3D Printing of Bone-Templated Scaffolds

JP Vanderburgh, S Lu, SJ Fernando, A Merkel, JA Sterling, SA Guelcher

Biomimetic 3D tissue engineered systems have been proposed for investigating molecular mechanisms of disease progression and drug screening. We have utilized 3D printing technology to investigate how the mechanical and topological properties of the bone microenvironment influence tumor progression to bone. Trabecular curvature, pore size and shape have been reported to affect the rate of new bone formation. Cells sense and respond to radii of curvature and the rate of new bone formation increases with the curvature of the surface, thus necessitating the creation of biomimetic 3D scaffolds. We reason that 3D scaffolds recapitulating the properties of bone at different anatomic sites will enable the development of 3D cell culture models for investigating the spatio-temporal dynamics of cancer progression in bone. We have developed a fabrication process in which templates of trabecular bone are prepared by a 3D inkjet printer and subsequently filled with reactive composite polyurethanes (PUR) to create scaffolds comparable to human bone. Human cadaver samples from the proximal tibia, proximal femur, and lumbar vertebrae were obtained from the Advanced Anatomy and Simulated Skills Program at Vanderbilt. Tissue samples were imaged using uCT technology from Scanco Inc. The scans were converted into STL images that were subsequently inverted to create a representation of the trabecular spacing using software provided by Solidscape Inc. and input into the 3D Printer for fabrication. The resulting wax molds were filled with a PUR/nanoparticle hydroxyapatite (nHA) composite comprising lysine diisocyanate, poly(É›-caprolactone) triol (300 Da), hydroxyapatite nanoparticles (diameter < 200 nm) and an iron acetylacetonate catalyst. The cured PUR composite was then extracted from the wax mold using acetone. The resulting scaffolds exhibited morphometric parameters and trabecular structure similar to that of bone, confirmed by SEM and Î¼CT analysis. Using a novel 3D inkjet printing approach, we have fabricated biomimetic scaffolds from synthetic polymers that recapitulate the mechanical and topological properties of bone. In future work, bone-templated scaffolds will be cultured with stromal cells, monocytes, and tumor cells to replicate the bone microenvironment in a bioreactor. These studies aim to investigate the effects of the bone microenvironment on the spatio-temporal dynamics of tumor progression in bone.

A Novel Platform Technology for Cytosolic Peptide Delivery with Endosomolytic Nano-Polyplexes Applied to Vascular Graft Intimal Hyperplasia

Evans BC, Hocking KM, Osgood MJ, Voskresensky I, Dmowska J, Kilchrist KV, Brophy CM, Duvall CL

Autologous vein grafts are commonly used for coronary and peripheral artery bypass but have a high incidence of intimal hyperplasia (IH) and failure. Here, a nano-polyplex (NP) approach is presented that efficiently delivers a MAPKAP kinase 2 inhibitory peptide (MK2i) to graft tissue in order to improve long-term patency by inhibiting pathways that initiate IH. In vitro testing in human vascular smooth muscle cells revealed that formulation into MK2i-NPs increased cell internalization, endosomal escape, and intracellular half-life of MK2i. This efficient delivery mechanism enabled MK2i-NPs to sustain potent inhibition of inflammatory cytokine production and migration in vascular cells. At the molecular level, MK2i-NPs blocked inflammatory and migratory signaling in human saphenous vein, as confirmed by reduced phosphorylation of the post-transcriptional gene regulator heterogeneous nuclear ribonucleoprotein A0 (hnRNP A0), the transcription factor cAMP element binding protein (CREB), and the chaperone heat shock protein 27 (HSP-27). Functionally, MK2i-NPs inhibited IH in human saphenous vein ex vivo. In a rabbit vein transplant model, a 30 minute intraoperative graft treatment with MK2i-NPs significantly reduced in vivo IH 28 days post-transplant ($p<0.01$ relative to untreated or free MK2i treated grafts). The decrease in IH corresponded to decreased cellular proliferation and a contractile vascular smooth muscle cell phenotype. These combined results confirm that MK2i-NPs potently reduce vein graft failure and highlight a new approach to the delivery of peptide-based therapeutics to cells and

tissue.

Remodeling of an injectable, settable, and cell-degradable composite bone cement with bone-like strength in a rabbit femoral plug defect model

Madison A.P. McEnery, Sichang Lu, Mukesh K. Gupta, Katarzyna J. Zienkiewicz, Daniel Shimko, Kerem N. Kalpakci, Craig L. Duvall, Scott A. Guelcher

Introduction: Calcium phosphate (CaP) ceramics are highly osteoconductive, but their use in weight-bearing applications is limited by their brittleness. Blending CaPs with polymers ideally enhances their mechanical properties while maintaining good osteoconductivity. We developed an injectable polythioketal urethane (PTKUR) that selectively degrades by reactive oxygen species generated by infiltrating cells during healing. Blending PTKUR with CaP particles yields a low-porosity (<5%) cement with mechanical properties exceeding trabecular bone. Current studies are focused on altering the CaP phase and degradation characteristics for superior bone regeneration. Methods: A thioketal (TK) diol was synthesized from thioglycolic acid and 2,2-dimethoxypropane. This was reacted with an excess of lysine triisocyanate to create a prepolymer. TK, prepolymer, and 55 wt% MastergraftÂ® (MG) (100-300 Î¼m) or 60 wt% hydroxyapatite (HA) (<200 nm) particles were mixed and catalyzed by iron(III) acetylacetonate for a PTKUR composite with a tack-free time <10 min. PTKUR films were immersed in a hydrogen peroxide-based accelerated oxidative media at 37Â°C. Samples were dried and weighed at various time points to determine degradation rate. 12 mm x 6 mm diameter cylindrical composites were compressed at 25 mm/min and the modulus calculated as the slope of the elastic portion of the stress strain curve and yield strength as the local maximum stress. Bilateral 6-8 mm x 5 mm diameter defects were drilled in the femoral condyle of the distal femur of 8 New Zealand White rabbits. PTKUR composites of MG or HA were injected and allowed to cure for 10 minutes prior to closing the wound and MG and HA alone were controls. ÂµCT and histology were used to evaluate healing and polymer degradation at 6 and 12 weeks. Results & Discussion: Degradation studies demonstrated nearly 100% degradation of PTKUR after 72 h in oxidative medium and minimal degradation in PBS. ÂµCT images of defects at 12 weeks showed densification and infiltration of trabeculae surrounding the defect indicating that the grafts were well integrated. Histological sections provide evidence of polymer degradation in the presence of cells. Mineralization is evident in the periphery of the scaffold indicating initiation of regeneration by creeping substitution. Conclusion: Injectable, settable, and cell-degradable PTKUR/ceramic composites have initial bone-like strength, degrade by cell-mediated oxidation, and remodel in vivo.

Local Delivery of siRNA from ROS-Degradable Scaffolds to Promote Diabetic Wound Healing

John R. Martin, Christopher E. Nelson, Mukesh K. Gupta, Fang Yu, Kyle Hocking, Scott A. Guelcher, Jeffrey M. Davidson, Craig L. Duvall

Introduction: Diabetics are predisposed to formation of nonhealing skin wounds that are prone to ulceration and infection, causing significant morbidity and an increased rate of limb amputation. The pro-angiogenic and pro-healing transcription factor hypoxia induced factor 1Î± (HIF1Î±) is known to be reduced in diabetic patients. Here, we applied a recently developed tissue scaffold-based platform for sustained, local release of siRNA nanoparticles (siNPs) against prolyl hydroxylase domain protein 2 (PHD2), a negative regulator of HIF1Î±. Our previous studies in a simple mouse subcutaneous implant model showed that PHD2 silencing promotes localized angiogenesis by activating HIF1Î±. Here we utilize a new poly(thioketal urethane) (PTK-UR) scaffolds chemistry with cell-mediated, ROS degradation mechanism for controlled release of PHD2 siNPs to promote angiogenesis and tissue regeneration in compromised diabetic rat excisional skin wounds.

siNPs were formulated by electrostatically condensing either scrambled (SCR) or PHD2 siRNA onto the surface of self-assembled, micellar nanoparticles pre-formed in dH2O. 3D porous scaffolds were formed from PTK polyols mixed with lyophilized siNPs and lysine triisocyanate (LTI) and implanted into excisional skin wounds in diabetic rats. Excised tissues (day 4 or 7) were evaluated by RT-PCR for PHD2 silencing, where PHD2 siNP-loaded PTK-UR implants demonstrated a significant decrease in PHD2 mRNA. Correspondingly, Western blot analysis of day 4 tissue samples displayed an increase in VEGF protein levels in PHD2 siNP scaffolds compared to SCR siNP scaffolds.This led to more robust wound healing with a significant increase in both blood vessel number and proliferating cells at day 7 as quantified by immunohistochemistry for Collagen IV and Ki-67, respectively. In summary, local delivery of PHD2 siNPs from ROS-degradable scaffolds has been utilized to kick start a pro-angiogenic, HIF1Î±-mediated growth program. This represents a promising, clinically translatable approach to treat non-healing skin wounds.

pH-Responsive Polyplex Nanovaccines for Enhancing MHC Class-I Presentation of Peptide Antigens

Feng Qiu, John T. Wilson

Peptide vaccines comprise essential antigenic short peptide fragments, or epitopes, derived from protein antigens. Compared with traditional vaccines, which are usually composed of whole organism or proteins that contain a high portion of epitopes that are non-immunogenic or worse divert the immune system from recognizing the intended target, peptide vaccines offer the advantage of generating more precisely targeted T cell responses. On the other hand, because peptide antigens are usually low molecular weight, they typically suffer from inefficient uptake by antigen presenting cells, rapid clearance with minimal accumulation in lymph nodes, and elicit very weak immune response by their own. Therefore, there is a need for delivery systems for peptide vaccines that overcome these problems.

