Injectable Polyurethane Composite Scaffolds Delay Wound Contraction and Support Remodeling in Rat Excisional Wounds

Elizabeth J. Adolph, Andrea E. Hafeman, Jeffrey M. Davidson, Lillian B. Nanney, Scott A. Guelcher

Injectable scaffolds present compelling opportunities for wound repair and regeneration due to their abilities to fill irregularly shaped defects and deliver biologics. In this study, we investigated the properties of injectable polyurethane (PUR) biocomposite scaffolds and their application in cutaneous wound repair. PUR scaffolds were synthesized by reacting a lysine triisocyanate-poly(ethylene golycol) prepolymer with a polysaccharide filler (hyaluronic acid [HA] or carboxymethylcellulose [CMC]) and a polyester triol comprising 60% caprolactone, 30% glycolide, and 10% lactide. The capacity of the scaffolds to facilitate dermal wound healing was evaluated in an excisional model in Sprague-Dawley rats. The groups investigated were blank wounds, PUR scaffolds with 15 wt% HA, and PUR scaffolds with 15 wt% CMC. The scaffolds have a minimal reaction exotherm and clinically relevant working and setting times. Moreover, they have mechanical and thermal properties consistent with rubbery elastomers. In the rat excisional wound model, scaffolds stented the wounds at early time points, resulting in a regenerative rather than a scarring phenotype at later time points. Measurements of wound length and thickness revealed that the treated wounds were less contracted at day 7 compared to blank wounds. Analysis of cell proliferation and apoptosis showed that the scaffolds were biocompatible and supported tissue ingrowth. Myofibroblast formation and collagen organization provided evidence that the scaffolds have a positive effect on extracellular matrix remodeling by disrupting the formation of an aligned matrix under elevated tension. In summary, we have developed an injectable polyurethane biocomposite scaffold that enhances cutaneous wound healing in a rat model.

Synthesis of Iron Oxide Nanoworms

Soubantika Palchoudhury, Yaolin Xu, Yuping Bao

Magnetic nanoparticles have shown great potential in targeted drug delivery, localized cancer therapy, and as contrast agents in MRI imaging. A potential challenge of using magnetic nanoparticles for bio-imaging is the low blood circulation time, because of the natural immune response of human body. Recently, it was reported that elongated iron oxide nanoparticles could effectively remain undetected by the phagocytes, in spite of their larger dimension. The increased blood circulation will allow them to reach the treatment targets without quick clearance. Here, we report the size controlled synthesis of iron oxide nanoworms via thermal decomposition of iron-oleate complex. These nanostructures are 10x200 nm, measured from a transmission electron microscope (TEM) image, and are highly crystalline. The crystal structure was confirmed to be maghemite by the x-ray diffraction scan (XRD). Because of the large dimension, these nanoworms are ferromagnetic at room temperature, indicated by the open hysteresis loop from alternating gradient magnetometer (AGM) measurement. The ferromagnetic property makes them less suitable for biomedical applications because of the potential aggregation issue because of the magnetic interactions. Our continuous efforts are to synthesize superparamagnetic nanoworms by increasing the aspect ratios. Magnetic resonance imaging studies on these nanoworms are in the process.

Synthesis of Magnetic and Fluorescent Bifunctional Nanoparticles

Yaolin Xu, Soubantika Palchoudhury and Yuping Bao

Magnetic nanoparticles (NPs) have significantly advanced cancer treatments through targeted drug delivery and localized therapy. Magnetic NPs further make simultaneous therapy and diagnosis possible as magnetic resonant imaging (MRI) contrast agents. Unfortunately, simultaneous therapy and diagnosis using magnetic NPs are limited by the expensive MRI equipments, which are not available to common research laboratories. Currently, fluorescence imaging remains the primary choice for bio-imaging because of its high sensitivity. Here, we reported magnetic-fluorescent integrated NPs using iron oxide as the magnetic component and metallic nanoclusters as the fluorescent component. Iron oxide NPs were first synthesized by a modified thermal decomposition method and then transferred into aqueous solution following a ligand exchange process. Subsequently, these iron oxide NPs were used as the seeds for the growth of Ag metallic nanoclusters. Around 12 nm iron oxide NPs were successfully produced, followed by attachment of fluorescent clusters with a broad emission in the range of 600-650 nm. Detailed structural analysis and physical property characterization are on-going. This work is funded by NSF-DMR 0907204.

Enhanced Bony Integration of An Engrafted Tendon In A Rat Model

Jinsong Huang; Tushar Jha; Jack Conoley; Tyler Cannon; Anobel Maghsoodpour; Frederick Azar; Richard Smith; Karen Hasty

Improving the integration of a tendon/ligament graft with bone resembling the native enthesis would have a high impact on the clinical outcomes in orthopaedic surgery.

In this study, we have established a rat model to study the healing and integration of peroneus longus tendon autograft into the tibia. Prior to insertion into a bone tunnel in the tibia, the tendon was wrapped with a type I collagen sheet rehydrated by platelet-rich-plasma (PRP) with or without β-tricalcium phosphate (β-TCP) granules.

Five weeks after transplantation, the histology showed minimal amount of bone surrounding the tendon fibers in controls compared to the graft wrapped with PRP rehydrated collagen mixed with TCP. The measurement of bone in-growth in the bony tunnel by microCT confirmed that there was more calcified tissue when the grafts were combined with collagen+PRP and collagen+PRP+TCP at 2 weeks post-operatively as compared to the tendon graft alone.

These preliminary results confirmed more calcified tissue inside the bony tunnel when tendon was wrapped with collagen sheet re-hydrated with PRP and mixed with TCP granules, especially at early stages, suggesting accelerated integration of the graft. Type I collagen is a natural component for both bone and tendon tissues. Platelet degranulation releases biologically active growth factors, such as PDGF, TGF-β, FGF, IGF, VEGF and others. By using collagen and β-TCP, combined with PRP, we enhanced both osteoconductivity and osteoinductivity at the tendon engraftment site. These auxiliary methods can be optimized to use in tendon/ligament repair clinically to reduce recovery time.

