineering because of its desirable biocompatibility, biodegradability, and drug delivery options. PLGA properties can be tailored for specific applications, such as regeneration of the growth plate following injuries. Currently the treatment for growth plate injury is use of a filler in place of the growth plate, containing no cartilage regeneration properties, leading to stunted growth or deformation. The success of the PLGA scaffold depends on several properties, including the rate of degradation, compressive strength, pore size and interconnectivity of pores for tissue ingrowth. However, these properties are codependent and require a unique combination to achieve the necessary conditions for implant success. This study focused on how varying the size (150 versus 250 μm) affects degradation and compressive modulus, both dry and wet, of porous PLGA scaffolds being developed for growth plate tissue engineering. The varying pore size concept comes from the different cell sizes and distributions that exist in the growth plate and what would be adequate to allow for ingrowth of tissue. Degradation affected pore morphology and reduced differences in compressive properties of PLGA scaffolds having dissimilar initial pore sizes. However, the degradation rates were not significantly different as measured using a nondestructive method. It was found that scaffolds with 150 μm pores initially had a higher compressive modulus, but five days into degradation, modulus of scaffolds with 250 μm pores size was significantly larger. Understanding the inter-relationships between mechanical properties, architecture, and degradation will be useful for creating scaffolds appropriate for growth plate tissue engineering. Additional work is being done using gradient scaffolds, which show similar degradation to uniformly sized scaffolds and need to be further tested to determine if there is any significant effect of mimicking the structural variation seen in the native growth plate.

(8) Biocompatibility Enhancement through Surface Adsorbed Antigenic Disguise Protein Tp0483 and Fibronectin

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Protein involvement is prominent in most immune responses. For example, in blood clotting adsorbed proteins undergo a conformation change resulting in thrombosis. Another example is phagocytosis where macrophages bind to common antigens found on the surface of harmful microorganisms and also secrete factors that coat foreign bodies allowing the cells to engulf and eliminate them. To address these issues the development of more biocompatible materials is essential. One possibility lies with antigenic disguise proteins. The ability of a microorganism to conceal itself and avoid detection in the body can be referred to as antigenic disguise. Treponema pallidum is one example that can remain dormant for many years. This property is thought to come from several surface proteins that bind host fibronectin (FN) and use it to shield the bacteria. This research focuses on a fragment of one of these proteins, Tp0483. The effect of Tp0483 with and without FN on the adsorption of common plasma proteins was examined. Tp0483/FN interactions were observed on COOH functionalized self-assembled monolayers (SAMs) on gold surfaces. The hemocompatibility of Tp0483 and Tp0483/FN layers was quantified by observing the binding of vitronectin (Vn), fibrinogen (Fg), and human serum albumin (HSA) using surface plasmon resonance (SPR). Proteins bound less to Tp0483 and Tp0483/FN than the unmodified surface and Tp0483/FN bound less than Tp0483 for all proteins studied.

(33) Hydrogel Nanocomposites: Heating Analysis, Modeling, and Simulations

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Hydrogels and hydrogel nanocomposites have been developed for a variety of biological applications including drug delivery, sensors and actuators, and hyperthermia cancer treatment. In previous studies, magnetic hydrogel nanocomposites of N-isopropylacrylamide (NIPAAm) have been demonstrated as pulsatile drug delivery systems and as microfluidic actuators with alternating magnetic field (AMF) at 293 kHz as a trigger. In order to achieve desired response from hydrogel nanocomposite system, it is very important to understand the heating induced by application of AMF and the resultant change in the hydrogel properties. The goal of this study was to analyze and predict the nanocomposite response based on AMF strength, nanoparticle loadings, hydrogel composition, and nanocomposite dimensions. Magnetic nanocomposites of poly (ethylene glycol) hydrogels were synthesized with iron oxide (Fe_3O_4) nanoparticles. Different nanocomposite systems were obtained by variation of nanoparticle loadings and hydrogel composition. AMF at 293 kHz was applied to the nanocomposite discs, and the surface temperatures were analyzed using infrared (IR) thermography. A heat transfer model was developed to account for the heat generation due to AMF as well as heat loss to surroundings. By fitting the temperature data, a correlation was obtained for the dependence of the heat generation on nanoparticle loadings and AMF strength. It was demonstrated that the model could successfully predict the resultant temperatures by using a hydrogel system with different swelling properties. COMSOL, a finite element modeling software, was used in the bioheat equation mode to predict the resultant nanocomposite temperatures. The effect on surrounding tissue temperatures was also analyzed for hyperthermia applications.

