

Oral Presentation Abstracts

Combinatorial library of ternary polyplexes enables identification of improved siRNA nanocarriers for rapid in vivo translation

Thomas A. Werfel, Martina Miteva, Taylor Kavanaugh, Kellye Kirkbride, Meredith Jackson, Rebecca Cook, Todd Giorgio, Craig Duvall

Many previous non-viral siRNA vector development efforts have yielded reagents effective for in vitro transfection but that have poor in vivo pharmacokinetics and bioactivity. The current work focuses on development of a siRNA nanocarrier optimized to overcome both cell-level barriers (uptake/endosomal escape) and systemic barriers following intravenous delivery (stability for long circulation time and small size for effective tissue penetration). To this end, a combinatorial library of ternary polyplexes was herein investigated to optimize formulations for siRNA delivery. The compositions tested build from our previous finding that balancing cationic and hydrophobic content in binary polyplexes can enhance both particle stability and endosome escape. Through this ternary complex/combinatorial approach, we were able to systematically study important structure-function characteristics such as polyplex surface PEGylation density, size, stability, and endosomolysis. Ternary polyplexes which were optimized to overcome multiple barriers to siRNA delivery achieved highest gene silencing and endosomolysis was identified as a crucial parameter for achieving siRNA silencing in vitro. Lead polyplexes were able to localize to tumors after intravenous administration and achieved target gene silencing of the model gene luciferase in vivo.

Copolymers induce increased stemness and pericyte phenotype in human mesenchymal stem cells

Dan Balikov, Boire TC, Crowder SW, Lee JB, Kirkbride KC, Gupta MK, Murthy S, and Sung HJ

The current state of culturing stem cells for regenerative medicine has inherent limitations in maintaining the potency of autologous and allogenic stem cells for human subjects. For mesenchymal stem cells (hMSCs), a core issue is maintaining a high stemness state as well as addressing the issue of how to make these cells behave as 'younger' cells when the primary donor is of advanced age. In this study, we have created a library of copolymers comprised of hydrophobic/protein-adsorbant PCL and hydrophilic/protein-repellent PEG where increase in stemness properties of hMSCs was observed. Specifically, pluripotency factor expression levels (SOX2 and Nanog) and phenotypic properties (low proliferation rates and decreased reactive oxygen species) exhibited by the hMSC on the copolymers relative to TCPS approached levels normally seen in in vivo stem cell niches. Moreover, hMSCs cultured on the copolymer substrates display pericyte-associated properties including an increased capacity to maintain endothelial cell tubulogenesis. Finally, we have begun interrogating the nanoscale properties of the copolymer films to identify a structure-function relationship that drives the phenotype alteration via surface repellency and nanoscale surface features of the copolymers and potential protein mediators integrin- α 2 and PECAM.

Animal models in cell-based therapies – Importance of cells and biomaterials

Madhu S. Dhar, Ph.D.

Combining stem cells with biomaterial scaffolds provides a promising strategy for engineering tissues and cellular delivery. The prospective clinical use of adult mesenchymal stem cells holds enormous promise for improved treatment of a large number of diseases in humans and companion animals. Although the use of bone-marrow-derived mesenchymal stem cells appears to be a popular therapy; the therapy suffers from the donor-to-donor variation in the quality and quantity of harvested cells. One critical biological factor that researchers and clinicians must take into account is this variability and how it may affect the clinical outcome in regenerative therapy. The focus of research in the Laboratory of Regenerative Medicine at the College of Veterinary Medicine is to understand

this variation in adult mesenchymal stem cells, and to test various combinations of biomaterials and cells in animal models of bone and cartilage damage. We have optimized in vitro molecular and cellular assays to isolate, characterize, and differentiate rat, horse and goat adult mesenchymal stem cells; we can generate an ex vivo model of a specific disease, and finally we can design a controlled animal (in vivo) study to test their biological function in regeneration. The goal of this three-step process is to improve clinical outcomes as well as increase our basic knowledge of stem cell function. Currently, we are carrying out experiments to test the efficiency of constructs generated by combining adult mesenchymal stem cells and novel biomaterials. We are investigating their potential in wound healing, treatment of corneal ulcers, bone and cartilage tissue engineering.

45S5 Bioactive Glass/Polyurethane Biocomposites for Repairing Weight-bearing Bone Defects

Andrew J Harmata, S Uppuganti, M Granke, CL Ward, K Zienkiewicz, JS Nyman, JC Wenke, SA Guelcher

Of the nearly 1.6 million bone graft procedures conducted annually to treat bone fractures in the U.S., ~25% of these fracture patients require rehospitalization due to graft failure. Injectable and settable synthetic bone grafts that possess initial quasi-static mechanical strength and dynamic fatigue resistance exceeding that of host bone and maintain properties comparable to bone while remodeling could improve the clinical management of a number of orthopaedic conditions. Ceramic/polymer composites have been investigated as weight-bearing bone grafts, but they are typically weaker than trabecular bone due to poor interfacial bonding. We hypothesized that entrapment of surface-initiated poly(ϵ -caprolactone) (PCL) chains on 45S5 bioactive glass (BG) particles within an in situ-formed polymer network would enhance the mechanical properties of reactive BG/polymer composites. The designed polyurethane (PUR) synthetic graft composite comprising PCL-modified 45S5 bioactive glass particles exhibited quasi-static compression and torsion, as well dynamic compressive fatigue, mechanical properties equal to or greater than those of native human trabecular bone and commercially available calcium phosphate cements. When injected into femoral condyle defects in rats and sheep, the composites supported new bone formation. The initial bone-like strength of BG/polymer composites and their ability to remodel in vivo highlight their potential for development as injectable grafts for repair of weight-bearing bone defects.

In situ cross-linkable gelatin hydrogels for vasculogenic delivery of mesenchymal stem cells

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Directing robust differentiation of mesenchymal stem cells (MSCs) to endothelial cells for regenerative medicine remains challenging, although not impossible. Gelatin is highly biocompatible, biodegradable, adhesive and non-immuno/antigenic, thus possessing desirable characteristics for tissue engineering. However, its application has been limited due to low melting temperature < 37°C. We recently developed injectable gelatin-based hydrogels by conjugating hydroxyphenyl propionic acid to gelatin (GHPA) that crosslinks in situ via a horseradish peroxidase (HRP)-mediated reaction. Interestingly, when encapsulated in GHPA, MSCs began to undergo extensive tubulogenesis and express distinctive endothelial cell markers without biological molecules supplementation in in vitro 3D culture and an in vivo murine subcutaneous implantation model. The pro-vasculogenic effects of GHPA on MSCs were demonstrated in vitro and in vivo. In particular, in vivo results showed that vasculogenesis was significantly enhanced with crosslinked GHPA gels, suggesting a causative role of the gelatin stability in retention and material-guided endothelial differentiation of delivered MSCs. The results are highly significant as these desirable effects were achieved without addition of any bioactive molecules. Studies to identify and elucidate a mechanism involved in this purely material-driven MSC differentiation to endothelial cells are currently under way. The preliminary results indicate a mechanistic role of integrin expression in the vasculogenic effect and necessitate further investigation into potential interplay of integrins with VEGF signaling, and downstream integrin signaling.

Targeted nanosomes for osteoarthritis in PTOA mouse model

Hongsik Cho, Karen A. Hasty

Osteoarthritis (OA) is one of the most prevalent causes of pain and disability in older individuals for which there are few therapies. OA is a complex process that develops over a long period of time. Post Traumatic Osteoarthritis (PTOA) is a prevalent form of OA commonly developing from joint injury. One complication with PTOA treatment is that it is difficult to detect cartilage damage before symptoms present and irreversible damage has already occurred. If a method of early cartilage degradation was available, then there might be great benefit in prompt treatment with pharmacologic intervention. In order to create cartilage degradation, we use a mouse model of mechanical loading. In this PTOA mouse model we use non-invasive and physiologically relevant loading to induce joint injury. Mechanically loaded models of PTOA have been shown to correlate with histological progression of OA in mice. This provides a valuable tool to researchers in establishing the degree of arthritic progression in a joint but requires the sacrifice of the specimen. A reliable method of quantifying articular cartilage damage without tissue removal could benefit both research and diagnosis of the condition. Antibody targeted to type II collagen(CII) has been shown to bind selectively to damaged tissue. Targeted nanosomes with encapsulated fluorescent tags can be readily detected in anesthetized mice using IVIS imaging. By correlating IVIS measurements of fluorescence intensity to histological damage in mechanically loaded mouse knees, we provide a non-invasive method of diagnosing PTOA in affected joints. Also, the CII targeted nanosomes will be provided as a vehicle for the delivery of drugs to the site of damaged cartilage.

