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

Gulf Coast Cluster for Regenerative Medicine Inaugural Symposium

October 3, 2014
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
Cindy Farach-Carson, Ph.D., Rice University
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

Sponsored by:

Gulf Coast Consortia for Quantitative Biomedical Sciences

brc BioScience Research Collaborative

Corporate Sponsors:

Synthecon Incorporated BOSE CELLTEX

Conference Sponsors

Gulf Coast Consortia for Quantitative Biomedical Sciences

www.gulfcoastconsortia.org

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, early disease detection, 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 and The University of Texas M. D. Anderson Cancer Center.

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.

Synthecon Incorporated

Synthecon, Inc.
Stephen Navron, Ph.D., Chief Scientific Officer
Lynn Robins, Technical Sales Associate
lynn@synthecon.com

BOSE

Bose Corporation – ElectroForce Systems Group
Mike Armstrong, Mike_Armstrong@bose.com

CELLTEX

Celltex Therapeutics
Madison Mauze, Vice President Business Development
mmauze@celltexbank.com

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

2014 Inaugural Symposium

Friday, October 3, 2014
BioScience Research Collaborative

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

	8:30 AM	Continental Breakfast and Poster Setup
	9:00 AM	*Introduction* GCC RM Steering Committee *Welcome Remarks* Bill McKeon, Texas Medical Center
SESSION 1 **Faculty and Trainee Short Talks**	9:15 AM	Session Chair: Hoang Nguyen, Baylor College of Medicine *The Use of Cord Blood in Regenerative Medicine* Elizabeth Shpall, UT MD Anderson Cancer Center *Developmental Gliogenesis: The Crossroads of Regenerative and Repair in the CNS* Hyun Kyoung Lee (for Benjamin Deneen), Baylor College of Medicine *Establishment of a Clinical Grade Amniotic Fluid-derived Mesenchymal Stromal Cell Bank* Fabio Triolo, University of Texas Health Science Center-Houston *Pluripotent Stem Cells for Studies on Human Pancreas Development and Disease* Malgorzata Borowiak, Baylor College of Medicine *Human CD34+ Cells Deficient in miRNA-377 Show Improved Cardiac Regenerative Ability After Myocardial Ischemia in SCID Mice* Darukeshwara Joladarashi, Houston Methodist Research Institute *Development of CRISPR/Cas9 Lineage Reporters for Differentiation and Purification of Transcription Factor-Defined Neural Progenitors from Human Pluripotent Stem Cells* Shenglan Li, University of Texas Health Science Center-Houston *Transdifferentiation of Fibroblasts to Endothelial Cells In Vivo* Shu Meng, Houston Methodist Research Institute *Reprogrammed Cardiac Fibroblasts to Rescue Heart Failure* Vivek Singh, Baylor College of Medicine *The Hippo Signaling and Mechanical Tension Coordinately Regulates Epicardial Progenitor Cell Fate* Yang Xiao, Texas A&M Health Science Center-Institute of Biosciences and Technology
KEYNOTE ADDRESS	10:45 AM	Session Chair: Ravi Birla, University of Houston *Injectable Biomaterials For Treating Cardiovascular Disease* Karen Christman, University of California San Diego
POSTER SESSION A	11:45 AM	Poster Viewing/Judging (BRC Event Space)
LUNCH	12:15 PM	Lunch and Networking (BRC Event Space)

Sponsors:

Gulf Coast Consortia for Quantitative Biomedical Sciences
brc BioScience Research Collaborative
Synthecon INCORPORATED
BOSE
CELLTEX

