Gulf Coast Cluster for Regenerative Medicine
a research cluster of the Gulf Coast Consortia for Quantitative Biomedical Sciences

Gulf Coast Cluster for Regenerative Medicine 2nd Annual Symposium

June 2, 2015
BioScience Research Collaborative

Organizers:
K. Jane Grande-Allen, Ph.D., Rice University
Charles S. Cox, Jr., M.D., University of Texas Health Science Center at Houston
Laura Smith Callahan, Ph.D., University of Texas Health Science Center at Houston
Elizabeth Olmsted-Davis, Baylor College of Medicine
James Dennis, Ph.D., Baylor College of Medicine
Ravi Birla, Ph.D., University of Houston
Hoang Nguyen, Ph.D., Baylor College of Medicine
Melissa S. Thompson, Ph.D., Gulf Coast Consortia

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The Gulf Coast Consortia (GCC), located in Houston, is a dynamic, multi-institution collaboration of basic and translational scientists, researchers, clinicians and students in the quantitative biomedical sciences who participate in joint training programs, utilize shared facilities and equipment, and exchange scientific knowledge. Working together, GCC member institutions provide a cutting edge collaborative training environment and research infrastructure, beyond the capability of any single institution. GCC training programs currently focus on **biomedical informatics, computational cancer biology, molecular biophysics, neuroengineering and pharmacological sciences.** GCC research consortia gather interested faculty around research foci within the quantitative biomedical sciences, and currently include **bioinformatics, chemical genomics, magnetic resonance, protein crystallography, translational pain research, neuroengineering, and translational addiction sciences, in addition to regenerative medicine**. Current members include Baylor College of Medicine, Rice University, University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, The University of Texas M. D. Anderson Cancer Center, and the Texas A&M Health Science Center's Institute of Biosciences and Technology.

brc BioScience Research Collaborative

The BioScience Research Collaborative is an innovative space where scientists and educators from Rice University and other Texas Medical Center institutions work together to perform leading research that benefits human medicine and health. More than just a building, it is an interdisciplinary, interinstitutional catalyst for new and better ways to collaborate, explore, learn and lead.

The symposium co-organizers would like to thank our corporate sponsors for their support of this event.

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2nd Annual Symposium

Tuesday, June 2, 2015
BioScience Research Collaborative

Unless otherwise noted, proceedings will take place in the Auditorium.

	9:00 AM	*Introduction* GCC RM Steering Committee
KEYNOTE ADDRESS	9:05 AM	Session Chair: Ravi Birla, University of Houston *Cell-synthesized ECM: Blood vessels and beyond* Nicolas L'Heureux, Cytograft Tissue Engineering
BREAK	9:50 AM	*Break*
SESSION 1	10:00 AM	Session Chair: Laura Smith Callahan, UTHealth *Amniotic fluid stem cells for repair of birth defects* Jeffrey Jacot, Rice University *Progress in using Mesp1+ progenitors in cardiac repair* Robert Schwartz, University of Houston *Injectable, high porosity bone grafts via emulsion templating* Elizabeth Cosgriff-Hernandez, Texas A&M Health Science Center-Institute of Biosciences and Technology Trainee Presentation *3D printing vascularized tissues: Closing the loop between computational and experimental models* Samantha Paulsen, Rice University
POSTER SESSION A	11:20 AM	Poster Viewing/Judging (BRC Event Space)
LUNCH	12:00 PM	Lunch and Networking (BRC Event Space)
SESSION 2	1:00 PM	Session Chair: Scott Olson, UTHealth *Hippo signaling in organ size control* James Martin, Baylor College of Medicine *Peripheral nerves provide essential cellular components for bone tissue engineering* Elizabeth Davis, Baylor College of Medicine *Pro-hematopoietic pathways activated by blood flow* Pamela Wenzel, UTHealth Trainee Presentation *Hippo signaling deletion in heart failure reverses functional decline* John Leach Baylor College of Medicine
POSTER SESSION B	2:30 PM	Poster Viewing/Judging (BRC Event Space)

Continued…

POINT-COUNTERPOINT DISCUSSION	3:15 PM	Point-Counterpoint Discussions *Growing engineered tissues in bioreactors vs the body* Moderator: Kurt Kasper, UTHealth Team Bioreactors: Stephen Navran, Synthecon Team Body: Jane Grande-Allen, Rice University *Stem cell topic: Autologous vs allogeneic stem cell transplantation* Moderator: Pamela Wenzel, UTHealth Team Autologous: Charles S. Cox, Jr., UTHealth Team Allogenic: Ian McNiece, UT MD Anderson Cancer Center
KEYNOTE (& Keck Seminar)	4:00 PM	Session Chair: Charles S. Cox, Jr., UTHealth *Bringing second-generation mesenchymal stem cell therapies closer to the clinic* Jan Nolta, University of California Davis
AWARDS	5:00 PM	Poster Award Ceremony
	5:15 PM	Reception and Poster Viewing (BRC Event Space)

Keynote Speaker Biography

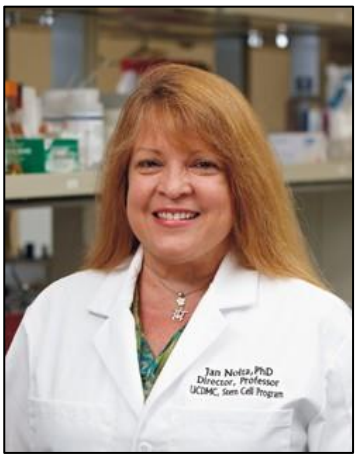
Nicolas L'Heureux, Ph.D.
Cytograft Tissue Engineering, Inc.

Tissue Engineering by Self-assembly, or TESA, is a method that can yield robust tissue-engineered constructs without the need for any synthetics or exogenous biomaterials. Strong sheets, composed of the natural ECM synthesized by normal human fibroblasts, can be rolled into vascular grafts that have burst pressures (3,490±892mmHg) that match or surpass that of diameter-matched human arteries. In a clinical study, such autologous vascular grafts were used as hemodialysis access grafts in end-stage renal disease (ESRD). With time points out to three years, we reported an average event rate of 0.7 events/patient-year, which represents a 4.2-fold decrease (compared to pre-implant rates for these patients). Recently, we have shown that a devitalized allogeneic construct (produced using a master cell line) does not elicit an immune reaction when implanted in three late-stage ERSD patients. A second-generation vascular graft has been developed that relies on the production of ECM threads that can be woven into tubes. This new textile-based approach can yield vascular grafts three times faster than with the rolling approach. In addition, this approach is highly tunable to achieve target diameter, burst pressures, suture retention strength, compliance, and wall porosity.

In a recent study, the cell-synthesized ECM was prepared as particles (< 180μm in diameter). The material was delivered subcutaneously as an injectable suspension to study the host response in a more controlled environment than the highly inflammatory and unstable hemodialysis access graft setting. In both nude mouse and human volunteers, the injected particles did not cause a significant inflammatory response and the particles were largely intact at the 3-month time point. We observed a positive remodeling process that included cellular repopulation, angiogenesis as well as adipogenesis. These observation confirm the unique biocompatibility expected from this unprocessed matrix and opens the door to the development of a first-in-class dermal filler product.

Biography:
Dr. L'Heureux holds a Bachelor's degree in Biochemistry, a Master's degree in Immunology and Microbiology, and a Ph.D. in Molecular and Cell Biology from the Laval University in Quebec City, Canada. He completed his training as a post-doctoral fellow of the American Heart Association at the Bioengineering Department at UCSD. In 2000, he co-founded Cytograft Tissue Engineering, Inc. (San Francisco) where he holds the position of Chief Scientific Officer. At a time when synthetic scaffolds were seen as the defining part of any tissue engineered construct, Dr. L'Heureux has been an early believer in the possibility of developing completely biological tissue engineering approaches that could produce constructs with appropriate biomechanical properties. With the creation of Cytograft Tissue Engineering, Dr. L'Heureux has sought to bring this vision to the clinic. This effort has led to the first clinical use of tissue-engineered blood vessels under arterial pressure. This approach can also been used to produce such diverse tissues as skin, heart valves, ligaments, nerve guides, and dermal fillers.

Keynote Speaker Biography

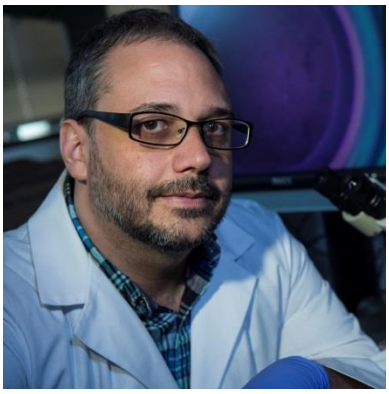


Jan Nolta, Ph.D.
UC Davis School of Medicine

Jan A. Nolta, Ph.D., is the Director of the Stem Cell Program at UC Davis School of Medicine, and directs the Institute for Regenerative Cures. The UC Davis Stem Cell Program has over 150 faculty members collaborating to work toward stem cell-related cures for a spectrum of diseases and injuries. The current research in Dr. Nolta's laboratory focuses on "bench to the bedside" research, and she has been involved in numerous clinical trials of gene and cell therapy. In 1994 she developed her passion for cellular therapy by performing the first cord blood stem cell gene therapy trials for newborns with "bubble baby disease", with her mentor Donald Kohn at the University of Southern California. A scientist with 25 years' experience with human stem cells, Dr. Nolta has published over 100 manuscripts in the stem cell field and has authored 25 book chapters. She has served on over 200 review panels for the National Institutes of Health and other grant-funding agencies, is Editor for the Journal "Stem Cells" and was editor of the Book "Genetic Engineering of Mesenchymal Stem Cells".

In 2012 Dr. Nolta received a prestigious five year Transformative Grant Award from the NIH office of the Director to study cell to cell communication. She is also funded as Co-Principal investigator on three major grants from the California Institute for Regenerative Medicine (CIRM) that have the goal of developing and delivering novel Phase 1 clinical trials. In these teams she works with physician/scientists who will deliver the therapies. In 2013 she was ranked as one of the "Global Top 50 Most Influential People in the Field of Stem Cells." Dr. Nolta is a native of northern California and is extremely happy to be back in the area, since being recruited back to UC Davis from Washington University in 2007.

Faculty Speakers



Jeffrey Jacot, Ph.D.
Rice University/Texas Children's Hospital

Jeffrey Jacot specializes in the study of congenital heart disease and heart defects, and in the translation of novel regenerative cardiac therapies for young patients of various stages in their growth and development.

As an assistant professor of bioengineering at Rice University and director of the Pediatric Cardiac Bioengineering Laboratory at the Congenital Heart Surgery Service at Texas Children's Hospital, Jacot works alongside surgeons, clinicians, radiologists and biologists to understand the clinical needs in congenital heart defect management and repair, analyze the mechanical and biological processes in heart tissue development, and develop novel biomaterials for tissue-engineered heart muscle.

Jacot's research focuses primarily on the influences of biophysical cues, such as stress, strain, shear, substrate stiffness and electrical stimulation, on the development and maturation of heart cells and tissues. He is the primary investigator on several projects that involve:

- Characterizing changes in the stiffness of cardiac tissue during development, and mechanisms connecting these changes to cellular behavior and responses;
- Analyzing differences in tissue mechanics and cellular responses in congenital heart defects
- Analyzing the effects of biophysical cues on the differentiation of stem cells into cardiomyocytes; and
- Evaluating the use of novel materials and constructs for tissue engineering or regenerative approaches.

Through a Medical Innovations Award by the Institute of Biosciences and Bioengineering (IBB) at Rice, a grant from the Virginia and L.E. Simmons Family Foundation Collaborative Research Fund, an NSF CAREER Award, and grants from the National Institutes of Health, the American Heart Association and the Gulf Coast Consortia, Jacot and his collaborators are working to develop heart tissue grown from stem cells taken from the amniotic fluid of patients' mothers. This tissue will be genetically identical to the child and will grow with the patient.

Jacot began modeling cardiac mechanics as a postdoctoral fellow in the Cardiac Mechanics Research Group at the University of California, San Diego. Working with Professor Jeffrey H. Omens and Professor Andrew D. McCulloch, he developed new approaches to explore the role mechanical forces play on the differentiation of embryonic stem cells into cardiomyocytes, or heart muscle cells, and the maturation of these cells. Jacot's work in this area continues to evolve, and he is actively seeking ways to apply the basic research toward the development of engineered cardiac tissue sheets that serve as a patch to repair ventricular defects.

<u>Research Statement</u>

Jacot's laboratories at Rice's BioScience Research Collaborative and on fourth floor of the Feigin Center at Texas Children's focus on the influences of biophysical cues, such as stress, strain, shear, substrate stiffness and electrical stimulation, on the development and maturation of heart cells and tissues. His research seeks to accomplish the following goals:

- Develop a cellularized cardiac patch for use in repair of congenital heart defects that is autologous, contractile and actively conductive;
- Investigate mechanotransductive pathways in cardiac maturation and the development of cardiac defects;
- Collaborate on clinical investigations and evaluation of therapeutic technologies for congenital heart surgery;
- Utilize primary human cells and tissues for most experiments, and focus on biophysical cues, such as strain, elastic modulus, shear stress and electrical stimulation; and
- Train graduate and undergraduate students for careers in medicine and engineering.

Faculty Speakers

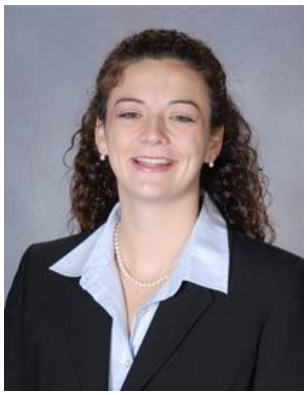
Robert Schwartz, Ph.D.
University of Houston/Texas Heart Institute

Dr. Schwartz is recognized for defining the regulatory paradigm in which nonmuscle contractile proteins are switched off during muscle differentiation and replaced by muscle specific contractile protein isoforms; thus launching the field of myogenesis in 1981. In the late 1980's Dr. Schwartz identified a highly conserved repeated element shared by the 3 alpha actin genes, later identified as the serum response factor binding site. Over the next 15 years, Schwartz provided the earliest evidence that SRF functioned as the master regulatory platform that directs myogenic gene expression programs through combinatorial interactions with other transcription factors and cofactors. In 1996, SRF was shown to associate with Nkx 2.5 the vertebrate tinman homologue that co-activated cardiac actin gene activity and then a few years later SRF was shown to partner with GATA4-6 factors. The combination of LIM-only proteins CRP1 and CRP2 with SRF and GATA factors were shown as potent cardiovascular differentiation cofactors.
Four years ago, Dr. Schwartz presented a gene switch mechanism that facilitated strong repression of SRF-dependent myogenic differentiation genes through phosphorylation of a specific evolutionarily conserved SRF residue in the MADS box, that allowed for activation of immediate early proliferation genes. In addition, Dr. Schwartz and colleagues identified more than 180 direct SRF gene targets that have roles in cellular contractility, movement and mesoderm formation and the newly discovered SM-HAT, a key histone acetyl-transferase that associates with SRF, CRP2 and myocardin directing de novo smooth muscle gene activity.

Conditional knockouts of SRF with Dr. Schwartz' early expressing Nkx2-5Cre completely blocked the appearance of smooth muscle and cardiac actin gene activity and sarcomere formation in the heart; thus, bringing full circle, after 25 years, the absolute proof for SRF obligatory role in muscle differentiation and the gene regulation of myogenic contractile proteins.
Finally, in human disease, Dr. Schwartz discovered caspase 3 cleavage of SRF generated a dominant-negative transcription factor responsible for driving depressed contractility in human heart failure and will serve as a new biomarker for human heart failure. SRF is also an important regulator of microRNA expression in the heart involved with silencing inappropriate genes and allowing for cardiomyocyte lineage specification. Schwartz continues to elucidate the chemical basis underlying the specification of cardiac muscle cell differentiation, which will provide opportunities for cell replacement therapy and heart regeneration in the future.

Robert J. Schwartz, Ph.D. is a Professor in the Department of Biology and Biochemistry and the Director of the Center for Molecular Medicine and Experimental Therapeutic. He previously was at Baylor College of Medicine in Houston, where he served as a tenured professor in the Departments of Cell Biology, Molecular and Cellular Biology, Medicine, and Molecular Physiology. He also was co-director of the Baylor College of Medicine Center for Cardiovascular Development. Schwartz also spent five years at the Institute of Biosciences and Technology where he was the Director of the Institute. During his more than thirty five years in Houston, Schwartz became widely recognized for his research on the developmental and genetic aspects of congenital heart disease. In this field he has received eleven US patents and co-founded three companies. He earned his B.S. from Brooklyn College and his Ph.D. in Biology from the University of Pennsylvania.

Faculty Speakers



Elizabeth Cosgriff-Hernandez, Ph.D.
Texas A&M Health Science Center – Institute of Biosciences and Technology

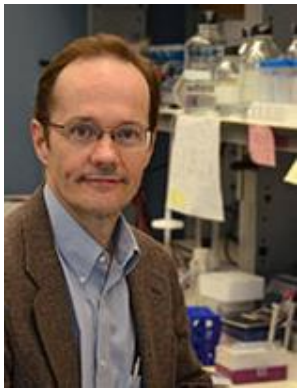
Dr. Elizabeth Cosgriff-Hernandez is assistant professor in the Department of Biomedical Engineering at Texas A&M University. Her laboratory specializes in the development of tissue-engineered scaffolds.