Cell protective, ABC triblock polymer-based thermoresponsive hydrogels with ROS-triggered degradation and drug release

Mukesh K Gupta, JR Martin, TA Werfel, T Shen, JM Page, and CL Duvall

A novel ABC triblock polymer poly[(propylenesulfide)-block-(N,N-dimethylacrylamide)-block-(N-isopropylacrylamide)] (PPS-b-PDMA-b-PNIPAAM) was synthesized to form injectable, biodegradable hydrogels. At ambient temperature, PPS-b-PDMA-b-PNIPAAM assembled into 66 nm micelles comprising a hydrophobic PPS core that can be loaded with therapeutic cargo. Upon heating to 37 °C, micelle solutions (at ≥2.5 wt%) sharply transitioned into stable, hydrated gels with concentration-dependent mechanical properties. These hydrogels demonstrated reactive oxygen species (ROS) triggered degradation and drug release based on the oxidation-dependent phase change behavior of PPS from hydrophobic to hydrophilic. The hydrogels were proven to have utility for cell encapsulation in vitro and to provide sustained, local drug release in vivo. These collective data support the broad potential biomedical use of dual thermo- and ROS-responsive PPS-b-PDMA-b-PNIPAAM hydrogels.

Implications of protein corona on physico-chemical and biological properties of magnetic nanoparticles

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Interaction of serum proteins and nanoparticles leads to a nanoparticle-protein complex formation that defines the rational strategy for a clinically relevant formulation for drug delivery, hyperthermia, and magnetic resonance imaging (MRI) applications in cancer nanomedicine. Given this perspective, we have examined the pattern of human serum protein corona formation with our recently engineered magnetic nanoparticles (MNPs). The alteration in particle size, zeta potential, hemotoxicity, cellular uptake/cancer cells targeting potential, and MRI properties of the MNPs after formation of human serum (HS) protein corona were studied. Our results indicated no significant change in particle size of our MNPs upon incubation with 0.5e50 wt/v% human serum, while zeta potential of MNPs turned negative due to human serum adsorption. When incubated with an increased serum and particle concentration, apolipoprotein E was adsorbed on the surface of MNPs apart from serum albumin and transferrin. However, there was no significant primary or secondary structural alterations observed in serum proteins through Fourier transform infrared spectroscopy, X-ray diffraction, and circular dichroism. Hemolysis assay suggests almost no hemolysis at the tested concentrations (up to 1 mg/mL) for MNPs compared to the sodium dodecyl sulfate

(positive control). Additionally, improved internalization and uptake of MNPs by C4–2B and Panc–1 cancer cells were observed upon incubation with human serum (HS). After serum protein adsorption to the surface of MNPs, the close vicinity within T1 (~1.33–1.73 s) and T2 (~12.35–13.43 ms) relaxation times suggest our MNPs retained inherent MRI potential even after biomolecular protein adsorption. All these superior clinical parameters potentially enable clinical translation and use of this formulation for next generation nanomedicine for drug delivery, cancer–targeting, imaging and theranostic applications.

R Guo, **Sichang Lu**, JM Page, AM Merkel, JA Sterling, S Basu, SA Guelcher

The properties of the extracellular matrix, including elastic modulus, porosity, pore size, and curvature, are known to regulate cell fate in a number of physiological processes. Biomimetic 3D systems are needed for investigating interactions between cell populations and the microenvironment. Three dimensional polyurethane (PUR) scaffolds with tunable rigidity (10-900 MPa) and pore size (423-557 μm) were fabricated by a new template-Fused Deposition Modeling (t-FDM) process to investigate the effects of substrate modulus and pore size on osteogenic differentiation of rat bone marrow-derived mesenchymal stem cells (BMSCs). Total protein assay indicated that modulus and pore size had minimal effects on cell proliferation. In contrast, BMSCs were more metabolically active and migrated faster on rigid substrates. Gene expression of osteogenic markers, including Runx2, Collagen-1, Fibronectin, and Osteopontin was up-regulated on rigid scaffolds. Mineralization of BMSCs on D21 was significantly up-regulated on rigid scaffolds with decreasing pore size, suggested by Alizarin Red S staining and SEM. These findings would guide the rational design of cell-responsive scaffolds that recapitulate the bone microenvironment for restoration and repair of bone damaged by trauma or disease.

Ormeloxifene nanoparticle formulation for pancreatic cancer

Subhash C Chauhana, Ph.D.

Pancreatic cancer is the fourth most prevalent cancer with about 85% mortality rate; thus, an utmost need exists to discover new therapeutic modalities that would enhance therapy outcomes of this disease with minimal or no side effects. Ormeloxifene (ORM), a synthetic molecule, has exhibited potent anti–cancer effects through inhibition of important oncogenic and proliferation signaling pathways. However, the anti–cancer efficacy of ORM can be further improved by developing its nanoformulation, which will also offer tumor specific targeted delivery. Therefore, we have developed a novel ORM encapsulated poly(lactic–co–glycolic acid) nanoparticle (NP) formulation (PLGA–ORM NP). This formulation was characterized for particle size, chemical composition, and drug loading efficiency, using various physico–chemical methods (TEM, FT–IR, DSC, TGA, and HPLC). Because of its facile composition, this novel formulation is compatible with antibody/aptamer conjugation to achieve tumor specific targeting. The particle size analysis of this PLGA–ORM formulation (~ 100 nm) indicates that this formulation can preferentially reach and accumulate in tumors by Enhanced Permeation and Retention (EPR) effect. Cellular uptake and internalization studies demonstrate that PLGA–ORM NPs escape lysosomal degradation, providing efficient endosomal release to cytosol. PLGA–ORM NPs showed remarkable anti–cancer potential in various pancreatic cancer cells (HPAF–II, BxPC–3, Panc–1, MiaPaca) and BxPC–3 xenograft mice model resulting in an improved animal survival. PLGA–ORM NPs suppressed pancreatic tumor growth via suppression of Akt phosphorylation and expression of MUC1, HER2, PCNA, CK19 and CD31. This study suggests that the PLGA–ORM formulation is highly efficient for the inhibition of pancreatic tumor growth and thus can be valuable for the treatment of pancreatic cancer in the future.

Cellular uptake and intracellular trafficking of MAPKAP kinase 2 inhibitor peptide delivered via endosomolytic nano–polyplexes

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

Coronary artery bypass grafting with autologous saphenous vein is the gold standard treatment for multivessel coronary artery disease. However, almost half of saphenous vein grafts fail within 18 months due to intimal hyperplasia (IH) and ultimately graft occlusion. The stress activated p38/MAPK signaling pathway is implicated as a key factor in IH: MAPK Activated Protein Kinase 2 (MK2) is a downstream effector of p38/MAPK and directly modulates inflammation, fibrosis, and cell migration in IH. We have previously demonstrated the ability of a cell penetrant MK2 inhibitory peptide (MK2i) that binds to MK2 and inhibits its ability to phosphorylate its downstream effectors. However, free MK2i suffers from entrapment in the endolysosomal pathway, causing poor cytosolic bioavailability. Electrostatic complexation of MK2i with poly(propylacrylic acid) triggers self-assembly into ~120 nm nanopolyplexes (MK2i-NP) that demonstrate enhanced uptake and retention relative to free peptide, facilitate endosomal escape and cytosolic delivery, and abrogate IH in human saphenous vein ex vivo. Understanding the mechanisms of MK2i-NP internalization into vascular smooth muscle cells is necessary to characterize the biological response to this drug delivery platform and enable its clinical translation. We have characterized MK2i peptide and NP uptake in the presence of various uptake inhibitors by flow cytometry and fluorescence microscopy. Our data suggest MK2i-NP binds to cell membrane via hydrophobic interactions and is internalized alongside membrane recycling through mechanisms representative of micropinocytosis. Interestingly, scanning electron microscopy and fluorescence microscopy demonstrate a lack of ultrastructural differences indicative of macropinocytotic upregulation in MK2i-NP treated cells. Future ultrastructural studies by TEM are required to fully confirm the mechanism(s) of MK2i-NP internalization.