In our current study, we have designed a facile approach to fabricate nanoparticles for peptide antigen delivery through electrostatic complexation of oligolysine (Kn) modified epitopes with the pH-responsive and endosome destabilizing polyanion, poly(propylacrylic acid) (pPAA). For proof-of-concept, a peptide comprising a model epitope (SIINFEKL) derived from the protein ovalbumin and an N-terminal K10 tail was used. Polyplex nanoparticles were fabricated by simply mixing K10-SIINFEKL with pPAA, offering a mix-and-go approach to particle-based vaccine formulation. DLS, TEM and gel electrophoresis demonstrated that stable polyplex nanoparticles (78.4Â±2.56 nm) could be obtained when pPAA and K10-SIINFEKL were mixed at a charge ratio of 2:1. An in vitro antigen presentation assay revealed that polyplex vaccines significantly enhanced MHC class-I antigen presentation of SIINFEKL epitope compared to polyplexes fabricated with poly(methacrylic acid) or the free K10-SIINFEKL peptide. We hypothesize that this is due to the enhanced cellular uptake of peptide associated with nanoparticle-based delivery as well as efficient cytosolic release of peptide to the classical MHC-I processing pathway mediated by the endosomal escape capability of pPAA. This approach may provide a simple, rapid, and adjuvant-free approach for enhancing T cell responses to peptide vaccines.

Release of Adenosine from Chitosan Beads

Allen Mamaril
Parwinder Singh
Ravi Patel
Joel D. Bumgardner
Jessica A. Jennings

This study was conducted to demonstrate the efficacy of chitosan microbeads as a delivery vehicle for adenosine. Recent literature has shown adenosine to reduce inflammation, increase proliferation and

matrix production, and promotes chondrocyte growth in osteoarthritic models. Chitosan beads were loaded with adenosine, and an elution study was conducted over the course of seven days. High concentrations of adenosine were released in the first two days of the trial, and the concentrations declined constantly over the next five days. Adenosine-loaded microbeads may provide effective controlled delivery of adenosine for applications in promoting healing of cartilage in osteoarthritic patients.

PSMA antibody functionalized docetaxel-loaded superparamagnetic iron oxide nanoparticles for prostate cancer therapy

Murali M. Yallapu, Prashanth K.B. Nagesh, Nia R. Johnson, Vijaya K.N. Boya, Pallabita Chowdhury, Bilal B. Hafeez, Shadi F. Othman, Vahid Khalilzad-Sharghi, Aditya Ganju, Sheema Khan, Stephen W. Behrman, Nadeem Zafar, Subhash C. Chauhan, Meena Jaggi

Objectives: Docetaxel (Dtxl) is currently the most common first-line therapeutic option for castrate resistance prostate cancer (PC). However, adverse side effects and problems associated with chemo-resistance limit use of docetaxel in clinical settings. Thus, using a targeted nanoparticle system to improve docetaxel targeted delivery and its activity at the tumor site could be an attractive strategy for PC therapy. PSMA is highly overexpressed in PC cells and thus is a highly attractive molecular target for PC therapy. Therefore, the objective of this study was to develop and determine the anti-cancer efficacy of a novel docetaxel loaded, PSMA targeted superparamagnetic iron oxide nanoparticle (SPION) (J591-SPION-Dtxl) formulation for PC therapy.
Methods: Physico-chemical characterization of SPION-Dtxl or MNPs-Dtxl was performed using TEM, DLS, FT-IR, TGA, X-RD, and MR imaging methods. Cell proliferation and colony formation assays were utilized to evaluate therapeutic efficacy of this unique nanoformulation in clinically relevant cell line models (C4-2, PC-3, and DU-145). Additionally, molecular effects of this formulation on apoptosis, anti-apoptosis, and drug resistant associated proteins were evaluated using immunoblotting assays. A tubulin stabilization study was performed using confocal immunofluorescence microscopy analysis. For active targeting, PSMA antibody (J591) conjugation to this formulation was achieved with the N-hydroxysuccinimide group containing PEG polymer. Active targeting potential of this formulation was evaluated in PSMA + (C4-2) and PSMA â€“ (PC-3) cell lines and their derived xenograft/orthotopic tumor models.
Results: The MNPs-Dtxl formulation showed optimal particle size and zeta potential, which can efficiently be internalized in PC cells. MNP-Dtxl exhibited potent anti-cancer efficacy via induction of the expression of apoptosis associated proteins and downregulation of anti-apoptotic proteins in PC cell lines. Interestingly, it also inhibited the expression of chemoresistance-associated proteins (PSMA and MDR1). J591-MNPs-Dtxl exhibited a profound uptake in PSMA+ cells (C4-2) compared to PSMA null (PC-3) cells. A similar targeting potential was also observed in ex-vivo studies in C4-2 tumors but not in PC-3 tumors, suggesting its tumor specific targeting potential.
Conclusion: PSMA antibody functionalized SPION/MNPs-Dtxl formulation can efficiently target PSMA+ PC cells and tumors.

Mechanism of Enhanced Cellular Uptake and Cytosolic Retention of MK2 Inhibitory Peptide Nano-polyplexes

Kameron V Kilchrist, Brian C Evans, Colleen M Brophy, Craig L. Duvall

Electrostatic complexation of a cationic MAPKAP kinase 2 inhibitory (MK2i) peptide with the anionic, pH-responsive polymer poly(propylacrylic acid) (PPAA) yields MK2i nano-polyplexes (MKi-NPs) that significantly increase peptide uptake and intracellular retention. This study focused on elucidating the

mechanism of MK2i-NP cellular uptake and intracellular trafficking in vascular smooth muscle cells. Understanding how NP physicochemical properties affect cellular uptake and trafficking is critical to the rational engineering of nano-scale constructs for biologic drug delivery. Small molecule inhibition of various endocytic pathways showed that MK2i-NP cellular uptake involves both macropinocytosis and clathrin mediated endocytosis, whereas the free peptide utilizes clathrin mediated endocytosis alone for cell entry. Electron microscopy studies revealed that MK2i-NPs, but not free MK2i peptide, induces cellular membrane ruffling and results in macropinosome formation. Consistent with the reported pH-dependent membrane disruptive properties of PPAA, MK2i-NPs demonstrated MK2i endo-lysosomal escape and cytosolic delivery. Finally, a novel technique based on recruitment of Galectin-8-YFP was developed and utilized to demonstrate that MK2i-NPs cause endosomal disruption within 30 minutes of uptake.

Cis 2-decenoic acid interacts with bacterial cell membranes to potentiate additive and synergistic responses against biofilm in orthopaedic pathogens

Elysia Masters, Michael Harris, Jessica Amber Jennings

Musculoskeletal infection is a major risk in all wounds; especially open fractures and surgical or orthopedic procedures such as implants or bone grafts. Bacteria attached to a surface can form a protective polymeric matrix called biofilm and enter a state of reduced metabolic activity. Causing as much as 80% of infection, biofilm limits the activity of antibiotics or immune cell attack, increasing the severity of infection and making it particularly difficult to treat. A fatty acid, cis-2-decenoic acid (C2DA) has been shown to both disperse bacterial communities as well as inhibit biofilm growth in polymicrobial communities. Interactions between antibiotics and C2DA were determined using checkerboard assays using varying concentrations of C2DA and the antimicrobials vancomycin, cefazolin, linezolid, tetracycline, chlorhexidine, ceftazidime and ciprofloxacin. Tubes containing tryptic soy broth (TSB), C2DA, and antibiotics were inoculated with 1x 104 colony forming units (CFU) of Staphylococcus aureus (UAMS-1) or Pseudomonas aeruginosa (PAO1), common gram-positive and gram-negative orthopaedic pathogens. The response of each antibiotic was quantified using the fractional inhibitory concentration index (FICI) calculated from minimum biofilm inhibitory concentrations (MBIC) by adding the ratios of MBIC for antibiotic to the MBIC in combination to the ratio of MBIC for C2DA. Bacterial membrane permeability was determined by a 1-N-phenyl-naphtylamine (NPN) uptake assay, in which a hydrophobic probe fluoresces in phospholipid environments where membrane is damaged but not in an aqueous environment. The antibiotics tetracycline, linezolid, and chlorhexidine were synergistic with S. aureus, while the antibiotics amikacin, ceftazidime, and ciprofloxacin produced synergistic effects in P. aeruginosa. NPN permeability assays indicated increased outer membrane permeability comparable to polymyxin B for increasing concentrations. While it is known that C2DA acts in biofilm dispersal, evidence in this study suggests that this fatty acid similar in structure to the outer membrane of bacteria, may incorporate into the membrane and increase antimicrobial action, particularly for those antibiotics that have internal mechanisms of action, including amikacin, tetracycline, linezolid, and ciprofloxacin. In ongoing and future studies local drug delivery systems that can effectively deliver both hydrophobic C2DA and antibiotics to the implant surface are being developed.