Biomaterial synthesis is complemented by the development of new fabrication strategies that improve the ability to manipulate 3D scaffold architecture. In addition to providing improved scaffolds for tissue repair, these innovative biomaterials and fabrication strategies provide new tools to probe the complex process of tissue remodeling in order to enhance the rational design of biomaterial scaffolds and guide tissue regeneration strategies.

The primary applications investigated by the Cosgriff-Hernandez laboratory include injectable bone grafts, multilayer vascular grafts based on bioactive hydrogels, wound dressings with tunable moisture control and bioactivity, and porous microspheres for drug delivery.

Dr. Cosgriff-Hernández received her B.S. in Biomedical Engineering and Ph.D. in Macromolecular Science and Engineering from Case Western Reserve University in Cleveland, Ohio. Her graduate research, under the guidance of Professors Anne Hiltner and James Anderson, elucidated key cell-material interactions and biodegradation mechanisms of biomedical polyurethane elastomers. She was awarded the UT-TORCH Postdoctoral Fellowship and conducted orthopaedic tissue engineering research with Professor Tony Mikos at Rice University. She joined the faculty of the Biomedical Engineering Department at Texas A&M University in 2007. Her laboratory specializes in the development of hybrid material systems that combine the advantages of synthetic and natural polymers to advance tissue engineering design. Biomaterial synthesis is complemented by the development of new fabrication strategies that improve our ability to manipulate 3D scaffold architecture. In addition to providing improved scaffolds for tissue repair, these innovative biomaterials and fabrication strategies provide new tools to probe the complex process of tissue remodeling in order to enhance the rational design of biomaterial scaffolds and guide tissue regeneration strategies.

Faculty Speakers



James Martin, M.D., Ph.D.
Baylor College of Medicine/Texas Heart Institute

Under the direction of physician-scientist Dr. James F. Martin, the Cardiomyocyte Renewal Laboratory (CRL) is focused on understanding how specialized signaling pathways are connected to adult tissue development and regeneration.

Major areas of research include atrial fibrillation (genetic basis), heart development (and associated congenital heart defects), and aortic valve disease (calcification process).

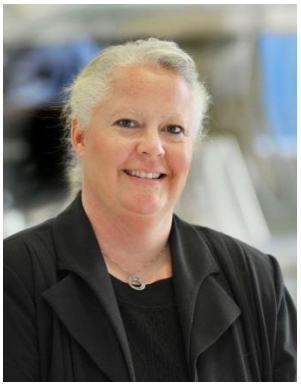
CRL is investigating genetic pathways (*Hippo*, *Wnt*, and *Bmp*) that have not only been linked to severe and/or widespread heart problems such as atrial fibrillation and pulmonary arterial hypertension, but that also are associated with congenital heart defects and the calcification process that leads to aortic valve disease.

Dr. Martin's goal is to obtain an in-depth understanding of these pathways in order to develop ways to regenerate heart muscle and prevent disorders such as atrial fibrillation and calcified aortic valves. Ultimately, these studies are designed to identify therapeutic options to promote normal tissue development and its regenerative capabilities.

Studies
Study areas in developmental pathways and adult tissue regeneration include the following:

- **Heart development.** Heart growth is a developmental process that is intricate and highly regulated. CRL has uncovered the involvement of several pathways (Nodal, Pitx2c) influencing discrete steps in heart formation (outflow and inflow tract development, cardiac septation and ventricular growth). Pitx2c is associated with human diseases (Rieger Syndrome I, atrial fibrillation). In addition, recent CRL discoveries indicate that there are inhibitory pathways, such as the Hippo pathway, that control heart size by regulating cardiomyocyte proliferation and may have a role in cardiomyocyte renewal. CRL's studies are providing mechanistic insight into these unique pathways in heart development and are focused on identifying new avenues for therapeutic intervention to promote cardiac regeneration.
- **Vascular and epithelial tissue development.** Bone morphogenetic protein (BMP) signaling has been implicated in human inherited disorders of blood vessel (vascular tissue) and tissues that line the structures of the body (epithelial tissue)
  such as juvenile polyposis and pulmonary artery hypertension. CRL is studying BMP signaling during vertebrate development and their research has provided insight into BMP function in congenital heart malformations, with the long-term goal of uncovering therapeutic avenues for human patients.
- **Aortic valve disease.** Aortic valve disease has become a major medical problem that continues to increase as the population ages. However, treatment options are currently very limited. The CRL research program is investigating the mechanisms underlying the valve disease that begins with narrowing of the aortic valve opening or "stenosis" due to deposition of calcium. This "calcification" process limits blood flow through the heart and can lead to valve hardening. CRL is focusing on the roles of the known regulators of calcification, the BMPs, in heart valve calcification. Dr. Martin's group was the first to provide genetic evidence that *Bmp2* is essential for atrioventricular valve development. CRL's recent discoveries indicate BMP signaling is involved in maintaining healthy valve structure and function (termed "homeostasis") by regulating poorly understood genes (*Smad8* and *microRNA 17-92*). CRL's goal is to determine how BMPs regulate development of valve calcification. CRL's studies are providing novel insights into the detailed molecular mechanisms behind BMP function and how different proteins in this pathway interact to either promote or block aortic valve calcification.

Faculty Speakers



Elizabeth Olmsted-Davis, Ph.D.
Baylor College of Medicine

Dr. Olmsted-Davis's research program encompasses the design of a cell based gene therapy system for targeted production of bone. One of the therapeutic goals for this system is to enhance bone repair both in long bone fractures, as well as offer a potential treatment for critical size defects resulting from bone trauma. These clinical problems are difficult challenges to orthopedic surgeons resulting in the need to introduce additional hardware or bone graft, and often in the case of trauma, result in the loss of the limb altogether. Our system is designed to rapidly form bone within two weeks after a single injection of our gene therapy system, thus significantly reducing both complications from potential infection as well as overall recovery time. This work is currently supported by the DOD-orthopedic trauma research program.

A second approach for the treatment of critical size defects is to tissue engineer the bone within a bioreactor which could then later be engrafted into the defect area. This work is a collaborative effort between our laboratory and several others within the Center for Cell and Gene Therapy, and Baylor College of Medicine. Our contribution to this project is to characterize the normal physiological processes of de novo bone formation. Using our gene therapy model, we have identified a novel stem cell population and characterized the micro-environmental signals that eventually lead to stem cell differentiation into cartilage and bone. This work is currently funded by the NIBIB.

The third focus of the laboratory is to develop a non-invasive approach for spine fusion. This project has been ongoing over the past three years and has resulted in an efficacious injectable system that results in fusion of the vertebral bones. We are currently completing the efficacy testing in rodent models. Once animal testing is completed, we propose to translate this project into a tentative clinical trial. This work is supported by the DOD-PRMRP.

Dr. Olmsted-Davis has also been involved with establishing a Vector Development Laboratory, a non-profit core facility at Baylor College of Medicine, which provides gene therapy vectors for use as gene delivery systems for preclinical and basic research. This organization has aided researchers in both the Texas Medical Center as well as the global research community.

Dr. Elizabeth Olmsted-Davis' lab is part of the Center for Cell and Gene Therapy (CAGT) research laboratories.

Faculty Speakers

Pamela Wenzel, Ph.D.
University of Texas Health Science Center at Houston

Mechanobiology of blood development: One arm of our research is designed to address how biomechanical force promotes the hematopoietic program in the embryo and how we might use this information to expand improved sources of hematopoietic cells for the clinic. A number of genetic and biochemical pathways are currently under investigation as key players mediating this signaling cascade, and we employ various approaches to evaluate their role in blood development, including microfluidics, pharmacology, mouse genetics, and transplantation assays.

Biomechanical modulation of anti-inflammatory genetic programs in mesenchymal stem cells: Shear stress, or frictional force, also modulates the behavior of mesenchymal stem cells, and impacts proliferation, cell survival, and fate decisions. A growing body of literature suggests that these types of cells can suppress unchecked inflammatory signaling and innate immune response in patients. Consequently, our second area of interest is to determine how mechanical force alters the biology of mesenchymal stem cells, including their ability to modulate anti-inflammatory programs and vascular permeability. We utilize culture-based assays, cellular phenotyping, and mesenchymal stem cell-based therapy models of traumatic brain injury as readouts of response to mechanical stimuli.

Role of force in initiation of metastatic programs: Finally, fluid flow and hydrostatic pressure have been implicated in tumor biology, but it remains unclear what role lymphatic or vascular shear stresses may play in modulating the gene expression programs or metastatic potential of cancer cells. Using custom microfluidics, we are modulating the shear stress present in the cancer cell environment and evaluating its impact on metalloprotease activity, invasive potential, and activation of oncogenic pathways.

Training:

Dr. Wenzel began her training as a stem cell biologist at The Ohio State University with Dr. Gustavo Leone. Her doctoral studies there focused on the developmental defects caused by genetic mutations of two classes of genes renowned for their relevance to cancer, Rb and E2f. She identified a critical role for Rb in regulating proliferation and differentiation of trophoblast stem cells by generating Rb-deficient trophoblast (placental) stem cell lines and a placenta-specific Cre-expressing transgenic mouse, referred to as CYP19-Cre. With these genetic tools, she showed that ablation of Rb in the stem cell compartment of the placenta was sufficient to cause death of otherwise normal fetuses and that the lethal placental phenotype resulting from loss of Rb induced non-cell autonomous defects in fetal tissues such as the central nervous system and red blood cells.

In parallel, she developed complementary systems to address the requirement for the E2f1, E2f2, and E2f3 transcription factors, the primary mediators of Rb transcriptional regulation, in initiation of the cell cycle. Using genetic ablation in mice and derivation of embryonic and trophoblast stem cell lines triply-deficient for E2f1-3 she demonstrated that, in stark contrast to dogma established by fibroblast-based assays, E2F activators are not required for cell cycle progression. In fact, expression of cell cycle regulatory genes modulated by E2F activators were upregulated in triply-deficient cells and embryos, suggesting that E2F activators possess repressor-type functions in vivo.

Dr. Wenzel moved to Children's Hospital Boston and Harvard Medical School to complete her post-doctoral training, mentored by a true pioneer in the field of stem cell biology and reprogramming, Dr. George Q. Daley. There she focused on hematopoietic stem cell biology. She studied a number of extrinsic factors, including biomechanical force, soluble molecules, and pharmacological compounds that endow hematopoietic precursors with the ability to contribute to the adult blood system. Conversely, she also studied the inhibitory role that adipocytes play in hematopoiesis of the adult bone marrow and showed that expansion of fatty marrow interferes with recovery of hematopoietic progenitors following radiation therapy in animal models.

3D Printing Vascularized Tissues: Closing the Loop between Computational and Experimental Models

Paulsen SJ[1], Miller JS[1]
1. Department of Bioengineering, Rice University, Houston, TX

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As tissue engineering advances from simple 2D structures to complex 3D organs, mass transport to cells becomes a paramount issue. 3D printing provides a method to establish a vascular space within large 3D tissues, as 3D printing can generate complex geometries using well defined materials. The objective of this work is to close the loop between computational and experimental models involving flow and mass transport in vascularized tissue engineered constructs. By developing reliable computational models for our tissue engineered systems, we can rapidly optimize vascular geometries to maintain cell viability throughout constructs on a physiological size scale. Furthermore, these models offer a high throughput means to adapt vascular geometry and flow to adjust for changes in bulk material or metabolic requirements of the cells.

We started by tracking the flow of fluorescent beads through our printed channels at a physiological flow rate of 10µL/min. After collecting a series of images with frame rates upwards of 300 frames per second, we used the particle tracking application TrackMate, part of the FIJI package, to calculate flow rates of beads within the channels. This step provides a basis against which we could compare our computational models. Then, to develop computational models for flow, we first used a Bruker micro-computed tomography scanner to scan the same channels we used in the bead tracking experiments. Using Mimics software, we used these scans to reconstruct 3D meshes for our printed channels. We imported these meshes into the computational fluid dynamics software COMSOL Multiphysics, to predict flow rates and patterns through individual channels. Preliminary evidence has demonstrated an agreement between the computational and experimental models, but that interruptions in flow in the experimental systems, such a bubbles, can result in flow aberrations that differ from predicted models. We predict that optimizing printing parameters to increase the size of channel junctions can reduce the effects of bubbles and result in more uniform flow between channels.

For future work, we plan to develop computational models for cell viability in large vascularized constructs, by estimating flow rates, oxygen diffusion, and carbon dioxide diffusion through large, cell-laden hydrogels. Reliable computational models are an invaluable resource in making high throughput optimizations in vessel geometries, flow rates, and cell seeding densities to ensure the viability of cells within a large scale tissue engineered construct.

Hippo Signaling Deletion in Heart Failure Reverses Functional Decline

Leach J[1], Heallen T[3], Zhang M[2], Rahmani M[3], Martin J[123]

1. Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas
2. Institute of Biosciences and Technology, Texas A&M, Houston, Texas
3. Cardiomyocyte Renewal Laboratory, Texas Heart Institute, Houston, Texas

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The heart has long been thought of as a static organ incapable of repair. Recent findings have challenged this view of the heart, and have demonstrated that mature cardiomyocytes are capable of re-entering the cell-cycle. However, there is still paucity in understanding endogenous mechanisms preventing cardiomyocyte self-renewal. During development Hippo signaling is a well-known regulator of intrinsic organ size by modulating cell cycle control. The mammalian Hippo pathway includes the Ste20-like serine/threonine kinases *Mst1* and *Mst2*, homologous to the *Drosophila Hippo* kinase. A subsequent kinase cascade leads to the phosphorylation of the transcription factor Yes associated protein (*Yap*). Phosphorylated *Yap* is sequestered in the cytoplasm, thus preventing transcriptional activation of proliferative genes.

Objective: We aim to apply developmental-mechanisms of cardiomyocyte cell-cycle control to the damaged heart. By altering the Hippo signaling pathway to coordinated cardiomyocyte proliferation we intend to establish a model of cardiac regeneration.

Methods: We have shown that Hippo-signaling controls cardiomyocyte proliferation during embryonic heart development. Both the Apex resection (AR) and Left Anterior Descending Coronary Artery ligation (MI) models of cardiac damage were used to determine if modulating Hippo signaling is *cardiac protective*. Current studies aim to determine if altering Hippo signaling can promote *regeneration*. We are taking a new approach to determine the effect of Hippo signaling deletion during heart failure (HF); by inducing Hippo deletion after cardiac damage when fibrotic scar formation has already occurred. To evaluate cardiac function we have performed echocardiogram over time to follow the progress of cardiac recovery.

Results: Indeed, in the AR and MI models *cardiac protection* was indicated by preserved cardiac function and reduced fibrotic scar formation. Additionally, these hearts display adult cardiomyocyte proliferation as marked by EdU incorporation, pHH3, AurkB, and Ki67 staining. ChIP sequencing and RNA sequencing revealed Hippo signaling control of a variety of genes involved in *cardiac protection* but also in genes that could be involved in *regeneration*.

Current studies looking at HF indicate functional recovery of the heart after inducible deletion of Hippo signaling. Furthermore the progression of fibrotic scar around the left ventricle is halted. Consistent with our AR and MI data, adult cardiomyocytes re-enter the cell cycle. Furthermore, using a double labeling pulse-chase experiment we have determined that a subset of Hippo deficient adult cardiomyocytes has stem-cell like growth and is capable of multiple rounds of cell division.

Conclusions: By altering Hippo signaling during heart failure and subsequently inducing cardiomyocyte proliferation we have promoted recovery of cardiac function. Furthermore, we have demonstrated that Hippo signaling acts a barrier preventing cardiomyocte renewal and subsequently regeneration. These results will greatly advance strategies to stimulate cardiac repair even after fibrotic scar damage has been established.

Funding Sources: NIH Cardiovascular Training Grant - 5T32HL007676-24

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Microfluidic Electrophysiology on Single Cells using Suspended Nanowire Electrodes

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The electrical properties of cells are essential for cellular metabolism, communication, and controlling the action potentials generated by excitable cells such as cardiomyocytes, neurons, and skeletal muscle. For over 40 years, most studies of electrical properties of cells have relied upon measurements performed using manual patch clamp electrophysiology. While capable of providing high fidelity recordings and precise control of membrane potential, conventional patch clamping is time consuming, requiring specialized equipment and a trained experimentalist. As a result, studies requiring electrophysiology on many cells often resort to measurements using less accurate proxies for membrane potential, such as calcium imaging. To permit precise studies of electrical properties across many individual cells, we have developed a technology to allow high throughput electrophysiology studies. We combine the flexibility of microfluidics with the scalability and precision provided by semiconductor fabrication techniques in a microfluidic chip incorporating suspended nanowire electrodes for electrical recordings and stimulation, along with planar electrodes for field stimulation when simultaneous stimulation and recording is desired. The ability to perform electrophysiology in a high-throughput manner will make it possible to perform experiments that would previously have been forbiddingly time consuming or difficult.