Phage display-mediated nanocarrier targeting to atheroprone vasculature

Lucas Hofmeister, Lee SH, Harrison DG, Sung HJ

In regions of the circulation where vessels are straight and unbranched, blood flow is laminar and unidirectional. In contrast, at sites of curvature, branch points and regions distal to stenoses blood flow becomes disturbed. Atherosclerosis preferentially develops in these regions of disturbed blood flow. Current therapies for atherosclerosis are systemic, and may not sufficiently target these atheroprone regions. In this study, we sought to leverage the alterations on the luminal surface of endothelial cells caused by this atheroprone flow for nanocarrier targeting. In vivo phage display was used to discover unique peptides that selectively bind to atheroprone regions in the mouse partial carotid artery ligation model. The peptide GSPREYTSYMPH (PREY) was found to bind 4.5-fold more avidly to the region of disturbed flow, and was used to form targeted liposomes. When administered intravenously, PREY-targeted liposomes preferentially accumulated in endothelial cells in the partially occluded carotid artery and other areas of disturbed flow. Proteomic analysis and immunoblotting indicated that Filamin A was preferentially bound by PREY-nanocarriers in vessels with disturbed flow. In additional experiments, PREY-nanocarriers were used therapeutically to deliver the nitric oxide synthase co-factor tetrahydrobiopterin (BH4), which we have previously shown to be deficient in regions of disturbed flow. This intervention increased vascular BH4 and reduced vascular superoxide in the partially ligated artery in wild-type mice, and reduced plaque burden in the partially ligated left carotid artery of fat fed atheroprone mice (ApoE^{-/-}). Targeting atheroprone sites of the circulation with functionalized nanocarriers provides a new approach for prevention of early atherosclerotic lesion formation.

Affinity-based delivery of neurotrophins in decellularized nerve allografts

Richard B. Boyer, David C. Riley, Alonda C. Pollins, R. Bruce Shack, Wesley P. Thayer

The gold standard therapy for nerve gap repair is autologous nerve grafting. In addition to significant donor site morbidity, donor nerve harvesting requires increased operative time and post-operative rehabilitation. Transplants of fresh cadaveric nerve allografts are precluded by the need for cytotoxic immunosuppressive drugs, such as tacrolimus and cyclosporine. Recently, acellular nerve allografts have become clinically available with

reduced antigenicity and without the requirement for immunosuppression. Similar to other decellularized organs, acellular nerve allografts are processed with specific detergents to remove the membranous and cytoplasmic components of the neuronal and non-neuronal cells present in peripheral nerve while preserving extracellular matrix proteins and microstructure of the basal lamina and endoneurium. Loss of pro-regenerative factors, such as Schwann cells and neurotrophic factors, during nerve decellularization limits the performance of acellular nerve allografts, particularly in proximal nerve injuries where target reinnervation may not occur prior to complete denervation muscle atrophy. In this study, we leveraged the preserved extracellular matrix proteins in acellular nerve allografts for affinity-based delivery of nerve growth factor (NGF) to regenerating axons following nerve injury. Under infinite sink conditions, Sondell detergent-decellularized nerve allografts were found to retain NGF over 21 days in vitro while maintaining bioactivity. In vivo rodent sciatic nerve injuries repaired with NGF-loaded acellular nerve allografts were found to increase mid-graft neurite regeneration. Immunohistochemistry and high-resolution diffusion tensor imaging indicated improved axon regeneration at early and late time points in NGF-loaded acellular nerve allografts. Affinity-based delivery of neurotrophic factors in acellular nerve allografts may provide a new tool for enhanced axon regeneration in proximal nerve gap injuries.

Preclinical evaluation of ROS-responsive, antioxidant therapies for diabetic peripheral arterial disease

Kristin M Poole, Kavanaugh TE, Nelson CE, Joshi RV, Madonna MC, Lee J, Skala MC, Duvall CL

A microparticle-based delivery system was synthesized from reactive oxygen species (ROS)-responsive poly(propylene sulfide) (PPS) and tested for "on demand" antioxidant therapy. PPS is hydrophobic but undergoes a phase change to become hydrophilic upon oxidation and thus provides a useful platform for ROS-demanded drug release. This platform was first tested for delivery of the promising anti-inflammatory and antioxidant therapeutic molecule curcumin, which is currently limited in use in its free form due to poor pharmacokinetic properties. PPS microspheres efficiently encapsulated curcumin through oil-in-water emulsion and provided sustained, on demand release that was modulated in vitro by hydrogen peroxide concentration. The cytocompatible, curcumin-loaded microspheres preferentially targeted and scavenged intracellular ROS in activated macrophages, reduced in vitro cell death in the presence of cytotoxic levels of ROS, and decreased tissue-level ROS in vivo in the diabetic mouse hind limb ischemia model of peripheral arterial disease. Due to the inherent ROS scavenging behavior of PPS, blank microparticles also showed therapeutic properties that were synergistic with the effects of curcumin in these assays. Functionally, local delivery of curcumin-PPS microspheres accelerated recovery from hind limb ischemia in diabetic mice, as demonstrated using non-invasive, quantitative optical imaging techniques. Ongoing work includes evaluating the functional response to delivery of a superoxide dismutase mimetic, TEMPO-benzoate, from PPS microspheres for more specific targeting of excessive superoxide levels associated with hyperglycemia. Overall, this work demonstrates the potential for PPS microspheres as a generalizable vehicle for ROS-demanded drug release and establishes the utility of this platform for improving local bioavailability of antioxidant molecules for treatment of chronic inflammatory diseases.

Prolonged protection against mitochondrial oxidative stress using Curcumin conjugated poly (β - amino esters) nanogels.

Prachi Gupta, Dr. Mihail Mitov, Dr. D. Allan Butterfield, Dr. J. Zach Hilt, Dr. Thomas Dziubla

Mitochondria are considered to be the energy power plants of the cell, but can also initiate and execute cell death, stimulated by oxidative stress (OS). OS induced mitochondrial dysfunction is characterized by a loss in oxygen consumption and reduced ATP production, and it has been linked to a wide variety of diabetic, cardiovascular, and neurodegenerative disorders. Curcumin is considered to be a potential drug to suppress mitochondrial oxidative stress, but rapid metabolism and aqueous insolubility prevent it from being an effective therapeutic. A lot of work has been done to incorporate curcumin into liposomal/micellar nanocarriers for improving the delivery of its active form. However, most formulations have limited net drug loading and exhibit significant burst release of curcumin.

To resolve the problem of unhealthy curcumin bolus dosage due to burst effect and deliver the antioxidant at therapeutic levels, self-precipitated curcumin conjugated poly (β -amino ester) (C-PBAE) nanogels in dilute reaction conditions were synthesized utilizing the Michael addition chemistry. Easy control over the nanogel size by varying the reactant concentrations and drug loading of 62% w/w was achieved employing this one-pot synthesis process. Upon hydrolytic degradation of the ester bond, these curcumin PBAE pro-drug nanogels showed uniform release of active curcumin over 30 hours under physiological conditions, enhancing the release rates with control over the initial burst release effect as well as structurally stabilizing the labile drugs for an extended period of time. Real time response analysis of mitochondrion bioenergetics like basal respiration, mitochondrial ATP production using Seahorse Bioscience XF96 analyzer, showed continuous prolonged protection against H₂O₂ mitochondrial oxidative stress confirming the uniform sustained release of active curcumin from pro-drug C-PBAE nanogel.