(32) Hemostatically Active Liposomes as Synthetic Platelet Substitutes

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Platelet transfusion plays a major role in therapeutic and/or prophylactic management of hemostasis in patients with hematologic and oncologic platelet disorders such as primary thrombocytopenia or chemo/radiotherapy-induced myelosuppression. The current clinical therapy of allogeneic platelet concentrates suffers from several risks such as febrile non-hemolytic transfusion reactions, infections, alloimmunization-induced refractoriness, and the possibility of transfusion-associated immunosuppression. The complex platelet-harvesting, processing, and storage methods, and the short shelf-life (5-7 days) of allogenic platelet concentrates further contribute to shortages in supply. Hence, designing a synthetic platelet substitute that can mimic platelet hemostatic biofunctionalities and can simultaneously provide advantages of large scale preparation, reproducible quality, long storage life, and absence of biologic infections, can have significant clinical benefit. The two most important hemostatic properties of platelets are (1) stable adhesion to specific matrix proteins (e.g. collagen and vWf) under physiological shear and (2) aggregation via fibrinogen-mediated inter-platelet bridging. Both functions require unique synergistic ligand-receptor interactions, and mimicking these interactions on a liposome platform provides a way to develop a synthetic platelet substitute. With this rationale, we are developing liposomes conjugated to a fibrinogen-mimetic peptide on its surface that can bind to GPIIb-IIIa receptors on other activated platelets (for the purpose of inter-platelet bridging), and a GPIbÎ± protein fragment that can bind vWf (for the purpose of matrix adhesion). The fibrinogen-mimetic peptide is developed by solid-phase peptide chemistry and the GPIbÎ± protein fragment is developed by recombinant methods in Chinese Hamster Ovary (CHO) cells. Our current research is directed at integrating these two properties on a liposome surface to study (a) liposome adhesion on vWf-coated surfaces under shear and (b) liposome-mediated aggregation of activated platelets. We envision that these hemostatically active liposomes can be potentially applicable for the treatment of thrombocytopenia and other bleeding disorders.

(9) Integrated Microchip Based Biosensor for Drug Delivery

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A controlled drug delivery system in which drug release is achieved by electrochemically actuating an array of polymeric valves on a set of drug reservoirs has been developed. For some patients it's extremely important to have a continuous monitoring of their physiological levels (for example, glucose levels in diabetic patients). In this work we developed polymer/gold microvalves, which can be reused and are suitable for both sensing and delivery. The system consists of an integrated biosensing element (for example, one for glucose based on. glucose oxidase) immobilyzed on a hydrogel deposited on an electroactive thin film formed by electrochemical polymerization of polypyrrole (PPy) on gold electrode surface. The properties of PPy allowed, by application of a potential, to open/close the valve of a device. Polypyrrole (PPy)-based microactuators hold a promise for a wide variety of engineering applications from robotics and microassembly to biosensors and drug delivery systems.

Enzymatic biosensor typically can work for several days before it deteriorates and should be replaced by a fresh biosensor. Our approach is to use an array of biosensors protected in cavities covered by individually-addressed PPy/Au valves. Protected biosensors do not deteriorate and can be stored for many months. The biosensors are opened sequentially; once the working biosensor starts to deteriorate, the fresh biosensor can be activated on-demand by opening the corresponding protected cavity. The microvalve lids are opened by the application of 1V bias. Thus, in-vivo biosensor platform operation can be extended from days to months; the platform lifetime is only limited by the number of biosensors in the array. An array of about 90 covered reservoirs is required for six months of continuous operation (if a fresh biosensor is activated every 48 hours). Such an array can be created on a chip of only several cm.sq since each protected cavity is only 200 microns in diameter. We will discuss sensor immobilization and initial test results. The proposed integrated protection system holds promise for implantable biomedical devices.

(10) Haloacid Bioremediation using a Hydrogel Encapsulated Thermophilic Enzyme

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Halogenated compounds have found numerous applications as pesticides, fungicides, and organic solvents. These compounds, however, are extremely hazardous environmental pollutants because of their organic composition, which causes them to be a persistent presence in nature. Dehalogenases are enzymes capable of breaking carbon-halide bonds, thus can be employed in the bioremediation of these halogenated environmental pollutants. Recently we have isolated and characterized such an enzyme from the thermophilic bacteria Sulfolobus tokodaii. Specifically, this enzyme belongs to the L-2-haloacid dehalogenase (L-2-HAD) family and has been shown to dehalogenate organochloride compounds at a pH range of 4.0 - 10.0 and at temperatures between 25 and 80 Â°C. The current goal of the work is to incorporate the enzyme into hydrogels with iron oxide nanoparticles and investigate their use in conjunction with a magnetic field as a remotely controlled bio-remediation system.

(31) Hemocompatibility Analysis of Biomedical Polymers

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Hemocompatibility implies favorable interaction between blood and materials in blood-contacting biomedical devices of which many are made of polymers. Blood clotting on the device surface reflects poor hemocompatibility and impairs performance and safety. Clotting involves adhesion, activation, and aggregation of platelets on the material surface and subsequent occurrence of the coagulation cascade, leading to a crosslinked fibrin mesh arresting aggregated platelets and other blood components. Hence, it is important to study platelet adhesion, activation, and coagulation processes on material surfaces in vitro, with human blood, in a dynamic flow environment to determine suitable materials for device applications. To this end, we are studying the adhesion and activation of platelets on biomedical polymers exposed to human blood at physiological shear stress ranges using a rotating disk system. We have obtained surface images using epifluorescence and scanning electron microscopy, and we have used Image J to quantify surface-adhered and surface-activated platelets at various shear stress values. Additionally, we have looked at the activation of the coagulation cascade on various surfaces, by monitoring the conversion of Factor X to Factor Xa and pro-thrombin to thrombin, using chromogenic assays. Our results provide critical insight into the hemocompatibility of several polymers relevant to biomedical applications.