Design of a Perfusion Bioreactor for Reproducible 3D Cell Culture

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Cell interactions with the surrounding environment vary greatly when the cells are cultured in a monolayer versus a three dimensional space. Furthermore, a 3D culture approach that mimics the physiological environment will promote in vivo-like cellular responses that may not occur during 2D culture. In many 3D culturing systems, perfusion flow is often implemented to support adequate nutrient delivery for cellular functions and to promote a homogeneous distribution of cells throughout the scaffold. However, many of these perfusion bioreactors create a large variability in the environmental conditions between different samples and from system-to-system. The scope of this project was to develop a perfusion bioreactor for 3D culture that reduces sample-to-sample variability while still allowing for sample-specific testing with independent flow loops. After optimizing a chamber design for reproducibility, cell-seeded scaffolds were cultured in the chambers for six weeks to assess long-term cell viability and sterility of the system.

The bioreactor was designed with an integrated reservoir chamber to standardize the flow loop from sample-to-sample. In the system, culture media is pumped from the reservoir to fill and surround the sample compartment. The fluid then exits the top of the sample compartment and flows through hollow shafts with porous platens that hold the sample in place. This ensures that media is passed directly through the sample before returning to the fluid reservoir. By standardizing this flow loop, each sample is exposed to similar flow-induced forces and levels of nutrient delivery. To perform the cell culture validation on the chamber design, bovine aortic smooth muscle cells (SMCs) were seeded onto polycaprolactone (PCL) scaffolds (5 mm thickness, 10 mm diameter). After a static adhesion period, the scaffolds were inserted into three bioreactor systems with porous platens (560 μm pore size) and culture media flowed through the system at a rate of 10 mL/min. Scaffolds were removed from the chambers at three time points (2, 4, and 6 weeks) and viability was assessed via confocal microscopy (calcein AM and ethidium homodimer-1)

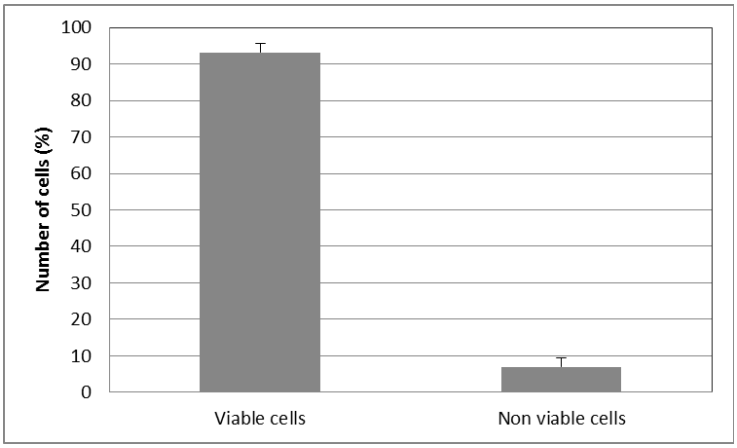

Figure 1: Viability of cells within the scaffolds after 6 weeks

After six weeks in culture, the SMCs had fully integrated into the scaffold microstructure and were shown to be viable (Figure 1). There was no evidence of microbial contamination at any point during the culture and post-culture biochemical analysis confirmed the absence of contaminants. The bioreactor chamber design was shown to maintain sterility in long term cell culture while maintaining cell viability.

Spontaneous Capillaries Formed with Mesenchymal Stem Cells supporting Endothelial Cells in Fibrin Gels

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Mature and stable blood vessel mimics are crucial to maintain viable tissue constructs for regenerative medicine. Cells within an engineered tissue cannot access essential mass transport beyond 500 microns of an oxygenated source through simple diffusion. Therefore, understanding the formation and maintenance of branching structures such as capillaries can bridge areas of the tissue without perfused blood to tissue regions with proximity to the oxygen source. One such component that influences the state of microscale, open channels is cell-cell interactions. Endothelial cells tightly line the blood vessel walls and function as a barrier to the blood fluid and tissue interface. However, endothelial cells alone do not perform functionally, as channels may delaminate, leak, or fail an aspect of its role without supporting cells or pericytes. Vessel networks that are recapitulated in a three-dimensional environment resemble the reality of a complex living tissue. Using fibrin, a natural extracellular matrix component often found in wound clots, as a scaffold, cell-cell interactions are observed in a three dimensional gel. It enables us to study capillary network formation in the fibrin model system as the cells are encapsulated three dimensionally, providing a more relevant environment. We asked whether the interaction of human mesenchymal stem cells (hMSCs) with endothelial cells (derived from induced pluripotent stem cells) promote a spontaneous and durable network of tubes. Such tubulation would suggest hMSCs perform a pericyte-like role. Overall, this key model system for studying capillary formation and pericyte function may address the appropriate orchestration of cellular activity for recapitulating critical vasculature required by tissues.

Optimal Activation of Innate Immunity is Necessary for Induced Pluripotent Stem Cell Generation

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Objective: We showed that activation of innate immunity is required for efficient nuclear reprogramming in generation of iPSCs (Cell 2012). Small molecule like polyinosinic:polycytidylic acid (poly I:C) stimulates toll-like receptor 3 (TLR3) to activate transcriptional pathways (mediated by NF-κb, IRF3 and IRF7) leading to global changes of epigenetic modifiers (downregulation of HDACs and upregulations of HATs). With respect to the role of innate immune signaling in reprogramming, we now provide evidence for an optimal range and duration of such signaling.
Methods: MEFs expressing doxycycline inducible polycistronic cassette of *Oct4*, *Sox2*, *KLF4* and c-*Myc* were treated with different concentrations of poly I:C (0,1,3,10,100,300,1000 and 10000 ng/ml) each day along with doxycycline (2 µg/ml) up to 7 and 12 days respectively. In separate studies, we have examined the effects of the duration of innate immune signaling on reprogramming efficacy.
Results: Our preliminary data indicates that there is an optimal range ("Goldilocks zone") of innate immunity activation for somatic cell reprogramming. The concentration of poly I:C which generated maximal colony formation was 30 ng/ml. On either side of optimal activation, the efficiency of iPSC generation is reduced. For example, reducing innate immune signaling with the addition of a decoy oligonucleotide against NFKb nearly abrogates colony formation. In addition, high levels of innate immune stimulation (eg. 1000ng/ml Poly I:C) also substantially reduces colony formation. Additional studies indicate that a period of about 6 days of innate immune signaling, in this model, is necessary for optimal colony generation. Shorter duration, or more extended duration of stimulation, reduces colony formation.
Conclusion: There is an optimal range and duration of innate immune signaling that is necessary for the greatest efficiency of nuclear reprogramming. Understanding what biological mechanisms are defining this "Goldilocks zone" may improve reprogramming efforts.

Optimization of Re-Endothelialization on Acellular Whole-Heart Rabbit Scaffold

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Introduction: With a growing number of people with heart failure and a limited number of donor hearts for transplant, tissue engineering is needed to create bio-artificial hearts (BAH) seeded with autologous cells. Past studies have shown promise in creating a small animal BAH optimized for minimal thrombogenicity[1,2]. The need for a larger animal heart model that parallels the human heart (infant), in size and disease progression, makes the rabbit model ideal to transition to creating a human compatible BAH[3]. Here, we hypothesize that acellular rabbit hearts can be seeded at distinct cell densities for optimal re-vascularization and re-endothelialization that avoids vessel occlusions from over seeding and thrombosis from lack of seeding endothelial cells (EC). In this study, re-endothelialization of rabbit hearts is assessed with varying seeding densities of EC.

Methods: Hearts (~8g) were isolated from New Zealand White rabbits and decellularized by Langendorff flow with gravity-driven (70mmHg) perfusion of 1% SDS solution. Residual dsDNA was quantified to ensure adequate decellularization of the scaffold. To recellularize, acellular hearts (AH) were sterilized and placed in a closed-loop Langendorff flow system with media. Rat Aortic Endothelial Cells (RAEC) were harvested at different densities and re-suspended in 2 mL of media for bolus injection into the AH. The cells were allowed to flow by gravity before low constant flow was introduced. The seeded heart was cultured for 7 days with media changes at day 1 and 3 after cell seeding. For each culture, non-adherent cells were quantified at day 1, 3, and 7 to assess the number of EC retained in the heart. Cell tracking with CMFDA (Life Technologies) was used to fluorescently image cell dispersion at the coronaries to assess EC coverage. H&E, Trichrome and CD31 antibody staining of the tissue was performed to assess the coverage of EC in 6 different cross-sections of the heart.

Results: Decellularized whole rabbit heart scaffold contained on average <4% residual dsDNA in 11 areas compared to native rabbit hearts. At all cell densities, RAECs perfused into the AH stained positive for CD31 within the left ventricle and vessels. Few to no negatively stained cells were seen. Seeding densities at or above 80 million RAECs showed cell loss of approximately 16% after day 1, less than 1% after day 3, and none at day 7. Cells seeded at densities below 40 million RAECs showed cell loss of approximately 22% after day 1, 4% after day 3, and less than 1% at day 7. Staining and imaging showed lack of RAECs seeded into the distal branching of the coronary arteries at seeding densities at or below 20 million cells.

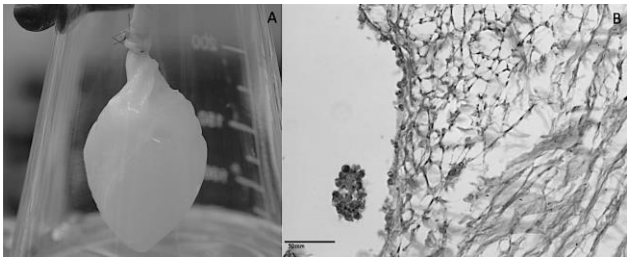

Figure 1**:** A) Decellularized Rabbit Heart
B) H&E Staining of Endocardial LV of RAEC Re-Endothelialized Rabbit AH.

Conclusion: Re-endothelialization of an AH, comparable in size to an infant human heart, is possible by utilizing 40-80 million endothelial cells cultured over 7 days. The number of cells needed for re-endothelialization of a decellularized rabbit heart is not directly scalable from the size of and the cell number required for re-endothelialization of the decellularized rat heart. This suggests that each animal model is unique in cell seeding density to adequately re-endothelialize. Overall, optimal seeding density of EC in rabbit hearts was crucial to keep from cell formed occlusions in vessels and possible thrombogenicity where EC are not present. Future studies include assessing functionality and anti-thrombogenicity of the newly lined vessels.

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References: 1. Robertson, M.J *PLOS ONE* 2014(9), e90406 2. Ott, HC *Nature Medicine* 2008(14), 213-221 3. Hasenfuss, G *Cardiovascular Research* 1998(39), 60-76

Combination with dECM improves the efficacy of cardiac cell based repair

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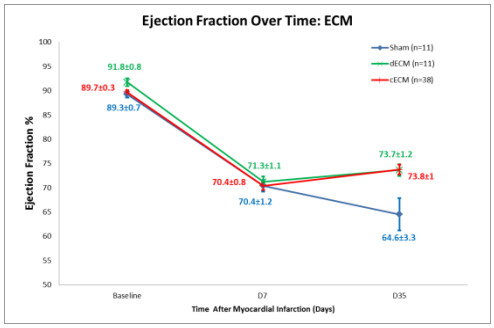
Background: Congestive heart failure remains a major health concern in the United States, with end stage heart failure two-year mortality rates of 70-80% affecting 60,000 patients annually. [1] Myocardial Infarction is one of the main causes of heart failure in western societies. Unfortunately, the availability of donor hearts dwarfs the clinical need, several lines of treatment for end-stage disease have been proposed: drug therapy, stem cell therapy, and biventricular assisted devices among them being a bridge to heart transplantation. Cell therapy has been used as an alternate treatment, but efficacy, retention and viability are still a challenge. Several preclinical trials[2] have demonstrated significant potential for heart regeneration and reverse remodeling. There is significant evidence that the extracellular matrix (ECM) retains significant information for stem cell differentiation[3] and that this can be preserved in native ECM by a process called decellularization. We hypothesized that a native extracellular matrix (ECM) patch cultured with cells could improve cell survival, homing and delivery of stem/progenitor cells to an ischemic damaged heart. By enhancing growth of functional cardiac tissue, treatment efficacy and regeneration of cardiac muscle in an immunocompromised murine myocardial infarction (MI) model.

Methods and Results: Sixty-six nude athymic rats underwent surgical left anterior coronary artery ligation. Seven days after, MI was corroborated by echocardiography and troponin I serum levels. Animals were randomly divided into sham, decellularized-ECM (dECM) and cellularized-ECM (cECM) surgical patch implantation; animals were follow-up by echocardiography on days 14, 21 and 35 after MI. There was an improvement in ejection fraction (EF) in those who received decellularized and cellularized patches compared to the Sham group. Immunohistochemistry analysis has shown cell recruitment, angiogenesis and neoformation of cardiac muscle in the patches.

Conclusion: We found differences in EF % after myocardial infarction finding a significant improvement of 9% EF in the cECM and dECM groups compared to the Sham group, P-value (0.02), as well as recruitment of cells in the patch observed by histology in animals with implanted ECM patches compared to animals with sham surgeries. Hence, we conclude that ECM facilitate the survival of stem/progenitor cells to damage myocardial tissue, and improves global systolic function after an acute ischemic attack. Further investigation in larger animal models should be pursue.

Fig 1. Ejection fraction, after acute MI procedure and implant of ECM patch.



References:

1. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) Trial Study Group. N Engl J Med 1987; 316(23):1429-1435.

2. The Role of Clinical Trials in Deciphering Mechanisms of Action of Cardiac Cell-Based Therapy.Viky Y. Suncion, Ivonne H. Schulman, and Joshua M. Hare. Stem Cells Transl Med. 2012 Hab: 1 (1):29-35.

3. Extracellular matrix: A dynamic microenvironment for stem cell niche. Francesca Gattazzo, Anna Urciuolo, Paolo Bonaldo,BBA 2014:1840(8):2506-2519.

Targeted Correction and Restored Function of *CFTR* Gene in Cystic Fibrosis Induced Pluripotent Stem Cells

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Cellular transplantation of lung stem/progenitor cells represent a potential therapeutic approach for a variety of inherited monogenic lung diseases. Crucial to the success of such a therapeutic strategy is that the transplanted cells and their progeny are corrected for the disease-causing mutation and that the transplanted cells do not elicit an immune response in the recipient. In order to satisfy these criteria, we are pursuing a patient-specific approach in which, starting with skin cells from patients with inherited lung disorders, autologous induced pluripotent stem (iPS) cells are first derived. Utilizing site-specific homology-direct repair (HDR), the disease-causing mutation is corrected in the endogenous chromosomal DNA sequence. Finally a direct differentiation approach will be employed to obtain highly purified populations of the relevant lung/progenitor cells from the corrected iPS cells for purposes of transplantation.

We have utilized this approach to generate corrected autologous iPS cells for patients with Cystic Fibrosis (CF). Starting with CF patient fibroblasts, we derived and extensively characterized iPS cell lines, confirming their pluripotency and normal karyotype. We then utilized Zinc Finger Nucleases (ZFNs), designed to target the endogenous *CFTR* gene, to mediate correction of the inherited genetic mutation in this locus via HDR. The corrected CF iPS cells, when induced to differentiate to epithelial monolayers *in vitro*, expressed the corrected *CFTR* gene; importantly, *CFTR* repair led to a readily detectable mature CFTR glycoform and evoked appropriate short circuit currents by Ussing chamber analysis, demonstrating functional *CFTR* correction. The methodology described here will be valuable for deriving new epithelial models (applicable to any CFTR2 mutation), for personalized medicine to CF drug discovery, including studies of missense, splice site and nonsense mutations, as well as the pathways contributing to mRNA utilization and nonsense mediated decay.

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Location Dependent *De Novo* Bone Formation In The Rat Model: Absence Of Activated Matrix Metalloproteinase-9

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The ability to rapidly regenerate missing bone at a targeted location in a variety of physiological settings would be a major advancement in the field of bone tissue engineering. Studies to harness heterotopic ossification or *de novo* bone formation that can occur in soft tissues have been the focus or much study. Using a cell based gene therapy approach that leads to physiological levels of BMP2 secreted at the target site revealed that in rats *de novo* bone formation is location dependent (figure 1). The location dependent nature of the bone formation in rats is very different from that in mouse, suggesting changes in the mechanics of *de novo* bone formation. In these studies, BMP2 induced bone formation was characterized in the rat model, and compared to the mechanisms that occur when the same system is applied to mice. To induce *de novo* bone formation, immunologically matched cells transduced with either a replication-defective adenovirus possessing bone morphogenetic protein-2 (BMP2) or an empty cassette was injected into the muscles in the rodent hind limb. In rats, cells that were injected near the skeletal bone resulted in *de novo* bone formation whereas cells injected into the same muscle group but distal from the bone, did not result in any bone formation. However, in both cases the bone appears to be distinct from the skeleton. When cells were injected in the same limb at both locations, HO was observed at both sites, demonstrating the potential for bone to form anywhere in the rat muscle. Characterization and comparison of the bone formation in rats and mice suggest that the progenitors have very similar phenotypes, although recruited from two different tissue sources, which may account for the location dependent bone formation. Comparison of the microenvironment at the location of bone formation between mouse and rat suggested a significant difference in the presence of active matrix metalloproteinase-9 (MMP9). In both models, MMP9 appears to be associated with brown adipose and peripheral nerves, however these model differ in the activation of this protein, where in the rat model, the majority of protein remains in an inactive form. Alternatively, active MMP9 is found significantly elevated in mice as early as 24 hours after induction of bone formation. MMP9 is made as a pro-protein that must be cleaved by plasmin for it to become an active protease. Plasmin the active form of plasminogen is recruited to tissues through platelet activation. We are currently measuring platelets and plasmin levels in blood and the tissues surrounding the site of new bone in both rats and mice. These studies provide novel insight into different mechanisms and pathways exhibited by different animals and species. This study highlihgts the idea that even closely related species can differ significantly in their biology, and biological considerations maybe needed to determine the best models for testing and development of systems for bone tissue engineering. Further, it provides novel insights as to the important considerations that must be made when choosing appropriate preclinical model and comparison of models for future therapies to manipulate bone formation. **Acknowledgements:** Thank you to the Department of Defense; W81XWH-13-1-0286 and W81XWH-12-1-0475.