Targeted Nanoparticles for Delivery of Short Interfering RNA for Prevention of Post-traumatic Osteoarthritis

Sean Bedingfield, Taylor Kavanaugh, Thomas Werfel, Christy Patterson, Karen Hasty, Craig Duvall

Osteoarthritis is characterized by the degeneration of cartilage, bone, and other tissues leading to chronic joint pain and debilitation. Post-traumatic osteoarthritis (PTOA) occurs after a traumatic injury

to the bone or soft tissue including ligament and meniscal tears, and there is currently no cure, only medications to relieve the pain. Traumatic injury exposes type II collagen (CII), and is reversible. Degradation of CII initiates a nonreversible, pathological cycle of inflammation and cell death that leads to permanent cartilage damaged and advanced OA. Matrix metalloproteinase 13/collagenase 3 (MMP-13) activity is significantly increased in human osteoarthritic cartilage and is known to be the primary mediator of CII degradation. Short interfering RNA (siRNA) catalytically binds and mediates degradation of its targeted mRNA through sequence-selective complementary base pairing, and is more potent than stoichiometric inhibitors (i.e. small molecule drugs). siRNA targeting MMP-13 could inhibit the progression of PTOA. As siRNA must be delivered to the cytoplasm to take effect, a carrier must be designed to induce cell uptake via endocytosis and then mediate escape from the endosome. The carrier presented is meant to target the cells in joint tissues, and activate when MMP-13 is present for â€œon demandâ€ delivery. In vitro and initial in vivo experimentation support the carrier design's ability to [1] complex nucleic acids into protective micelles, [2] target damaged cartilage, and [3] enable knockdown of MMP-13 expression.

A Shape Memory External Stent to Prevent Dialysis Graft Failure

Timothy Boire, Byron Smith, Kyle Hocking, Eric Wise, Christy Guth, Colleen Brophy, Hak-Joon Sung

Hemodialysis is the primary lifeline for patients with end-stage renal disease (ESRD), but arteriovenous graft (AVG) failure imposes significant morbidity, mortality, and financial impositions. Failure rates of 50% after 1 year and 75% after 2 years are reported in hemodialysis patients that utilize polytetrafluoroethylene (PTFE) dialysis grafts. Stenosis at the venous anastomosis ultimately leads to compromised blood flow, necessitating vascular interventions (e.g. balloon angioplasty or stents) or re-do access surgeries. Major financial impositions ensue for patients, insurers, and dialysis clinics, while blemishing hospital records with unwanted, expensive patient readmissions.

The leading cause of failure at the venous anastomosis is neointimal formation triggered by venous responses to surgical injury resulting from PTFE implantation and arterial flow.

We are developing an external stent that can minimize neointimal formation by providing mechanical support, promoting outward instead of inward vein remodeling in the arterial circulation, and eluting anti-neointimal therapeutics. Existing external mesh supports applied in other settings, such as to saphenous vein grafts in heart bypass grafting surgeries, have demonstrated some promise but cannot be applied in hemodialysis vascular access surgeries due geometric complexities. Our novel shape memory polymers (SMPs) are moldable at body temperature to enable facile wrapping of the external support around the venous anastomosis, the most critical area for vein failure. The material is biocompatible, slowly biodegradable, and mechanically compliant to provide mechanical support for the vein over the critical first few months of remodeling, while permitting normal vein pulsation and ultimately being resorbed.

Preliminary data in an ex vivo AVG model with human saphenous veins (HSV) indicates the potential of these external supports to reduce neointimal formation and, in turn, obviate the subsequent adverse clinical repercussions. To determine the role that arterial hemodynamics plays in vein remodeling and treatment effects in a flow-relevant system, treatments are currently being applied in an AVG flow model with arterial-mimetic pressures. From this ex vivo flow work, device characterization, and in vitro vascular smooth muscle cell phenotyping, an optimal treatment will be derived and applied in a clinically-relevant porcine AVG model.

Utilizing Poloxamer 407 As A Nucleus Pulposus Regeneration Scaffold

Nicholas A. Temofeew, Katherine R. Hixon, Scott A. Sell

Degeneration of the nucleus pulposus (NP) is the primary cause of back pain in almost 80% of the world population. The current gold standard treatment for a degenerated NP is a spinal fusion surgery which is costly, extremely invasive and is a temporary solution that can lead to further complications. Research has been moving towards minimally invasive methods to lessen the collateral damage created during surgery and has encouraged the rise of a regenerative medicine solution. The use of an injectable scaffold has the potential to promote a healthy and hydrated environment to regenerate the NP. Cryogels are unique polymeric scaffolds composed of a highly connected, macroporous structure, and capable of maintaining stability under high deformations. For this project, cryogels have been developed using gelatin and poloxamer 407 (P407) at varying ratios to determine the ideal combination of stability, water retention and pore size. P407 is an amphiphilic, tri-block polymer with thermoreversible properties. Its molecules also aggregate into micelles as the temperature rises which increases its mechanical strength. For the application of NP regeneration, a gelatin-P407 cryogel should be both stable and a well hydrated carrier. The cryogels created varied from a 1:1 gelatin to P407 ratio to a 10:1 ratio with an addition of a 50 mM EDC crosslinking agent. All samples were divided into cylindrical samples between 7 and 15 mg and lyophilized. The inclusion of P407 in the cryogels resulted in a significant increase in hydrophilicity after just one hour of swell tests. At the end of the 24 hour swell test, each of the gelatin-P407 cryogels increased in weight at least 2000% while the gelatin cryogel only increased 1721% the original dry weight. P407 increases the water retention capabilities of gelatin cryogels. There was no difference in pore diameter between the differing ratios of gelatin-P407 cryogels with averages between 70 and 108 micrometers. At the time of abstract submission, mechanical evaluations, degradation tests, and cellular interactions were being performed to further understand and evaluate the gelatin-P407 cryogels as a possible NP regeneration scaffold.
Previous studies have concluded that a loss of water in the intervertebral disc (IVD) is a huge contributor to NP degradation. An injectable cryogel that is stable and able to maintain a hydrated environment in the IVD could potentially regenerate the natural tissues in the IVD.

Development of a Silk Fibroin/Poloxamer Electrospun Scaffold for Skin Tissue Engineering

Parin U. Kadakia, Scott A. Sell

The goal of this study is to create a novel, inexpensive off-the-shelf dermal regeneration template (DRT) with amplified moisture/permeability properties. The presence of moisture is important for optimal burn wound healing since it creates an environment for re-epithelialization and minimizes the risk of infections. To form such a scaffold, the polymers of silk fibroin (SF) and Poloxamer 407 (P407) were electrospun into a nanofibrous mesh. SF is a biodegradable/biocompatible polymer with RGD binding sites that enhance both cellular attachment and proliferation. Furthermore, the hydrophilic nature of P407 is expected to increase scaffold wettability and moisture retention.
For this preliminary study, various ratios of SF:P407 were dissolved in hexafluoroisopropanol at a total polymer concentration of 10% (w/v) and then electrospun. To observe surface morphology, fiber diameters and pore sizes were measured from SEM images. Furthermore, uniaxial tensile testing was performed on both dry and hydrated samples at a strain rate of 10.0 mm/min to failure. Swelling kinetics were investigated by submerging air-dried scaffolds into DI water and measuring their weights at subsequent time points. Contact angle measurements were completed by dropping 3 Î¼L of DI water from a certain height onto each scaffold and taking a picture 2 s after the water droplet landed on the surface.
With regards to porosity, pure SF scaffolds had the largest pore sizes (261Â±203 Âµm^2) and fiber diameters (2.7Â±0.8 Âµm), and an increase in P407 concentration caused both of these properties to decrease. Tensile testing demonstrated that the average strain at break was significantly larger and the average modulus was significantly smaller for the hydrated scaffolds as compared to the dry samples.