(30) Mucoadhesive Patches Delivering Imiquimod for Treatment of Oral Dysplasia

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Imiquimod is an immune response modifier that strengthens the immune response against infected cells and basal and squamous cell carcinomas. Although it may be useful for treating oral mucosal dysplasia, it is not commonly applied in the oral cavity for lack of an appropriate delivery method. The aim of this study was to design mucoadhesive films that can act as useful vehicles for imiquimod delivery. However, the hydrophobic nature of imiquimod makes homogeneous distribution challenging. This study compared drug release and uniformity in three possible formulations of imiquimod-loaded mucoadhesive films and films with differing ratios of film-forming and mucoadhesive polymers. In the first method, sonication was used to disperse imiquimod in the polymer solution. In the second, linoleic acid was used to enhance the solubility of imiquimod in the solution. In the third, imiquimod was complexed with hydroxypropyl-β-cyclodextrin (HPβCD) with a co-evaporation process. Substantial differences in imiquimod release between samples formed by sonication suggested nonuniformity of the films, which show the method to be in sufficient. Even though linoleic acid increased solubility of drug and its uniformity of distribution, variable fluorescence readings suggested interactions between drug and linoleic acid. In contrast, HPβCD was successfully used to establish more uniform distribution of the drug throughout the mucoadhesive film without any compromise of patch properties. Unlike 2:1 PVP:CMC films, sustained release was obtained from 1:2 PVP:CMC films, which is more desirable for treatment of precancerous lesions. Degradation studies showed that while release patterns for 1:2 patches were governed mostly by erosion, release from 2:1 films was determined by both mucoadhesive erosion and diffusion of imiquimod from the material. The sustained release and homogeneous distribution of drug in cyclodextrin complexed 1:2 PVP:CMC films made them as best device for treating precancerous lesions.

(11) Mineralized Bone Particle/Polyurethane Composites for Bone Tissue Engineering

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The healing of bone is a multifaceted process that includes the restoration of both anatomy and physiology, and there are numerous biomaterials available to aide in bone healing. Typically, such biomaterials incorporate interconnected pores to facilitate bone formation. However, the initial strengths of these materials are not adequate for weight-bearing applications. We are synthesizing a two-component mineralized bone particle (MBP)/polyurethane (PUR) composite system suitable for bone tissue engineering. These MBP/PUR composites are non-porous and are comprised of 79 wt%(67.9 vol%) MBP (100-500 microns in size), providing a percolated, osteoconductive pathway for cellular infiltration. In this study, we have manipulated the molecular weight of the polyol used to synthesize MBP/PUR composites as well the surface chemistry of the MBP. We have characterized the MBP/PUR composites as well as studied its remodeling capability in vivo. SEM micrographs show the exposure of collagen fibrils on the surface of SDBP, while XPS shows a decrease in both calcium and phosphorus peaks. Strengths of 165 and 112 MPa were achieved for composites synthesized from 300 and 600 g/mol polyols, respectively. Histology shows that MBP/PUR composites are biocompatible without interruption to the normal bone healing process including the onset of new bone formation. MBP/PUR plugs synthesized with higher molecular weight triols exhibited greater allograft bone resorption allowing for greater cellular infiltration into the implant cavity. The data from these studies suggests that nonporous MBP/PUR composites are promising biomaterials for bone tissue engineering. A particulated phase of allograft bone particles allows cellular infiltration as the bone is resorbed. Mechanical properties can be tuned by manipulating the polyol MW. Also, preliminary histomorphometry suggests that the rate of bone particle resorption/celluar infiltration can be controlled by polyol MW. There was a modest amount of new bone formation in the implant cavity. However, MBP/PUR composites could be a suitable vehicle for the delivery of angiogenic and osteogenic growth factors. Growth factors such as rh-BMP2 would be an ideal candidate to balance resorption and re-mineralization.

(12) Mathematical Modeling of a Tunable Drug Delivery Platform Based on High Affinity Molecular Interactions

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Mathematical modeling plays an important role in the design of biomaterials for tissue engineering and drug delivery by identifying key release mechanisms and parameters. Our group has developed a model for an affinity-based drug delivery system using Î²-cyclodextrin. Due to its unique physical properties, Î²-cyclodextrin has been widely used to form inclusion complexes with hydrophobic molecules such as antibiotics, hormones, chemotherapeutics, and other small molecule drugs. Using this platform we have demonstrated the capacity to offer a defined release rate by binding the drug to a single cyclodextrin pocket, or we can achieve exponential decrease in release rate by modifying the drug complex allowing it to bind to multiple cyclodextrin pockets. This could be particularly useful for increasing the therapeutic lifetime of drug delivery implants and limiting the need for multiple implants in the treatment of slow-growing tumors such as prostate cancer. With our mathematical model we are exploring the transport processes underlying the loading and release of drug from our affinity-based drug delivery system. Dimensionless governing equations are developed to model two key processes: diffusion of the drug and the association and dissociation of drug from cyclodextrin. Three parameters are identified as key candidates for affecting loading and release kinetics. Lastly computer simulations are generated for loading and release profiles, as well as release rate and total mass of the drug released. The results from our simulations are then compared to experimental loading and release profiles to evaluate our modelâ€™s validity.