Poster Presentation Abstracts

1. Asafo-Adjei, Theodora– University of Kentucky, *Synthesis and Characterization of a Simvastatin Polyprodrug*

Poly(lactic-co-glycolic acid) and poly(ϵ -caprolactone) are commonly used in drug delivery due to bioinertness and tunable degradation. However, drug payload limitations can often exist. By directly polymerizing bioactive agents into the polymer backbone and changing the comonomer ratio, drug loading can be controlled. Simvastatin, which has anti-atherosclerotic, osteogenic, anti-inflammatory, and angiogenic properties, is being copolymerized into a biodegradable polymer by ring-opening polymerization of its lactone ring. Simvastatin was mixed with 550, 2000, or 5000 Da monomethyl ether poly(ethylene glycol) (mPEG) at a 100 to 1 molar ratio with 1 wt% of triazabicyclodecene (TBD) at 150°C for 24 hr. Reactions using stannous octoate and 5 kDa mPEG were run at 230°C. Small mPEG-block-poly(simvastatin) disks were incubated in saline for degradation studies. Gel permeation and high performance liquid chromatography, nuclear magnetic resonance (NMR) and ultraviolet spectroscopy, and mass spectrometry were used for analysis. The molecular weight (MW) of mPEG-block-poly(simvastatin) was 10, 15, and 18-29 kDa using 550, 2000, and 5000 mPEG, respectively. NMR measurements showed a MW of 21 kDa. After 4 weeks, 64 μ g and 100 μ g of free simvastatin were released from the copolymer made via TBD and stannous octoate, respectively. Mass spectrometry of the degraded products revealed trimers, dimers, and open and closed-ring forms of simvastatin. Successful polymerization of simvastatin was seen along with slow drug release and identified forms of the drug in the degradation products. Poly(simvastatin) may be useful in tissue regenerative applications.

2. Bailey, Mark – University of Kentucky, *Curcumin Poly(β -amino ester) as a Targeted Radioprotectant for Lung Cancer Treatment*

In the current clinical environment, regimens of combined radiotherapy and chemotherapy are used to treat lung cancer. Although both of these therapies have been proven to be effective, they are both heavily limited in dosing due to peripheral toxicity to healthy tissue. In this work it, the antioxidant curcumin was used as a radioprotectant drug during radiation treatment. Curcumin is a powerful antioxidant which can scavenge reactive oxidative species. Curcumin also down regulates the NF- κ B protein complex which protects healthy endothelial cells by reducing inflammatory damage. Curcumin has been limited in use however due to its poor bioavailability. In order to avoid problems associated with bioavailability of free curcumin, curcumin poly(β -amino ester) (CPBAE) was synthesized. CPBAE is a polymer that is hydrolytically degradable due to the presence of ester bonds. When these ester bonds break, the active drug curcumin is released. To deliver this curcumin to the endothelium in the lungs, CPBAE was formed into nanoparticles. Through intravenous injection, these nanoparticles can be targeted to the lungs with anti-platelet cell adhesion molecule (PECAM-1) antibodies coating. PECAM1 is expressed at a higher density in the lungs compared to the rest of the body's vasculature which leads to preferential targeting towards lung endothelial cells for delivery. By administering nanoparticles before radiotherapy, pneumonitis may be able to be reduced in patients.

3. Chun, Young Wook –Vanderbilt University, *Combinatorial Tailored Polymers to Enhance Maturation of Human Cardiomyocytes*

Cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CMs) hold great promise as a tool to model human heart diseases. However, iPSC-CMs used in studies over the past several years morphologically and physiologically resemble immature embryonic myocytes and therefore do not adequately recapitulate the native adult cardiomyocyte phenotype. As the extracellular matrix (ECM) plays an essential role in heart development and cardiomyocyte maturation in vivo, we reasoned that a synthetic culture matrix could be engineered which would enhance cellular and functional maturation of human iPSC-CMs in vitro in a similar fashion. In this study, we employed a library of combinatorial polymers comprising of three functional subunits - poly- ϵ -caprolactone (PCL), polyethylene glycol (PEG),

and carboxylated PCL (cPCL) – as synthetic substrates for culturing human iPSC-CMs. Of these, iPSC-CMs cultured on 4%PEG-96%PCL (each % indicates the corresponding molar ratio) exhibited the greatest contractility and highest inner mitochondrial membrane potential. These functional enhancements were also associated with increased expression of cardiac myosin light chain-2v and integrin alpha-7, markers associated with ventricular myocyte maturation. Interestingly, culturing iPSC-CMs on 4%PEG-96%PCL had significantly increased expression of genes encoding intermediate filaments that transduce integrin-mediated mechanical signals to the myofilaments. Taken together, our study demonstrates that synthetic culture matrices engineered from combinatorial polymers can be utilized to promote in vitro maturation of human iPSC-CMs through the engagement of critical matrix interactions.

4. Cook, Andrew –Fisk University, *Developing a Raman-Based Biosensor to Identify Circulating Tumor Cells Using Functionalized Zinc Oxide Nanowires*

Circulating tumor cells (CTCs), which are tumor cells that metastasize through blood, can potentially serve as markers for cancer progression and treatment response. The ability to detect CTCs has particular application for treating metastases, which are the primary cause of most cancer-related deaths. Low, clinically relevant CTC concentrations are difficult to detect, however. Most tumors are also of epithelial origin, so distinguishing between cancerous and healthy cells is immensely important. Near-infrared Raman spectroscopy (RS) can be employed to differentiate CTCs from healthy epithelial cells, yet RS's signal-to-noise ratio limits sensitivity to low CTC concentrations. ZnO nanowires are a strong candidate to detect CTCs due a high surface area to volume ratio and transparency to visible and infrared light. Additionally, by coating high quality ZnO nanowires with specific thicknesses of MgO, an insulating material with a lower refractive index, they become highly efficient optical cavities for chosen wavelengths. Resultant "core-shell" ZnO/MgO nanowires can capture and guide Raman signal from adsorbed CTCs, reducing signal loss. By decorating core-shell nanowires with gold nanoparticles, surface-enhanced Raman scattering (SERS) can further enhance the Raman signal. SERS occurs when surface plasmon oscillations in metal nanoparticles resonate with incident light. Finally, by coating the nanowires in antibodies to select epithelial cells, CTCs and healthy epithelial cells can be immobilized, then differentiated using RS. Current efforts involve growing uniform nanowires, depositing specific MgO thicknesses for Raman-resonant cavities, and quantifying enhancement of the Raman signal.

5. Elkhenany, Hoda –University of Tennessee, *Graphene based scaffold for stem cell delivery in cortical bone defect: A preliminary study*

Bone healing cannot be guaranteed by grafting scaffolds alone, unless fresh cells are recruited to the bone failure sites. A type of the cells that can be recruited are represented by mesenchymal stem cells (MSCs). Adult MSCs are desirable for bone tissue regeneration not only because of their capability of self-renewal and potential for osteogenic differentiation, but also due to their availability from a wide variety of adult tissues. In the current study, we tested the in vitro proliferation and osteogenic differentiation of caprine adipocyte derived MSCs (cAdSCs) on graphene surfaces. Graphene is an inert, derivative of carbon and is recently identified as a nanoparticle and shown to affect osteogenesis. To test the in vivo biocompatibility and the bone-forming potential of the nanoparticle and MSCs, cells were xenogenically implanted into a 2 mm diameter unicortical tibial defect in rats. In each rat, the right tibia was used as the treated group, and the contralateral tibia served as an unfilled control. Bone response was assessed with computed tomography at days 0 and 45. Results demonstrate that in vitro, the AdSCs could proliferate and undergo osteogenic differentiation on both of the graphene surfaces. To measure bone healing in vivo, the regions-of-interest were marked and the area was calculated using the CT scans and compared amongst different groups. The cAdSCs + graphene treated limbs showed significant roughly 4 fold higher healing compared to graphene alone. The density, however, was not significantly different. Between the 2 groups. In conclusion, graphene is a potential non-toxic nanomaterial that can be used as a delivery system for stem cells.