Engineering MMP-9-Activatable Contrast Agents for Imaging of Vulnerable Atherosclerotic Plaques

Shann S. Yu, W.G. Jerome, David J. Maron, James H. Dickerson III, and Todd D. Giorgio

Ruptured atherosclerotic plaques are a leading cause of strokes and heart attacks, but there is currently no robust noninvasive method to discriminate stable plaques from "vulnerable" plaques. In addition to macrophage infiltration, vulnerable plaques exhibit pathologic concentrations of matrix metalloproteinase-9 (MMP-9). We have therefore synthesized and characterized "proximity-activated" ultrasmall

superparamagnetic iron oxides (PA-USPIOs) coated with two ligands—MMP-9-degradable polyethylene glycol (PEG) chains, and peptides targeting the macrophage scavenger receptor (SR). In circulation, the MMP-9-degradable PEG chains mask the SR-targeting ligands. In the presence of macrophage activity and

a proteolytic environment, characterized by a pathologic concentration of MMP-9, the PEG chains are cleaved, unveiling the underlying SR-targeted ligands. PA-USPIOs were characterized by FT-IR, DLS, and TEM. MMP-9-dependent PA-USPIO binding was quantified using colorimetric iron assays in cultures of human THP-1 monocyte leukemia cells. Further, co-administration of PA-USPIOs

with fucoidin, a ligand specific for the SR, led to a dose-dependent decrease in PA-USPIO internalization, demonstrating specificity of PA-USPIO binding. These studies show promise in the development of a novel vulnerable plaque-specific MR contrast agent.

An in vitro Analysis of Angiogenic Processes in Human Endothelial Cells on PEG Hydrogels

AM Porter, CM Klinge and AS Gobin

Angiogenesis is the process by which new blood vessels emerge from the pre-existing vasculature. Endothelial cells are involved in three key cellular processes during angiogenesis: increased cell proliferation, degradation of the extracellular matrix during cell migration, and the survival of apoptosis. The above biological processes depend upon the presence of growth factors, such as Vascular Endothelial Growth Factor isoform 165 (VEGF165), which is released from the extracellular matrix as it is being degraded, or secreted from activated endothelial cells. The goal of the current study is to develop a system with a backbone of polyethylene glycol (PEG) and grafted angiogenic signals to compare the angiogenic response of Human Umbilical Vein Endothelial Cells (hUVEC) or Human Microvascular Endothelial cells (hMEC). Adhesion peptide sequences (PEG-RGDS) for cell attachment and PEG-modified VEGF165 (PEG-VEGF165) are grafted into the hydrogels to encourage angiogenic response. Data suggest that our biomimetic system is as effective in inducing all of the angiogenic processes in hMEC as compared to the response observed in hUVEC.

Measurement of Thermally Stimulated Luminescence in Gamma irradiated Ultra High Molecular Weight Polyethylene in the presence of Vitamin E

Dereje Abdi, M. Shah Jahan, BenjaminWalters

The thermoluminescence of medical grade ultra high molecular polyethylene (UHMWPE), GUR1020(Ticona) after gamma irradiation at room temperature(220 C) in air or nitrogen was detected using a commercial TSL apparatus (HARSHAW QS3500) by subsequent heating from 220 C to 2990C. A typical polyethylene (GUR1020 without vitamin E, previously irradiated in nitrogen) TSL glow curve exhibited three major peaks near 1160C, 2000C and 2500C. An additional glow peak is observed near 1400C in GUR1020-E (UHMWPE containing 0.1% vitamin E (α -T)). The full width at half maximum and the activation energy of each peak are 1160C, 400C, 1.2eV; 2000C, 240C, 1.0eV and 250oC,56oC, 1.5eV. The glow peaks seem to flow a kinetic order of 1.5. While the peak near 1160C is produced in all irradiated samples, the peak near 2000C and 2500C is produced only when irradiation is performed in air, suggesting that these peaks are associated with oxygen-centered species and the glow peak near 1160C is associated polyethylene free radicals. The peak near 1400C which is associated with the presence of vitamin E is unaffected by the presence of air and this peak could result from thermal break down of the vitamin E radical (α-T-O*). Varying dose (30, 65, or 100KGy) simply changes the TSL intensity without affecting the TSL characteristic.

Effect of high dose on residual radicals in open air irradiated α-T UHMWPE resin powder

Malik Mehmood, Muhammad Jahan, Sanjay Mishra, Benjamin Walters

Polyethylene(PE) radicals generated in ultra-high molecular weight polyethylene (UHMWPE) by radiation sterilization and crosslinking processes are believed to be precursors to oxidative degradation of polyethylene components in total joint replacements. Better understanding of these radicals can be important in the field of orthopedic science and medicine. The purpose of this study was to determine the effects of radiation dose on theses radicals in vitamin E-blended UHMWPE resin powder. The powder was irradiated to doses of 30-, 65-, and 100-kGy (60Co) doses, creating primary alkyl and allyl radicals which then decayed in air while the carbon-centered polyenyl R1 (-˙CH-[-CH=CH-]m-, m≥3) and the oxygen-centered di- or tri-enyl R2 (-˙OCH-[-CH=CH-]m-, m≤3) residual radicals grew. These residual radicals were measured for eight weeks using an electron spin resonance spectrometer. Both R1 and R2 grew significantly in the 65-kGy sample while they grew only slightly in the 30-kGy and 100-kGy samples. Dependence of the residual radical concentration on the three-phase morphology of UHMWPE matrix was also examined. The results may be explained by Raman spectroscopic data which suggests the 65-kGy samples had a higher percentage of amorphous region when compared to the 30-kGy or 100-kGy (21.7 compared to 15.7 or 17.9) and also suggest a lower percentage of interfacial regions (16.4 compared to 25.6 or 17.5) and a lower level of structural disorder (0.26 compared to 0.44 or 0.27).

Development of A Novel Cell-Internalized Smart Polymer Platform for Intracellular Peptide Delivery

Brian C. Evans, Craig L. Duvall

Cell-internalized peptides show potential as research tools and pharmaceuticals in a variety of biological and disease processes. However, substantial hurdles exist against their cytosolic delivery relative to small molecule drugs, limiting their therapeutic potential. Intracellular peptide delivery can be achieved using cell-penetrating peptide (CPP) sequences, but cytoplasmic bioactivity is limited due to sequestration within intracellular vesicles that are trafficked for exocytosis or lysozomal degradation. To overcome this barrier, we propose a CPP-internalized smart polymer (CISP) platform that contains both a CPP component and a pH-responsive, endosomolytic component. The pH-responsive, smart polymer component can 'sense' the acidic, endosomal pH and abruptly transition into a hydrophobic, membrane-disruptive state. The smart polymer also contains spatially-defined attachment sites for a peptide therapeutic that can be reduced upon exposure to the cytoplasm in order to avoid steric hindrance of peptide bioactivity. A CPP-functionalized, macro-chain transfer agent has been verified through ESI-MS and liquid chromatography to be used in RAFT polymerization of the smart polymer. Furthermore, the coupling efficiency of the conjugation reaction has been optimized (~90%) and smart polymer mediated hemolysis has been demonstrated at endosomal pH. We propose to validate this platform technology using a peptide inhibitor of MAPKAP kinase II (MK2), a signaling molecule known to be involved in activation of heat shock protein 27 (HSP27), progression of intimal hyperplasia (IH), and graft failure following coronary artery bypass grafting. We hypothesize that efficient pharmacological inhibition of MK2 will prevent IH and provide further insight into autologous graft disease progression.