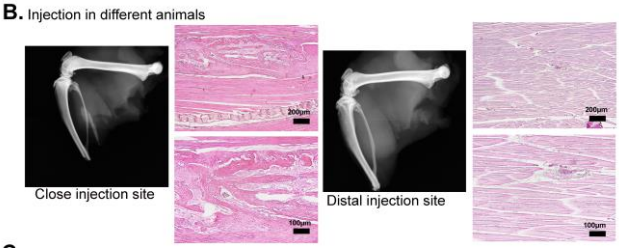

Figure 1: Representative photo-micrographs of *de novo* bone formation in rats.

An Automatized *ex-vivo* Bioreactor System with Programmable Perfusion Flow and Loading Capabilities for Bone Ingrowth Culturing

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Background

Many implantable orthopedic devices and reparative procedures show promise translation to clinical applications through advances in materials and manufacturing processes. However refinement of new designs and strategies is hindered by the cost and complications of animal studies, particularly for evaluating candidate materials and early iterations in devices. Culturing organs in a bioreactor seems to be a promising approach for evaluating new methods and materials. Several bioreactors have been developed for bone tissue engineering and bone ingrowth studies, but none is able to provide programmable fluid flow, mechanical loading, and media exchange in an automated fashion.

Objective

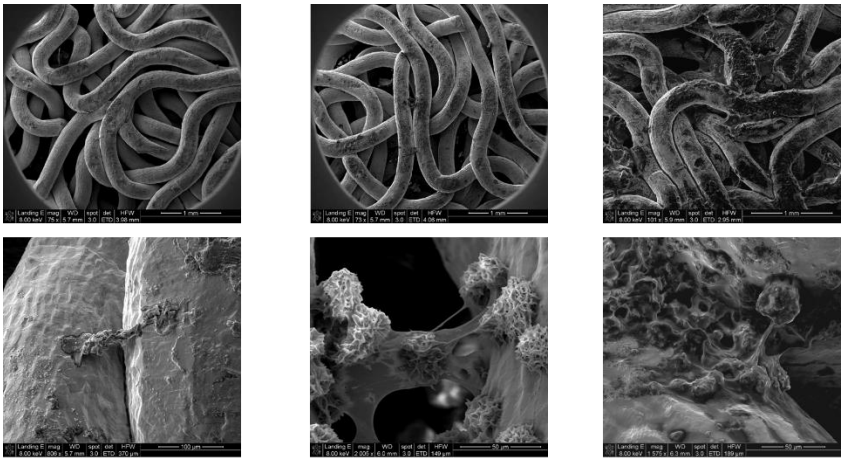
To construct an *ex-vivo* organ culture system to enable empirical testing of iterative design changes in an economical manner

Methods

Porcine bone blocks with metal geometry were incubated in three different conditions for 7 weeks. Group 1 in static culture, group 2 in static culture with weighted metal geometry and group 3 in dynamic culture in bioreactor with flow rate 1ml/min and cyclic loading at 0.5 Hz for 15 mins every 8 hours. The histologic and morphologic assessment was conducted using H&E staining and scanning electron microscopy at week 7.

Results

Histology results demonstrated that the bone morphology was better maintained in the bioreactor system when compared to the static groups. SEM images for different groups as shown in figure 1. clearly demonstrated that there was an increased production of extra cellular matrix within the pores of the metal geometry for the bioreactor group.



Static culture | **Static culture with weights** | **Dynamic culture in Bioreactor**

Figure 1

Conclusions

An *ex-vivo* organ system was constructed with some of the salient features including quadrangular shaped specimen chambers to induce laminar fluid flow, autoclavable polysulphone materials, programmable perfusion flow rate, sterile media exchange system, and programmable pneumatic actuators that can provide adjustable intermittent mechanical stimulus. The described *ex-vivo* bone culturing bioreactor system provided a means to empirically test implantable orthopedic devices during the design process in a cost effective manner. This system can also serve as an indispensable tool in the study and development of orthopedic devices requiring fixation through ingrowth.

Morphometrics of Calcification and Relationships to Fibrous Cap in Carotid Endarterectomy Tissue

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Background: Arterial calcification plays a significant role in the pathogenesis of a carotid atherosclerotic plaque development. The aim of this study was to provide a detailed analysis of calcification morphology based on histological features in carotid endarterectomy (CEA) specimens. Emphasis has been focused on calcium particle size and shape, particularly their relation to tissue anatomical location, lumen morphologies and fibrous cap thickness. These studies will help to understand the mechanisms of the arterial calcification process and elucidate the vulnerability fibrous cap.
Materials/Methods: Representative frozen sections were cut from the common, bifurcation, internal and external segments obtained from 20 CEA samples. Histological Von Kossa stains were used to visualize the calcific particles and tissue compositions. The digitized images of sections were warped when necessary to achieve closure of the vessel wall. Image analysis was to determine the morphologies of calcium particles (size, shape and perimeter), lumen (area and perimeter), tissue (area and compositions) and fibrous cap thickness using ImageJ.

Results: Calcified particles ranged in size from smaller than $0.1mm^2$ to larger than $1.0mm^2$. Particles could be found in each of the 20 CEA tissues. Calcified areas were most abundant in the bulb segment followed by the internal segment and the common segment ($p=0.001$). The lumen area in the bulb segment deviated from a patent circular shape. The thin fibrous caps were associated with larger calcium particles, but the fibrous cap in the bulb segment was thicker.

Conclusions: Calcified areas of various sizes were found in all CEA tissues. These areas were predominantly located at/near the bifurcation. The internal branches were more prone than external branches to calcification. As calcified area increased in size, the area shape became more elongated. The presence of various sized calcified particle, suggests that very small particles coalesce with neighboring particles to form larger patches ($p=0.0001$). The larger calcified particles were accompanied with thin fibrous caps.

Study of The Effect of Hypoxic Preconditioning on Engraftment and Self-renewal of Human Myoblasts in a Mouse Model for Duchenne Muscular Dystrophy

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Objectives: Cell transplantation for tissue repair is one of the main goals of regenerative medicine. In case of muscular dystrophies such as Duchenne Muscular Dystrophy (DMD), cell transplantation can repair damaged fibers and restore the expression of missing protein (dystrophin). However, previous studies have demonstrated inefficiency of the cell transplantation in DMD patients due to poor survival and limited migration capacity from the site of injection. These are mainly attributed to ischemic condition of the dystrophic muscle which induces severe cell death after transplantation. Meanwhile, recent studies have demonstrated significant benefits of hypoxic preconditioning on cell survival, self-renewal and their in vivo migration and regeneration. Current study is designed to evaluate the effect of hypoxic preconditioning on human primary myoblasts in vitro as well as after transplantation into a novel immunodeficient mouse model for DMD (NSG-mdx).
Methods: Human primary myoblasts were grown in hypoxic (1%) vs. normoxic (20%) conditions for 6 days and then allowed to differentiate. Cells were counted every 2 days and growth curve was compared among groups. Gene expression assays were done using RT-PCR for pathways associated with myogenesis, angiogenesis, cell survival, cell fusion and hypoxic signaling. Cells were also stained for myosin heavy chain (MHC) and myogenin (MYOG) and were statistically analyzed for fusion index and myotube area using ImageJ software. For in vivo transplantation, tibialis anterior (TA) of NSG-mdx mice was damaged with cardiotoxin and control vs. hypoxic treated cells were transplanted in the right and left TA respectively.
Results: Growth curves showed no difference in cell proliferation after 2 days in hypoxic conditions. Post 2 days, normoxic conditions continued to grow exponentially while hypoxic conditions exhibited diminished proliferation. Gene expression data in hypoxic group demonstrated significant up-regulation of genes which promote cell survival, angiogenesis, and myogenesis. Anti-apoptotic BCL2 gene was also up-regulated in in hypoxic conditions. Interestingly, chemotactic receptor CXCR4 was also highly upregulated in hypoxic conditions suggesting conditioned cells might have an increased capacity to mobilize when transplanted into muscle. Hypoxic conditions also promoted vascular endothelial growth factor (VEGF) as well as erythopoietin (EPO) and nitric oxide synthase 2 (NOS2) which are important for angiogenesis. Matrix metalloproteinase-2 (MMP2) was also upregulated in the hypoxic cell culture which is important for fusion of myoblasts. Finally increased terminal differentiation markers (MyoD, MYOG, MHC) in hypoxic conditions indicated their superior differentiation potential in vitro.
Conclusion: In vitro gene expression data suggests hypoxic preconditioning of human myoblasts can promote angiogenesis, myogenesis, and chemotaxis and improves their terminal differentiation into myotubes. Especially increased expression of chemotaxis and angiogenesis factors might play a significant role in their in vivo survival and engraftment which are being evaluated in NSG-mdx mice (in progress).

Tissue Engineered Human Cartilage Sheets are Significantly Stiffer When Re-Differentiated at Low Oxygen Tension

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Introduction: Human articular cartilage has poor intrinsic repair capacity. Tissue engineering approaches are being investigated for their ability to produce a functional cartilage replacement. Oxygen tension is a significant factor in chondrogenic differentiation, with improvements in biochemical composition reported. Although there is still some controversy depending on the cell type, time period and construct format. There is a lack of data on the functional consequence of a more physiological O_2 on mechanical properties. To address this, we investigated the effect of physiological O_2 (5%) on the biochemical composition and mechanical stiffness of scaffold free, tissue engineered, human cartilage sheets.
Methods: Human chondrocytes (6 donors) were expanded under low (5%) or atmospheric (20%) O_2 on devitalized porcine synoviocyte matrix. Cells were seeded at high density ($4.4x10^6$ cells/cm^2) in a custom biochamber, switched to serum-free differentiation medium, and cultured at either low or atmospheric oxygen tension for 3 weeks (21 days) and 7 weeks (46-56 days). At the end of this culture period, 5 mm biopsy punches were taken for biochemical (glycosaminoglycan (GAG), hydroxyproline (Hyp), collagen cross-link content (hydroxylysyl pyridinoline + lysyl pyridinoline; HP+LP; moles/mole collagen)), histological, and mechanical assessment (equilibrium modulus).
Results: Low O_2 supported solid scaffold-free tissue engineered cartilage sheet formation from all donors (6 of 6) at both time points, whereas atmospheric O_2 supported solid sheet formation in only half of the donors at week 3 and two-thirds of the donors by week 7. Differentiation at low O_2 significantly increased tissue stiffness of engineered cartilage at both time points studied (Fig. 1). GAG content was increased 3.1-fold over atmospheric controls by re-differentiation at low O_2 at week 3, and 4.4-fold at week 7. Collagen content was also increased at week 3 (1.2-fold) and at week 7 (1.8-fold). Collagen crosslink density increased with increased culture duration (7 Weeks vs. 3 Weeks) to a greater degree at low O_2 (3.7-fold) than at atmospheric O_2 (2.4-fold). Histologically, sheets stained strongly for glycosaminoglycan and collagen type II (Fig. 2). Sheets were also thicker at low O_2 at week 3 (0.77 ± 0.33 mm vs. 0.52 ± 0.17 mm) and at week 7 (1.00 ± 0.42 mm vs. 0.52 ± 0.19 mm).
Discussion: Cartilage has remained a challenge for the tissue engineer. Although cartilage is composed of a single cell type, it is clear that not all chondrocytes are created equal. Each donor was seen to respond similarly to a more physiological oxygen tension in terms of both biochemical and mechanical properties. This increase in mechanical properties under low O_2 correlates best with the increase in glycosaminoglycan content, but increased amount of collagen and its maturation, through cross-linking, are important. For a functional tissue engineered cartilage replacement, sufficient compressive strength must be achieved. The effect of low O_2 on mechanical properties and how they relate to the biochemical components is lacking in human chondrocytes. We demonstrate here, that significant increases in stiffness are achieved through growth and differentiation of human chondrocytes at low O_2. The use of a scaffold-free system shows a direct effect of oxygen tension on *de novo in vitro* tissue formation. This research is another step forward in the pursuit of a functional, biological, joint resurfacing solution.

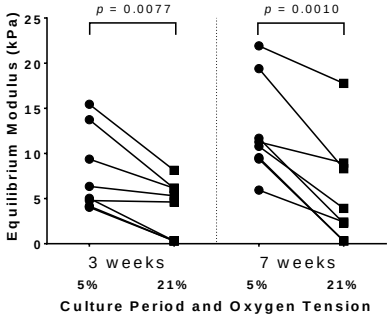

Figure 1
Symbols show the average modulus for sheets from each donor/experiment and lines connect sheets from the same donor/experiment.

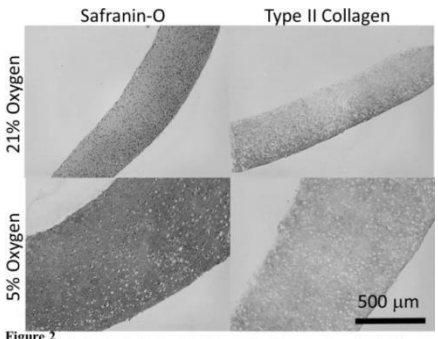

Figure 2
Histology for GAG (Safranin-O) and type II collagen at 3 weeks, both sheets from the same donor/experiment.

Open-source Selective Laser Sintering (OpenSLS) of Nylon and Biocompatible Polycaprolactone

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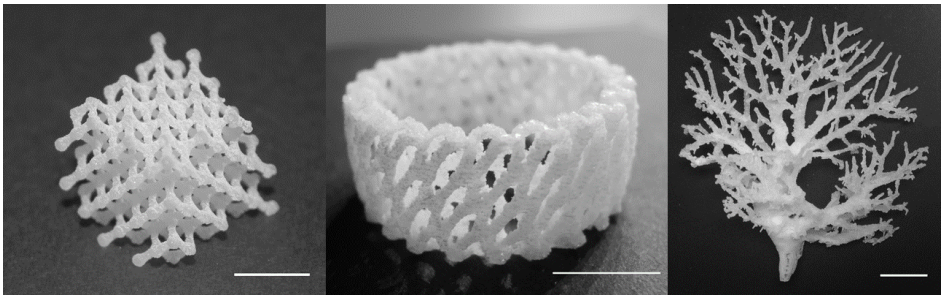
Selective Laser Sintering (SLS) in an additive manufacturing process which uses a laser to form powdered starting materials into solid 3D structures. Despite the potential for fabrication of complex, high-resolution structures with SLS using diverse starting materials (including biomaterials), prohibitive costs (>$200,000) of commercial SLS systems have hindered the wide adoption of this technology in the scientific community. Here, we developed a low-cost (<$10,000), open-source SLS system (OpenSLS) and demonstrated its capacity to fabricate structures with sub-millimeter features and overhanging regions in nylon (Figure 1). For example, we fabricated a highly branched geometry derived from a microcomputed tomography scan of mouse liver vasculature (Figure 1, right).



Subsequently, we demonstrated fabrication of polycaprolactone (PCL) into a diamond lattice structure (Figure 2a). There has been widespread interest in using PCL for bone tissue engineering, suggesting that PCL lattices may be relevant as a model scaffold geometry for engineering bone. Since the powder size of PCL led to very rough sintered surfaces, we also introduced a simple vapor-smoothing technique to modify the surface smoothness of sintered PCL structures (Figure 2b,c) as well as a sacrificial templating strategy where sintered PCL is used as a template for perfusable fluidic networks within a silicone matrix.

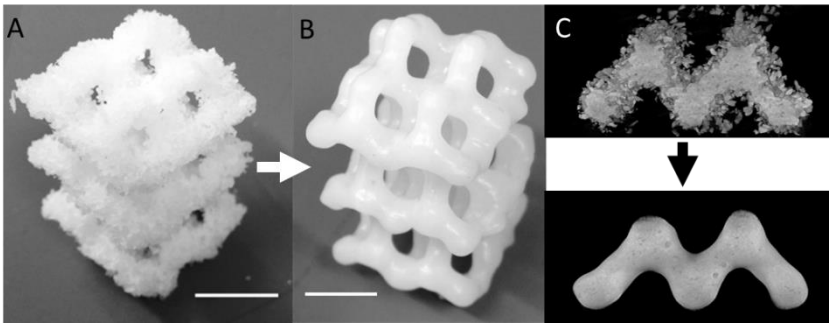


Figure 2: A) Biocompatible PCL sintered into a diamond lattice exhibits a high degree of surface roughness. B) Exposure to dichloromethane vapor smoothed sintered PCL surfaces (scale bars = 1cm). C) Microcomputed tomography scans illustrate the change in surface roughness due to smoothing.

Finally, we report that human mesenchymal stem cells were able to adhere and spread on sintered PCL surfaces, suggesting that OpenSLS has the potential to produce PCL scaffolds useful for cell studies. We believe that OpenSLS could serve the scientific community as an accessible platform for fabrication of, and experimentation with, structures composed of a wide range of materials, including non-traditional materials not supported by commercial SLS suppliers.