POINT/ COUNTERPOINT DISCUSSION	1:15 PM	*Cell vs Non-Cell Based Therapies* *Moderator: Brian Davis, University of Texas Health Science Center-Houston* Non-Cell Based Therapies: John Cooke, Houston Methodist Research Institute Cell Based Therapies: Elizabeth Davis, Baylor College of Medicine
	1:35 PM	*Synthetic vs Native Scaffolds* *Moderator: Doris Taylor, Texas Heart Institute* Synthetic Scaffolds Team: Elizabeth Cosgriff-Hernandez, Texas A&M University and Jordan Miller, Rice University Native Scaffolds Team: Ravi Birla, University of Houston and Andrea Gobin, Texas Heart Institute
SESSION 2 Faculty and Trainee Short Talks	2:00 PM	Session Chair: K. Jane Grande-Allen, Rice University *Biomaterials for Tissue Engineering* Antonios G. Mikos, Rice University *Gradient Approaches to the Optimization of Biomaterials for Stem Cell Differentiation* Laura Smith Callahan, University of Texas Health Science Center-Houston *Silk Fibroin Architecture And Applications* Anshu Mathur, UT MD Anderson Cancer Center *Scaffold-free Tissue Engineering for Tracheal Reconstruction* James Dennis, Baylor College of Medicine *Development of a Model Surface for Bioprosthetic Heart Valves* Monica Fahrenholtz, Rice University *Fabricating a Bioartificial Heart by Seeding a Rat Heart Scaffold with Neonatal Cardiac Cells* Ze-Wei Tao, University of Houston *Condition Media From Human Lung Fibroblast Grown on 4D Model Inhibit Cancer Metastasis* Dhurva Mishra, Houston Methodist Research Institute *Three-Dimensional Enteroids As Novel Systems For The Study of Enteric Infections* Luz Vela, Baylor College of Medicine *Polyethylene Glycol Hydrogels Functionalized with a Continuous Ile-Lys-Val-Ala-Val Concentration Gradient for Optimizing Neural Differentiation of Murine Embryonic Stem Cells in 2D* Yueh-Hsun Yang, University of Texas Health Science Center-Houston
POSTER SESSION B	3:30 PM	Poster Viewing/Judging (BRC Event Space)
KEYNOTE (& Keck Seminar)	4:00 PM	Session Chair: Charles S. Cox, Jr., University of Texas Health Science Center-Houston *The California State Stem Cell Agency Experience in Product Development and Future Directions* Ellen Feigal, California Institute for Regenerative Medicine
AWARDS	5:00 PM	Poster Award Ceremony
	5:15 PM	Reception and Poster Viewing (BRC Event Space)

Sponsors:

Gulf Coast Consortia for Quantitative Biomedical Sciences

brc BioScience Research Collaborative

Synthecon INCORPORATED

BOSE

CELLTEX

Keynote Speaker Biography

Ellen Feigal, M.D.

Senior Vice President, Research and Development
California Institute for Regenerative Medicine

Dr. Ellen G. Feigal is the Senior Vice President, Research and Development at the California Institute for Regenerative Medicine (CIRM). Prior to joining CIRM in January, 2011, Dr. Feigal was Executive Medical Director, Global Development, at Amgen, where her primary focus was in clinical development of therapeutics in hematology/oncology. She also led the scientific/clinical interface with patient advocacy organizations, formalized the company's policy on expanded access to therapies for those with limited or no treatment options, and led the cross-functional teams to the company's first collaborative research and development agreement with the National Cancer Institute. From 2007 until joining CIRM, Dr. Feigal was adjunct professor and founding Director of the American Course on Drug Development and Regulatory Sciences, UC San Francisco, School of Pharmacy. The course, developed under her leadership as a collaborative effort with the FDA, UCSF's Department of Bioengineering and Therapeutic Sciences, its Center for Drug Development Sciences and the European Center of Pharmaceutical Medicine at the University of Basel, was launched in 2007. It is taught in Washington, D.C. with a separate parallel course in San Francisco.

Prior to joining Amgen in 2008, she worked in clinical research and drug development in positions at the Federal Government, non-profit and for-profit institutes and companies. She was Chief Medical Officer, Insys Therapeutics 2007-2008, Director of Medical Devices and Imaging at the Critical Path Institute, and Vice President of Clinical Sciences and Deputy Scientific Director at the Translational Genomics Research Institute from 2004 to 2007. She directed the National Cancer Institute's (NCI) Division of Cancer Treatment and Diagnosis from 2001 to 2004, served as Deputy Director from 1997 through 2001, and as senior investigator in the Cancer Therapy Evaluation Program, NCI from 1992-1997. Dr. Feigal earned a BS in biology and a MS in molecular biology and biochemistry from the UC Irvine, and her MD from the UC Davis. She completed her residency in internal medicine at Stanford University, and her fellowship in hematology/oncology at the UC San Francisco. She was on the faculty at the UC San Francisco, and UC San Diego before joining the NCI.

Keynote Speaker Biography

Karen L. Christman, Ph.D.

Associate Professor of Bioengineering
Sanford Consortium for Regenerative Medicine
University of California, San Diego

Cardiovascular disease remains the leading cause of death in the western world. Two major types of cardiovascular disease, myocardial infarction and peripheral artery disease, have few available treatments and therefore numerous patients continue to decline towards heart failure for the former and amputation for the latter. Current clinical trials have focused on cell therapies; however it is largely acknowledged that these cells act via paracrine mechanisms to recruit endogenous cells to help repair and regenerate the tissue. In animal models, it has been established that cellular recruitment to the damaged tissue can also occur via implantation of biomaterial scaffolds. Injectable materials are particularly attractive since they have the potential to be delivered minimally invasively, thereby requiring less recovery time and reducing the chances of infection. This talk will cover recent developments and translational progress with the use of injectable hydrogels for treating cardiovascular disease.