These outcomes can be explained via a tradeoff between elasticity and strength as the scaffolds become hydrated. Swelling kinetics showed that with or without P407, all scaffolds swelled to over 250% of their dry weights in 4 h. Finally, as the P407 concentration increased, contact angle measurements decreased (from 71Â° to 11Â°). Overall, these results indicate that the inclusion of P407 does indeed increase scaffold wettability, which should in turn promote cellular adhesion and attachment. At the time of abstract submission, the following investigations were in progress: (1) mechanical evaluations of degradation and (2) human dermal fibroblast (hDF) adhesion, proliferation, and infiltration studies.

Altering hydrophilic siRNA polyplex corona chemistry by zwitteration or sialylation to improve intravenous pharmacokinetics

Meredith A. Jackson, Eric A. Dailing, Zoe E. Johnson, Thomas A. Werfel, Todd D. Giorgio, Craig L. Duvall

Traditional siRNA polyplex nanoparticles designed for intravenous delivery employ the use of PEGylation to reduce protein adsorption and prevent aggregation. However, polyplex circulation half-lives are still often less than 20 min and most particles are quickly destabilized or taken up by macrophages in the liver as a direct result of serum opsonins and the complement system. To improve the circulation time and passive targeting of siRNA polyplex-based therapeutics, the current work explores the use of either zwitterionic coronas or sialic acid-based coatings within an endosomolytic, pH-responsive siRNA delivery system.

Polymers were synthesized by RAFT polymerization to contain one block of hydrophilic corona polymers (variable) and a second random copolymer block of (2-dimethylamino)ethyl methacrylate (DMAEMA) and butyl methacrylate (BMA) with an optimized 50:50 ratio of DMAEMA and BMA. Hydrophilic corona chemistries included a zwitterionic phosphocholine based monomer or branched PEG derivatives. For sialylation, poly(oligoethylene glycol) methacrylate monomers containing an azide function were reacted with alkyne-functionalized sialic acids. Polyplexes were assessed for siRNA complexing, uptake, in vitro knockdown, endosomal escape, and thermodynamic protein adsorption.

Overall, zwitterionic corona polymers outperformed traditional linear PEGylated architectures in terms of increased cell uptake and reduced protein adsorption. However, in vitro all polyplexes showed similar levels of knockdown of the model gene luciferase and low cytotoxicity. Similarly, all polyplexes showed similar levels of endosomolysis except for sialylated polyplexes, which proved too hemolytic at physiological pH levels. Further investigation revealed that incomplete reaction of azide-functionalized monomers may have contributed to increased hemolytic capacity. Based on isothermal titration calorimetry studies of albumin absorption, zwitterionic polyplexes proved to be thermodynamically least favorable to protein adsorption, implying that greater energy is required to break apart the hydration shell of zwitterated polyplexes than PEGylated coatings. Further characterization is needed for sialylated surfaces.

In conclusion, the zwitterionic coronas show the most promise thus far for improved in vivo pharmacokinetics. In vivo studies are an important next step to confirm importance of corona chemistry for circulation time.

Protection of Pancreatic Islets in ROS Sponge Hydrogels for Type 1 Diabetes Therapy

Bryan Dollinger, Mukesh Gupta, John Martin, Craig Duvall

Type 1 Diabetes (T1D) is an autoimmune disorder that results in the destruction of insulin-producing Î²-cells within the pancreatic islets of Langerhans. The current gold standard treatment for T1D requires invasive peripheral monitoring and regulation of glucose levels via daily insulin injection and glucose supplementation, resulting in reduced quality of life. Transplantation of primary islets or engineered glucose-responsive/insulin-producing cells represents a potential route for a T1D cure. However,

transplanted cells suffer from poor survival post-transplant due to usage of non-cytocompatible cell implant sites and host production of high levels of cytotoxic reactive oxygen species (ROS) associated with â€œinjuryâ€ to the implant site.
Co-loading a degradable, â€œROS spongeâ€ hydrogel with antioxidant drugs and islet conducive matrix proteins will promote islet survival and function, providing an improved T1D therapy. This therapeutic approach leverages a thermoresponsive hydrogel technology, which transitions from a liquid to a solid gel state as its temperature is increased from ambient to physiological temperature. This hydrogel comprises the ABC triblock polymer poly(propylene sulfide-block-dimethyl acrylamide-block- N-isopropylacrylamide) (PPS-PDMA-PNIPAAM). Hydrophobic PPS causes this polymer to self-assemble into micelles at room temperature in aqueous solutions, hydrophilic PDMA ensures swelling/hydration of the resulting hydrogel, and PNIPAAM triggers a thermally-induced transition of micelles into a supra-assembled 3D hydrogel at its the lower critical solution temperature (LCST), which occurs at ~34Â°C. Prior work has proven that PPS has multiple functions: (1) encapsulation and sustained release of hydrophobic drugs (2) transition from hydrophobic to hydrophilic due to reaction with ROS, providing a hydrogel degradation mechanism (3) irreversible reaction with ROS (especially H2O2) making PPS a cell-protective â€˜spongeâ€™ for cytotoxic ROS species. With the addition of a collagen matrix, various cell lines will remain viable long-term in this hydrogel at concentrations of H2O2 where unprotected cells would not. This offers great promise that this system will be an exceptional platform for therapeutic cell delivery as a treatment for T1D.

Adenosine Increases Chemotactic Migration of Stem Cells

Mamadou Diallo, J. Amber Jennings

The high self-renewal capacity of Mesenchymal Stem Cell (MSC) and their ability to differentiate into different tissues including bone makes them the most targeted cells in tissue regeneration. Studies show that high adenosine concentration may be present at the site of injury, and more particularly in bone injuries. Previously, increased proliferation of cells has been observed when exposed to adenosine concentrations ranging from 7-1000 Âµgâ€¢ml-1. The role of adenosine in the function of mesenchymal stem cells (MSCs) is not fully understood. In this study we analyzed the effect of adenosine on migration of stem cells (MC3T3 preosteoblast cells) through a transwell migration assay. Groups included no-additive negative control, 10% fetal bovine serum positive control, 1% serum control, and concentrations of adenosine at 50, 25, and 12.5 Âµgâ€¢ml-1 . Results indicate that 50 Âµgâ€¢ml-1 of adenosine stimulates significantly more migration of preosteoblast stem cells compared to no additives or low concentrations of serum, and is comparable to 10% serum. This suggests that biomaterials releasing adenosine may stimulate stem cell migration and cell proliferation for better healing through recruitment of stem cells and increased proliferation. Delivery of adenosine from calcium sulfate or chitosan matrices may promote healing in severe bone defects without using growth factors.

Incorporation of Platelet Rich Plasma into Silk Fibroin Electrospun Scaffolds to Stimulate Wound Healing

Andrew J. Dunn, Paul N. Richard ,Scott A. Sell

Non-healing, chronic pressure ulcers are a constant threat for elderly patients and spinal cord injury (SCI) patients. The restoration of blood supply to ischemic tissues can initiate a positive feedback loop of inflammation creating these debilitating wounds. Through creating an off-the-shelf dermal regeneration template (DRT), we hope to be able to promote accelerated healing of these wounds by providing a template for regeneration while simultaneously modifying the local cellular environment. Platelet rich plasma has been found to stimulate regeneration in chronic pressure ulcers, and the purpose of this study was to develop an electrospun DRT which incorporates PRP and evaluate the in vitro wound

healing potential of these scaffolds.
Silk fibroin (SF) of varying concentrations (5%, 7%, 10%) with and without a preparation rich in growth factors, (lyophilized PRP, PRGF), were dissolved into hexafluoroisopropanol solutions and electrospun. Scaffold fiber and pore diameter was determined through SEM images. Scaffold degradation and mechanical properties were collected through a PBS incubation study. At the time of submission, quantification of growth factor and cytokine release from scaffolds are underway. Fibroblasts proliferation and infiltration studies are also ongoing.
Average fiber diameter and pore area increased with increased SF concentration. The incorporation of PRGF further increased fiber diameters. Most notably, the mechanical and degradation characteristics of SF scaffolds were significantly changed with the incorporation of PRGF. While the inclusion of PRGF decreased mechanical strength and accelerated degradation, this was not seen as a negative result. The rate of degradation of the SF/PRGF constructs (between 14-28 days) would be appropriate for use in a dermal wound. Additionally, this fiber breakdown allows for PRGF release in a relevant fashion to create a chemotactic gradient for reparative cells.
The results of this study reveal that silk scaffolds infused with PRGF elute pro-healing growth factors into surrounding fluids, supporting these scaffoldsâ€™ utility in chronic wound applications. Future studies include studies on keratinocytes and macrophages, as well as an in vivo murine ischemia/reperfusion model testing. In order to promote greater porosity of scaffolds, SF will be electrospun onto an air-impedance mandrel to physically inhibit fibers from tightly packing by injecting air throughout the scaffold as it forms.