Sustained Local delivery of siRNA from "smart" nanoparticles and injectable scaffolds

Christopher E. Nelson, Mukesh K. Gupta, Elizabeth J. Adolph, Scott A. Guelcher, Craig L. Duvall

Small interfering RNA (siRNA) mediate specific knockdown of target genes, however, delivery barriers prevent sustained local delivery to pathological sites where siRNA can have a significant clinical impact (i.e. chronic diabetic wounds). We have created a two component delivery system composed of siRNA carrying polymeric nanoparticles (NPs) loaded into polyurethane (PUR) scaffolds to achieve sustained, local delivery of siRNA. SEC and NMR confirm the synthesis of a diblock copolymer (DMAEMA70-b-PAA63-co-DMAEMA63-co-BMA125). DLS and TEM verify ~70nm micelles before and 60nm micelles after electrostatically condensing siRNA. GAPDH siRNA was used to validate NP activity showing significant reduction (80%) in GAPDH mRNA with no significant toxicity. Confocal microscopy indicated a homogenous distribution of siRNA-containing NPs throughout the PUR. NP release from PUR scaffolds was characterized by a burst release followed by a sustained release over a 21 day period with ~100% released by the 21st day. TEM of releasate confirms the presence of micelles indicating stability of NPs to PUR fabrication. Here we present a novel PUR-NP system for sustained, local siRNA delivery that could be used to efficiently transfect cells seeded onto the scaffold in vitro or in cells infiltrating the porous material in vivo. NPs mediate efficient knockdown in multiple cell lines and are released intact from PUR for 21 days. Once verified and optimized, this novel combination of PUR and NPs will provide a template for cell in-growth, ease of delivery, multiple levels of tunability, as well as the ability to optimize siRNA activity for specific pathological applications.

Bulk Mechanical Properties of a Novel Titanium Foam

Dirk Scholvin, Richard Obert, Michael Carroll, Jon Moseley

Introduction:
Titanium metal foam is a prospective material for orthopedic implants and coatings. In this study, mechanical properties of a novel open porous titanium foam material were determined.

Methods:
Metal parts of porosity 67-71% were manufactured from commercially pure titanium (Wright Medical Technology). Pore cell sizes were around 530µm with ~200 µmD interconnecting pores. Porous tantalum parts (Trabecular MetalTM, Zimmer Inc., Warsaw, IN) were obtained for comparison.

Compression tests were performed per ASTM D695. A total of 32 cylinders (8mmD x 12 mmL) were machined from 6 lots of bulk foam. Seven cylinders were machined from porous tantalum parts. Specimens were loaded at a strain rate of -0.333mm/sec.

Four-point bend testing was performed on (7.5 mm)3 titanium foam blocks per ASTM D790. A total of 15 samples were cut from three lots also tested in compression. Specimens were loaded at a strain rate of -0.333mm/sec, using a four point bend fixture.

Results:
Even though the metal foam had a higher average compressive strength, ANOVA failed to show statistical significance compared to porous tantalum (P-value = 0.154). The flexural strength and modulus were found to be higher compared to the compressive properties.

Conclusions:
As expected, lower percent porosity resulted in greater compressive strength of the titanium foam. Titanium foam with 68-69% porosity showed equivalent compressive strength and modulus compared to the tantalum parts. All porosity ranges of the titanium foam as well as the porous tantalum demonstrated excellent mechanical integrity and showed strength sufficient for use in many load-bearing orthopedic applications.

Correlation Between Contact Stresses and Wear Damage in a Total Knee Replacement

David A Knox, John L. Williams, Matthew G. Teeter, David W. Holdsworth, & William M. Mihalko

Throughout the life of a total knee arthroplasty (TKA) implant repeated loading causes wear on the contact surfaces. Polyethylene wear changes kinematics and contract stresses, and the effects of these changes are not fully understood. This study examines a method for predicting where implant wear will occur, and how the wear alters the kinematics of the implant. A cruciate-retaining knee (Natural-Knee, Intermedics Orthopedics, Inc., Austin, Texas) was retrieved from a donor at the MERI. The polyethylene insert was scanned and models were generated for simulations. KneeSIM (LifeMOD/KneeSIM, San Clemente, California) was used to simulate a gait cycle comparing worn versus unworn inserts. Using information gained through KneeSIM, ANSYS finite element analysis software (ANSYS, Inc., Canonsburg, Pennsylvania) was used to find loading and contact stress patterns on the insert. These contact stress areas on the insert are hypothesized to be indicative of actual wear damage on the implant. Contact stresses after an implant is damaged by wear were found to be three times higher than those in a new implant. Application of loading conditions to ANSYS showed a correlation between predicted contact stress areas, and physical wear damage on the implant. TKA designs should keep in mind the altered characteristics of TKA implants caused by wear and what effects these changes may have on the biomechanics of the joint over time.

Combinatorial Microfluidic Reactor to Decouple Hemodynamic Cues on Endothelium

Lucas Hofmeister, Chad Augusty, Aidan Boone, Ian Baird,John Edd, and Hak-Joon Sung

The vascular system is locally specialized to accommodate widely varying hemodynamic forces and tissue structure, indicating that the phenotype and function of vascular cells are adapted to the vascular environment from which they originate. For example, arterial cells are exposed to cyclic transmural tension and pulsatile shear, whereas venous cells are exposed to steady tension and shear. The pathophysiological effects of each hemodynamic parameter on vascular cells have been studied extensively. However, complex coupling of hemodynamic parameters hampers clear elucidation of their role in pathogenesis. To decouple three major hemodynamic parameters: stretch type, flow type, and matrix stiffness, we have developed a novel combinatorial microfluidic reactor by employing an advanced bioreactor and set of microfluidic devices coated with polymeric materials that can tune matrix properties. A PDMS microfluidic device coated with polymers having the general formula x%PEG-co-y%CPCL-co-z% PCL has been fabricated to control oxidative environments and matrix stiffness. With this device ECs can be exposed to programmed fluid shear and cyclic strain. Finite element modeling was used to show the strain dynamics of PDMS microfluidic devices. Under 10% cyclic strain, downregulation of ICAM-1 in human aortic endothelial cells (HAEC) was observed as opposed to human umbilical vein ECs (HUVEC), demonstrating differences in endothelial cell type response. Additionally, Monocyte recruitment to HUVECs was shown to increase with cyclic strain as well as with DMSO damage. These results demonstrate the importance of decoupling the effects of different mechanical properties on mechanotransduction-mediated inflammatory activation in human vascular endothelial cells.