Combinatorial treatment with propranolol and mesenchymal stem cells improves spatial learning and memory after experimental traumatic brain injury

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Over the past 20 years, there hasn't been a clear decrease in mortality or improvement of outcome after traumatic brain injury (TBI), the leading cause of death and disability in individuals ages 1 to 44. Despite extensive efforts to develop neuroprotective therapies for TBI, there have been no successful outcomes in human clinical trials to date. Retrospective studies have shown that beta-adrenergic receptor blockers, such as propranolol, significantly decrease mortality of TBI through mechanisms not yet fully elucidated, but thought to counterbalance a hyperadrenergic state resulting from a TBI. Conversely, cellular therapies have been shown to improve TBI outcome, likely by reducing inflammation. Given the non-redundancy in their therapeutic mechanisms, we hypothesized that a combination of acute propranolol followed by mesenchymal stem cells (MSC) would have additive effects in treating a rodent model of severe TBI. In here, the combinatory treatment using propranolol followed by MSC, but not either treatment alone, significantly improved cognitive and memory functions 120 days following TBI. Combined with the increasing use of propranolol for severe TBI patients, and the successful use of MSCs in pre-clinical models and the safety of MSCs clinically, the results here strongly supports the therapeutic potential of the combinatory treatment proposed here.

Funding from the TIRR Foundation and Mission Connect Award #13-110 and Glassell Family Pediatric Stem Cell Research Fund.

Evaluation Of Polyelectrolyte Hydrogels Incorporating Poly(L-Lysine) As A Stimulant Of Chondrogenic Differentiation For Cartilage Tissue Engineering

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Introduction: While materials-assisted therapies for osteochondral defect repair are gaining significant traction, inconsistencies in therapeutic efficacy still existent between various regenerative strategies indicate that ideal conditions for unlocking the full regenerative potential of mesenchymal stem cells (MSCs) still remain largely unknown. Indeed, the normal homeostasis of articular cartilage *in vivo* is regulated by a myriad of signaling pathways that simply cannot be recapitulated by using traditional growth factors. Hence, this study focuses on the use of polycationic polypeptide poly(L-lysine) (PLL), which has been shown to up-regulate biochemical signals controlling mesenchymal condensation during developmental skeletogenesis *in vitro*, as an early chondrogenic stimulant of MSCs. Specifically, we characterized the effect of PLL incorporation on the swelling and degradation behavior of oligo(poly(ethylene) glycol) fumarate) (OPF)-based hydrogels as functions of PLL size and incorporation amount. Furthermore, we investigated the effect of PLL incorporation on the early chondrogenesis of hydrogel-encapsulated MSCs.

Methods: OPF hydrogel constructs were fabricated by dissolving 100 mg of OPF (synthesized with poly(ethylene glycol) (PEG) of either 10,000 g/mol or 35,000 g/mol nominal molecular weight) and 50 mg of PEG-diacrylate (3,400 g/mol) in PBS and combined with thermal radical initiators ammonium persulfate and *N,N,N',N'* – tetramethylethylenediamine for a final polymer solution of 22% (w/v). PLL (50 or 225 kDa) was dissolved in the polymer precursor solution prior to crosslinking for the fabrication of PLL-laden hydrogels (500 ng/construct or 1 μg/construct). At days 1, 7, 14, and 28, the swelling ratio and the mass loss of hydrogels incubated in PBS were determined (n=4) using the following equations: swelling ratio = $(W_s - W_d)/W_i$ and mass loss = $(W_i - W_d)/W_i$, where W_i, W_s, and W_d represent the weight of dried hydrogel immediately after fabrication before swelling, the weight of swollen hydrogel, and the weight of dried hydrogel after swelling, respectively. The amount of PLL released and retained were determined at those time points using FITC-conjugated PLL and a spectrofluorometer. For the cell study, rabbit marrow-derived MSCs were encapsulated at a density of 10 million cells/mL precursor into PLL-laden hydrogels, cultured *in vitro* in defined chondrogenic media, and retrieved for biochemical and gene expression analyses at various time points over 28 days.

Results: The swelling ratio of OPF hydrogels was significantly influenced by the incorporation of cationic PLL during fabrication, with PLL-containing groups exhibiting decreased swelling compared to blank hydrogels by day 28. Such differences in swelling behavior were magnified in the presence of collagenase in the incubation medium. A main effects analysis revealed that increasing the size of PLL led to increases in OPF swelling for PBS conditions but resulted in the opposite effect when collagenase was present. Additionally, the main effects of changing PLL incorporation amount only influenced swelling in the presence of collagenase. Overall, PLL incorporated into OPF hydrogels during fabrication remain largely retained at approximately 84.6% over 28 days, even when the initial incorporation amount was varied from 500 ng/construct to 20 μg/construct. When MSCs were encapsulated, the incorporation of PLL of 225 kDa resulted in early (day 7) enhancements of type II collagen gene expression and type II/type I collagen gene expression ratios when compared to negative controls. This effect was not observed for lower molecular weight (50 kDa) PLL.

Conclusions: In MSC-laden hydrogels, PLL can function as an inductive factor that primes the cellular microenvironment for early chondrogenic differentiation but may require additional biochemical factors for the generation of fully functional chondrocytes. Further, the incorporation of PLL into synthetic OPF hydrogels influences their swelling behavior, which may be leveraged for the development of constructs with desirable swelling properties for cartilage tissue engineering applications.

Acknowledgements: This study was supported by the National Institutes of Health (R01 AR048756)

Influence of Porcine Heart Orientation on Its Decellularization Efficiency

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Introduction: Creating a functional heart with decellularized porcine heart scaffold populated with autologous cells would provide a novel treatment for patients with end stage heart failure. However, perfusion decellularizing whole heart without sacrificing structural integrity and mechanical properties of the scaffold remains a challenge. We hypothesize that an inverted orientation of porcine heart during decellularization could improve its decellularization efficiency while maintaining scaffold integrity.

Materials and Methods: We compared conventional decellularization of porcine heart (Langendorff method with the heart physiologically oriented and retrograde perfused through the brachiocephalic artery (BA), upright orientation) with decellularization in an inverted orientation (apex pointing up at approximately 45° with retrograde perfusion through descending aorta (DA) by using decellularization solutions at constant 60mmHg aortic root pressure. Aortic valve patency tests were performed during the decell process at different times by measuring flow dynamics (inflow into DA, BA and outflow from pulmonary artery (PA) in response to aortic pressure at 30, 40 50 and 60mmHg). In three sets of experiments, the heart appearance, turbidity of outflow solution containing cell debris and perfusion efficiency (ratio of outflow flowrate from PA to inflow flowrate into DA or BA) were monitored throughout the decellularization process. Anterior images of decellularized hearts suspended in Langendorff mode were subjected to rectangle image fitting to obtain heart shape index, defined as ratio of horizontal to vertical axis length. Aortic valves were isolated from decellularized hearts and imaged with nonlinear optical microscopy (NLOM) to obtain microstructure of collagen and elastin fibers via signals of second harmonic generation (SHG) and two photon fluorescence (TPF) [1]. Finally, DNA, glycosaminoglycan (GAG) and detergent level in decellularized hearts were compared between the two orientations.

Results: Compared to the conventional upright orientation decellularization method, the inverted orientation method allowed higher coronary perfusion efficiency, Figure 1. Throughout the decellularization process, higher infusion flowrate into BA and outflow flowrate from PA were observed in upright heart than inverted heart with more turbid outflow solution (i.e., more cell debris) from PA in the inverted heart. NLOM imaging revealed that more collagen and elastin remained inside aortic valve of inverted decellularized heart than upright heart. At process end, inverted hearts were able to retain their shapes better than upright heart as evidenced by the higher heart shape index in the inverted hearts. Finally, the inverted hearts were found to have a lower DNA content than the upright orientation; however GAG level was comparable between them.

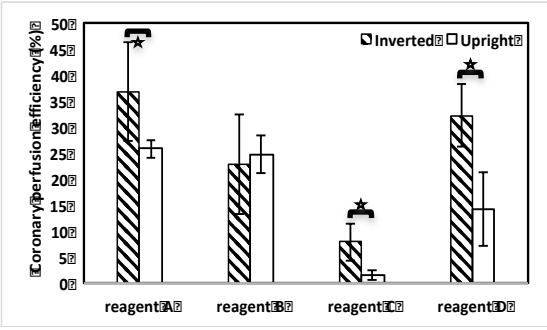

Figure 1. Higher coronary perfusion efficiency was observed in inverted hearts than upright hearts during infusing with different reagents. Sample number = 3 hearts for each group. Star symbol represents for significance difference with $P<0.05$

Conclusions: We believe this is the first study to investigate how orientation influences decellularization efficiency of whole human-sized (porcine) heart. Overall, inverting the heart during decellularization not only improved decellularization efficiency but also maintained better scaffold integrity.

Reference:

1. Ferruzzi, J., et al., Mechanical assessment of elastin integrity in fibrillin-1-deficient carotid arteries: implications for Marfan syndrome. Cardiovasc Res, 2011. **92**(2): p. 287-95.

Osteochondral tissue repair using a bilayered hydrogel composite delivering spatially-guided dual growth factors

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The osteochondral unit is a multiphasic tissue comprised of two main tissue types: the articulating cartilage and the subchondral bone. The health of knee cartilage is linked to the health of the subchondral bone and joint homeostasis will ultimately rely on the functional restoration of the entire osteochondral unit. As a result, successful repair of osteochondral tissue will require strategies to address both layers of tissue.

The present work investigated the use of biodegradable hydrogel composite scaffolds, based on the macromer oligo(poly(ethylene glycol) fumarate) (OPF), to deliver growth factors for the repair of osteochondral tissue in a rabbit model. In particular, bilayered OPF composites were used to mimic the structural layers of the osteochondral unit, and insulin-like growth factor-1 (IGF-1) and bone morphogenetic protein-2 (BMP-2) were loaded into gelatin microparticles and embedded within the OPF hydrogel matrix in a spatially controlled manner. Three different scaffold formations were implanted in a medial femoral condyle osteochondral defect: 1) IGF-1 in the chondral layer, 2) BMP-2 in the subchondral layer, and 3) IGF-1 and BMP-2 in their respective separate layers.

The quantity and quality of osteochondral repair was evaluated at 6 and 12 weeks with histological scoring and micro-computed tomography (micro-CT). While histological scoring results at 6 weeks showed no differences between experimental groups, micro-CT analysis revealed that the delivery of BMP-2 alone increased the number of bony trabecular islets formed, an indication of early bone formation, over that of IGF-1 delivery alone. At 12 weeks post-implantation, minimal differences were detected between the three groups for cartilage repair. However, the dual delivery of IGF-1 and BMP-2 had a higher proportion of subchondral bone repair, greater bone growth at the defect margins, and lower bone specific surface than the single delivery of IGF-1. These results suggest that the delivery of BMP-2 enhances subchondral bone formation and that, while the dual delivery of IGF-1 and BMP-2 in separate layers does not improve cartilage repair under the conditions studied, they may synergistically enhance the degree of subchondral bone formation.

Correlation analysis was also performed between cartilage histological scores and bone micro-CT measurements to examine relationships between the repair of cartilage and bone over 6 and 12 weeks. In particular, significant correlations were consistently seen between bone formation parameters and cartilage surface regularity. In addition, the strength of correlations changed from 6 to 12 weeks depending on the growth factor(s) delivered. This analysis suggests that bone and cartilage repair at different rates and that the growth factors delivered have different effects on overall osteochondral repair over time. Overall, bilayered OPF hydrogel composites demonstrate potential as spatially-guided, multiple growth factor release vehicles for osteochondral tissue repair.

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Discovery and Characterization of a Novel Transcription Factor for Endothelial Cell Development and Lineage Specification

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Background: We have developed bi-species heterokaryons [generated by fusion of mouse embryonic stem cells (mESC) and human endothelial cells (hEC)] as a model system for discovery of novel factors required for endothelial lineage. Our preliminary RNAseq data suggests that the determinants of endothelial phenotype in the hEC act on the mESC to recapitulate endothelial ontogeny. Furthermore, novel transcription factors in endothelial specification were implicated, such as POU domain-containing transcription factor (POU3F2), also called BRN2 or N-Oct3. This study assessed the role of POU3F2 in endothelial cell differentiation and in zebrafish vascular development.

Methods: We used mESC to study differentiation towards endothelial lineage. EC differentiation was induced by culture of mESC with growth factors (VEGF, bFGF and BMP4). POU3F2 loss-of-function was induced by lentiviral shRNA in mESCs. FACS was used to analyse cell lineage. Tg(Fli1:eGFP) zebrafish embryos were used to analyze vascular development following caged morpholino (MO) knockdown of POU3F2. Injected caged morpholino was activated at 6 or 24 hour post fertilization by exposure of embryos to UV light. *In situ* hybridization with POU3f2 RNA sense (control) and antisense probes was used to localize POU3f2 mRNA in the zebrafish embryo. Real time PCR and western blotting were used for a semi-quantitative analysis of gene and protein expression respectively.

Results: POU3F2 knockdown in mESCs reduced the $Flk1^{+}CD144^{+}$ cell population during differentiation of mESCs. POU3F2 knockdown also reduced endothelial cell markers in this mESC derived $Flk1^{+}CD144^{+}$ cells, including Kdr, Cdh5, Nos3, Tie2 and Lmo2; and reduced EC tube formation in matrigel. In zebrafish embryos, micro-injection of MOs targeting POU3F2 reduced POU3F2 mRNA detected by *in situ* RNA probes and protein at 24 and 48 hpf. This was associated with an embryo phenotype characterized by severe vascular aberrations.

Conclusion: Our heterokaryon studies implicated the transcription factor POU3F2 in endothelial cell development. We validated the role of POU3F2 in the reprogramming of pluripotent stem cells to EC lineage. In addition, we provide data that POU3F2 is required for normal vascular development in the zebrafish.

Funding sources: This work was supported by grants to Dr. Cooke from National Institutes of Health (U01HL100397, RC2HL103400). Dr. Wong was supported by a NIH PCBC Jump Start Award (PCBC_JS_2012/1_02) and American Heart Association Scientist Development Grant (13SDG15800004). Dr. Matrone was supported by a NIH PCBC Jump Start Award (PCBC_JS_2014/2_01).

Electrical Stimulation of Artificial Heart Muscle Constructs

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Development of tissue engineered heart for treatment of myocardial infarction or biological pacemakers has been hampered by the production of mostly arrhythmic or insynergistic constructs. Electrical stimulation of these constructs has been shown to produce tissues with greater twitch force and adrenergic response, with the drawback of losing spontaneous contractility. Gene expression and structural analysis has also shown improved electrophysiological and contractile protein phenotype and myocyte sarcomeric organization, which tends to diminish overtime more rapidly for unstimulated constructs in culture.

In order to further our understanding of the mechanisms underlying the effect of electrical stimulation, we fabricated a bioreactor capable of delivering continuous or intermittent waveforms of various types to multiple constructs simultaneously. In this study, we examined the effect of an intermittent biphasic square wave on our artificial heart muscle (AHM) composed of neonatal rat cardiac cells and fibrin gel. Twitch forces, spontaneous contraction rates, biopotentials, gene expression profiles, and histological observations were examined for the electrical stimulation protocol, illustrated in Figure 1 below, over a 12 day culture period.

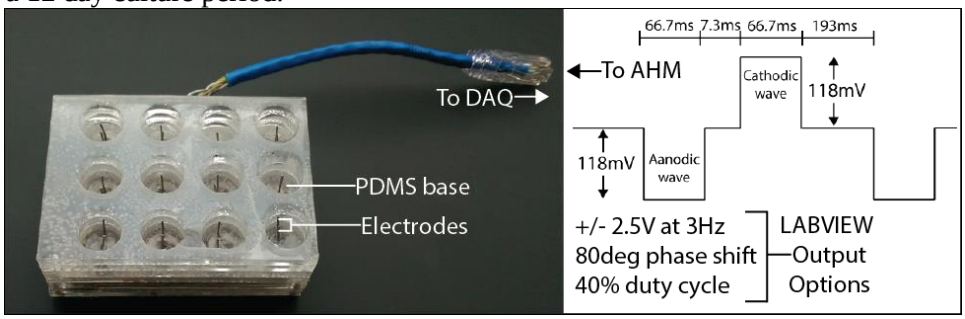

Figure 1. Electrical stimulation bioreactor consisting of stainless steel electrodes (left) and parameters of the biphasic square waveform (right) applied to artificial heart muscle (AHM) composed of neonatal rat cardiac cells and fibrin gel. AHM were subjugated to intermittent stimulation, 1 hour on, and 1 hour off for a period of 12days.

Results show improved consistency between samples for twitch force and contraction rate, although higher twitch force amplitudes were not observed. Gene profiles showed a peak at day 4 of culture, 2 days after the initiation of electrical stimulation for most of the electrophysiological and structural proteins examined. These results will be used for optimization strategies to establish protocols for producing AHM capable of replacing damaged heart tissue in either a contractile or electrophysiological capacity. This study will also expand to tissue engineered heart constructs composed of various cell sources and matrices.