6. Evans, Brian—Vanderbilt University, *A Novel Platform Technology for Cytosolic Peptide Delivery with Endosomolytic Nanopolyplexes Applied to Vascular Graft Intimal Hyperplasia*

Autologous vein grafts are commonly used for coronary and peripheral artery bypass but have a high incidence of intimal hyperplasia (IH) and failure. We present a nanopolyplex (NP) approach that efficiently delivers a mitogen-activated protein kinase (MAPK)–activated protein (MAPKAP) kinase 2 inhibitory peptide (MK2i) to graft tissue 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. In intact human saphenous vein, MK2i-NPs blocked inflammatory and migratory signaling, as confirmed by reduced phosphorylation of the posttranscriptional gene regulator heterogeneous nuclear ribonucleoprotein A0, the transcription factor cAMP (adenosine 3',5'-monophosphate) element–binding protein, and the chaperone heat shock protein 27. The molecular effects of MK2i-NPs caused functional inhibition of IH in human saphenous vein cultured ex vivo. In a rabbit vein transplant model, a 30-min intraoperative graft treatment with MK2i-NPs significantly reduced in vivo IH 28 days posttransplant compared with untreated or free MK2i–treated grafts. The decrease in IH in MK2i-NP–treated grafts in the rabbit model also corresponded with decreased cellular proliferation and maintenance of the vascular wall smooth muscle cells in a more contractile phenotype. These data indicate that nanoformulated MK2 inhibitors are a promising strategy for preventing graft failure.

7. Faley, Shannon—Vanderbilt University, *Development and Validation of Microfluidic, Free–standing Gelatin Hydrogel Cell Scaffolds as 3D Vascular Model Systems*

At the forefront of emerging tissue engineering lies the design and implementation of 3D, multicellular constructs, teeming with exciting potential to facilitate cutting-edge scientific discovery and revolutionize regenerative medicine. Biomimetic scaffolds approaching physiological scale, whose large size and cellular load exceed the limits of diffusion, require incorporation of a fluidic means to achieve adequate nutrient/metabolite exchange. Despite recent engineering advances in scaffold fabrication, remarkably little attention has been devoted to the critical interface between the microchannel network and the external fluidic system necessary for perfusion. In this report, we describe the use of melt-spun, sacrificial polymeric fibers to generate a perfusable network of microchannels in gelatin-based hydrogels, along with a thorough analysis of methods for establishing robust connections between freestanding tissue scaffolds and external pumping systems. Confocal imaging confirms this fabrication method yields a complex, interconnected lumen varying in scale from 5 to 200 μm in diameter. Subsequent evaluation comparing efficacy of various direct-connect and adhesive mediated fluidic interface modalities encompassing mechanical burst pressure, tensile strength, and cell-compatibility assays within the confines of custom manufactured test devices composed of 7-12% gelatin resulted in identification of a novel combination of DOPA-modified gelatin and enzymatically cross-linked, cold-water fish-gelatin as a rapid and promising adhesive to use with ports for long-term, continual perfusion of microfluidic gelatin hydrogels. Finally, we discuss ongoing efforts to rapidly seed endothelial cells (HUVECs) throughout the microchannel network to achieve a confluent, vascular-like lumen. This work establishes a foundation upon which to build thick tissue constructs and physiologically-inspired models of biological systems.

8. Gordon, Andrew—Vanderbilt University, *Gold Nanorods as Optical Coherence Tomography Contrast Agents*

Improved retinal imaging capabilities enhance our capacity to detect early retinal disease, monitor its progress, and measure its response to therapy. Accordingly, we have designed and fabricated biocompatible, targeted gold nanorods (GNRs) with a surface plasmon resonance (SPR) tuned to 800-900 nanometers as exogenous contrast agents for use with the optical coherence tomography (OCT) imaging modality. Additionally, we employ novel techniques for improved native imaging of these contrast agents with existing OCT systems. We sterilized and stabilized our GNR by adding a surface coating of relatively biologically inert polyethylene glycol (PEG), which was modified to include a carboxyl moiety for

further addition of targeting ligands. We confirmed successful surface functionalization using mass spectrometry and bicinchoninic acid (BCA) assays. In vivo OCT imaging was performed of GNRs injected intravitreally, to confirm the presence of contrast enhancement while allowing full visualization of the retina. Ex vivo immunohistochemical analysis showed successful delivery of GNRs to retinal tissue. Further work with GNRs in tissue phantoms showed our ability to detect GNRs based on their spectral signature. We have demonstrated an OCT contrast agent that can be successfully delivered and visualized in the eye, while generating sufficient spectral signature for detection in tissue phantoms. Our results suggest that detoxification, stabilization, and appropriate SPR tuning of GNRs prior to administration is necessary for effective OCT imaging of GNR-based contrast in the eye. This technology will allow us to utilize the unparalleled resolution of OCT imaging to visualize contrast targeted to specific cell types or disease markers.

9. Jackson, Meredith—Vanderbilt University, *Facile preparation of a hydroxyl-functionalized RAFT copolymer of DMAEMA and BMA for branched polyglycidol chemistry*

RAFT, or reversible addition fragmentation chain transfer polymerization, is a well-known method of living free radical polymerization with important applications in the field of polymeric micelles for drug and gene delivery. Previously, our lab used RAFT to optimize a random copolymer of 50% DMAEMA and 50% BMA, which, when copolymerized with PEG, forms micelles with high stability due to well-balanced cationic and hydrophobic content. This core architecture may be further stabilized by branched polyglycidols in the micelle corona, but current RAFT polymers are not amenable to further polyglycidol modification without hydroxyl functionalization. We therefore compared two methods for creating hydroxyl-functionalized RAFT polymers. In the first method, using 2-(benzylsulfanylthiocarbonylsulfanyl)ethanol (BSTSE) as a chain transfer agent (CTA), polymers were very polydisperse and did not contain the characteristic trithiocarbonyl group, even under varying initiator and CTA amounts. For the second method, we synthesized 4-cyano-4-(ethylsulfanylthiocarbonyl)sulfanylpentanol as an alternative CTA. In one step, this CTA successfully polymerized BMA and DMAEMA at the appropriate ratios and provided a hydroxyl functionalization on the end of the polymer that is not sterically hindered, opening the door for future chemistry involving branched PEG analogues.

10. Jayaram, Rohith—University of Kentucky, *Development of a Local, Sustained Delivery Vehicle for Zoledronic Acid to Treat Tumor-Mediated Bone Resorption*

Osteolysis and the related pain negatively impact the quality of life for cancer patients with skeletal metastases. Zoledronic acid (ZA) is a third-generation bisphosphonate that inhibits bone resorption. Intravenous delivery, however, is inefficient and is associated with side effects that include osteonecrosis of the jaw. The purpose of this study was to improve delivery of ZA to the local tumor environment. ZA was polymerized into poly(methyl methacrylate) (PMMA) bone cement at drug loadings of 0, 1, 2, and 5% by mass. Cylindrical samples were incubated in phosphate-buffered saline to measure drug elution for 8 weeks. Alternatively, ZA was incorporated into poly(lactic-co-glycolic acid) (PLGA) microspheres that were consolidated into thin films for drug release studies. ZA concentrations were measured by high performance liquid chromatography. Drug elution from both materials progressed over 8 wk, but release rate was highest during the first day. For ZA release from PMMA, ANOVA showed a significant ($p < 0.05$) increase in release for increased drug loading. The PMMA samples with the highest drug loading, 15.2 mg ZA, released a total of 2.6 mg (17%). PLGA films containing 13.3 mg ZA released a total of 3.5 mg (26%) over 8 wk. Therefore, PLGA may provide a better local delivery vehicle for ZA compared to PMMA. This research was funded by a Peter and Carmen Lucia Buck Clinical and Translational Research Grant from the Markey Cancer Center, University of Kentucky.