In Vitro and In Vivo Evaluation of a Slower Resorbing Calcium Sulfate (CS) Cement

Jonathan McCanless, Jon Moseley, Robert Urban, Thomas Turner, Deborah Hall, Michael Carroll

Introduction:
While surgical grade CS materials offer high initial strength and consistent bone replacement, slower material resorption is desired for some applications. A new, slower-resorbing CS composite was characterized.

Methods:
A calcium sulfate/calcium phosphate composite (SR) was compared to a calcium sulfate material (X3) (MIIG®X3, Wright Medical Technology).

Diametral tensile strength (DTS) was measured for cylindrical specimens in polyurethane foam. Specimens were cured for 1 and 24 hours in ambient air or 37ºC calf serum and transversely loaded to failure at 5mm/min.

Dissolution was assessed for pellets in distilled water at 37ºC. Water was changed and specimens were dried and weighed daily for 30 days and every 5 days thereafter until mass was <5%.

SR or X3 pellets were implanted in transverse defects in humeri of three dogs for 6 weeks. Bone healing and implant resorption were assessed from radiographs bi-weekly, and from undecalcified histology sections and contact radiographs at 6 post-sacrifice.

Results:
A significant difference in DTS was seen between 1 and 24 hour cure times for SR cured in air but not in serum. Average dissolution rates for SR and X3 were 8.2%/day and 18.5%/day, respectively.

In vivo, both types of pellets were replaced with osteoid and new bone. New bone area fraction at 6 weeks with SR pellets and X3 pellets was 35.9+6.1% and 26.7+10.0%, respectively. More residual SR implant material remained vs. X3.

Conclusions:
The composite CS cement demonstrated slower resorption and consistent setting, strength, and in vivo characteristics similar or superior to the control.

The Effects of Iron Oxide Nanoparticles on Drosophila Development

Sarah Boyd, Gavin Daigle, Janis O'Donnell, Yuping Bao

Iron oxide nanoparticles (Fe2O3 or Fe3O4) are widely used in biomedical fields, for purposes such as targeted drug delivery and contrast agents for magnetic resonance imaging. With the increased use of nanoparticles in biomedical settings, there is a concern about the potential toxicity of these nanoparticles. In particular, the biological responses of whole organisms to these nanoparticles are not well studied; most studies employ single cell survival assays. Here, using polyacrylic acid-coated iron oxide nanoparticles to develop a whole organism toxicological model, we have tested for toxicity and other physiological effects of these particles in Drosophila. The nanoparticles were mixed with yeast-sucrose solutions at concentrations ranging from 1-10 μg/ml and then fed to early 3rd instar wild type larvae over a 24 hr period. The larvae were then examined for lethality, and survivors were transferred to normal Drosophila medium. Subsequently, the larvae were tested for ability to pupate, survival to adult stage, and fertility of the female survivors. We found that the nanoparticles cause low, but reproducible levels of larval death, as well as long-term effects for the larvae that survive the initial feeding. These include decreased ability of larvae to enter the pupal stage, decreased hatching rates, and reduced fertility of surviving females at lower concentrations. Surprisingly, we also observe maximum larval deaths at 3 μg/ml with improved survival at higher concentrations, due to the induction of the innate immune response. We also visualized an apparent innate immune cell migration in the larvae exposed to nanoparticles, using confocal microscopy.

Low Porosity Injectable Allograft Bone/Polyurethane Composites Incorporating rhBMP-2 Enhance Bone Remodeling in a Rabbit Femoral Plug Model

Prieto, E.M., Dumas, J.E., Holt, G., Bible, J., Guelcher, S.A.

As an alternative to autograft bone for the treatment of bone defects, our lab has developed an injectable non-porous composite putty with sustained release of recombinant human bone morphogenetic protein-2 (rhBMP-2). The release mechanism of rhBMP-2 is responsive to the surrounding cellular environment. The putty comprised a lysine triisocyanate-PEG prepolymer, polyester triol, allograft bone (70 wt%), amine catalyst, and rhBMP-2 (two doses: 110 and 440 micro-g/ml). Bilateral defects were drilled in the metaphysis of the distal femurs of NZW rabbits and filled with the biocomposites. The materials exhibited compressive strengths comparable to trabecular bone. Histological sections (Sanderson's rapid bone stain counterstained with Van Gieson) of the biocomposite without rhBMP-2 revealed extensive cellular infiltration and new bone deposition. mCT images were characterized by extensive remodeling. Incorporation of rhBMP-2 enhanced new bone formation as evidenced by the decrease in allograft particles. However, approximately 30% of the samples incorporating a high dose of rhBMP-2 displayed extensive areas of osteoclast-mediated resorption. Similar regions have been reported for doses of rhBMP-2 exceeding by a factor of 3 the recommended dose delivered on an ACS sponge in a sheep femoral condyle plug model (Toth JM, Spine 34(6), 2009). However, in our study the high dose was the recommended dose for rabbits, suggesting that the release mechanism of rhBMP-2 from the biocomposite may reduce the minimum effective dose required to enhance bone healing. Thus the allograft/polymer biocomposites may be a promising approach for developing injectable biomaterials that maintain their initial mechanical properties during the bone remodeling process.