Stimulating Pulmonary Immunity with pH-Responsive Nanoparticle Vaccines that Enhance Intracellular Delivery

Frances C. Knight, Pavlo Gilchuk, Sema Sevimli, Sebastian Joyce, and John T. Wilson

Stimulation of immunity through vaccination at mucosal surfaces (e.g., in the lungs) is important because this is a route of entry for many pathogens. Vaccinating here mimics natural infection and is more likely to trigger a protective immune response directly at the site of pathogen encounter. Pulmonary immunization with subunit vaccines is an attractive approach because they are safer than vaccines based on live or attenuated microbes; they are less likely to trigger inflammation and damage in the lung tissue and cannot revert to a virulent form. However, subunit vaccines are also typically less immunogenic than live or attenuated vaccines. They are particularly inefficient at generating CD8+ T cells (CD8T), which are necessary for defense against many intracellular pathogens (e.g., viruses), as well as for treating or preventing cancer. To address this, we have developed a pH-responsive nanoparticle (NP) delivery system that can be loaded with subunit protein antigen (Ag) and nucleic acid adjuvant (Adj). The system uses endosomal acidification after uptake to release Ag into the cytosol, where it can be processed by the MHC-I pathway, resulting in a CD8T immune response. Approximately 20 nm diameter NP were self-assembled from endosomolytic diblock copolymers synthesized by RAFT polymerization. NP were covalently conjugated to a model Ag, ovalbumin (OVA), and electrostatically complexed with CpG DNA Adj. Complete formulation (OVA-NP/CpG) was administered intranasally to C57BL/6 mice. OVA-specific CD8T in the lungs, bronchoalveolar lavage fluid, and spleen were quantified using MHC-I tetramer staining. Absolute count of OVA-specific CD8T was significantly higher in groups receiving complete formulation vs. control groups that received partial formulations (NP, OVA-NP, NP/CpG, or NP/CpG + soluble OVA). Mice showed no weight loss or signs of morbidity, and pathology analysis revealed modest local inflammation and no damage to the lung tissue. Flow cytometry was also used to characterize cellular uptake of fluorescently labeled complete formulation in the lungs, which was found to be highest in alveolar and interstitial macrophages and CD103+ and CD11b+ dendritic cells. Finally, ELISA was used to assess OVA-specific IgG antibody responses in sera. Complete formulation

elicited a higher IgG response than control groups after administration of a single dose. This work has implications in developing vaccines for respiratory infections and lung cancer.

Measuring and Modeling Elution of Cis 2-Decenoic Acid from Phosphatidylcholine coatings

Michael Harris, Elysia Masters, Ravi Patel, Jessica Amber Jennings, PhD

The need for innovative therapies for prevention of implant-associated infections is becoming more urgent as the mortality due to such infections has increased, with implant infection rates as high as 17-21%. A novel phosphatidylcholine (PC) carrier matrix can be applied to the surface of an implant material to leave a degradable, biocompatible matrix that can be loaded with various antibiotics and antimicrobials for local drug delivery. In this study, we have incorporated cis-2-decenoic acid (C2DA), a known antibiofilm agent, and Alizarin Red S, a water soluble dye used as a model antibiotic, into PC matrices to develop initial models of drug release. Five coating types were fabricated by mixing powdered Alizarin Red S and C2DA into purified PC (Phospholipon 90G) and applied to titanium coupons. Coupons were each placed in 2 mL phosphate buffered saline and incubated at 37Â°C. Samples were taken every 24 hours with complete media refreshment at each time point. We observed elution of C2DA from PC to follow a pattern of burst release during the first 24 hours with an additional burst on day 6. At least 60% of the total C2DA was released over the course of one week at levels above inhibitory concentrations. Alizarin eluted from the PC in a burst response indicating first-order release kinetics and 100% had eluted over 7 days. An antibiotic and C2DA loaded PC coating could serve as an effective local drug delivery device for preventing infection on or around orthopedic implants.

In Situ Crosslinked Endosomolytic Polymer Vesicles for Versatile Delivery to Cytosolic Immune Surveillance Receptors

Daniel Shae, Anna Caldwell, Sema Sevimli, John T. Wilson

Cyclic dinucleotides (CDNs) are a recently emerging class of vaccine adjuvants that have shown particular promise in cancer immunotherapy for their ability to elicit the production of type I interferons and trigger the generation proinflammatory leukocyte phenotypes in immunosuppressive tumor microenvironments. Ligands of the stimulator of interferon genes (STING) cytosolic protein sensor, the endogenous 2â€™3â€™ cyclic guanosine monophosphate-adenosine monophosphate (cGAMP), and more generally CDNs in general, have shown to be a promising immunotherapeutic in eliciting tumor regression. However, poor cytosolic localization remains a significant barrier to the efficacy and translatability of these emerging immunotherapeutics. We have developed polymeric vesicles that actively enhance endosomal escape of CDNs for efficient activation of cytosolic STING protein. Reversible addition-fragmentation chain transfer (RAFT) polymerization was used to synthesize poly[(ethylene glycol)-b-(butyl methacrylate-co-diethylamino ethyl methacrylate-co-pyridyl disulfide ethyl methacrylate)] (PEG-b-DBP) amphiphilic diblock polymers that self-assemble into surface neutral pH-responsive vesicles with d<100 nm in physiological conditions. Pyridyl disulfide (PDS) moieties were exploited for post-assembly crosslinking of the vesicle membrane via partial reduction of disulfide bonds. Particles exhibited a drop in hydrodynamic radius as pH was reduced from 7.4 to 5.8 corresponding to particle disassembly, release of encapsulated cargo, and transition to a membrane destabilizing state. Crosslinking was found to increase the effective average molecular weight of polymer chains, resulting in enhanced membrane-destabilizing activity in an erythrocyte lysis assay. Vesicles encapsulated the cGAMP with efficiencies exceeding 25% and significantly enhanced potency, eliciting type I interferon responses between one and three orders of magnitude higher than control doses of free cGAMP in human monocytes and murine dendritic cells, allowing for over 100 fold cGAMP dose sparing. Additionally, encapsulated cGAMP triggered murine macrophage repolarization from an

immunosuppressive M2 phenotype to the proinflammatory M1 phenotype. Collectively, these data demonstrate that endosomolytic polymer nanoparticles enhance drug delivery to the cytosolic STING sensor and suggest that vesicle encapsulated cGAMP formulations may prove to be a powerful cancer immunotherapeutic.

Evaluation of Chitosan Paste as a Local Antibiotic Delivery Alternative

Christopher Alexander

The risk of infection of musculoskeletal injuries is a major problem within the medical industry. Additionally, injuries to avascular regions of the musculoskeletal system, such as tears to tendons or ligaments, have an increased chance of being infected since the bodyâ€™s immune system canâ€™t effectively access the wounded area. Recently, chitosan, a derivative of arthropod shells, has proven to be an effective carrier of antibiotics due to its uniform elution of antibiotics, mucoadhesive properties, and biodegradability. A paste form of chitosan is appealing in the treatment of infection because a biomaterial with high plasticity will cover more surface area of the wound compared to current treatment options such as chitosan sponges or polymethylmethacrylate beads. Antibiotic elution studies were performed on groups of chitosan paste and sponge. The results illustrated the pasteâ€™s long term, uniform elution characteristics. Vancomycin was uniformly eluted by the chitosan paste after 72 hours at a rate ranging from 60-20 Âµg/ml; Amikacin was being eluted from the paste up to 48 hours post injection. The paste outperformed the chitosan sponge samples which stopped eluting antibiotics in less than 10 hours. Future research involves creating chitosan paste via different formulations and evaluating the drug delivery and biocompatibility qualities in order to determine the most effective option in preventing infection.

Novel Interaction of HER2 with MUC13 in Aggressive Pancreatic Ductal Adenocarcinoma

Subhash C. Chauhan, Sheema Khan, Mara C. Ebeling, Mohammad Sikander, Murali M. Yallapu, Tomoko Ise, Satoshi Nagata, Nadeem Zafar, Stephen W. Behrman, Jim Y. Wan, Hemendra M. Ghimire, Peeyush Sahay, Prabhakar Pradhan, Meena Jaggi

MUC13, a transmembrane mucin, is aberrantly expressed in pancreatic ductal adenocarcinoma (PDAC) and correlated with increased expression of HER2. This study demonstrates the co-localization and physical interaction of MUC13 with HER2 expression in PDAC cells (reciprocal co-immunoprecipitation, immunofluorescence, proximity ligation, co-capping assays) and tissues (immunohistofluorescence), which signifies cross-talk of MUC13 and HER2 in PDAC progression. Our results showed that stable expression of MUC13 leads to the activation of HER2 with an increased phosphorylation (pY1248-HER2). This modulates HER2 downstream signaling as indicated by increased phosphorylation of extracellular signal-regulated kinase 2 (ERK1/2), focal adhesion kinase (FAK) and AKT, thus, regulating growth, motility and invasion of PDAC cells. We also demonstrate that MUC13 upregulates HER2 signaling that triggers a rapid stimulation of p21-activated kinase 1 (PAK-1) activity leading to reorganization of actin remodeling. This result was further confirmed by MUC13 knockdown in PDAC cells. Additionally, our observations suggest that interaction of MUC13 with HER2 might be preferentially mediated through first and second EGF-like domains of MUC13, as their deletion failed to promote MUC13 induced proliferation and invasion in PDAC cells. Interestingly, MUC13-HER2 co-localization was observed in PDAC tissues which was found to be associated with increased morphological distortion strength Lmd, representing an increased degree of disorder and cancer aggressiveness. This data suggest a putative clinical relevance of MUC13-HER2 interaction. In conclusion, our results provide a compelling evidence of MUC13-HER2 interaction and suggest potential clinical implications of this novel interaction that may help identifying a subset of patients harboring an aggressive form of PDAC.