11. Kavanaugh, Taylor—Vanderbilt University, *Targeted Microparticles for Sustained, On-Demand Delivery of Anti-inflammatory Agents for Prevention of Post-traumatic Osteoarthritis*

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. Reactive oxygen species (ROS) are elevated at sites of joint injury and can cause cell-damaging and pro-inflammatory “oxidative stress” that propagates the tissue degenerative process of PTOA. Targeted, sustained, and “on demand” delivery of TEMPOL, a powerful superoxide dismutase mimetic, by targeted, H₂O₂ “sponge” microparticles could prevent onset of PTOA after a joint injury. These particles encapsulate TEMPOL in poly(propylene sulfide)(PPS) microparticles via oil-in-water (OW) emulsion method; PPS serves both as a H₂O₂ “sponge” and also as a bioresponsive depot that triggers release of therapeutic cargo in response to ROS. TEMPOL loading has been quantified through HPLC and both optical and electron microscopy. The ROS scavenging ability of TEMPOL and PPS has been confirmed through ROS and H₂O₂ assays. Surface functionalization, performed post particle fabrication, will provide targeting and will be performed using a tri-block polymer, pluronic f-127, to conjugate an antibody against type II collagen. Finally, a luminescence reported mouse will be utilized for a model of PTOA to validate these microparticles in vivo. The elimination of early oxidative stress experienced after an injury will prevent the progression of PTOA. Combining these particles with a targeting ligand will lead to greater targeting and local delivery of our therapeutic agent.

12. Kendall, Peggy—Vanderbilt University, *Antigen Specific Targeting of Autoreactive B Lymphocytes by siRNA Nanoparticles to Prevent T1D*

Type 1 Diabetes (T1D) occurs when T cells destroy pancreatic β cells. Autoreactive B cells are essential to present autoantigen to T cells. The ideal treatment would eliminate autoreactive cells while leaving normal subsets intact. We have shown that insulin-specific B cells internalize antigen via their B cell receptors (BCRs) for presentation to autoreactive T cells, while non-insulin binding B cells do not. We therefore conjugated insulin to newly designed siRNA nanoparticles (siNPs) to specifically target these B cells. These si-NPs have a polyethylene glycol (PEG) coating that blocks adsorption of proteins, inhibits hemolysis, avoids immune stimulation, and protects the siRNA from degradation. A key feature is a pH responsive core that becomes lipophilic in the acidic environment of lysosomes, triggering it to merge with the lysosomal membrane and deliver siRNA into the cytosol. Si-NPs were optimized for in vivo proof-of-principle in their non-specific form, and fluorescently labeled versions showed successful delivery to the spleen. The si-NPs also silenced a house-keeping gene in the spleen with no discernable toxicity. We find that anti-insulin B cells efficiently internalize insulin-siNPs in vitro, compared to non-insulin binding control B cells, macrophages, and dendritic cells. We are currently optimizing a variety of siRNA targets, including CD79a, CD86, and BTK, focusing on genes most relevant to B cells to further minimize potential toxicity to other tissue types. The development of targeted si-NPs will have clinical significance for autoimmune diseases like T1D by selecting for and silencing autoreactive B cells while avoiding global immune suppression.

13. Martin, Timothy—University of Memphis, *Changes Of MSC Proliferation On Chitosan Coatings Due To DDA And Protein Addition.*

Rapid integration of implant devices is essential to decreasing patient recovery times and the opportunity for complications to occur during healing. Chitosan coatings have been shown to support growth of osteoblast cells; however, more data is needed on their effect on MSC recruitment, proliferation, and differentiation. Our objective was to determine the effect of degree of acetylation and the addition of the proteins collagen and fibronectin on the proliferation of MSCs. Chitosans of varying DDAs (60%-100%) were solution cast at 2 wt% in 2% v/v acetic acid into TC plastic and were neutralized after drying using a 0.05 M NaOH solution in 80% EtOH for 1 hour. These dishes were then seeded with human MSCs at 5000 cells/cm². At days 1, 3, and 7, the relative density of cells in each well was measured using a Cell Titer Glo Assay. Lower DDA chitosans showed decreased cell attachment and proliferation and an abnormal round morphology. An increase in the DDA positively correlated to the attachment and proliferation, and an increase in proper spindle shaped morphology of the MSCs. The best results were observed in the dishes with a DDA higher than 90%. Three groups of cpTi coupons were then coated

using silane with 95%DDA chitosan, 95%DDA chitosan blended with 5wt% Collagen I, and 95%DDA chitosan adhered with 5ng of fibronectin. These groups were also seeded with hMSCs and observed for 7 days. An increase of cell proliferation, attachment, and morphology was observed in both the collagen and fibronectin laced chitosan plates when compared with chitosan alone and a Ti control. Therefore, a combination coating of high DDA chitosan combined with collagen/fibronectin may lead to quicker osseointegration of an implant. A further study of the gene expression of MSCs on these coatings is currently underway.

14. Miller, Sinead—Vanderbilt University, *A Nanoparticle–Based Model to Guide Therapeutic Design for Bacterial Sepsis*

The Giorgio Lab is developing a new strategy for targeting multi-drug resistant (MDR) *Acinetobacter baumannii* that avoids the nephrotoxic and neurotoxic side effects associated with high dose colistin, a cationic bactericide. To circumvent negative side effects associated with colistin, we will separate pathogenic bacteria from whole blood through ex vivo processing in an innovative mesofluidic device. The approach is performed by functionalizing magnetic nanoparticles (NPs) with colistin, a ligand to *A. baumannii* surface features. The consequent magnetic labeling allows for separation of the pathogenic bacteria from whole blood, while avoiding bacterial cell lysis. The initial task of this proposal was performed by first conjugating and characterizing colistin-decorated gold NPs. Later, this helped guide the fabrication, conjugation, and characterization of gold-shelled magnetic NPs. In summary, a nanoparticle-based model has been successfully developed and utilized to guide the therapeutic design of bacterial sepsis targeted magnetic nanoparticles.

15. Najarzadeh, Amir—University of Kentucky, *Fabrication and characterization of functionally graded hybrid scaffolds*

The ultimate goal of this research was to fabricate and characterize hybrid polymeric scaffolds composed of at least two components to mimic natural tissue structure at specific defect sites. Using salt particles and degradable hydrogel particles 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. The system comprised poly(lactic-co-glycolic acid) (PLGA), poly(β -amino ester) (PBAE), and salt particles. In the present study, homogenous and layered scaffolds were examined to determine the compositional relationship, mass loss, pore size and pattern of porosity development to design application-based scaffolds.

This study demonstrated controlled pore opening with degradation as a function of time through different HG porogens. Spatiotemporal modulation of hybrid scaffold properties, e.g., pore opening, pore size, and mechanical and structural characteristics, can be used to design scaffolds for specific applications.

16. Nelson, Christopher—Duke University, *CRISPR/Cas9-Targeted Gene Correction of Duchenne Muscular Dystrophy in Mice*

Duchenne muscular dystrophy (DMD) is a monogenic and fatal genetic disorder characterized by muscle wasting, loss of ambulation, and death by the age of 20 due to the loss of functional dystrophin - an essential musculoskeletal protein. Gene therapy has held promise for treating monogenetic diseases, however, delivery barriers and the requirements for costly repetitive treatments are a few of the challenges of technologies in the therapeutic pipeline. Recently, the CRISPR/Cas9 system has been adapted for genome editing to make specific modifications in the host genome. This technology would enable permanent genome corrections and a potentially curative approach to DMD.