Dr. Christman received her B.S. in Biomedical Engineering from Northwestern University in 2000. She then moved to California for both her graduate work and postdoctoral studies. She received her Ph.D. from the University of California San Francisco and Berkeley Joint Bioengineering Graduate Group in 2003 under the direction of Dr. Randall J. Lee at UCSF, where she examined in situ approaches to myocardial tissue engineering. She was a NIH postdoctoral fellow under the direction of Dr. Heather D. Maynard at the University of California, Los Angeles in the fields of polymer chemistry and nanotechnology. During this time, she developed novel methods for site-specifically patterning proteins into 2D and 3D structures. Dr. Christman is currently an Assistant Professor in the Department of Bioengineering and a member of the Institute of Engineering and Medicine at the University of California, San Diego. She is a recipient of the NIH Director's New Innovator Award and the Walter H. Coulter Foundation Early Career Translational Research Award.

Faculty Speakers

Elizabeth J. Shpall, M.D.

Professor and Deputy Department Chair, Director, Cord Blood Bank
Department of Stem Cell Transplantation
The University of Texas MD Anderson Cancer Center

As a nationally recognized expert in stem cell transplantation, Medical Director of the GMP Cell Therapy Laboratory since 2002**,** and the Director of the Cord Blood Bank (CBB) at M. D. Anderson Cancer Center since 2004, Dr. Shpall's research covers a range of applications, among them: leukemia, lymphoma treatment, anti-tumor immunity enhancement, and tissue repair. Many of her studies are designed to address the safety and efficacy of stem cell transplantation for patients. It is, however, Dr. Shpall's leadership in the area of umbilical cord blood collection, banking and transplantation that has helped to move M. D. Anderson to the forefront in this new and important field. Under her watch, the CBB has collected over 15,000 cord blood units from voluntary donations since its establishment in April 2005, coordinating these efforts with several hospitals in Houston. The MD Anderson Stem Cell Transplant Program has transplanted more than 200 patients with cord blood using novel strategies such as ex vivo expansion of the cord blood cells before infusion, to make it work better in patients.

Dr. Shpall's influence is felt beyond the Texas Medical Center. She is the current vice-president of FACT – Netcord, having chaired their Cord Blood Standards Committee since 1999, and she was the founding president and has been a board member of the Foundation Accreditation of Hematopoietic Cell Therapy since 1995. Internationally, she has been a member of the International Association for Breast Cancer Research (1992-2000) and the International Society for Cellular Therapy (1992-present).

Dr. Shpall's academic career started with an undergraduate degree from Brown University in 1976, Dr. Shpall completed her medical degree at the University of Cincinnati College of Medicine in 1980. She was a resident at Beth Israel Medical Center inNew York for three years. From 1983 through 1985, she was a fellow in the Department of Neoplastic Diseases at Mount SinaiMedical Center, also in New York, serving as chief fellow in her final year. Before coming to M. D. Anderson in 2002, she was the director of the Cord Blood Bank at the University of Colorado Health Sciences Center, a program she started. She has authored or co-authored hundreds of research abstracts, articles and editorials.

Benjamin Deneen, Ph.D.

Associate Professor
Department of Neuroscience, Center for Cell and Gene Therapy, Center for Stem Cells and Regenerative Medicine
Baylor College of Medicine

Glia cells comprise approximately 60% of the central nervous system (CNS), play diverse roles in the functioning CNS, and consequently are associated with numerous neurological disorders and malignancies. In spite of their vital role in CNS physiology and pathology the molecular mechanisms that control their development and diversity remain poorly defined; accordingly how these processes contribute to CNS pathology also remains enigmatic. Therefore, our laboratory is mainly focused that how to apply developmental gliogenesis to these associated disease. In particular, we will discuss myelin disorders by presenting the project on Daam2 to demonstrate how we bridge developmental and regenerative mechanisms in the CNS.