Designing Electrospun Scaffolds for Skin Regeneration

Paul Bonvallet, Matthew Phipps, Bonnie Culpepper, Susan Bellis

Engineering full thickness skin substitutes by electrospinning is a technique by which a scaffold consisting of nanofibers resembling native human skin tissue is constructed. 30% polycaprolactone (PCL) and 70% collagen type I were electrospun in a layer-by-layer fashion, a method critical for the inclusion of fibroblasts embedded throughout the entirety of the scaffold. Confocal images of DAPI stained fibroblasts expressing GFP were captured for the purpose of visualizing the viability of cells. Layer-by-layer scaffolds were grown in culture over 21 days to quantitate the proliferation of fibroblasts within the scaffold by assessing ATP and DNA quantities. The results showed the fibroblasts adopted a spread morphology typical of fibroblasts, and displayed mitotic properties as early as 2 days. Also, 21 days post fibroblast seeding, both ATP and DNA quantities had increased by more than 2-fold. Additionally, an in vitro full thickness skin model was constructed by seeding keratinocytes at an air-liquid interface on top of a layer-by-layer scaffold containing embedded fibroblasts. Fluorescent stains BRDU, CK10, and DAPI were used to demonstrate this model forms a stratified epidermal layer upon a dermal matrix. Finally, a PCL/collagen scaffold was constructed and implanted in a rat where a full thickness skin wound was created. Results from in vivo experiments show that an implanted electrospun matrix not only recruits endogenous keratinocytes, but also assists in stratification to form an epidermal layer. The obtained results suggest these scaffolds are similar to native human dermal tissue and have high potential for accelerating the wound healing process.

Chitosan nanofibrous membranes crosslinked with genipin for GTR applications.

Drew Norowski, Joel Bumgardner

Guided tissue regeneration (GTR) is used to direct the formation of bone and exclude soft tissue from the graft space. Current biodegradable GTR membranes suffer from high exposure rates, inadequate bone regeneration, premature degradation, and high infection rates. Electrospun chitosan nanofibrous membranes have shown great potential but may possess inadequate degradation kinetics to last for 4-6 months, in vivo. This study investigates the natural crosslinker genipin as a method to crosslink electrospun chitosan membranes and evaluates the chemical, mechanical, and biological properties in vitro. Results show that tensile strength is increased, degradation is hampered, and bond structures are altered with genipin crosslinking. Swelling and cell viability did not significantly change with crosslinking (α=0.05), and osteoblastic cells maintained a high level of viability on the membrane over the 5 day study. Genipin crosslinked chitosan membranes have potential to be used in GTR applications, and further investigations are warranted.

Pro- and Anti-Oxidative Material to Direct Stem Cell Fate

Crowder SW, Zachman AL, Liang Y, Gupta MK, Sung H-J

Microenvironmental cues can be generated by changing the physicochemical properties of the extracellular matrix and can influence cell fate by modulating cell-cell and cell-matrix interactions. Biomaterials have been applied to mimic these processes. Poly(ethylene glycol) (PEG) has been found to generate residual peroxides, a type of reactive oxygen species (ROS), in response to external stimuli. We have found that changes in both the PEG and the negatively charged units of polymeric substrates impact cellular ROS mechanisms. ROS have been implicated in various human diseases including cancer. In this study, we employed a library of combinatorial polymers as culture substrates for human mesenchymal stem cells (hMSCs). Each polymer contained a different molar ratio of three polymeric subunits: (1) PEG, (2) a hydrophobic unit, and (3) a negatively charged unit. The incorporation of a negative charge into the surface chemistry counteracted the pro-oxidative effects of PEG in both extra- and intracellular ROS levels, indicating an anti-oxidative activity. Cell transformation was induced in vitro through prolonged exposure to carcinogens. Transformation of hMSCs was modulated by ROS levels which were controlled by changing the ratio of PEG to the negatively charged unit in the polymer design. The results from biological experiments indicate two directions of lineage change in stem cells upon transformation: normal endothelial cell differentiation and abnormal mesenchymal to epithelial transition. This approach has been applied to identify a niche to control transformation and differentiation of stem cells.

Composite Nanomaterials for Magnetothermally-Activated Drug Release

James B. Bennett, Amanda L. Glover, Medhat Farahat, David E. Nikles, Jacquelyn A. Nikles, Christopher S. Brazel

Cancer remains a leading cause of death, stressing the need for advances in therapy methods and techniques. Chemotherapy and hyperthermia cancer treatments are effective but have undesired side effects on healthy tissues. Many chemotherapy side effects are due to the release of anti-cancer drug into the bloodstream before reaching the cancer cells. One way to improve chemotherapy is to trigger drug release to occur only after localization of the drug in affected tissue. Likewise, localized hyperthermia minimizes damage to healthy cells. Our group is investigating the use of a magnetic induction hyperthermia field to heat magnetic nanoparticles to activate release from a temperature-sensitive drug carrier. A diblock poly(ethylene glycol)-co–poly(ε-caprolactone), P(EG-b-CL), micelle encapsulates anticancer drug doxorubicin and ɣ-maghemite nanoparticles into the semi-crystalline core. The magnetic nanoparticles are custom synthesized in our labs to achieve sufficient magnetic heating to melt the PCL core of the micelle. The effect of nanoparticle size and magnetic field properties on heating was determined for aqueous solutions of magnetic nanoparticles. The system design allows for thermally- or magnetically-activated drug release, which when combined with targeting antibodies permits localized hyperthermia combined with chemotherapy.

Modeling the magnetic properties of superparamagnetic iron oxide nanoparticles in ordered clusters

Ryan A. Ortega, Shann S. Yu, Todd D. Giorgio

Superparamagnetic iron oxide nanoparticles (SPIONs) have undergone much investigation as MRI contrast agents, molecular sensors, and magnetic separation agents. A current trend in SPION research is the utilization of clustered particles as activatable contrast agents and sensors. These SPION clusters are designed to disperse in the presence of some predetermined stimulus: the presence of a certain molecule or the activity of a biological event. Predicting the behavior of these nanomaterials before they are fabricated will help save research time and other resources that might otherwise be spent for high throughput research and material characterization aimed at discovering the ideal SPION size and cluster arrangement for a given application. To that end, we have developed a novel model of SPION magnetic properties, derived from first principle magnetic theory and experimental relationships.

The model shows quantitatively that the magnetic behavior of SPIONs differ from bulk iron oxide due to the presence of nanoscale magnetic phenomena. This effect is observed in multiple magnetic parameters, including magnetic susceptibility and total magnetization. We modeled hexagonally close-packed clusters of SPIONs and used the model to predict the magnetic interaction of these clusters with water. The model predicts the behavior of three key cluster parameters: maximum cluster field strength, cluster field uniformity, and total number of SPIONs interacting with a water molecule. The field uniformity and number of interacting SPIONs increase as individual particle size decreases while maximum field strength increases as particle size increases.