Previously we adapted CRISPR/Cas9 for targeted deletions of regions of the human dystrophin gene that restore expression of a functional protein (Ousterout et al., *Nature Communications* (in press)). The delivery of Cas9 with single guide RNAs (sgRNAs) targeting the introns surrounding exons 51 and 45-55 created double strand breaks which are repaired through non-homologous end joining to create genomic

deletions of these regions. These deletions restored the reading frame and recovered dystrophin expression in cultured patient-derived myoblasts. Therapeutically, this approach could be used to convert the fatal DMD phenotype to the milder phenotype associated with partial dystrophin function characteristic of Becker muscular dystrophy. Here we utilize adeno-associated virus (AAV) to deliver the CRISPR/Cas9 system to the mdx mouse model of DMD. AAV vectors were designed to package and deliver genes encoding the *Staphylococcus aureus* Cas9 (SaCas9), due to its smaller size (3.2 kb) compared to the widely used Cas9 from *Streptococcus pyogenes*. Two single guide RNAs (sgRNAs) were designed to target sites flanking exon 23 of the dystrophin gene and validated to direct SaCas9-mediated deletion of this exon, which contains a premature stop codon in this model. Mice were anesthetized and AAV1 containing the SaCas9 and sgRNA expression cassettes was injected into the gastrocnemius muscle of 8 week old mice. At 4 and 8 weeks post-injection, the gastrocnemius was harvested and genomic DNA and mRNA were extracted. PCR of the genomic DNA demonstrated the expected ~1200 bp deletion of exon 23. Further, RT-PCR of the extracted mRNA showed removal of the 216 bp encoding exon 23. Furthermore, sgRNAs compatible with SaCas9 have been developed and validated for the deletion of human exon 51. This work demonstrates proof-of-principle of CRISPR/Cas9-mediated genome editing in skeletal muscle in an adult mammal, opening up new possibilities for gene therapy and the study of gene function. Optimization of delivery and activity is underway to improve the therapeutic potential of this approach. Additional work to characterize functional improvement in the mouse hind limb is also ongoing. This work establishes CRISPR/Cas9-mediated genome editing as a potential therapeutic approach to DMD.

17. Pasek, Raymond—Vanderbilt University, *Therapeutic Delivery of CTGF with PLGA Microspheres to Induce Beta Cell Proliferation*

Type 2 diabetes is characterized by impaired insulin signaling in peripheral organs and insufficient insulin production by pancreatic beta cells. Individuals with Type 2 diabetes have reduced beta cell mass compared with body mass matched non-diabetic controls. While stimulating beta cell proliferation with therapeutic compounds could alleviate diabetes, beta cells are often refractory to replicative stimuli, and drug delivery methods to the beta cells are currently very limited.

Studies from our lab have revealed that Connective tissue growth factor (Ctgf) is a previously unrecognized player in beta cell proliferation. During development, Ctgf is expressed in pancreatic ducts, vasculature, and islets, where it promotes proliferation of beta cells. After birth, beta cell proliferation slows and Ctgf expression is silenced. Our data indicates that Ctgf is reexpressed in maternal beta cells during pregnancy, and pregnant mice with Ctgf haploinsufficiency display impaired glucose tolerance. Pregnant Ctgf haploinsufficient mice display a defect in the increase in beta cell proliferation that normally occurs during pregnancy. Notably, treating adult mouse or human pancreatic islets with Ctgf *ex vivo* also stimulates beta cell proliferation, potentially indicating that Ctgf can be used as a therapeutic to increase beta cell mass in individuals with diabetes. In the present study, we are using poly(lactic-co-glycolic acid) (PLGA) microspheres to deliver recombinant human CTGF (rhCTGF) to mouse and human beta cells *ex vivo*. Additionally, we will transplant human beta cells under the kidney capsule of recipient mice to determine if rhCTGF can stimulate human beta cell proliferation in an *in vivo* setting.

18. Sarett, Samantha—Vanderbilt University, *Conjugation of trivalent, cyclic RGD to siRNA for improvement in gene silencing*

We recently reported biomaterial scaffold-based delivery of siRNA using pH-responsive, endosomolytic polymeric nanoparticles (NPs) for local gene silencing to promote tissue regeneration. The current work is aimed at gearing this system towards clinical translation by eliminating the polymer nanocarrier by modifying the siRNA via direct conjugation and leveraging the phenomenon of substrate-mediated transfection. Conjugation of hydrophobic and peptidic moieties have shown promise as a means of improving the pharmacokinetic properties of siRNA; in particular, trivalent cyclic RGD (tcRGD) conjugated to siRNA has been proven to improve siRNA stability and to mediate cellular uptake and gene silencing of otherwise naked siRNA without eliciting an immune response. Using the promising scaffold-based delivery platform to deliver tcRGD-siRNA is expected to allow therapeutic efficacy without the

potential deleterious side effects associated with polymer carriers. The current study was designed to assess the effect of siRNA conjugation to tcRGD on target gene silencing in cells relevant to wound regeneration. Thus far, the tcRGD-siRNA conjugate has been generated and purified, and studies comparing the uptake of the tcRGD-siRNA to that of unmodified siRNA are in progress.

19. Song, Min—University of Kentucky, *Ongoing study to characterize the dependence endothelial sprout formation rates on hydrostatic pressure*

Recently, we provided evidence that pressure may be a stimulus for microvascularizing tissues by showing that endothelial sprouting rates are selectively upregulated by 20, but not 40, mmHg. But these studies only examined sprout formation after 3 days of exposure to only 2 pressures. This is insufficient to determine how pressure-sensitive endothelial sprouting is. Moreover, the study in question had used a 3-D endothelial bead model that may be a challenge to translate to a tissue engineering application. The present investigation seeks to adapt to using endothelial spheroid cultures into our pressure studies which we believe is a more suitable tissue engineering strategy. Simultaneously, we are identifying the operating pressures and exposure times that can be used to control endothelial sprouting rates. For this purpose, we are using a custom system and 3-D bovine aortic endothelial cell (BAEC) spheroid models to identify the minimum pressure durations required for accelerating endothelial sprouting under 20 mmHg. We are also characterizing the pressure dependence of BAEC sprouting by exposing cultures to 0, 5, 10, 20, and 40 mmHg. So far, our data suggests that exposure to 20 mmHg for 1 or 2 days is inadequate to enhance sprouting. Moreover, a 5 mmHg stimulus appears to be inhibitory. Based on these pilot data in conjunction with the results from our prior study, BAEC sprouting appears to exhibit a complex pressure dependence that one may exploit in order to fine-tune microvessel formation rates in tissues. But more work is needed to further support this possibility.

20. Unlu, Gokhan—Vanderbilt University, *Mechanism Regulating Synchronous Collagen Expression and Trafficking during Stem Cell to Chondrocyte Differentiation*

Transition from the stem cell phase to a differentiated cell type is characterized by retooling cellular machinery, including regulatory molecules, structural proteins, and their transport machinery. To understand how the differentiation process provisions for synchronized changes in cellular functions during development, we used a robust in vivo model of zebrafish neural crest stem cells, which differentiate into chondrocytes and then produce, transport, and secrete procollagen II. Although procollagen synthesis and trafficking are well studied, little is known about how collagen-specific transport machinery and collagen cargo are made synchronously available during vertebrate development. We hypothesize that a stem cell factor temporarily restricts both collagen expression and trafficking machinery in the undifferentiated stem cell state. In silico analyses suggested that a neural crest factor may bind to the promoter region of Creb3L2 to suppress collagen secretion as well as the promoter of a major chondrogenic factor Sox9 to inhibit collagen expression during cartilage development. Using in vivo mosaic analysis, we find that overexpression of the stem cell factor in differentiated zebrafish chondrocytes and human fibroblasts downregulates both collagen expression and secretion. We demonstrate that overexpression of the stem cell factor leads to reduction in CREB3L2 promoter activity as detected by luciferase-based assays as well as decreased transcript levels of CREB3L2 and components of ER-to-Golgi (COPII) trafficking machinery, especially SEC23A and SEC24D. The changes at the transcript levels are matched by intracellular accumulation of type-I and type-II collagens. Our data support a model in which a neural crest stem cell factor acts as a master regulator of collagen expression and intracellular trafficking machinery, and its developmentally driven downregulation leads to synchronous upregulation of collagen cargo and its transport machinery during differentiation.