(29) Effects of Surface Composition on the Mechanical Properties and Biocompatibility of both Polyurethane/Allograft Bone and Polyurethane/Tricalcium Phosphate Composite Cements

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Injectable polyurethane cements offer a minimally invasive solution to treat bone defects. While these materials biodegrade to non-cytotoxic compounds, support the attachment and proliferation of cells, and integrate with host tissue, their mechanical properties are lower than those of allograft bone. To enhance the mechanical properties, composite cements comprising a mineralized particle phase and a polyurethane binder were synthesized and characterized. Five different fillers were investigated: defatted mineralized bone particles (DFMBP), surface demineralized bone particles (SDMBP), β-tricalcium phosphate (TCP), TCP with adsorbed polyethylene glycol on the surface (TCP-PEG), and TCP with grafted polycaprolactone on the surface (TCP-PCL). The polyurethane (PUR) was obtained from the reaction of a lysine triisocyanate-PEG prepolymer with a poly(ε-caprolactone-co-DL-lactide-co-glycolide) triol in the presence of an amine catalyst. Cements with filler composition between 0.45 and 0.60 vol% were prepared and their surface composition, thermal, and mechanical properties were analyzed. The compressive properties of PUR/DFMBP and PUR/TCP composites were relatively independent of composition at volume fractions below 0.55, and decreased dramatically at higher volume compositions. Despite the differences in surface composition between DFMBP and SDMBP, there were no significant differences in compressive properties between the PUR/DFMBP and PUR/SDMBP composites. In contrast, the compressive modulus of PUR/TCP-PCL composites increased compared to those of the PUR/TCP material, suggesting an increase in reactivity of TCP by grafting PCL on its surface. Attachment and viability of MC3T3 cells on the composites have been measured using a Live/Dead kit, while cell proliferation and differentiation will be assessed using a CyQuant assay kit and the measurement of Alkaline Phosphatase levels, respectively. These results will be used to assess the biocompatibility of the composite cements.

(28) Polymeric Micelles for Tunable Release of Doxorubicin in Cancer Tumors

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Purpose: To develop the groundwork of a drug delivery system that can achieve concurrent and sequential delivery of multiple drugs to tumor sites while minimizing toxicicity and maximizing efficacy. Methods: Ring-opening polymerization of Î²-benzyl L-aspartate N-carboxy anhydride (BLA) was conducted by using a-methoxy-w -amino-poly(ethylene glycol)(PEG) as a macroinitiator in anhydrous DMSO at 45ÂºC for two days. By increasing the amount of BLA, three compositions of block copolymers were synthesized containing 5, 15, and 35 pBLA repeating units. This provided PEG-poly(β-benzyl L-aspartate) block copolymers whose side chains were deprotected using 0.1 N NaOH making poly(ethylene glycol)-poly(aspartic acid) block copolymers [PEG-p(Asp)]. PEG-p(Asp) was chemically modified with spacers (e.g. glycine methyl esters (Gly) and methyl 4-aminobenzoate (Abz)) using HBTU in DMF at 40ÂºC overnight. The methyl esters of the spacers were replaced with hydrazide (Hyd) making PEG-p(Asp-Gly-Hyd) and PEG-p(Asp-Abz-Hyd) block copolymers. In DMSO, at 30ºC these block copolymers were conjugated with doxorubicin (DOX) containing a ketone group through the hydrazone linkage to achieve pH-sensitive release of each drug with differential hydrolysis rates at the acidic intratumoral or intracellular environment (pH 4-6.8). Block copolymer-drug conjugates were used to prepare polymer micelles using dialysis. Block copolymers were characterized with 1H-NMR. Size distributions of the micelles were analyzed by dynamic light scattering (DLS) measurements. Results: 1HNMR analysis provided confirmation of each step of the synthesis process. Six different block copolymers were synthesized consisting of 12kDa PEG and pASP (5, 15, and 35 repeating units) with Gly or Abz conjugated were synthesized, which were in turn conjugated with DOX. This was confirmed using UV-VIS spectroscopic analysis at 480nm. DLS analysis showed that polymeric micelles ranged between 50-100 nm. Conclusion: A collection of six micelle-forming block copolymers, possessing drug binding linkers with different hydrolysis rates became available for further in vivo development of effective combination cancer chemotherapy.

(13) Poly(ethylene oxide)-silicate Cross-linked Bio-nanocomposite with Incorporated Chitosan for Bone Repair

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The development of biomaterials for bone repair has recently focused on bioactive nanocomposite materials that can induce favorable cellular responses. Considerable research interest in polymer nanocomposites arises from the possibility to synergistically combine the properties of individual biomaterials. Here we investigate the physical and biological properties of a series of silicate cross-linked poly (ethylene oxide) (PEO) nanocomposites containing chitosan. Addition of chitosan results in the formation of ionic complexes within the physically cross-linked networks. Chitosan not only improves the mechanical strength and physiological stability of the nanocomposites but also retards the release of encapsulated protein. In vitro degradation/dissolution of nanocomposite films was accelerated by enzyme-mediated degradation of chitosan. Moreover, addition of chitosan results in enhanced adhesion and spreading of preosteoblast cells. All of the nanocomposite films support cell proliferation and exhibit high cell viability during in vitro culture. The bioactivity of the nanocomposites was evaluated by analyzing apatite formation on their surfaces. These results indicate that nanocomposites containing a combination of chitosan/PEO/silicate hold great promise for bone repair.