Source of Funding: NIH ROl-EBOl 1516

Cardiac Regeneration by Hippo Pathway and Dystrophin Glycoprotein Complex

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Regeneration of the mammalian heart is limited in adults. In rodents, endogenous regenerative capacity exists during development and in neonate but is rapidly repressed after birth. We are elucidating the mechanisms responsible for regenerative repression and applying this knowledge to reactivate cardiac regeneration in adult hearts. We have previously shown that the Hippo pathway is responsive for regenerative repression, however, the molecular and cellular mechanism responsible remain unclear.

The molecular analysis identified components of dystrophin glycoprotein complex (DGC) as downstream targets of Hippo pathway. The DGC anchors the cytoskeleton and extracellular matrix and is involved in prevention of muscle damage. Furthermore, we found that downstream effector molecule of the pathway, Yap, and the components of DGC directly interact. The studies using the muscular dystrophy mouse model, mdx, reveals that DGC is required for endogenous cardiac regeneration. Our studies provide insights into the mechanisms leading to repair of damaged hearts from endogenous cardiomyocytes and novel information into DGC function.

Funding Sources: This work was supported by grants from the NIH (5T32HL007676-23 to J.L., HL 118761 to J.F.M.), the Vivian L. Smith Foundation (J.F.M.), and the American Heart Association (AHA) (AHA10POST4140029 and AHA12POST11760019 to T.H., AHA NCRP SDG 0930240N to Y.M., and AHA13POST17040027 to G.T.)

Determination of the Best Methodology for Preparation of an Acellular Pig Salivary Gland

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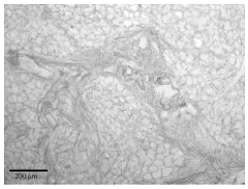
Introduction: Xerostomia or dry mouth occurs when the salivary gland does not make sufficient saliva. This can be a result of diabetes, Parkinson's disease or Sjogren's syndrome. It also frequently occurs after radiation therapy for head and neck cancer. Currently, the only treatments available for xerostomia are transient and usually consist of saliva substitutes[1]. A better treatment would be the creation of a functional salivary gland from the patient's own cells. Hence we hypothesize that an acellular salivary gland can be achieved.

Methods: The salivary gland of the miniature pig has a similar maxillofacial system to that of the human[2] and may be an ideal scaffold for reconstruction with human cells. With this in mind, the mandibular glands of adolescent pigs were obtained for the creation of an acellular scaffold. The glands were divided into 4 pieces for various treatments to remove the cells without compromising the architecture of the gland. Methods one and two consisted of several freeze/ thaw cycles in either water (method one) or a hypertonic solution (method two). Method three used detergent only and the fourth method used a sequential application of hypertonic, hypotonic and detergent solutions[3]. Analysis consisted of histological examination and biochemical assessment.

Results: All methods maintained the outline of the previously existing acini. The freeze/ thaw methods resulted in either high DNA content (method two) or possible compromise of the extracellular matrix as denoted by the poor uptake of aniline blue, a dye that binds to collagen (method one). Method three showed the second highest DNA content but cellular debris was located in the periphery of the tissue while method four had the lowest DNA content measured and the debris was located mainly between the lobes of acini. Therefore, methods three and four are the best candidates for creating an acellular scaffold for reconstitution with human cells.



An acellular pig salivary gland as a result of undergoing freeze/ thaw cycles while immersed in water (method one).

Conclusion: Determination of the best method that results in the least amount of cellular debris but maintains the integrity of the extracellular matrix is detrimental to the reconstitution of the organ. If the matrix is compromised there is a greater likelihood that cells will not attach and function properly. This is the first step in creating a functional salivary gland from a patient's own cells. To achieve an optimal acellular pig salivary gland, methods two and three will be refined and further characterization of the matrix will be ascertained by biochemical means as well as by seeding with salivary gland cells.

References:

1. Jorku A, Sullivan CA, Yoo J and Atala A (2007) *Differentiation* **75**:318-324.
2. Wang S, Liu Y, Fang D and Shi S. (2007) *Oral Diseases* **13**:530-537.
3. Ott HC, Matthiesen TS, Goh S-K, Black LD, Kren SM, Netoff TI, and Taylor DT. (2008) *Nature Medicine* **14**(2): 213-221

Production of One Billion Bovine Aortic Endothelial Cells By Automated Quantum Cell Expansion System

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Introduction: Cardiovascular disease is one of the major causes of death in United States. Patients with end stage heart failure are in need of a heart transplant, while only a limited number of patients receive a new donor heart. Regeneration of a beating rat heart was first demonstrated in 2008 by our group[1]. However, translation into a human-sized heart will require production of an extensive number of primary cells. Here we show the efficacy of expansion and culture of isolated primary bovine aortic endothelial cells (BAEC) by an automated cell expansion system. Our objective is to use a scale-up system to efficiently and timely produce large number of cells. The system chosen was a Quantum® cell expansion system developed by Terumo BCT.

Methods: BAECs were isolated from cows, recently exsanguinated and cultured in culture flasks until adequate system seeding density was achieved. The Quantum cell expansion system consists of hollow fibers providing 21,000 cm^2 cell surface growth area. Quantums were tested to assess the production of BAECs. Either 50.0 $x10^6$ or $100x10^6$ BAECs were used to culture each run. For controls, seeding densities similar to Quantum were used in T-175 flasks. Lactate levels were measured prior to cell loading and then once per day thereafter for control and Quantums cultures. The lactate level regulated the system flow rate to maintain the level below 7 mmol/L. Cells were cultured with DMEM (10% FBS, 1% Penicillin/Streptomycin, 2 mM L-glutamine, 0.05 U/ml Heparin) growth media at an internal environment at 37°C with mix oxygen gas (75% N, 20% O, 5% CO_2) introduced. After culture, cells were removed from system or culture flasks and analyzed for purity and functional study by flow cytometry and Dil_AC-LDL fluorescence assay, respectively.

Results: To determine optimal cell density, $50x10^6$ and $100x10^6$ cells were cultured per Quantum. In order to reach the goal of 1 billion cells, the above mentioned cell density were cultured for 7 and/or 8 days. Results showed that $50x10^6$ cells cultured for 7 days yielded a higher number of cells than $100x10^6$ during 8 days. An average of 1.0 x 10^9 BAECs were harvested from all six runs. Flow cytometry data showed that the cells were similar to control flasks cells with about 70% positive for CD31 cell surface marker. However, remaining 30% of the cells might be contaminates from other aortic cell types such as fibroblast. In addition, cells from control flask and Quantums both maintained the capability for LDL-uptake as shown by Dil_AC-LDL fluorescence assay (Figure 1).

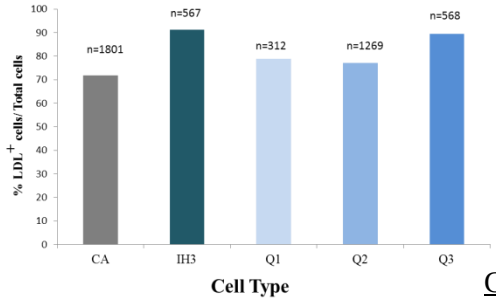

Figure1: Functional analysis of BAEC by fluorescent LDL-uptake assay.

Conclusion: Our results from the 6 test runs with bovine aortic endothelial cells showed the potential of the system to produce one billion cells within seven days. The comparison to grow a similar amount of cell in T-1000 culture flasks would have required 15 flasks. In addition functionality was maintained. Future studies will investigate the use of the expansions systems with other cell types needed for cardiac regeneration.

Reference: 1. Ott HC *Nature Medicine* 2008(14), 213-221

In Vivo Assessment of Engineered Cardiac Patches for Repairs of Congenital Heart Defects

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Introduction: Congenital heart defects generally require surgical placement of a patch across the right ventricular outflow tract (RVOT) in an area that normally consists of contractile myocardial tissue. Current materials for RVOT repair induce an inflammatory foreign-body response, resulting in the material encased by a fibrous scar-like tissue that does not degrade, grow with the child's heart, nor provide contractile force. These patch materials also disrupt electrical conduction, and serve as a nexus for calcification. As a result, patients with heart patches have an increased risk of heart failure, arrhythmias, infection and aneurysm.[1] The objective of this study was to investigate a cardiovascular tissue engineered device using *in vivo* methodological approaches and to develop novel therapies to treat cardiac defects by reducing the drawbacks associated with current cardiac patches.

Materials and Methods: Three different patches, each 6 mm in diameter, were prepared: (1) gelatin (type-A)-chitosan composite hydrogel,[2] (2) decellularized porcine myocardium (referred as heart matrix)-chitosan coated on polycaproactone core[3] and (3) SJM™ Pericardial patch for the control. Full thickness RVOT repair surgery on the Sprague-Dawley rat (200-300g) model was performed. Briefly, the purse-string was applied to the surface of RVOT and right ventricle (RV), then the RV wall was resected. The patch was sutured along the margin of the purse-string suture to cover the resected region in the RV. Cardiac MRI imaging was performed for detailed assessment of cardiac function of all hearts treated with the three different patches at three time points (2, 4, and 8 weeks post-surgery) and images were compared with those taken from a native heart. Images were taken from the LV and RV throughout systole and diastole. Cardiac function was assessed by calculation of the ejection fraction and end diastolic volume adjacent to the implanted graft using Amira Imaging software. Rats were sacrificed after 8 weeks and hearts were fixed and sectioned. Immunohistochemistry analysis was also performed to evaluate cell immigration from the native tissue and tissue regeneration using haematoxylin and eosin, Masson's Trichrome, CD31 (endothelial cells), Cx43 (gap junction), and α-smooth muscle actin (α-SMA).

Results and Discussion: MRI results showed that there was no RVOT obstruction due to the surgery. Left ventricular ejection fractions (LVEF) of patched hearts were reduced significantly after 2 weeks post surgery. LVEF of both gelatin and heart matrix patched hearts were normalized after 8 weeks while Pericardial patch samples maintained reduced LVEF (Fig. G). Newly grown tissue covered the patched area, and no significant areas of clotting or fibrosis were observed on the patch (Fig. A-C). The connective tissue surrounding the heart matrix patch had a continuous endothelial layer on the surface. Both engineered patches promoted aligned cardiac smooth muscle cell formation though the patched area (Fig. E&F) whereas the pericardial patch had no cell immigration (Fig. D). Further, engineered patches promoted neovascularization through the patched region and localized in the same area (Fig. H&I). Furthermore, positive staining for cTnT was observed through the patch and connective tissue. However, gap junction formation (cx-43) was observed in only connective tissue area.

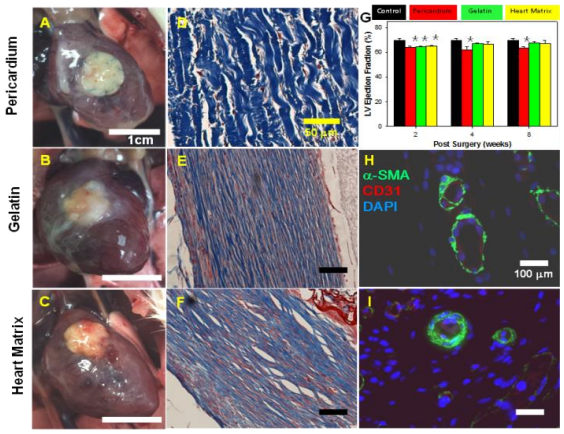

Figure. (A-C) Representative patched heart images, (D-F) Masson's Trichrome stained patches, (G) left ventricular ejection fraction after 2, 4 and 8 weeks and (H-I) antibody stained patches. (*; $p<0.05$)

Conclusions: These results demonstrate that an engineered patch promotes cell immigration and neo-endothelialization though the patched area and promotes native tissue remodeling, demonstrating improved function as a full-thickness cardiac patch in RVOT repair for cardiac defects. Future research will involve testing cellularized patches to improve cardiomyocytes maturation through the patched area.

References: [1]Santibanez-Salgado JA, et al. European Cells & Materials, (2010).
[2]Pok S, et al. Acta Biomater. (2013). [3]Pok S, et al. Tissue ENGR. Part A (2014)

Mesenchymal Stems Cells to Treat Neuropathic Pain in Spinal Cord Injury

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Introduction: Chronic neuropathic pain affects approximately 64-82% of all patients living with a spinal cord injury (SCI). Despite years of research, a thorough understanding of the underlying mechanisms involved in the development and maintenance of SCI-dependent neuropathic pain remains elusive, with no effective treatment. Inflammation plays a significant, dynamic role in the development of such pain, and is a prime target for therapeutic intervention. With potent anti- inflammatory and immune-regulatory characteristics, mesenchymal stem cells (MSC) may provide a safe and effective method for modulating these processes and attenuating neuropathic pain.

Objective: To determine whether early, acute delivery of MSC via intravenous and intrathecal routes will prevent the development of neuropathic pain.

Methods: Adult, male, Sprague-Dawley rats received a moderate, spinal contusion injury at thoracic level 10. All injured subjects and age-matched controls were subjected to neurosensory assessments of mechanical allodynia in hind paws, as well as the thoracic girdle region (von Frey filaments). Thermal hyperalgesia in hind paws was also assessed (Hargreaves test). All neurosensory assessments were performed prior to injury and again at 7 day, 14 days, 21 days and 28 days post-injury. MSC were suspended in sterile PBS at a concentration of 50×10^6 cells/ml for intravenous and intrathecal delivery at 24 hrs post-injury.

Results: SCI animals receiving MSC treatment were compared to SCI animals receiving vehicle injections and revealed significantly decreased mechanical allodynia in hind paws at 7 days ($p<0.0001$), 21 days ($p<0.001$) and 28 days ($p<0.05$) post-injury. Thermal hyperalgesia was not significantly decreased in MSC-treated animals compared to vehicle-treated animals. MSC-treated SCI subjects exhibited significant increases in mechanical allodynia in the torso at 14 days post-injury ($p<0.05$). Two-way ANOVA with Tukey's multiple comparison posttests were performed for all behavior data.

Conclusions: MSC treatment after CNS injury is appealing due to the immune-regulatory and anti-inflammatory characteristics of MSC. The data generated in this study suggests that intravenous and intrathecal administration of MSC after spinal contusion injury partially prevents neuropathic pain associated with mechanical allodynia in both the hind paws and the torso. These results support our hypothesis that MSC are an effective cellular therapy approach to treat SCI-dependent neuropathic pain.

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Flow Perfusion Effects on Three-Dimensional Culture and Drug Sensitivity of Ewing Sarcoma

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Use of three-dimensional (3D) tissue-engineered tumor models holds great promise for *in vitro* drug testing in light of the inherent limitations associated with monolayer cultures. 3D tumor models accurately describe different aspects of the tumor microenvironment and, accordingly, are readily available for mechanistic studies of tumor biology. Nevertheless, these systems often overlook biomechanical stimulation, another fundamental driver of tumor progression.

To address this issue, we cultured Ewing sarcoma (ES) cells on an electrospun poly(ε-caprolactone) (PCL) 3D scaffold within a flow perfusion bioreactor, where flow-derived shear stress provided a physiologically relevant mechanical stimulation. We hypothesized that flow-derived shear stress would influence ES cell phenotype and drug sensitivity, with emphasis on the IGF-1/IGF-1R signaling transduction and biological targets against this pathway. We further hypothesized that perfusion bioreactors would better promote ES cellular proliferation and distribution compare to static conditions through an improved mass transfer.

Electrospinning was used to fabricate cylindrical PCL scaffolds with an average fiber diameter of 10 μm. Each scaffold was seeded with 35,000 human ES TC71 cells and cultured up to 10 days either in static conditions (S), or into a flow perfusion bioreactor, using to three different flow rates: 0.04 (B-04), 0.08 (B-08), and 0.4 mL/min (B-40). ES cells were exposed either to chemical drugs or to biologically targeted therapeutics against IGF-1R, analyzed based on their proliferation- and apoptosis-responsiveness, profiled for biomarkers controlling the IGF-1R-crosstalk signaling pathways, and examined by immunofluorescent microscopy.

Comparison of DNA levels showed no statistical difference among all groups except for group B-40, which exhibited a significantly higher DNA content at each time point. The CD99, a cell surface diagnostic biomarker of ES cells, was steadily expressed in all groups of bioreactor cultures, which confirm that our bioreactor cell culture parameters preserve a typical ES-like phenotype. The spatial distribution of ES cells within the constructs was assessed by immunofluorescent staining of the cells for Dapi. The presence of a convective flow promoted cell infiltration within the construct in a flow-dependent fashion, while cells in static conditions were confined to scaffold outer edges. Flow perfusion significantly promoted insulin-like growth factor-1 (IGF-1) ligand production by ES cells. This finding is particularly relevant, given the central role of IGF-1/IGF-1R pathway in ES tumorigenesis and as a promising clinical target. Additionally, flow perfusion enhanced in a rate-dependent manner the sensitivity of ES cells to IGF-1R inhibitor MK-0646.

In conclusion, this study demonstrated shear stress-dependent ES cell sensitivity to MK-0646, highlighting the importance of biomechanical stimulation in bone tumors like ES. Furthermore, flow perfusion increased nutrient supply throughout the scaffold, ultimately enriching ES culture over static conditions. Our use of a tissue engineered model, rather than human tumors or xenografts, enabled precise control of the mechanical forces experienced by ES cells, and in so doing provided at least one explanation for the remarkable antineoplastic effects observed by some ES tumor patients to IGF-1R targeted therapies in stark contrast to the lackluster effect observed in cells grown in conventional monolayer culture.