21. Zayed, Mohammed—University of Tennessee, *Synovial fluid-derived mesenchymal stem cells as a cell source for cartilage tissue engineering*

To date no drugs or therapies are available to regenerate the affected tissues in cases of arthritis because of low regenerative capacity of articular cartilage. Adult mesenchymal stem cells (MSCs) have been suggested as an alternative solution for cartilage tissue engineering due to their proliferation and chondrogenic capacity. Since, MSCs derived from bone marrow, have been shown to undergo hypertrophy during chondrogenesis, it is important to identify an alternate source of MSCs that do not undergo hypertrophy while maintaining chondrogenic potential. We hypothesize that synovial fluid-derived MSCs (SFMSCs) may be superior to bone marrow-derived MSCs. Prior to their application in clinical cases, however, their biological properties should be evaluated. To prove our hypothesis, synovial fluid was collected aseptically from normal joints and MSCs were isolated in the Laboratory of Regenerative Medicine in the Department of Large Animal Clinical Sciences at the University of Tennessee. Cell proliferation was assessed using the CellTiter 96 Aqueous Non-Radioactive (MTS) assay. Nuclear/cytoplasmic staining with wheat germ agglutinin and TO-PRO-3 iodide were used for evaluating cellular morphology and viability throughout the expansion process. Adipogenesis, osteogenesis and chondrogenesis was monitored microscopically and was confirmed by cell-specific staining. To further understand the mechanism of chondrogenesis, we used indirect immunofluorescence and immunoblot analyses to investigate the expression of key chondrocyte progenitor proteins. All data show that SFMSCs adhere to the tissue culture plastic, proliferate and have higher chondrogenic potential. The results suggest that synovial fluid represents a potentially attractive source of MSCs which may have utility for cartilage repair therapies in trauma and arthritis.

Undergraduate Students:

1. Black, Maggie—University of Memphis, *Effects of size on release of molecules from phosphatidylcholine coatings*

A novel coating material has been developed using phosphatidylcholine that can be loaded with antibiotics or other therapeutics and applied directly to an implant surface to release high concentrations of antimicrobial locally. In this study, the elution profiles of the antibiotics amikacin, vancomycin, and a combination of both were characterized using spectrophotometry. In addition, dyes of various molecular weights and hydrophobicity were added to the coating to model the effect of these characteristics on release kinetics. Coatings were applied to titanium and stainless steel coupons and then placed in buffered saline under constant shaking. Samples were taken every day for 7 days and the elution media was completely replaced each day. First day release of amikacin and vancomycin varied depending on antibiotic, with the smaller molecule amikacin releasing at much higher concentrations. There was also a “second burst” effect at high loading levels after 5 days of elution. The patterns of release can be used to modify the loading concentration of antibiotics or other therapeutic agents loaded to remain active without reaching levels toxic to tissue. Future studies are planned to evaluate drug release in and biocompatibility in preclinical models.

2. Duncan, Elizabeth—University of Memphis, *Proliferative and Migratory Responses of Mesenchymal Stem Cells Exposed to Adenosine*

Current treatment for bone fractures or non-union of bone can include expensive growth factors or an additional painful surgical procedure, an autograft, to facilitate bone healing. Adenosine has been shown to increase the proliferation rate of fibroblasts and osteoblast precursor cells. Adenosine could be a useful therapeutic to modify stem cell proliferation and migration to improve repair of tissues. The aim of this study was to determine how adenosine affects mesenchymal stem cell (MSC) proliferation and migration. Passage 8 rat MSCs were subjected to media containing adenosine ranging in concentration from 125 µg/ml to 1,500 µg/ml (n=8 in each group). Cell viability and proliferation were determined via a Cell Titer Glo (Promega) assay. After 48 hours, proliferation of MSCs was significantly higher than 5% FBS controls for cells with adenosine added in concentrations from 250 uM to 1000 uM. MSCs were seeded into transwells with 0.8µm pore membranes and allowed to migrate for a period of 17 hours. A non-chemotactic DMEM media with 0.2% FBS and Normocin was used to seed the cells and as a

negative control. The chemotactic media involved three variable groups: 500 μ M adenosine with 0.2% FBS, 5% FBS, and a combination of both with n=3. Cells that migrated were fixed and stained with Dapi for capturing images and performing cell counts. The results confirm the hypothesis that adenosine increases MSC proliferation but cannot confirm that adenosine stimulates increased migration over the negative control. Adenosine may not be chemotactic for MSCs, even in the presence of serum.

3. Shen, Tianwei—Vanderbilt University, *Environmentally-responsive Composite Porous Silicon Nanoparticles for Improved Delivery of PNA Therapeutics*

Peptide nucleic acids (PNA) are synthetic oligonucleotides that are potent inhibitors of disease-associated microRNA (miRNA). Two major hurdles preventing the clinical application of PNA are its poor cellular uptake and rapid renal clearance. Packaging PNA into nanoparticles is a promising method for overcoming these intracellular delivery barriers. We have previously shown that PNA can be encapsulated into a biocompatible, biodegradable material known as porous silicon (PSi). In this work, we introduce a novel means of functionalizing PSi particles with environmentally responsive, “smart” polymers, consisting of hydrophilic poly[(ethylene glycol)] (PEG), pH-responsive (dimethylamino) ethyl methacrylate (DMAEMA), and hydrophobic butyl methacrylate (BMA). We hypothesize that this polymer/PSi composite will have enhanced stability in blood, and improved PNA delivery to the cytosol where target miRNA are located. We therefore made and characterized a library of composite particles with differing amounts of polymer and PSi. Select composites were tested for cytotoxicity and endosomal escape ability. We conclude that PNA-loaded PSiNPs can be effectively coated by electrostatically condensing positively charged PEG-(DMAEMA-co-BMA) onto the negatively charged PSi nanoparticles at pH 5.5, then raising the pH to 7.4. These composite particles are biocompatible (exhibiting only a 7% reduction in cell viability at a particle dose of 1 mg/mL). Using a hemolysis assay for endosomal escape, we found that composite particles possess the pH-dependent, membrane disruptive ability of the smart polymer, whereas naked PSi particles show no membrane disruption at any pH. Taken together, these studies show that these composites possess improved functionality for PNA therapeutic application in vivo.

4. Wells, Carlos—University of Memphis, *Release of Tobramycin and Vancomycin from dual-loaded local delivery systems*

Effective treatment of infections resulting from bacteria strains presents clinical challenges that are ongoing. These infections can result in failure of orthopedic implants. Eradicating biofilm-forming bacteria once attached to implant has proven to be an arduous and costly excursion. Two of the more common pathogenic strains are methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. Antibiotics have shown success against one or the other, but using single antibiotics may lead to overgrowth of unsusceptible bacteria and further complications. The combination of vancomycin (Gram-positive specific) and tobramycin (Gram-negative specific) is being investigated for possible synergism against a polymicrobial mixture consisting of both strains. Antibiotic loaded sterile sponges—polyethylene glycol (PEG) and chitosan, acetic and lactic acid and chitosan, and a commercially available chitosan sponge (Sentrex, Bionova Medical—Memphis, TN)—were included with polymethylmethacrylate (PMMA) bone cement (Simplex P with tobramycin—Kalamazoo, MI) as the investigative sample groups. All samples were loaded with tobramycin and vancomycin at concentrations determined from previous investigations. Eluates from all samples were obtained at predetermined time points ranging between 1 h – 240 h. Analysis of the concentration in eluates of tobramycin and vancomycin was completed using spectrophotometric methods. The commercially available sponge displayed the highest initial burst release for both antibiotics of all samples. The vancomycin elution profile for all sample groups was steady \geq 20 μ g/mL. Tobramycin concentration in eluates declined after initial release in all sample groups except PMMA beads. Future studies will compare these delivery vehicles in a polymicrobial model of wound contamination.