(14) Multicenter Trial of CPE for Maxillofacial Prosthetics

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A controlled, randomized, prospective, double-blind, single crossover, multicenter Phase III clinical trial was performed to determine the noninferiority of thermoplastic chlorinated polyethylene elastomer (CPE)(experimental) compared to silicone rubber (80% Silastic Adhesive A/20% MDX4-4210A)(control). CPE is much less expensive than silicone rubber and has application in developing countries. It is molded by laying up colored layers of CPE in a gypsum/polymer flask and heating under pressure to 110C, followed by surface coloring. Of the 42 patients enrolled, 71% were male; 82% were Caucasian, 2% Asian, 2% Hispanic, and 1% African-American; mean age was 62 years (range 34-82); 75% had cancer, 14% trauma, and 11% birth defects. Patients wore a custom-made prosthesis from each material for 4 months in random order. At the end of each study arm, patients were asked to rate how satisfied they were with their prosthesis on a 0-10 scale (10=completely satisfied). Many other evaluations were made throughout the process, including quality of life measures. Twenty-eight patients completed the study (22 at M.D. Anderson Cancer Center, 6 at Toronto Sunnybrook Regional Cancer Centre); of these, 68% had used silicone prostheses previously. Fourteen patients needed ear prostheses, 10 nose, and 4 orbital (extraoral, full or partial). Overall, patients rated the silicone prosthesis higher than the CPE (8.4+/-2.2 vs. 6.2+/-3.1 [mean+/-SD]), difference=2.2, 95% CI=0.9 to 3.6, p=0.017). Previous prostheses users had a stronger preference for silicone (difference=3.3, 95% CI=1.7 to 4.9, p=0.001), while the 9 patients new to maxillofacial prostheses rated the two materials the same (difference=0.0, 95% CI=â^'2.1 to +2.1, p=1.00). Previous users had a strong preference towards silicone, the material with which they were familiar. However, new users, i.e., those with no previous experience with prostheses, scored the prostheses made with silicone and CPE exactly the same. Supported by cooperative agreement U01 DE014543 from the NIDCR/NIH.

(27) Endothelial Cell Culture in a Ceramic Microfluidic Device

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Microfluidic devices possess many qualities that make them attractive to research. First, their small dimensions make it possible to carry out reactions and other processes with a minimal amount of starting materials and waste. Their compact dimensions also make it possible to create self-contained devices that can be portable enough for fieldwork or implantable in the human body as a sensor. Finally, these devices can be fabricated to mimic vascular and cellular dimensions. By recreating the conditions within the body one could more accurately model the natural environment for cells or other tissue. To date, microfluidic devices have been fabricated almost exclusively from two materials: Polydimethylsiloxane (PDMS) and glass. Glass has the disadvantage of requiring extremely hazardous hydrofluoric acid to etch the channels. PDMS also has some less desirable properties. For instance, its flexible structure gives the fabrication an inherent lack of reproducibility and durability, which would preclude its use in the field or as an implantable device in the body. To take microfluidics from an interesting research subject to a commercial product will require a durable, rigid, uniform substrate with a high precision, reproducible fabrication technique. In addition, the substrate needs to be suitable for some type of analytical measurement technique, primarily optical or electronic. Low temperature co-fired ceramic (LTCC) materials and processing methods meet these criteria. The goal of the current study is to show that LTCC materials can be used to construct a microfluidic device containing a viable cell culture grown on gold electrodes. This device will incorporate trans-endothelial electrical resistance (TEER) measurements allowing potential applications in flow based cell culture studies and cell based sensors. Initial results suggest that it is possible to bind HUVEC cells to gold electrodes. In the near future, this method will be implemented to achieve a microfluidic device containing a viable cell culture.

(26) Optimizing in Situ Forming Drug Delivery Implants to Deliver Low Molecular Weight Drugs

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In situ forming drug delivery implants (ISFI) have shown promise in delivering adjuvant chemotherapy following minimally invasive cancer therapies. Because of the injectable nature of these implants, they can be placed in strategic locations throughout a tumor volume to overcome limitations in drug penetration. These ISFI systems exist as a polymer solution that undergoes a process of phase inversion whereby the solution solidifies into an implant that releases drug in a time dependent manner when injected into an aqueous environment. The polymer solution was comprised of poly(D,L-lactide-co-glycolide), small MW drug sodium fluorescein (376 Da), and excipient (pluronic P85) dissolved in a polar organic solvent, 1-methyl-2-pyrrolidinone (NMP). To aid in the design of an optimal ISFI system, the effect of varying the formulation components such as the PLGA MW, concentration of excipient, and drug loading dosage was examined. Solubility studies were also conducted to determine the critical water concentration required for phase inversion. Our results demonstrated that the PLGA MW was the most significant factor in modulating drug release from the ISFI systems. ISFI polymer formulations comprised of a lower MW PLGA had a significantly lower ($p<0.05$) percent mass of burst drug release. Critical water concentration studies also demonstrated that formulations with lower MW PLGA had increased solubility in water; therefore these formulations may require more time to phase invert and release the drug. These experimental studies when combined with a corresponding computational model can help us design an optimal implant formulation that will quickly reach therapeutic levels followed by a period of steady drug release.