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Culture Models for the Study of Hypoxia in Aortic Valve Disease

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Objectives: Calcific aortic valve disease (CAVD) is a serious progressive valve disease that involves the thickening and fibrosis of the aortic leaflets. Currently, the only effective treatment is valve replacement through invasive surgery. As the aortic valve normally thickens with age and loses vascularity, cells within the valve are more likely to experience hypoxia. These cells, known as valvular interstitial cells (VICs), are essential regulators of extracellular matrix (ECM) turnover and are major factors in the progression of CAVD. While uncommon in the adult valve, angiogenesis is frequently observed in the diseased valve. Due to the relationship between the activation of the hypoxia inducible factor (Hif) pathway and angiogenesis, there is growing interest in the valve field to understand the effect of hypoxia on VIC behavior and calcification. Here, novel culture systems are presented for examining the effect of hypoxia on VICs and aortic valves, and 2D diffusion models are shown to predict the level of oxygen throughout a variety of leaflets.

Methods: A custom hypoxic chamber and flow loop system were created to study the effect of hypoxia on aortic valves and calculate the diffusion coefficient (DO_2) of oxygen in the valve. The hypoxic chamber was created by using gas cylinders with varying gas compositions (21%, 13%, and 5% O_2) to control oxygen levels within a sealed glass desiccator that acted as the sample incubator. Whole aortic leaflet cultures and 3D VIC cultures were cultured for 2 weeks in the chamber and then analyzed with immunohistochemistry. The DO_2 of oxygen was determined using a flow loop system with a custom-built flow chamber that passed solution below the valve leaflet. Once recorded, the DO_2 and valve thickness measurements were used to create a 2D model of oxygen diffusion in normal and diseased aortic leaflets in Mathematica.

Results: Using the flow loop system, the average oxygen DO_2 for aortic valves in the relaxed state (low pressure) and the stressed state (80 mmHg pressure) was calculated. Using the relaxed DO_2, computational models were created in Mathematica for oxygen diffusion in a range of normal valve thicknesses. 30 year old human (Fig. 1B top) and normal 70 year old human (Fig. 1B bottom) valve models are presented here. The older valve model showed lower oxygen partial pressures within the valve. Hif-1α staining of whole leaflets cultured in a hypoxic chamber had greater Hif-1α expression in the low oxygen conditions (5% and 13% oxygen) compared to normal oxygen levels (21% oxygen).

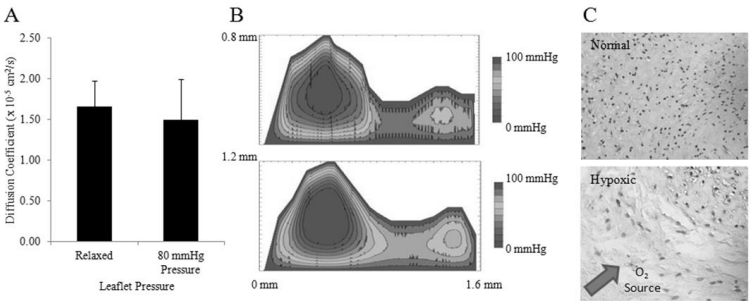

Figure 1. A) DO_2 measurements of aortic valves in relaxed and stressed states. B) Computational models of oxygen diffusion profiles in a 30 year old human valve (top) and a 70 year old valve (bottom). C) Hif-1α staining in 2 week culture in normoxic conditions (top) and hypoxic conditions (bottom) at 13% oxygen.

Conclusions: DO_2 measurements of aortic valves were high for tissue, likely as a result of the large water content in the spongiosa layer of the valve. Computational models of valves of different ages showed that oxygen levels decreased in the center of the valve as the valve aged. However, even in older valves, most of the valve tissue had oxygen levels above 0 mmHg, supported by the observation that adult aortic valve tissue is largely avascular. Hif-1α staining in valves cultured in low oxygen environments had high expression of Hif-1α. Although there may be a link between Hif-1α expression and angiogenesis in diseased valves, future studies are needed to more closely examine this potential factor in CAVD.

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Isl1 Directs Cell Fate Decisions in the Pancreas by Specifying Progenitor Cells Towards Different Endocrine Lineages

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During pancreatic development, different endocrine cells are specified in a temporal and spatial manner before differentiating into mature hormone producing cells that play critical roles in regulating energy homeostasis. The master regulator Ngn3 specifies endocrine progenitors (EPs) and has biphasic expression, with early Ngn3 expression leading to more alpha and delta cells and later Ngn3 expression leading to more beta and gamma cells. The transcription factor Isl1 is necessary for the formation of functional endocrine cells, and has dynamic expression during pancreatic development; first expressed in pancreatic mesenchyme and epithelial cells while later only expressed in the endocrine producing epithelial tissue of the pancreas. Here, we show that Isl1 is alternatively spliced in a developmentally regulated pattern in various mouse tissues, including the brain and the pancreas, resulting in two distinct protein isoforms, Isl1a and Isl1b. To understand the impact of Isl1 on the maturation of progenitors into functional beta cells, an *in vitro* approach using human embryonic stem cell derived pancreatic cells, mouse *ex vivo* cultures, and mouse pancreatic organoids was applied. Isl1a and Isl1b-dependent transcriptional targets were analyzed through shRNA knockdown and overexpression using the pINDUCER system during different developmental windows. Single cell sequencing of murine cells in the EP stage was performed to provide more detailed information about progenitor cell heterogeneity.

Using these tools, we show that pancreatic progenitors (PPs) and EPs are not homogenous, with PP potential depending on dorsal/ventral patterning during the primary transition and EP lineage determined by the transcriptional landscape and temporal commitment of the cells. We show EP cells are not multipotent and that Isl1 directs cell fate decisions during pancreatic development by modulating Ngn3 expression and shifting temporal competence windows. When Isl1 is lost, Ngn3 expression increases 86 fold while overexpression of Isl1 represses Ngn3. This change in expression also led to a shift in endocrine lineage commitment, with more alpha and delta cells as well as ghrelin expression when Isl1 is lost and more beta and gamma cells when Isl1 is overexpressed. From this study we have a new understanding of pancreatic development. In addition, we have shown that the dorsal and ventral pancreas are distinct and have different potential to develop into different mature endocrine cells. This information is critical to *in vitro* differentiation of stem cells into beta cells or other pancreatic endocrine cells for regenerative therapies, as understanding how to specify progenitors with the proper potential and direct cell fate decisions is essential in efficiently producing beta cells.

Clindamycin-releasing Porous Poly(methylmethacrylate) space maintainers for Prevention of Mandibular Infection

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Infection is a common and devastating consequence of injuries to the Craniomaxillofacial region. Because of the vast microbiome present in the oral cavity, injuries to the mandible are at risk of becoming infected, derailing the efforts of tissue engineering strategies to regenerate the region. In order to address this difficulty, antibiotic-releasing porous space maintainers have been developed to address multiple aspects of staged reconstructions: 1) infection prevention, 2) soft tissue healing, 3) prevention of later dehiscence, and 4) maintenance of the defect contours for later reconstruction.

The objective of this study is to evaluate the effects of antibiotic release kinetics and dose on soft tissue healing over a porous poly(methylmethacrylate) space maintainer implanted in critical size infected rabbit mandibular defect.

Three groups of clindamycin-releasing porous space maintainers were fabricated for in vivo testing: Burst Release (Burst), High Dose Sustained Release (PLGA High), and Low Dose Sustained Release (PLGA Low). Poly(lactic-co-glycolic acid) (PLGA) microparticles were used in the PLGA groups in order to extend the release of antibiotics. The space maintainers were implanted into a 10 mm bicortical rabbit mandibular defect after inoculation of the defect with the anaerobic bacteria *Prevotella melaninogenica* (n = 9-10/group). After 12 weeks, mandibles were harvested and evaluated by gross observation, qualitative culture, microcomputed tomography, and histology.

Release of clindamycin from the Burst group occurred over 7 days, after which no further release was noted; release of clindamycin from the PLGA groups occurred over 28 days. The inoculated bacteria was not recovered from any animal at any timepoint. At 12 weeks, cultures from the surfaces of all space maintainers grew anaerobic bacteria, but none grew the inoculated bacteria. Gross healing over the defect was similar between all groups ($p > 0.05$). However, the PLGA High groups exhibited significantly less dehiscence than either the Burst or PLGA Low groups ($p<0.05$).

PLGA microparticles can be leveraged to reduce burst release and extend duration of antibiotic release from porous PMMA space maintainers. While it appears that the release kinetics have no effect on the healing of soft tissue over the space maintainers, the results from this study indicate that a high dose extended release of antibiotic may be useful in preventing dehiscence of healed mucosa.

This work was supported by the AFIRM II effort (W81XWH-14-2-0004). Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense. In conducting research using animals, the investigator(s) adheres to the laws of the United States and regulations of the Department of Agriculture. SRS would like to acknowledge support from a Ruth L. Kirschstein Fellowship from the National Institute of Arthritis and Musculosketal and Skin Diseases (F30AR067606).

Development of Multicolor Immunophenotyping Protocols for Rat Blood, Spleen and Brain

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Rat models of human conditions are often superior to mouse models, particularly in the areas of physiology, toxicology, cardiovascular research (stroke/hypertension), diabetes, behavioral studies, regenerative medicine, and neuroscience. Recent advances with rat ES cells hopefully foreshadow the long-awaited development of robust genetic targeting methodologies like those that in recent decades made the mouse the preferred rodent model, especially for immunology, but due to limitations such as our relatively rudimentary understanding of the rat immune system and the scarcity of anti-rat antibody clones (mostly available in a restricted selection of fluorophores), a literature search for "rat flow cytometry" reveals mostly basic two- and three-color analyses. However, availability of reagents has recently increased, and we are developing multicolor immunophenotyping protocols for rat blood, spleen and brain to characterize inflammatory responses in our translational research on cellular therapies for traumatic brain injury (TBI).

Our workflows typically involve high numbers of rats, so we prefer relatively simple and quick sample prep protocols (without sacrificing good cell yields) and avoid enrichment steps where possible. Accustomed to human and mouse samples, we were surprised upon beginning rat work to see some of the most basic subset-defining cell surface markers for mouse/human leukocytes expressed on different populations and became frustrated by the presence of nucleated erythrocytes and the fact that rat monocytes won't separate from lymphocytes along a side scatter axis. We optimized and deployed PBMC and spleen protocols in a long-term study of the effects of treatment of TBI with propranolol and mesenchymal stromal cells (MSCs), and initial attempts at flowing the injured rat brain have also been successful in identifying key leukocyte populations.

We adapted a published rat bone marrow differential panel and created our own lymphoid and myeloid panels for spleen and PBMCs. A 4-color differential panel (CD45, CD71, LDS750, Ghost Dye) allows enumeration of viable nucleated erythrocytes, mature erythrocytes, and myelo-lymphoid cells; an 8-color lymphoid panel (CD3, CD4, CD8a, CD11b/c, CD25, CD45RA, RT1B, Ghost Dye) identifies viable B cells, T cells (CD4 TH & CD8 TC) plus $RT1B^{+}$ ($MHCII^{+}$) and $CD25^{+}$ (activated) subsets within those populations. An 8-color myeloid panel (CD4, CD8a, CD11b/c, CD43, CD161, CD172a, HIS48, Ghost Dye) allows discrimination of monocyte subsets and NK cells (CD161bright) among other populations. We are particularly interested in tracking the ratio of $CD43^{+}$ to $CD43^{++}$ monocytes and levels of expression of CD4 and CD8 (shown to vary on rat monocytes after inflammation), and CD161 (most sensitive marker of rat monocyte activation) on these cells after TBI/treatment.

For the brain, we have an 8-color M1/M2 panel in development (CD11b/c, CD32, CD45, CD163, CD200R, HIS48, RT1B, Ghost Dye) which discriminates neutrophils, monocytes/macrophages, microglia, and lymphocytes from other cells and should also reveal the activation and/or polarization states of those cells. Finally, we have also developed a semi-automated low-volume (100µl) whole blood "lyse/no wash" method capable of generating absolute cell counts and suitable for serial blood draws of large numbers of rats, which we will use with 7- and 8-color modified versions of the lymphoid and myeloid panels described above. These methods will be useful to others interested in the inflammatory status of their rats.

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Using the Body to Regrow the Body: *In vivo* Bioreactors for Craniofacial Tissue Engineering

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The reconstruction of large bony defects in the mandible presents many challenges to surgeons, such as sourcing appropriate tissue for transfer and successfully restoring facial aesthetics. In order to circumvent donor site morbidity and also produce tissue with geometries that conform to the mandible, we have designed a strategy for producing tissue-engineered specimens at an ectopic in vivo site distal to the mandibular defect. Bioreactors, or chambers with custom-designed geometry, can be implanted with an open face against the periosteum of the rib. After several weeks, progenitor cells from the periosteum migrate into the bioreactors and generate tissue to fill their dimensions. This tissue is vascularized and can be transferred as a free flap to the mandibular defect. In an ovine model, we compared the use of morcellized autograft (AG) to synthetic ceramic graft (SG), as well as a 50/50 mixture (Mix) as scaffold material for the bioreactors by analyzing chamber contents by microcomputed tomography (bone volume/total volume, trabecular number, trabecular separation, trabecular thickness) and RT-qPCR for osteogenic markers after 9 weeks of bioreactor implantation. Tissues generated with SG were transferred into a large mandibular defect as either a vascularized flap (n=3) or avascular graft (n=3) for reconstruction. We demonstrated that AG resulted in significantly greater bone volume/tissue volume (BV/TV) within bioreactors compared to SG-containing groups by microcomputed tomography analysis after 9 weeks of implantation (Fig. 1).

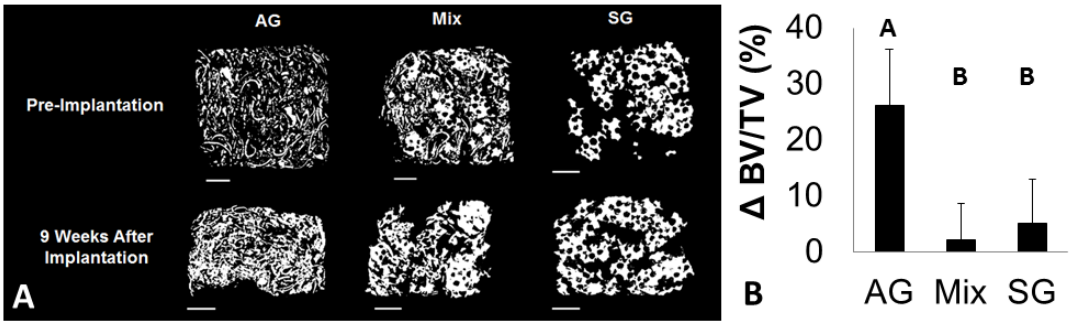

Figure 1. A: Two-dimensional cross-sections of tissues pre- and post-implantation (scale bar = 2 mm). B: Change in BV/TV over 9 weeks of implantation (groups which do not share the same letter are significantly different, $p < 0.05$).

From a clinical standpoint, sheep tolerated mandibular reconstruction using these engineered tissues as either vascularized flaps or avascular grafts. These data validate this strategy in a clinically-relevant large animal model.

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Development of Human Lung Model to Study Pathogenesis of Lung Disease

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Introduction: Tissue-engineered human lung models serve as experimental tools to study initiation and progression of disease caused by respiratory pathogens. Our human lung models are being used to determine the role of influenza A virus infection in development of lung fibrosis. Of interest is the role of infection of alveolar epithelial cells (AEC) and macrophage subsets (classically activated and alternatively activated) in response to lung injury.
Methods: Human lung constructs we developed are produced using acellular human scaffolds from natural lung following a decellularization protocol in which all cells are removed from the tissue with minimal disruption of the extracellular matrix and conservation of the lung ultrastructure. Recellularization of scaffolds involved the use of primary or immortalized human lung cells which include mixtures of AEC type I and II, fibroblasts, smooth muscle cells and endothelial cells. Blood was collected from healthy human donors with informed consent and by an IRB approved protocol. Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. Triplicate cultures of human lung constructs were exposed to bleomycin or Influenza A virus. Fibrosis development was determined in the presence or absence of macrophages in living human lung tissue constructs using H&E staining, immunofluorescence staining and multiphoton-microscopy (MPM) with second harmonic generation imaging (SHG).
Results: Before exposure, macrophages added to the model migrate to the interstitial spaces of lung tissue. Following exposure to bleomycin or Influenza A virus, macrophages move out of the interstitial regions and into alveolar spaces. Data suggests macrophages play a significant role in lung fibrosis formation. At 12-24 hours post exposure, we observe AEC apoptosis, followed by proliferation of fibroblasts and deposition of collagen type I. Controls did not show apoptotic cells or increase in collagen. Fibrosis never developed in bleomycin or virus exposed human lung constructs unless macrophages were present. Fibrosis was scored using quantitative evaluations of collagen content.
Conclusion: This model allows for deconstruction of complex interactions involved in pathogenesis of influenza A virus and development of pulmonary fibrosis by selectively adding cell types of interest and simplifying responses that would otherwise be hard to recognize in a dynamic *in vivo* microenvironment.