Increased Porosity of Electrospun Scaffolds to Facilitate Cell Infiltration

Phipps MC, Clem WC, Bonvallet PP, Culpepper BK, Bellis SB

The development of a synthetic bone graft provides hope in replacing the need to harvest patients' bone tissue to repair critical size defects in their skeletal system. Through the process of electrospinning, our lab has developed a bone-like matrix composed of the polymer polycaprolactone (PCL), type-I collagen (col), and hydroxyapatite nanoparticles (HA). Previous work in our lab has shown that these bone-like matrices support greater human Mesenchymal Stem Cell (MSC) attachment, proliferation, and integrin activation than PCL or PCL/HA scaffolds. Further, TRI scaffolds were found to facilitate increased bone formation in vivo when implanted into a rat tibia defect. However, it was observed that cell infiltration into the electrospun scaffolds was minimal, which will ultimately delay the healing process by preventing angiogenesis, MSC infiltration, and degradation of the scaffold. To address this issue we are investigating the use of sacrificial fibers of Poly (ethylene oxide), which can be incorporated into the scaffolds during fabrication and later washed away in order to increase scaffold pore size. In this study, we show that the removal of sacrificial fibers of PEO increases the pore sizes of PCL/col/HA scaffolds as measured through fluorescent imaging and permeability experiments. Furthermore, the increased pore sizes facilitated cellular infiltration of MSCs in vitro. Ultimately, this should lead to an improved scaffold-tissue interface, vascularization of the scaffold, and replacement of the scaffold with new bone.

Synthesis and Characterization of Multilayered Nanostructured Diamond Coatings for Biomedical Implants

L. Booth, S. A. Catledge, A. W. Eberhardt, Y. K. Vohra

In order to reduce wear and potentially increase the service lifetime of total joint replacements, alternative surface treatments of implant articulating surfaces are currently being explored. Nanostructured diamond coatings synthesized by microwave plasma chemical vapor deposition feature many desirable properties, including biocompatibility, low surface roughness, high hardness, and wear resistance. Multilayered structures have been shown to improve toughness and adhesion for a variety of coating materials on metallic substrates. To further optimize the properties of nanostructured diamond coatings, a multilayered coating with alternating layers of nanostructured diamond and microcrystalline diamond has been developed. It is hypothesized that these coatings may allow for improved toughness and adhesion over the single-layer nanostructured diamond coating. Preliminary work has been focused on synthesis and characterization of multilayered coatings on titanium alloy surfaces with varying numbers of layers and thickness ratios of nanostructured and microcrystalline diamond. Structure and morphology of multilayered coatings have been characterized by Raman spectroscopy, X-ray diffraction, and atomic force microscopy. Thickness of the microcrystalline diamond layer was shown to directly influence average grain size and surface roughness. Hardness and elastic modulus of multilayered coatings, as determined by nanoindentation, were similar to those of single-layer nanostructured diamond. Rockwell indentation of multilayered coatings indicated qualitatively that the adhesion of multilayered coatings were similar to single-layer nanostructured diamond. Ongoing work includes progressive load scratch adhesion tests to further evaluate and compare the mechanical properties of select multilayered and single-layer nanostructured diamond coatings.

Aerogel-based nerve repair: A Feasibility Study

George-Rudolf M. Tamula III, Dr. Firouzeh Sabri

When a peripheral nerve is transected, surgical intervention is required to reapproximate the severed ends so that the organelles within the neurons can establish a connection. After some form of repair is implemented, assessments of nerve recovery must be made.

Currently, the gold standard for peripheral nerve repair is based on suturing techniques. Complications associated with this technique are hemorrhaging and misalignment of the repaired nerve terminals. The practiced technique is also most effective for a limited number of connecting components.

In this study, the feasibility of a sutureless aerogel-based peripheral nerve implant referred to as Neuronal Printed Circuit Board (NPCB) is assessed. The implant relies on mechanisms other than suturing to immobilize the implant in vivo and the nerve terminals onto the implant. The NPCB is also intended to enable redirection of multiple severed terminals simultaneously. Transection of the sciatic nerve branching site was performed on Sprague-Dawley rats, and the implant was inserted at the designated location. The gaits of four groups of rats were monitored—an intact nerve (no surgery), suture repair (gold standard), severed nerve with no repair (control) , and aerogel-based implant repair groups. The Bain-Mackinnon-Hunter Sciatic Function Index Formula (BMH Formula) was applied to the gait prints from each test group to quantify sciatic nerve functional recovery through hind leg performance. The effectiveness of the NPCB repair technique is reported here as compared to the current practices.

β-Cyclodextrin-based Nanoparticles Containing Quaternary Amine for Drug Delivery to the Retina

Linfeng Wu, Junjie Zhang, Tao L. Lowe

Beta-Cyclodextrin-based nanoparticles containing quaternary amine groups (QA-β-CD) have been developed in our lab [1]. The purpose of current work is to investigate the feasibility of using these nanoparticles for drug delivery to the retina through different administration methods.

QA-β-CD nanoparticles were synthesized through one-step condensation polymerization by using beta-cyclodextrin (beta-CD), epichlorohydrin (EP) and choline chloride (CC) as the precursors, and characterized using 1H-NMR, dynamic light scattering, atomic force microscope, and zeta potential measurements.

For ex-vivo and in-vivo studies, QA-β-CD nanoparticles were labeled with dichlorotriazinylaminofluorescein (DTAF). DTAF labeled QA-β-CD PBS solutions were administrated into the rats through different routes. Ex-vivo permeability study is conducted using a side-by-side diffusion apparatus. Either cornea or sclera is mounted in the apparatus.

The in-vivo studies showed that many QA-β-CD nanoparticles entered into the retina of the injected eye after intravitreal injection as compared with the other tissues in the same eye. More ocular biodistribution study is in progress in order to find the better administration route for drug delivery to the retina.

The ex-vivo permeability provides information about how permeable the QA-β-CD nanoparticles are to the cornea and sclera, thus helping us to understand the ocular biodistribution of QA-β-CD nanoparticles via different administration routes.

The developed QA-β-CD nanoparticles have shown great potential for delivering drugs to retina via intravitreal injection to treat retina diseases.