(15) Enzyme Electrode Interfaces for Biofuel Cells

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Enzyme electrode interfaces are designed based on glucose oxidase (GOx) and laccase to study their potential uses in biofuel cells. Biofuel cells use electrochemical reactions that involve biochemical pathways to transform chemical energy to electrical energy. GOx, from the fungus Penicillium amagasakiense, is being studied for use in the anode of the biofuel cell. GOx catalyzes the oxidation of β-D-glucose to D-glucono-1,5-lactone. The use of wild type GOx is problematic because oxygen competes with the mediator for electrons that come from the oxidation of glucose. A mutation was introduced into GOx to prevent this loss of electrons to oxygen. The mutated site is believed to be the oxygen-binding site of the enzyme. It is believed that without the oxygen binding to GOx, the electrons produced during oxidation will be carried to the electrode more efficiently. Laccase, from Thermus thermophilus HB27, is incorporated into the cathode and is capable of accepting electrons from the anode while it reduces O_2 to H_2O. This particular laccase is a thermophilic enzyme that is notable for having faster kinetics when it is exposed to higher temperatures. The goal is to utilize both of these enzymes in the development of biofuel cells.

(16) Development of Degradable Hydrogels for Growth Plate Regeneration

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The growth plate, or epiphyseal plate, is the site of new bone growth at the ends of bones in growing children. The importance of this area to normal growth and development makes it especially prone to having lasting effects in the case of an injury. Many times an injury can result in formation of a bone bridge that can lead to growth arrest, which could eventually create angled growth and deformity. Here, the goal is to develop a hydrogel construct that displays the mechanical properties inherent to the natural growth plate, as well as a degradation profile that would allow new tissue to form and repair the damaged physis. In this project, poly-β-amino-esters were synthesized and characterized to determine their viability in this application. It was found that the hydrogel systems used provided a large range of tunable properties (e.g., degradation and mechanical properties) that can be altered through a change in the functionality of the chemicals used or the relative ratios of these chemicals that were reacted in the macromer synthesis. Single phase hydrogel samples were developed, and the degradation and compressive moduli were studied. The relative toxicities of all macromer systems were studied using MTT analysis. Multiphase hydrogel composites were also created by incorporating fast-degrading hydrogel particles into a slower degrading hydrogel matrix. Degradation and drug release studies were carried out for these systems.

(25) Biomimetic Synthesis of Pd Nanoparticles for the Stille Coupling Reaction

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In response to the projected exhaustion of fossil fuels, new eco-friendly and energy efficient catalytic systems are required. These catalysts should be designed such that they employ environmentally friendly conditions, which include the use of a water-based solvent at room temperature with low catalyst loadings. In contrast to traditional toxic catalytic conditions, nature has developed methods for the generation of highly active inorganic nanostructures via biomineralization processes. These methods can be exploited for materials fabrication, which can produce functional structures that operate under green conditions. Unfortunately, the number of naturally derived materials is limited; however, bio-based selection methods have been developed to isolate peptides with the ability to nucleate, grow, and passivate a variety of technologically interesting materials. We have employed such techniques to prepare highly active spherical Pd nanoparticles using peptides isolated via combinatorial phage display. The Pd nanoparticles are nearly monodisperse and have an average diameter of 1.9 ± 0.3 nm. These particles are of such small dimensions that maximize the surface-to-volume ratio, which is desirable for catalytic applications. To test the functionality of these materials, and their use as model green catalysts, their reactivity for C-coupling via the Stille reaction was probed in water at room temperature. Pd loadings of 0.005 mol% produced quantitative yields for this reaction that was analyzed over a variety of chemical functional groups and halogen moieties. Turnover frequencies for this reaction were determined to be 3,201 ± 269 mol product / (mol Pd h). Together, by using the peptide-derived materials, a 100 to 1000-fold enhancement in reactivity and Pd loading were achieved, as compared to comparable system, suggesting that biomimetic methods may be able to address technologically derived goals.

(24) Calcium Sulfate/ Hydrogels as Space-Making Composites for Controlled Release of Bioactive Molecules

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For dental implants to be successful, the jawbone needs to have a sufficient amount of bone for the implant to anchor to. Sometimes loss of bone or narrow ridge is caused by disuse, severe periodontal disease, trauma, or birth defects. Bone augmentation is performed to build up these defects, may it be a large hole, back filling a ridge to make it wider or however, bone will need to be added to achieve a desired platform. To enhance the augmentation process, a calcium sulfate hemihydrate (CS) based composite is being developed that will act as a "tenting" barrier to soft tissue proliferation while allowing the delivery of bone growth molecules from degradable hydrogel particles to promote bone regeneration. The composite consists of CS (structural matrix) and varying weight percentages (1 - 10%) of All/1.4 hydrogel particles (delivery component) with an average diameter of 150-250 μm. Compression tests were performed to determine mechanical properties of the different composite formulations. Destructive degradation testing was performed to characterize degradation of the composites. It was demonstrated that CS has a predictable, steady degradation, regardless the weight percentage of gel particles used. Preliminary microCT imaging shows uniform distribution of particles within the CS matrix. Combining previous results, release studies using lysozyme as a "mock growth factor are being performed to determine the protein delivery potential of these composites, and allow for adjustments concerning dosage and loading of hydrogel particles needed to optimize the delivery capabilities of the construct.