Analysis of the Deposited Matrix on Synthetic Scaffolds Subjected to Mechanostimulation to Improve Tendon Injuries at the Enthesis

Jonathan Tapp, Mamadou Diallo, Christopher Alexander, Joel D. Bumgardner, PhD , Warren Haggard, PhD, Jessica Amber Jennings, PhD University of Memphis, Memphis, TN, USA.

Introduction: Native tendon has similar mechanical characteristics to synthetic fabrics and has been used in tendon repair. Using mechanostimulation to deposit extracellular matrix (ECM), a bioreactor environment was created to mimic the bodyâ€™s tissue to house fibroblast and osteoblast cells on synthetic scaffolds. The purpose of this experiment is to determine whether the presence of applied strain on these cells promotes cell proliferation. The deposited matrix of scaffolds subjected to tensile forces and static conditions will be compared.
Methods: A PET fabric scaffold was sterilized by immersion in 2% bleach solution and 70% ethanol, followed by thorough rinsing with DI water. Fabric scaffolds were submerged in media (DMEM) containing 10% FBS and 100 Âµg/ml Normocin antibiotic. A 5% strain was applied to the side of the scaffold containing fibroblasts for 5 hours daily for 7 days, while the MC3T3 region was static. The control group was scaffolds in petri dishes without force or media circulation. The DNA present on the scaffolds was quantified using a Picogreen Assay, which detected the number of nuclei present. Collagen content was detected using a Sirius red collagen assay while GAG was detected using an Alcian Blue assay. The amount of collagen and GAG content was normalized to DNA content and represented as a ratio. Statistical differences between groups were detected using one-way ANOVA with Holm-Sidak post-test at [Symbol]=0.05 level of significance.
Results: The fibroblast cell regions exposed to daily periods of force exhibited higher concentrations of GAG than the cell regions exposed to static conditions with fluid flow and the control group. Collagen concentrations were significantly higher on scaffolds within the bioreactor exposed to constant media flow and no applied strain.
Discussion: The higher deposits of GAG within cell regions exposed to strain compared to those without strain means that cell growth can be promoted using an applied force. These deposits of GAG are important in the formation of tendon ECM. The decreased collagen production under cyclical strain can be explained since the cells may be in a proliferative phase at such an early timepoint. Future studies will include longer studies with more timepoints. The efficacy of cell-seeded scaffolds with conditioned ECMâ€™s from these periods of cyclical strain will be observed in promoting tendon formation and enthesis repair.

Use of calcium phosphate- silver nanoparticles in chitosan coatings on titanium and for drug delivery

Gillen Gonzales

Introduction: Calcium-phosphate (CaP) nanoparticles decorated with silver (Ag) have shown potential to kill infectious bacteria in vitro. A coating that localizes the antibacterial CaPÂ Ag nanoparticles using osteoconductive chitosan may provide a means to inhibit bacterial biofilm formation and subsequent infectious complications for these implants. This work evaluated antimicrobial, cytocompatibility, and protein delivery properties of CaPÂ Ag in chitosan coatings.
Materials and Methods: Porous CaP nanospheres with 0, 15, 37, or 50% Ag were fabricated using a hydrothermal synthesis of CaP followed by microwave heating of CaPÂAgNO3 for Ag decoration on CaP. Chitosan coatings with 30wt% CaPÂAg particles were made via silane Âsolution casting on to cpTi and sterilized using ethylene oxide gas. Coated and uncoated cpTi coupons were incubated with 2X104 NIH 3T3 cells for 24 hrs and viability determined using CellTitreÂGlo Assay. For protein release, 20mg of

CaPÂAg particles were incubated in 3mL of 1mg/mL Î±-chymotrypsin, used as an analogue to BMPÂ2, for 24 hrs at 37C under gentle rocking. Samples were incubated in 3mL PBS at 37C and eluates were collected with complete PBS every 2 days for 3 weeks.
Result: There was a significant and dose related decrease in viability of all bacteria with increasing Ag content on CaP nanoparticles with the CaPÂ50%Ag particles having the largest antibacterial properties. The total loading and percent release of protein was not different for the different CaPÂAg particles This may be due to the similarly large negative Zeta potential of the particles Protein release pattern was also similar for the different CaPÂAg particles showing an initial burst release followed by a sustained release of approximately10 ng/Âµl from day 3 to 21.
Discussion: Cell viability was reduced 100Âfold on all chitosan coatings incorporating CaPÂAg particles regardless of the %Ag in the particles. Part of reduced viability of the fibroblasts may be due to early release of Ag from particles due to the acetic acid used in the coating process.
Conclusion: Increasing concentration of Ag in CaP particles in chitosan coatings exhibited a dose dependent antibacterial effect on fibroblast viability. The CaPÂAg particles exhibited high protein loading and sustained protein release profile that may be advantageous for the local delivery of growth factors or other agents that require low and extended release profiles.

Chitosan Paste as Local Delivery Device to Lower Diffusion Distance of Antibiotics

Joel Martin Berretta

Antibiotics are often introduced to the body through a shot, IV or pill but because of the diffusion distance this is not always effective. The antibiotic is diffused throughout the body and reaches the wound in a less concentrated state. With a complex musculoskeletal wound not only is the soft and hard tissue damaged but the vasculature is also impaired. With little to no blood supply it can be hard for the antibiotics to even be able to reach the bacteria in the wound. People who have undergone a joint replacement surgery and have gotten an infection have a 20% chance of dying in the next 5 years. To help solve this problem, local drug delivery options have been developed. Bone cement beads and chitosan sponges can be loaded with antibiotics and applied directly to the wound but they do not offer complete wound coverage. We are working on chitosan paste which can be loaded with antibiotics and injected into a wound. Being in paste form, the chitosan paste can seep into the wound bed, offer complete wound coverage, and contact bacteria that would otherwise not be reached. The chitosan paste is mixed with polyethylene glycol (PEG) to aid in degradation. There are three chitosan/PEG variations that we are testing: 50:50 acidic/neutral combination paste and 1%:1% and 1%:0.5% paste with a v/v acetic acid solution of 0.85%. Based on studies we have conducted, the paste variations have been shown to be degradable, cytocompatible, and able to elute active antibiotics in doses that could combat bacteria levels in a musculoskeletal wound. Based on the design of the paste and the studies performed, we hope to achieve our goal of infection prevention.

Optimizing pH-responsive Nanoparticles for Delivery of Immunostimulatory Nucleic Acids to Cystosolic Immune Surveillance Pathways

Max Jacobson, Katie Bumila, Sema Sevimli, and John T. Wilson

Cells sense invading pathogens using a vast array of molecular sensors called pattern recognition receptors (PRRs). Several PRRs recognize viral RNA which stimulates the production of type-I interferon (IFN) and proinflammatory cytokines to generate an anti-viral state and elicit anti-viral immunity. These sensors, which are mostly cytosolically localized, have emerged as promising targets for vaccine adjuvants and cancer immunotherapeutics. However, nucleic acid ligands that bind to these receptors currently have low therapeutic efficacy because of nuclease degradation, poor cellular uptake, and,

critically, inefficient cytosolic delivery. In order to address these challenges, we propose using â€œsmartâ€ pH-responsive nanoparticles (NPs) to enhance intracellular delivery of nucleic acids to cytosolic PRRs.
Initial investigations utilized amphiphilic diblock copolymers originally designed for siRNA delivery composed of a cationic dimethylaminoethyl methacrylate (DMAEMA) first block and a pH-responsive second block that destabilizes endosomal membranes. These polymers self-assemble in aqueous media and electrostatically complex with nucleic acid cargo. In these studies, we used 5â€™ppp dsRNA, a ligand for the cytosolic PRR retinoic acid-inducible gene 1 (RIG-1), and demonstrated that NP formulations increased the production of IFN in a 5â€™ppp dsRNA-dependent manner.
To develop a system with increased biocompatibility specifically optimized for 5â€™ppp dsRNA delivery, we used reverse addition-fragmentation chain transfer (RAFT) polymerization to synthesize a small series of polymers with a polyethylene glycol (PEG) first block and a second block copolymer comprising variable DMAEMA composition and a methacrylate monomer of variable alkyl chain length. We determined the effect of second block composition on NP self-assembly, RNA complexation, and pH-dependent membrane destabilization and found that polymers with a second block comprising 70% DMAEMA and 30% hexyl methacrylate displayed the most potent membrane disruptive behavior at early and late endosomal pH values. Selected NP formulations were used to deliver 5â€™ppp-RNA and increased the production of type-1 IFN in a 5â€™ppp dsRNA dependent manner. These studies establish that endosomolytic polymer nanoparticles enhance delivery to the cytosolic RIG-I pathway and establish structure-activity relationships for endosomolytic carriers that can be developed for optimal delivery to cytosolic immune surveillance pathways.