References:

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Broad applicability of a bone-binding domain (E7) to functionalize Hydroxyapatite-containing materials

Bonnie K. Culpepper and Susan L, Bellis

With more than 500,000 bone grafting procedures performed yearly in the United States alone, the need for improving current treatment options is evident. Hydroxyapatite (HA) is an osteoconductive material that makes up the main component of native bone. HA has been historically utilized as coating for metal implants and a bone filler. A significant challenge associated with the use of HA is the limited methods for functionalizing these surfaces with additional bioactive factors. To circumvent this issue, we have attempted to model the mechanism that native bone binding proteins use to associate with native HA in bone. These proteins have binding regions that carry a net negative charge, presumably interacting with positively charged Ca2+ present in native HA. Therefore, we have developed a coupling technique using a heptaglutamate domain (E7) that improves anchoring on both bulk HA and electrospun HA scaffolds as well as enhances initial peptide loading. We found that addition of the E7-domain to both DGEA and BMP-2 peptides improves osteoblastic differentiation in vitro as well as bone formation in a rat tibial osteotomy model. Importantly, results from this study suggest that modification of peptides with an E7 domain facilitates retention to HA-containing materials in vitro and for at least 2 months in vivo. We believe that the broad applicability of the E7 domain to many different peptides speaks to the potential benefit this strategy presents for enhancing response to a variety of HA-containing materials.

Progress Towards Targeted Magnetically Triggered Drug Delivery: Micellization of Poly(ethylene glycol-b-caprolactone) with Crystalline Core

Amanda L. Glover, Sarah M. Nikles, James B. Bennett, Medhat S. Farahat, Jacqueline A. Nikles, Christopher S. Brazel, David E. Nikles

We are interested in designing a targeted magnetically triggered drug delivery system using poly(ethylene glycol-b-caprolactone) diblock copolymers. The final assembled micelles will contain a payload of drug and nanoparticles in the crystalline polycaprolactone core of the micelle. We have synthesized four diblock copolymers from poly(ethylene glycol) (molecular weight of 2000) and varying polycaprolactone having a degree of polymerization varying from 5, 10, 20, to 40. Micellization of the copolymers was studied using three different preparation techniques: direct dissolution of the copolymer in water and solvent evaporation using THF as the universal solvent. The polycaprolactone core is crystalline and melts in the range of 41˚C to 44˚C as evidenced by melting endotherms present in differential scanning microcalorimetry. Dynamic light scattering showed the micelle size varied depending on the different preparation techniques and was dependent on whether or not the micelle solutions are filtered prior to measurement.

Effect of Architecture on the Long Term In Vivo Degradation of Designed PLLA Porous Scaffolds

Eiji Saito, Yifei Liu, Francesco Migneco, Scott J. Hollister

Porous scaffold architecture plays a critical role not only for bone tissue regeneration but also in scaffold degradation. Degradation studies of many porous scaffolds have been previously reported, but the wide range of pore diameters, poorly or non-connected pores have made it difficult to interpret the relationship between architecture and degradation. The goal of this study was to determine the influence of scaffold architecture, specifically pore size, strut size, porosity and surface area, on scaffold degradation in vivo to improve scaffold design for a desired degradation. We designed and fabricated 3 types of poly (L-lactic acid) (PLLA) porous scaffolds with designed pore channels (Diameter = 280, 550, and 820μm) using computer based design and indirect solid freeform fabrication methods, and bulk PLLA cylinders were also fabricated. Then, they were subcutaneously implanted into mice for 6, 12 and 21 weeks. The relationship between the implants' degradation and their architectures were determined by changes in weight, compressive modulus and polymer crystallinity. The bulk cylinder had faster weight loss and crystallization than the porous scaffolds due to advanced degradation. The weight loss was faster in larger strut sizes in the scaffold groups. Furthermore, good correlation between the surface area and weight loss were found at 12 (R2=0.681) and 21 (R2=0.671) weeks. Mechanical properties of scaffolds decreased along to degradation time, and still maintained around the lower range of the human trabecular bone around 50MPa. Overall, this study shows that porous scaffold degradation can be controlled by compuataional designs.

Response of Osteoblast Precursor Cells to Cyclic Compressive and Tensile Strain on a Titanium Substrate

Andrew Noblett, Joel D. Bumgardner, Warren O. Haggard

Five commercially pure (cp) titanium plates were wet ground to 1200 grit SiC and fitted with customized culture dishes with a biocompatible silicone rubber sealant to create twelve culture areas on each plate. W-20-17 cells, a mouse stromal line, were seeded into each well at a density of 5x104 cells/cm2 in mineralizing media; DMEM high glucose supplemented with 10% FBS, 1% AM/AB, 50ug/ml ascorbic acid, and 10mM B-glycerophosphate. The plates were then subjected to cyclic strains of 800ue at a rate of 1Hz for 30minutes a day for 6 days with a custom built pneumatically controlled 4-point bend machine according the loading schemes shown in Table 1. One plate was strained under tension, another was strained under compression, and the third plate was not strained and served as the control. Cells were lysed with RNAase free water after 0, 1, 3, and 6 days of straining. Cell proliferation was assessed by DNA quantification of the lysates using a Picogreen® assay kit, total cellular protein levels were evaluated by BCA Total Protein assay, and alkaline phosphatase (ALP) enzyme levels were measured by a p-nitrophenol assay.

Cells exposed to cyclic tensile strains proliferated significantly less than the unstrained control, but produced the most ALP by the end of the experiment. Compressive strains had little effect on proliferation but did increased ALP production. There were no significant differences in intermittent vs. continuous loading, and fluid motion could not be eliminated as a factor of the system. From the data it was clear that The cyclic mechanical strains effected the proliferation and ALP production of the osteoblast precursor cells vs. the unstrained control. Tensile strains strongly stimulated differentiation over proliferation. Compressive strains also stimulated differentiation but less strongly. Variations in the responses to intermittent and continuous loading might be more pronounced with longer durations of straining.

The MultiStrata Nanoparticle: A FeOx/Au Core/Shell Encapsulated in a Silica-Gold Shell

Charleson S. Bell, Shann S. Yu, Todd D. Giorgio

Emerging materials and methods in biomedical imaging and biophotonics are improving patient outcomes. However, optical, magnetic resonance imaging (MRI), and computed tomography (CT) based imaging contrast of pathologic tissues, therapeutic localization at the site of action at the cellular level, and the inability to unite diagnosis and treatment into a single entity continue to limit the practical power and application of these technologies. In order to relieve these limitations and couple diagnostics and therapeutics into a single theranostic material, we have prepared an enhancement technology via the synthesis of a novel, multi-functional, multilayered nanomaterial. The Au MultiStrata nanoparticle (Au-MSNP) is inspired by the material characteristics of its predecessors, the FeOx/Au nanoparticle and Au-Nanomatryushka. Designed to exhibit MRI contrast, X-ray contrast for CT, photonic contrast for optical coherence tomography (OCT), absorbance in the near-infrared (NIR) spectrum for photothermal therapy (PTT), tunability of extinction characteristics during fabrication, theranostic potential, easy surface modulation for cellular targeting and biocompatibility and a nanostructure diameter of <60nm to support vascular extravasation ability, the fabrication of each 'primary' and 'subsidiary' strata – or functional layer – must be carefully controlled through fabrication methods. We report here our preliminary findings regarding the multi-step fabrication and initial characterization of the Au-MSNP. Furthermore, we specify methods necessary to fabricate extremely thin shells (as small as 1-2 nm; to maintain an overall particle diameter less than 60 nm) and to ensure magnetic material retention throughout the fabrication process. Relaxometry characterization, suggesting MRI contrast capacity, is also reported.