In Vitro and *In Vivo* Mineralization and Osteogenesis of Injectable Stem Cell Laden Hydrogels

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Reconstruction of craniofacial bone defects remains a clinical challenge due to limitations of current treatments, such as allografts and autografts, and associated risks. A minimally invasive tissue engineering alternative is a thermoresponsive hydrogel system that undergoes a sol-gel phase transition at body temperature, allowing for injection in and contouring to a defect site, as well as localized delivery of stem cells or growth factors. Previous work has shown that an injectable dual-gelling hydrogel system composed of poly(*N*-isopropylacrylamide) (PNiPAAm)-based thermogelling macromers (TGM) and polyamidoamine (PAMAM) crosslinkers can undergo rapid chemical and physical gelation without shrinking and demonstrates tunable physicochemical properties, degradation to bioresorbable soluble products, and most promisingly, mineralization due to protein and ion adsorption on the hydrogel surface [1]. The aim of this study was then to first, investigate the osteogenic capacity of the hydrogels, with and without the incorporation of gelatin microparticles (GMP) as sites for cell attachment, to support the osteogenesis of mesenchymal stem cells (MSC) *in vitro,* and second, to evaluate the regenerative potential of the hydrogel-cell constructs for bone formation *in vivo*.

Methods: TGMs and PAMAM crosslinkers were synthesized from established polymerization protocols [1]. 50-100 μm diameter GMPs with 10mM glutaraldehyde were made as previously described [2]. Following Rice University IACUC approved animal protocols, MSCs were harvested from the long bones of 6 week old Fisher 344 rats and cultured for 6 days before encapsulation. To evaluate the effect of polymer wt % and GMP loading on encapsulated MSC viability and osteogenic differentiation, a factorial study was performed using 10 or 20 polymer wt % (w/v) hydrogels with 0 or 20 wt % (w/w) GMP loading. Hydrogels were cultured in dexamethasone-containing complete osteogenic media for 0, 7, 14, and 28 days (n=4) following established protocols [3]. At each timepoint, samples were analyzed by Live/Dead confocal imaging, calcium biochemical assay and histology. For the *in vivo* evaluation, 20 wt % hydrogels with 0 or 20 wt % (w/w) GMP loading were implanted in an 8 mm critical size rat cranial defect for 4 and 12 weeks as previously described [4]. At each timepoint, samples were harvested and analyzed via microcomputed tomography and histological staining.

Results: Injectable, physically and chemically crosslinking hydrogels were successfully fabricated from the mixing of TGMs, PAMAM crosslinkers, GMPs, and MSCs. Hydrogels with loaded GMPs significantly supported encapsulated MSC viability and differentiation *in vitro*. Both GMP incorporation and MSC encapsulation led to significant bone formation and bone tissue ingrowth *in vivo.*

Conclusions: The results show that incorporation of MSCs and GMPs in injectable, dual crosslinking hydrogels can be successfully created to support cell viability and direct osteogenesis both *in vitro* and in an orthotopic defect. These self-mineralizing injectable hydrogels show promise as a minimally invasive strategy for stem cell delivery in craniofacial tissue engineering.

References: [1]Vo et al. *Biomacromolecules*, 15(1):132-142 (2014) [2] Holland TA et al. *J Control Release.* 2003;91:299-313. [3] Klouda L et al. *Acta Biomaterialia*. 2011;7:1460-1467. [4] Spicer P et al. *Nature Protocols*. 2012;7:1918-1929.

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Characterisation of Injured Muscle-Derived Stem Cell-Like Cells

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Tissue repair after injury is a complex biological process, which involves the activation of tissue-resident precursor cells, stem cells, and infiltrating cells in response to local and systemic signals. Mammalian skeletal muscle regeneration relies on the activation and proliferation of the residue muscle precursor cells, e.g. the original satellite cells or muscle stem cells (MuSCs), which are a group of mononucleated cells located between the basal lamina and sarcolemma of muscle fibers. Accumulating evidence points to functional heterogeneity of MuSCs, which indicates differences in their proliferation rate, expression profile, and in their self-renewal, clonogenic and differentiation ability.

We recently discovered a population of naturally existing stem cells that can be obtained from the injured murine skeletal muscles. Mouse injured muscle-derived stem cell-like cells (iMuSCs) were isolated from the injured tibialis anterior (TA) muscles of *C57BL/6J* (3-8-week-old female) mice four days after laceration injury, while control MuSCs were isolated from the uninjured TA muscles. Characterisation of iMuSCs was performed by applying standard *in vitro* and *in vivo* assays.

Our results show that these iMuSCs are partially reprogrammed from differentiated myogenic cells and display an approximate pluripotent state. IMuSCs have stem cell properties, natural improved migration, and multiple, including myogenic and neurogenic, differentiation *in vitro*, and muscle engraftment ability *in vivo*. IMuSCs express several pluripotent and myogenic stem cell markers; have the capability to form embryoid bodies and teratomas, as well as to differentiate into all three germ layers. Moreover, blastocyst microinjection showed that iMuSCs contributed to chimeric embryos but could not complete germline transmission.

These results indicate that iMuSCs do not regress completely to pluripotency and possibly hold an epigenetic memory of their myogenic tissue origin. Thus, the key conclusion from our study is that changed environmental factors upon skeletal muscle injury partially reprogram terminally differentiated myogenic cells into an approximate pluripotent state.

Dynamic Mechanical Forces Affect Intestinal Epithelial Physiology

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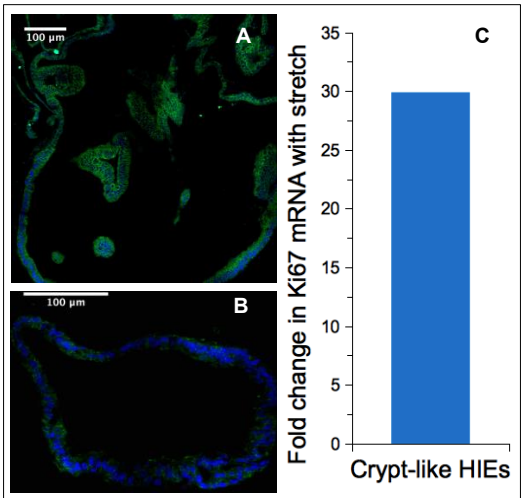
The intestinal epithelium experiences significant mechanical forces, including cyclic stretch, compression, and shear. These forces are known to be important regulators of stem cell biology in many types of tissue; however, their effect on the intestinal epithelium is poorly understood. Human intestinal enteroids (HIEs) are a primary human cell line isolated from bariatric surgery specimens and endoscopic biopsies. They contain all major cell types present in the intestinal epithelium, and, depending on their culture conditions, are able to represent the major cell types present in the intestinal crypts or on the villi. In this study, HIEs were grown in mixtures of collagen and matrigel to simulate the native extracellular matrix, and exposed to physiological magnitudes and frequencies of cyclic stretch using a Flexcell FX-5000 Tension System. The effect of stretch on intestinal epithelial homeostasis and function were examined by IHC and qRT-PCR. Cyclic stretch increases the proliferation of predominately undifferentiated, crypt-like HIEs, and alters the function of predominantly differentiated, villus-like HIEs. These results demonstrate that physical forces are an important component of the intestinal environment in vitro, and should be considered in future regenerative medicine efforts.



The effect of mechanical stimulation on HIEs. Immunofluoresence for E-cadherin (green) and DAPI shows that crypt-like HIEs stretched for 72 hrs (A) are significantly larger than static controls (B). Stretch also causes a 30-fold increase in Ki67 expression (C) in crypt-like HIEs.

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Effective Gene Delivery to Heart Valve Cells Using Adeno-associated Virus

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Introduction: The current lack of curative therapy for heart valve disease drives research in therapeutics, valve replacements, and basic science. Much research in these areas uses porcine aortic valves as a model tissue. In studying valve disease and engineering new valve therapies, it would be useful to perform genetic manipulation of primary valvular interstitial cells (VICs). However, primary VICs are challenging to transfect, and there is little information reported about effective gene delivery methods for VICs. Moreover, adeno-associated virus mediated gene delivery to VICs has not been reported. In this work, non-viral and viral vectors were tested to assess their efficacy for gene delivery in primary porcine VICs.

Materials and Methods: Valvular interstitial cells were harvested from suckling pig aortic valves obtained from a commercial abattoir within 24 hours postmortem. VICs were isolated after swabbing the valve leaflets to remove cells, mincing the leaflets, and digesting with collagenase.

Gene delivery vectors were compared by measuring the delivery of cytomegalovirus (CMV)-green fluorescent protein (GFP) transgene to VICs, HEK293T cells (a highly permissive cell line), and primary human dermal fibroblasts (data not shown) using Lipofectamine LTX (Invitrogen), linear 25,000 MW polyethylenimine and recombinant adeno-associated virus (rAAV). Gene delivery vectors were added to cells at 90% confluency in 12-well plates. GFP expression was measured with flow cytometry and analyzed using FlowJo software. Gene delivery efficacy was measured by transfection/transduction index (*TI*), where *TI = (%GFP-positive cells)*(geometric mean FITC intensity)*.

Transfection using Lipofectamine LTX was optimized by varying the amount of liposome LTX reagent added (0, 0.4, 0.7, 1 ul) per well (n = 2). PEI-mediated transfection was optimized by testing a range of nitrogen/phosphate (NP) ratios (10, 15, 20, 30) (n = 2). rAAV serotypes 1 through 9, and 12 were compared for gene delivery using a multiplicity of infection (MOI) of 5×10^3 viruses per cell (n=1). AAV2 transductions at MOI of: 5×10^3, 1×10^4, 2.5×10^4, and 5×10^4 were compared (n = 3).

Results and Discussion: Lipofectamine and PEI-mediated gene delivery resulted in low GFP expression in all conditions. Whereas PEI-mediated transfection showed minimal GFP expression across the range of NP ratios tested, lipofectamine-mediated transfection efficiency trended upwards with increasing concentrations of LTX reagent.

Based on the AAV serotype screen ,AAV2 appeared to be the most effective serotype compared to other serotypes (TI = 22964 vs. 44 to 255, respectively). Moreover, AAV2 transduction efficiency scaled with increasing MOI. Of the gene delivery vectors tested, AAV was most effective in % GFP-positive cells and overall gene expression levels.

Conclusions: AAV2 infects primary porcine VICs with moderately high efficiency and appears to be a more effective gene delivery vector than polyethylenimine and Lipofectamine for these cells. Thus, AAV2 is a promising gene delivery vehicle with applications for valvular cell biology and disease therapy.

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Building Salivary Cell Mini-Modules: A First Step Toward Reconstruction of the Human Salivary Gland

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Our tissue engineering strategy to reconstruct a functional human salivary gland to benefit patients suffering from radiation-induced xerostomia requires the generation of salivary cell-laden bioactive, 3D hydrogels that can reconstitute secretory units of the gland: the salivary cell mini-modules (MMs). Our strategy integrates four complementary approaches: 1) creation of designer biomaterials that dial in optimal cues mimicking native extracellular matrix; 2) encapsulation of primary human salivary progenitor cells along with logically presented growth factors that can induce morphogenesis to create complex structures; 3) inclusion of myoepithelial and ductal cells to form multi-layer cell assemblies; 4) provision of neurotransmitter stimuli that can solicit coupled secretion responses. All components ultimately must be human-compatible and suited for implantation.

An interdisciplinary team including clinicians, salivary biologists and bioengineers, materials scientists and physiologists was assembled for this endeavor. The team realized the biomaterial matrix must have correct mechanical properties to allow salivary progenitor cells to freely migrate, proliferate and self-assemble into acini-like spheroids. MMs also must contain biological recognition units and ECM-derived peptides that mimic the salivary basement membrane (BM). MMs we developed combine a thiolated hyaluronic acid (HA) polymer with a commercial polyethylene glycol-diacrylate (PEGDA) to produce soft, porous hydrogels that allow 3D salivary spheroids to form. This system is designed to incorporate acrylated BM-derived peptides that provide cues to organize and guide the behavior of primary salivary cells. Implanted MMs in rat showed no inflammation or an obvious tissue reaction.

Human salivary acinar-like cells (hSACs) grown in our 3D MMs self-assemble into spheroids and elicit fluid and protein secretory responses to neurotransmitters. Protein and RNA analysis of hSAC spheroids grown in MMs showed they express stem/progenitor cell markers KIT, Musashi, K5, and K14, revealing that hSACs have the potential to undergo morphogenesis into lobular structures. With FGF7/10 treatment lobulo-tubular structures with lumens formed as well as ductal elements. Engineered growth factor gradients within the hydrogels for temporally and spatially controlled release will be employed to direct organized tissue formation.

Human salivary myoepithelial cells (hSMECs), isolated and analyzed for biomarker expression to confirm phenotype, had the ability to contract collagen gels upon stimulation to reveal a functional role during salivary secretion. hSMECs successfully co-assembled with hSACs in 3D, and is the subject of ongoing work where both populations respond to neurotransmitters. Production and secretion of salivary-specific proteins and fluid by the cell assemblies in the cell MM is a necessary step toward attaining complete functionality. Experiments continue to further refine the salivary cell MM system as a first step to reconstruct the salivary gland for relief of xerostomia.

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Study of Skeletal Muscle Differentiation in hES/ iPS Cells Using MYF5 Knock-in Reporters

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Characterized by progressive skeletal muscle weakness and loss of muscle mass, muscular dystrophies are among the most common types of muscle disorders. Despite great advances in understanding the pathophysiology of these disorders, there is no effective treatment so far. Meanwhile, stem cells are considered as one of the best options due to their unique differentiation potentials. So far, few approaches such as myogenic gene over-expression have been developed for derivation of skeletal myogenic precursors from human embryonic stem cells (hES cells) or induced pluripotent stem cells (iPS cells). However, these approaches can't be used for clinical purposes due to viral integration, low efficiency or impurity of the myogenic cells.

We hypothesize that high efficient myogenic differentiation of hES/iPS cells can be achieved by using a primary myogenic regulation factor, MYF5, knock-in reporter human ES/ iPS cell lines to allow for chemical screening (HTS) for myogenic inducers. We will further isolate and identify surface makers for purification of early skeletal myogenic precursors from differentiating iPS cells.

To generate the MYF5 knock-in reporter hiPS cell line, we designed a homology recombination (HR) targeting vector incorporating a polycystronic 2A-GFP reporter right before the stop codon of MYF5 gene. In order to improve the HR efficiency, a pair of RNA-guided cas9 nickase was applied to generate a double strand break (DSB) near the stop codon. After two weeks' selection, 55.6% positive colonies were successfully targeted and 11.1% were double-allele targeted colonies. The expression of GFP and MYF5 in the MYF5 knock-in reporter hiPS cell lines could be initiated by transcriptional activation using a catalytically inactive Cas9 (dead Cas9) activator. Moreover, the GFP positive myogenic precursors derived from the MYF5 reporter hiPS cell line could successfully be differentiated into myotubes in vitro.

Our final goal is to develop an efficient protocol for myogenic differentiation and prospective isolation of early myogenic progenitors from human iPS cells which can be used for disease modeling as well as cell based therapies in degenerative muscle disorders.

Proton Pump Inhibitors Impair Vascular Function By Accelerating Endothelial Senescence

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Objective: Aging, vascular senescence and endothelial dysfunction are a major risk factor for cardiovascular disease (CVD) and atherosclerosis. Proton pump inhibitors (PPIs) like Esomeprazole (Nexium) are widely used drugs sold over the counter for the treatment of gastroesophageal reflux disease (GERD). Recently, our laboratory discovered that the PPIs inhibit the enzyme dimethylarginine dimethylaminohydrolase (DDAH) which is a key regulator of cardiovascular system. Inhibition of DDAH by the PPIs elevates the plasma levels of asymmetric dimethylarginine (ADMA). ADMA is an endogenous inhibitor of endothelial nitric oxide synthase (eNOS) and a risk factor for cardiovascular mortality. In addition, our analyses of large clinical databases indicate that PPI use is associated with cardiovascular risk. We believe that PPIs pose a major risk for CVD by impairing endothelial function. The aim of my project is to investigate the mechanistic basis by which the PPIs increase vascular dysfunction.

Methods: Human microvascular endothelial cells (HMVECs) were treated with clinically relevant concentration of esomeprazole and passaged over time prior to assessing the expression of molecular markers that indicate endothelial dysfunction.

Results: Long term exposure of endothelial cells (ECs) to clinically relevant concentration of PPIs accelerated premature endothelial cell senescence by shortening telomere length. PPI treatment increased superoxide levels, decreased nitric oxide production, and decreased DDAH, eNOS and iNOS gene expression. Functionally, PPIs impaired the angiogenic and proliferative capacity of ECs as confirmed by Matrigel tube formation and cell proliferation assays. Investigation of the molecular pathways involved in PPI-induced endothelial dysfunction revealed that plasminogen activator inhibitor-1(PAI-1); a gene commonly associated with EC dysfunction, was strongly up-regulated by PPI treatment. This data was confirmed using several orthogonal assays. Furthermore, PPIs down regulated all genes involved in Shelterin complex (a set of genes involved in regulation and maintenance of telomere function and also responsible for the regulation and signaling of DNA damage response pathways) providing a mechanistic evidence for the role of PPIs in accelerating endothelial senescence.

Conclusion: We provide molecular and mechanistic evidence that PPIs accelerate endothelial senescence and endothelial dysfunction through, in part by activation of PAI-1 protein and telomere shortening. Given the widespread use of PPIs in the absence of medical supervision, our data raises a concern for people who are on long-term PPI therapy.

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