Regulation of Isocitrate Dehydrogenase 1 (IDH1) Activity using Smart Nanomaterials Delivering CRISPR Technology

Kavya Sharman, Gerardo Valadez, Michael Cooper, Todd Giorgio

Isocitrate dehydrogenase (IDH) is a vital enzyme that catalyzes the oxidative decarboxylation of isocitrate and exists in three isoforms: IDH1, IDH2, and IDH3. Missense mutations in IDH1 have been implicated in several types of human gliomas, specifically WHO grades II and III astrocytomas and oligodendrogliomas and grade IV secondary glioblastomas. Current efforts to rectify this mutation involve competitive NADPH inhibitors, siRNA-targeted knockdown, and enzyme modulating drugs. For the lattermost method, a model system for testing the drugs is needed because the glioma cells harvested from human gliomas do not retain their mutation over time; they either revert back to wild-type or are negatively selected against in vitro. The purpose of this investigation is to develop a model system in which cultured glioma cells retain the mutant IDH1. The genomic IDH1 sequence of glioma cells will be modified using an adaptive molecular modification system consisting of clustered regularly interspaced short palindromic repeats (CRISPR). This study involves constructing CRISPR plasmids, which carry the wild type or mutant IDH1 and also encode the Cas9 molecular machinery responsible for recombination, and transfecting them into glioma cells to determine the effects of wild-type or mutant IDH1 enzyme activity. Preliminary results show that the CRISPR plasmids do contain the appropriate machinery and are capable of inserting the desired IDH1 sequence into cells. Upon successful gene editing using CRISPR, smart nanoparticles will be constructed to deliver the CRISPR plasmid to the glioma cells. These will be constructed using diblock polymers composed of a hydrophobic core that will encompass the CRISPR plasmid. Furthermore, the nanoparticles will have a pH responsive core and surface targeting that will enable tumor-specific uptake. The ultimate goal is to develop an in vivo glioma-specific treatment for with reduced side effects and a decreased rate of remission.

Release and Activity of Adenosine Incorporated Into Calcium Sulfate.

Ravi Patel

This study characterized the elution profile of adenosine incorporated into CaSO4 pellets and its activity on MC3T3 osteoblast precursor cells. Adenosine-loaded CaSO4 could provide a locally delivered bone graft substitute to improve bone healing rates and may be an alternative to growth factor delivery. Our preliminary study shows increases in bone cell proliferation by controllable release of active adenosine from bone graft substitutes. Results suggested that elution that occurs at one percent loading does significantly increase cell proliferation and differentiation. Future studies include investigating in vivo models for the promotion of bone growth at several selected adenosine concentrations incorporated into CaSO4 material.

Alternative and accelerated degradation method to increase physiological representation

Carlos M. Wells, Michael Harris, Marmadou Diallo, Warren Haggard, Jessica A. Jennings

Int: Local delivery of antibiotics has proven to effectively reduce the risk of bacterial infection on orthopedic implants or traumatic wounds. The most common method is through the use of polymethlymethacralate (PMMA) beads to deliver local antibiotic. These beads must be removed surgically, leading to investigations of alternative devices. Chitosan (CS) sponges are being investigated as a biodegradable local drug delivery device, but simulating the physiological degradation process has shown to be an illusive task. This investigational study examined 2 methods accepted by the FDA seeking higher instances of clinical relevant results. Met: The degradation of CS in a lysozyme and antibiotic/antimicrobial (AB/AM) mixture was tested against a cobalt chloride ($CoCl_2$) and hydrogen peroxide (H_2O_2) solution. CS sponges of various compositions, 2% CS (C), 1.5% CS 0.5% PEG (P), and a commercially (CA) available sponge were cut to 9 cm^2, hydrated, and tested. Sponge samples were placed in 30 mL [1 mg/ml lysozyme, 1% AB/AM] solution and incubated. Samples were taken at 2, 4, 6, 8 and 10 days with complete refreshment at time point. A similar set of sponges was prepared and placed in 30 mL [0.1 M $CoCl_2$, 3% H_2O_2] solution. Samples were taken at ~4, 7, 11, 14 and 18 h. Initial and post degradation masses of samples were compared. Res: Sponges from the lysozyme degradation showed minimal degradation over 10 days, with a maximum degradation of 26% in one sample, while 35% of the samples showed an increase in mass. CA and C sponges from the accelerated degradation test showed an overall downward degradation trend over the course of the 18 h study, with mean degradation >50% achieved at 7.2 h. Samples composed of P virtually degraded by the first sample point. Dis: The lysozyme degradation test showed no discernable degradation pattern. The accelerated $CoCl_2$, H_2O_2 degradation method provided a more predictable rate of degradation for the C and CA sponges. There was deviation from a linear rate of degradation that might be explained by the large mass discrepency between sponges. The 3.6 h time intervals may be reduced for the accelerated method to model degredation of the P sponges. This acclerated approach can be used to efficaciously model CS degradation in the laboratory. Con: Accelerated degradation studies of chitosan can be performed in vitro using a solution of $CoCl_2$ and H_2O_2 similating degradation of chitosan in surgical applications or traumatic wounds.

Photosensitizer-loaded Gold Nanorods for Near-Infrared Photodynamic and Photothermal Cancer Therapy

Ryan T O'Connor, Saheel Bhana, and Xiaohua Huang

Photodynamic therapy (PDT) is a type of cancer treatment that uses noninvasive light. As the treatment isnoninvasive, localized, portable, inexpensive, and simple to operate, it has received considerable attention for many years and used in the clinic for the treatment of certain cancers such as esophageal

cancer and non-small cell lung cancer[4]. However, conventional PDT is limited bythe poor water-solubility and non-specificity of most PSs as well as the inherent photobleaching issue of the PS agents. To improve the delivery efficiency and tumor specificity, substantial efforts have been made on the development and use of nanocarriersduring the past decades Au NRs have attracted a great deal of attention because of several advantages: (1) Compact size (around 50 nm in length), (2) ease of preparation, (3) excellent stability (shelf life more than one year), and (4) long circulating property, with the half-life more than 10 h when modified with poly (ethylene) glycol (PEG) Here we report a compact nanocomplex for simultaneous PDT and PTT under a single NIR laser irradiation using Au NR as the PS carrier/PT agent and silicon 2,3-naphthalocyanine dihydroxide(SiNC) as the PS agent. PSs were trapped in the hydrophobic pocketwith high density on the surface of Au NRs that is formed byPEG covalently linked with alkyl-thiol segmentwith certain chain length. Highly efficient PS release and cellular uptake was achieved through partition of the hydrophobic PSs between the nanocomplex and cell lipid membrane. We further studied the effect of We have demonstrated through in vitro studies that the nanocomplex allows for complete eradication of cancer cells with low intensity of NIR light, superior to PTT or PDT alone. The nanocomplex has great potential to deliver high concentration of PS and PT agents into tumor, which may lead to complete ablation of tumors and thus prevent tumor reoccurrence.

Effect on Degrade Rate of Chitosan with Removal of Residual Materials

Matthew Weaver, Osheana jenkins

Chitosan is a natural biocompatible polymer derived predominantly from shells of arthropods, and has many uses in the field of biomedical sciences, especially in the area of medicine. Chitosan is commonly used in implants that are designed to degrade over a period of weeks to months as tissues heal and new tissue gradually replace the implant. This ability to be degraded by the human body completely also removes the risk of infection due a second procedure to remove the implant. Due to its natural source, chitosan contain residual materials; such as residual animal protein and mineral (calcium carbonate) that are not completely removed during processing. These substances have been known to cause allergic reactions in some patients. The objective of this study was to treat the chitosan to remove residual protein and ash and determine effect on biodegradable properties of the chitosan polymer. The degree of deacetylation of the chitosan used was 78.0% and molecular weight was 244.6 g/mol. Quadruplicate samples of original and treated chitosan, 98.3 mg to 101.9 mg, were placed into four plates, one for each week. The solution used to induce the degradation of the chitosan consisted of type IV lysosome at a concentration of 400 Î¼g/ml. To ensure the consistent concentration of the lysosome solution, the solution was replaced every 48 hours Â± 2 hours. At the end of each week the plate was removed and samples dried in an oven. Each specific sampleâ€™s mass was the measured and percent change in mass recorded. Data showed that the removal of the residual materials had no significant effect on the degradation rate of the chitosan. While the treatment had no effect on degradation, additional studies are underway to determine if the treatment effects chitosan - cell/tissue interactions.