Osteoinductivity of rhBMP-2 Released From Composite Chitosan-Calcium Phosphate Scaffolds in Rat Muscle Pouch

Benjamin Reves, Joel Bumgardner, Judith Cole, Warren Haggard

Of the seven million bone fractures that occur in the U.S. each year, 5-10% of these injuries will not heal properly. Bone regeneration scaffolds can be used to enhance the body's natural healing cascade and help prevent the occurrence of inadequate healing in severe fractures. These scaffolds are designed to degrade gradually and be replaced with new bone, and they can be used as vehicles for local drug delivery. For instance, the scaffolds can be used to deliver growth factors such as BMP-2 that will augment the healing process. Chitosan, a biopolymer derived from the shells of crustaceans, has been shown to be osteoconductive. Calcium phosphate, in the form of hydroxyapatite, is the main inorganic component of bone, and has been used to enhance the osseointegration of bone implants. Our labs have demonstrated the in vitro potential of composite chitosan-calcium phosphate scaffolds in augmenting fracture repair. The objective of this study was to investigate the in vivo osteoinductive properties of composite chitosan-calcium phosphate scaffolds loaded with rhBMP-2 in a rat muscle pouch model.

Evaluation of Two Sources of Calcium Sulfate for a Local Drug Delivery System: A Preliminary Study

Ashley Parker, J. Keaton Smith, Harry Courtney, Warren Haggard

Local drug delivery has demonstrated substantial infection prevention benefits over systemic delivery, including reduced toxicity, antibiotic resistance, and time to reach the wound. Calcium sulfate (CaSO4) has been studied for local drug delivery and two types are commercially available, but the differences have not been reported. The differences were determined between two sources of CaSO4 and a K2SO4 catalyst's presence on the degradation, daptomycin elution and activity against Staphylococcus aureus. Pellets were formed with two different types of CaSO4, synthetic and naturally sourced from gypsum, and loaded with 5% daptomycin and 3% or 0% K2SO4. In vitro experiments were used to determine the daptomycin concentration and degradation profiles over 10 days. Turbidity assays were used to evaluate the activity of the daptomycin eluates against S. aureus. All pellets exhibited a bolus release with the highest daptomycin concentration on Day 1 with the sourced CaSO4 pellets. The synthetic CaSO4 pellets with 3% K2SO4 exhibited a slower drug release compared to the synthetic CaSO4 pellets with 0% K2SO4, which degraded and eluted daptomycin too quickly to be effective. Turbidity assays demonstrated that all CaSO4 pellets inhibit S. aureus for expected lengths of time. This preliminary in vitro data suggest differences in the degradation, elution, and activity properties between naturally sourced and synthetic CaSO4 pellets. From this study, the addition of K2SO4 appeared beneficial when using synthetic CaSO4. Synthetic CaSO4 may be effective when slow degradation and longer elution times are needed.

Polyionic Drug Carriers via Stereocomplexed Polylactide Hydrogels

Daniel G. Abebe, Tomoko Fujiwara

Polymer gels have been applied to biomedical field with remarkable advance in the therapeutic and clinical devices. Their applications involve cell culture, tissue engineering, drug delivery system (DDS), medical sensor, and so on, in which the bio-compatibility and safety of the gels are extremely important as well as the physicochemical properties. Since in most of these in vivo and ex vivo applications the polymer gels must immediately be removed by the metabolic system after completing their temporal functions, they are expected to consist of bioabsorbable or biodegradable polymers. Fujiwara et al, reported a novel thermo-responsible formation of a hydrogel in an enantiomeric mixture of the A-B-A type triblock copolymers, poly(L-lactide)-block-polyoxyethylene-block-poly(L-lactide) (PLLA-PEG-PLLA) and poly(D-lactide)- block-polyoxyethylene-block-poly(D-lactide) (PDLA-PEG-PDLA), in which the stereo-complexation of the PLLA and PDLA segments acted as the driving force for the gel formation.

In the present study, we report on thermo-responsive stereocomplexed hydrogels incorporating charged polymers. The incorporation of such charged polymers is either through a block copolymer design or in a micelle solution before and/or after gel assembly. The charged polymers used are polyethyleneimine (PEI) and polyglutamic acid. In recent years PEI has received much attention as a promising vector for non-viral gene therapy. PEI and DNA form polyion complex through the interaction of the positively charged amino groups of PEI with the negatively charged phosphate groups of DNA. In a similar mechanism polyglutamic acid can form complex with cationic proteins. We present the effect of polyions or polyplex on PLA hydrogel formation and its physical properties.

Preliminary Characterization of Drilled Fracture Fixation Implant Particles In Vitro and In Vivo

Jessica A. Jennings, Vernon Hartdegen, Richard Smith, Joel Bumgardner, Warren Haggard

Polymeric materials have many practical advantages for bone fixation implants. A surgeon's discretion in where fixation screws are placed would afford additional utility in these implant applications, as opposed to implants with pre-drilled holes for screws. In this preliminary study, particles generated by drilling PEEK implant materials were characterized for size, morphology, and biocompatibility. The majority of particles were above 100 microns in size, with an irregular shape. Particles did not induce expression of inflammatory cytokines interleukin-1 alpha or tumor necrosis factor alpha in a mouse moncytic cell line. Implants drilled in situ after placement in a rabbit femoral defect were compared to predrilled implants. An increased amount of particulate was found in when implants were drilled in situ, but this particulate did not induce more inflammatory response or have any negative effects on bone healing. Drilling implants in situ is a feasible way to achieve custom orthopedic fixation with biocompatible materials.

Tissue Engineering of Tendon to Bone Interface

Jared Cooper

Tissue interfaces are increasingly becoming a target of tissue engineering. There is a need to choose a scaffold material and morphology that can accommodate multiple tissues and transfer mechanical loads between those tissues. This work focuses the selection of a tendon to bone scaffold based on promotion of cellular attachment and proliferation as well as mechanical characterization.