(17) Mechanical Loading for Increasing Bone Responsiveness in Organ Culture

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In conjunction with developing bioactive materials for bone repair, replacement and regeneration, significant benefits may be obtained by optimizing the mechanical environment. Understanding the response of bone to mechanical loading is the first step toward exploiting mechanical loading in regenerative medicine and bioreactor development to enhance growth, maintenance, and function of the material, such as a cell-impregnated graft or scaffold. In this work, using neonatal bone organ cultures, we investigated the role of physiologic levels of mechanical strain on bone and bone cell activity. Specifically we isolated femoral pairs from five day old Wistar rats and maintained them for one week in culture. During this period, one bone from each pair was strained according to one of two loading regimes. The first involved applying a 2% linear strain to the bone shaft for 2 hours on day 1 (Grp I); the second involved applying the same strain magnitude and duration on days 1, 3, and 5 (Grp II). At 1 week, the strain effect was assessed comparing the strained bones to their contralateral controls. Three-point bending was used to determine structural properties. In both loading regimes, the strained femurs had an increased failure load (9.15% Grp I, 18.85% Grp II), decreased failure displacement (-1.45% Grp I, -11.49% Grp II), and an increased stiffness (31.28% Grp I, 53.12% Grp II). For Grp II, the differences in failure load ($p<0.01$) and stiffness ($p<0.001$) were significant. This data suggest that the cells in organ culture are responsive to linear strain. Organ culture models provide a unique biomimetic environment in which to study the response of bone cells to controlled stimulation. Given that these isolated systems, not unlike their in vivo counterparts, are capable of being mechanically manipulated, they provide valuable tools for enhancing bone cell responsiveness in regenerative medicine applications.

(18) Reagentless Fiber Optic Biosensors for the Continuous Monitoring of Glucose

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The development of versatile, highly sensitive and selective sensors with long term stability able to provide fast detection of glucose continues to be a challenge in the management of diabetes. In that regard, there is still a need to develop technologies capable of continuous in vivo glucose monitoring in the hypoglycemic, normal, and hyperglycemic range. To that end, herein we report the design and development of a catheter containing a reagentless fluorescence biosensor for glucose employing a fiber optic coated with a miniature hydrogel that incorporates Glucose Binding Protein (GBP). GBP is a hinge-motion binding protein that in the presence of glucose undergoes a significant conformational change. We have previously developed biosensors for glucose by strategically attaching a fluorescent probe on GBP such as to allow monitoring changes in fluorescence when glucose binds to GBP. In order to miniaturize and integrate the biosensors into a catheter, we prepared a hydrogel by free radical polymerization of acrylamide and functionalized GBP labeled with a fluorescent probe, directly on the tip of a fiber optic cable. This results in the entrapment of the glucose recognition element, GBP, in the bulk of the optically transparent acrylamide hydrogel. The development of the hydrogel GBP biosensor allows to shift the range of detection of glucose from the μM range typical of our GBP solution-based biosensing systems to the desired physiological mM range by incorporation of a diffusion barrier provided by the hydrogel matrix. The fiber optic glucose biosensor demonstrated a fast response time (~ 1 min) at different physiological temperatures ranging from 35.5 Â°C to 42.5 Â°C. Moreover, the biosensor was capable of glucose detection in different samples, including human serum and pig blood, without any pretreatment of the sample, thus, demonstrating its potential use in the monitoring of glucose.

(23) Magnetic Hydrogel Nanocomposites for Chemotherapy and Hyperthermia-Based Treatment of Cancer

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Hyperthermia, the heating of tissue to 41 to 45 °C, has been shown to improve the efficacy of cancer therapy when used in conjunction with irradiation and/or chemotherapy. This can be done through the utilization of biomaterials which can be delivered at tumor sites and remotely heated from outside the body while simultaneously controlling the delivery of therapeutic drugs. One such potential class of materials is hydrogel nanocomposites which are composed of biocompatible polymeric systems and magnetic nanoparticulates capable of heating upon exposure to an electromagnetic field. Hydrogel nanocomposites have been developed that can be implanted or injected within a tumor to deliver both heat and chemotherapeutic agents. The nanocomposites studied include a stealth, poly(ethylene glycol) (PEG)-based system and a poly(β-amino ester) (PBAE)-based biodegradable system. Iron oxide nanoparticles were physically entrapped in these materials during polymerization to provide the remote heating mechanism of these systems.

Thermal analysis showed the capability of the hydrogels to be heated in an alternating magnetic field at various temperatures depending on the strength of the field. Swelling analysis was done and showed temperature-responsive behavior of PEG gels in their swollen state. These gels were determined to be non-toxic to murine fibroblasts indicating their potential biocompatibility. M059K glioblastoma cells were heated to thermoablative temperatures (above 55°C) via gels exposed to the AMF and the resultant cell death was observed. Current PBAE degradable gels are being evaluated that will have the ability to heat via the iron oxide nanoparticles and have controlled release of chemotherapeutic drugs. The degradation profile of these gels can be tailored by the ratio of macromers used, and again, remote heating has been successfully demonstrated. In summary, these systems have the ability to be remotely heated and to deliver drugs to specific sites, which make them viable systems for treating deep-seated or reoccurring tumors.