Characterization of poly(simvastatin)-containing copolymers and blends

T.A. Asafo-Adjei1, T.D. Dziubla2, and D.A. Puleo1
1 Department of Biomedical Engineering
2 Department of Chemical and Materials Engineering
University of Kentucky, Lexington, KY, USA

Poly(lactic acid) (PLA), poly(lactic-co-glycolic acid), and poly(Îµ-caprolactone) are commonly used as drug carriers in regenerative engineering due to their tunable degradation characteristics. However,

drug loading capacity limitations often exist. Polymerizing the bioactive agent into its respective polymer counteracts this disadvantage and allows control of drug loading via molar ratios of drug to initiator chosen for synthesis. Simvastatin, which has osteogenic, anti-inflammatory, and angiogenic properties, was copolymerized because its lactone ring is amendable to ring-opening polymerization. PLA copolymers were blended with a slow-degrading poly(simvastatin) copolymer and different mPEG initiators were used to assess their effect on modulating degradation.

Simvastatin or D,L-lactide was reacted with 5000, 2000, or 550 Da monomethyl ether poly(ethylene glycol) (mPEG) at a 100:1 molar ratio using triazabicyclodecene or stannous octoate as catalyst, respectively, to synthesize their respective copolymers. Poly(simvastatin)-mPEG (5 kDa) was mixed with each PLA-mPEG copolymer at 80:20 w/w or at 60:40 w/w with the PLA-mPEG (5 kDa) copolymer. Blends were dissolved in dichloromethane and pipetted onto Teflon to make ~20 mg samples to incubate at 37 ËšC in phosphate-buffered saline, pH 7.4, for 8 weeks. Poly(simvastatin) copolymers underwent degradation for 44 days. Mass loss was measured gravimetrically, and simvastatin release was analyzed via high performance liquid chromatography.

At 60 days, the 80:20 blend with 5000 and 550 Da mPEG incorporated, retained the most mass at 55Â±3.1%, followed by poly(simvastatin) â€“ mPEG (5kDa), and the copolymer blended with PLA-mPEG (2 kDa) or PLA-mPEG (5 kDa) with 50Â±5.6, 39Â±2.6, and 37Â±3.6% of mass remaining, respectively. The 60:40 blend had the lowest mass remaining (25Â±2.5%) and simvastatin amount released. The 80:20 blend, with solely 5 kDa mPEG, released the most simvastatin. At 44 days, poly(simvastatin) copolymers retained 71Â±7.6, 72Â±8.1, and 93Â±6.5% of initial mass as the mPEG initiator molecular weight (MW) decreased in the copolymer, correlating with a decreasing amount of simvastatin released.

The more hydrophilic samples, with the highest MW of mPEG incorporated, exhibited faster degradation, leading to altered rates achieved by blending with PLA-mPEG and copolymerization with mPEG. Tunable poly(simvastatin)-based copolymers and blends may be useful in tissue regeneration applications.

Simvastatin release from poly(lactic-co-glycolic acid) scaffolds incorporating poly(Î²-amino ester) microspheres

Amir Najarzadeh

Introduction

The ultimate goal of this research was to fabricate and characterize polymeric scaffolds composed of at least two components to mimic natural tissue structure at specific defect sites. Using degradable hydrogel microspheres (MS) as porogens allows for controlled pore opening after implantation as well as the potential for drug release during degradation. The controlled pore opening also allows the scaffolds to withstand the necessary mechanical properties at the implant site while degrading at a rate consistent with tissue regeneration. In the present study, systems composed of poly(lactic-co-glycolic acid) (PLGA) and poly(Î²-amino ester) (PBAE) loaded with simvastatin were examined to determine the drug release, mass loss and pattern of porosity development to design application-based scaffolds.

Materials and Methods

PLGA (50:50, IV: 0.55-0.75 dL/g, acid-terminated; Durect Corporation) MS were fabricated using a water/oil/water double emulsion technique. The resultant microspheres were sieved to <250 Âµm. The hydrogel (HG) macromer was synthesized through a step-wise reaction between poly(ethylene glycol) diacrylate (PEGDA; Polysciences), and isobutylamine (Sigma-Aldrich) at a 1.2:1 diacrylate:amine molar ratio at 85 Â°C for 120 hrs. To create cross-linked hydrogel MS, the macromer was weighed and

combined with 0.2 wt% 2,2-dimethoxy-2 phenylacetophenone (initiator) in 80 wt% dichloromethane (solvent). All percentages are based on the initial macromer mass. Microspheres were then made by creating a single emulsion of macromer followed by UV photopolymerization. Simvastatin was loaded directly to the HG MS by dissolving simvastatin in reagent alcohol at three different concentrations of 100, 150 and 200 mg/mL and pipetting drug solution over HG MS at a ratio of 5 Î¼L per mg of HG MS. The drug loaded lyophilized HG MS were then mixed with PLGA microspheres at a 400:100 wt:wt ratio and heated in novel compression mold system capable of producing radially- and axially-graded scaffolds. Scaffolds were fabricated with simvastatin-loaded HG MS incorporated in PLGA matrix. All three sets of samples were incubated in 3 mL phosphate-buffered saline (PBS), pH 7.4, on a plate shaker at 37Â°C for almost 40 days. Samples were removed at predetermined time points to measure swelling, mass loss, pore formation, and drug release.

Investigation of Delamination Resistant Bio-Laminates Using Finite Element Modeling Methods

Matt Nelms, Dr. Wayne Hodo, Dr. Arunachalam Rajendran

During the last few decades, research on biological materials such as abalone shell, fish armor, turtle shell, and human bone revealed a carefully arranged multilayered structure whereby each layer comprises unique subscale structures to achieve properties of high strength, high ductility, and light weight, which are far superior to any man-made materials and systems. In this research, experimentally driven finite element modeling was used to investigate the delamination resistance for the bio-laminate structures. The Atractosteus spatulas (Alligator gar) was used as the model structure.

The Alligator gar possess a flexible dermal armor consisting of overlapping ganoid scales. Each scale is a bilayer consisting of hydroxyapatite and collagen-based bio-laminates and is thought to be used for protection against its predators. The exoskeleton fish scale is comprised of a stiff outer ganoine layer, a characteristic â€œsawtoothâ€ pattern at the interface and a compliant bone inner layer with all materials exhibiting a decreasing elastic modulus, yield strength and density through the thickness. Optical microscope and scanning electron microscope (SEM) images of the cross section of garfish scales revealed a two-layered structure. Nanoindentation tests correlated to SEM with energy dispersive X-ray (EDX) analysis show that the hardness and modulus exhibit a grading of both mechanical and elemental properties across the scaleâ€™s layers. The main objective of this investigation is to quantify the effects of the material grading as a function of depth as well as the influence of the geometrically anisotropic interface between the ganoine and bone layers on the homogenized elastic properties. The fish scale was modeled using an representative volume element based finite element method. The numerical model was used to infer the effects of material variations response in terms of in terms of material symmetry and stress redistribution at the ganoine-bone interface. The results indicate that at the ganoine-bone interfaces stress is reduced and local property variations produce orthotropic symmetry.

Growth plate cartilage microstructure under physiological and hyperphysiological conditions

Bhavya Vendra

The growth plate is a highly specialized structure responsible for long bone growth. The proliferative and hypertrophic zone chondrocytes form a tubular structure called a chondron. Abnormal mechanical loading of growth plate is implied to be one of the causes of idiopathic scoliosis. The purpose of this study is to examine the cellular deformations at the chondron level within an intact growth plate sample

under physiological and hyper-physiological compressive loading. The growth plate nuclei were stained and imaged under fluorescence microscope to obtain a 2d stack of images and were then characterized for chondron deformation.

Spinal Implant's Vancomycin Elution Analysis

Parwinder Singh

Spinal implants are vulnerable to contamination during surgical procedure. To maintain sterility and prevent infection to the patient, spinal implants could be soaked in an antibiotic solution of vancomycin. An elution study was conducted on the implants over the course of three hours to determine the rate of release of the vancomycin. The elution study showed that the concentration peaked at around 2.25 hours and steadily decreased in rate of elution. We can conclude that vancomycin is an effective antibiotic to soak spinal implants. Changing the concentration of the antibiotic and duration of the elution study could provide further benefit.