(22) Delivery of the Photosensitizer Pc 4 in PEG-PCL Micelles for in Vitro PDT Studies

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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.

(19) Protein Transport Through Stimuli-responsive Hydrogel-based Nanoporous Membranes

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Stimuli-responsive hydrogels containing proteins, such as, calmodulin, are important for molecular separations, sensing and drug delivery applications. Calmodulin is known to have affinity for phenothiazine antipsychotic drugs (e.g., chlorpromazine). As previously demonstrated, covalently immobilized calmodulin within a hydrogel network interacts with chlorpromazine or calcium ions, in a three stage process, resulting in the swelling or shrinking of the hydrogel. Such hydrogels have been efficiently incorporated in a microfabricated and implantable drug delivery systems. In order to achieve device miniaturization and high performance, we have developed a method to incorporate stimuli-responsive hydrogels containing calmodulin inside a nanoporous template (AAO) resulting in a hybrid membrane. Further studies were focused on utilizing these hybrid membranes for the transport of biomolecules. The pore size of the hybrid membranes was 5.34 ± 0.44 nm under swollen condition of the hydrogel. Diffusion-based experiments were performed to study the transport of biomolecules, such as, proteins and DNA based on their sizes, charges, and conformations. In addition, these membranes were used for encapsulation of insulin within the nanoporous hydrogel and its subsequent release in the swollen and shrunken states was monitored. We envision that such smart membranes may find applications in membrane-based separation methods and drug delivery systems.

(20) Comparison Between the Migration Rates of Bone Marrow Stromal Cells and Tendon Derived Fibroblasts on Random and Electrochemically Aligned Collagen Constructs

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Introduction: Tissue engineering is a viable approach to treat damaged tendons. Existing biomaterials lack the mechanical strength needed for tendon tissue engineering applications. We have previously developed and reported a novel methodology to form densely packed electrochemically aligned collagen (ELAC) threads with mechanical properties converging upon the natural tendon. In tissue engineering strategies, expediting the cellularization of constructs carry clinical importance. In this study, we investigated the effects of the cell type (bone marrow stromal cells "MSCs" vs. tendon derived fibroblasts "TDFs") and fabric orientation (ELAC vs. random collagen) on the population rates of collagen constructs. Methods: ELAC was synthesized by loading dialyzed monomeric collagen solution between two electrodes. On application of an electric current for 12 hours, the collagen molecules align and form highly oriented collagen threads. Random collagen threads were made by gelling a mixture of dialyzed monomeric collagen solution and 10X PBS (pH - 7.4) at 37 ºC for 45 minutes. MSCs and TDFs were isolated from Long-Evans rats and cultured in DMEM supplemented with 10% FBS. Both the MSCs and the TDFs were subcultured three times before being used for the migration studies. An in vitro cell migration model that allowed the unidirectional population and migration of cells onto collagen threads was used. Results and Discussion: The TDFs migrated a significantly longer distance on both ELAC and random threads compared to their respective MSC counterparts. More importantly, the MSCs migrated significantly faster on the ELAC compared to the random threads. High magnification images of the ELAC showed that the cytoskeletons of both the MSCs and the TDFs were aligned along the long axis of the thread. We conclude that the ELAC is more receptive to cell migration compared to the traditional random collagen.

(21) Dual Delivery of Growth Factor and Antibiotic from Polyurethane Scaffold Improves Tissue Regeneration in Infected Bone Wounds

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Bioresorbable polyurethanes have been used extensively in tissue engineering to serve as both a supportive scaffold and drug delivery system due to their biocompatibility and biodegradability. We have fabricated highly porous polyurethane scaffolds by two-component liquid molding of hexamethylene diisocyanate trimer (HDIt) and polyester triol. BMP-2 has been delivered from polyurethane scaffolds to facilitate cell differentiation and new bone formation. To maximize function of the growth factor, long-term delivery is desired. We have successfully encapsulated BMP-2 into 1μm PLGA microspheres which were subsequently embedded into the scaffolds. The PLGA microencapsulation approach resulted in a lower burst and more sustained release profile. The scaffolds have been tested in a rat femoral plug model, and the release of BMP-2 promoted significantly more new bone formation at weeks 2 and 4 relative to the negative control as evidenced by both μCT imaging and histomorphometry. Sustained release of tobramycin for up to 21 days from polyurethane scaffolds has been reported. In order to extend the period of release, we chose a more hydrophobic molecule, vancomycin hydrochloride, which resulted in more extended release. Conversion of vancomycin hydrochloride into vancomycin free base before incorporation into polyurethane eliminated the burst release and achieved a more sustained release. The scaffold disks containing vancomycin were bioactive as demonstrated by Kirby-Bauer assay. The combination of growth factors with antibiotic incorporated within polyurethane scaffold is expected to promote infected bone wound healing through the synergistic effects of promoting cell differentiation by BMP-2 and anti-bacteria ability by the antibiotic.