Wnt signaling plays an essential role in developmental and regenerative myelination of the CNS, however contributions of proximal regulators of the Wnt receptor complex to these processes remain undefined. To identify components of the Wnt pathway that regulate these processes, we applied a multifaceted discovery platform, spanning chick and human, finding that Daam2-PIP5K comprise a

novel pathway regulating Wnt signaling and regenerative myelination. Using dorsal patterning of the chick spinal cord we found that Daam2 promotes Wnt signaling and receptor complex formation through PIP5K-PIP_2. Analysis of Daam2 function in oligodendrocytes (OLs) revealed that it suppresses OL differentiation during development, after white matter injury (WMI), and is expressed in human white matter lesions. These findings suggest a pharmacological strategy to inhibit Daam2-PIP5K function, application of which stimulates remyelination after WMI. Put together, our studies integrate information from multiple systems to identify a novel regulatory pathway for Wnt signaling and new therapeutic target for WMI.

Fabio Triolo, D.d.R., M.Phil., Ph.D.

Associate Professor, Department of Pediatric Surgery, Program in Regenerative Medicine
Director, cGMP Facilities
The University of Texas Medical School at Houston

The maturation stage of a cell-based therapy depends on the cell source and spans technologies ranging from proof-of-concept phase (e.g., embryonic stem cell-based) still far from the clinic, to those used on millions of patients yearly in routine medical practice (e.g., hematopoietic stem cell-based). Between the two extremes there are several non-hematopoietic adult stem cell based (NHASC) technologies in intermediate maturation phase being experimented worldwide. While cell banking procedures are well structured and established for the more mature therapies, there is great need for standardization for emerging NHASC-based technologies, especially in academia where knowledge on product development, regulations and quality control and assurance is generally very limited. To address this, we describe a model approach used to establish a clinical-grade amniotic fluid-derived mesenchymal stromal cell bank at UTHealth.

Dr. Triolo, an expert in clinical cell therapy manufacturing for regenerative medicine applications, directs the Judith R. Hoffberger Cellular Therapeutics Laboratory and the Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory within the Program of Regenerative Medicine. The Hoffberger laboratory is focused on translating, scaling-up, validating and supporting IND applications (CMC section) of promising new therapeutic technologies developed by scientists and physicians at a preclinical level into clinical-grade processes that can be used to manufacture cell-based and/or tissue engineered products for clinical applications. The Griffin Laboratory is an FDA-registered facility where tissues and organs are processed to produce cells for clinical applications in compliance with current Good Manufacturing Practice (cGMP).

Malgorzata Borowiak, Ph.D.

Assistant Professor
Stem Cell and Regenerative Medicine Center, Cell and Gene Therapy Center, Molecular and Cellular Biology Department, of Baylor College of Medicine
McNair Medical Institute

The pluripotent stem cell potential to generate any cell of interest forms a basis for regenerative medicine. Diabetes type one where only one component, producing insulin beta cell is lost stands as good candidate for cellular therapy. Our current approaches to coax pluripotent stem cells into becoming beta cells include a recreating of pancreatic niche, small molecules and overexpression of selected genes, lead to the

generation of single hormonal, glucose sensitive beta cells though not identical to naïve human beta cells. We are using the directed differentiation of pluripotent stem cells as platform to study human endocrine cells maturation and we uncovered the function of sympathetic nervous system during the islet formation. The details function along molecular mechanism will be presented as an example of studies undergoing in out laboratory.

Dr. Borowiak, an expert in stem cell and regenerative medicine, joined BCM in January of 2012. She earned a master's of science degree from the Polish Academy of Sciences in Poznan, Poland, followed by a Ph.D. from Free University and Max-Delbrueck Centrum for Molecular Medicine in Berlin, Germany. There she studied tyrosine kinase receptor signaling during liver regeneration and homeostatic. Prior to joining BCM, Dr. Borowiak trained under Dr. Douglas Melton, renowned leader in stem cell biology, at Harvard University where she employed small molecules and cell-cell interaction to generate pancreatic progenitors. She continues to work on human pancreatic development and pluripotent stem cell technology application towards cellular therapy for type 1 diabetes at BCM.

Antonios G. Mikos, Ph.D.

Louis Calder Professor, Bioengineering and Chemical & Biomolecular Engineering
Director, John W. Cox Laboratory of Biomedical Engineering
Director, Center for Excellence in Tissue Engineering
Rice University

Biomaterial-based strategies for tissue engineering span a vast spectrum from the production of scaffolds tailored with appropriate mechanical properties and degradation kinetics to serve transiently as a bridge to tissue formation to the leverage of biomaterials for the controlled delivery of biological signals to regenerate tissue in specific sites in the body. For example, our laboratory has developed a variety of biodegradable polymers for the controlled delivery of bioactive agents and/or stem cell populations to promote regeneration of tissues such as bone and cartilage. We have also applied engineered culture of cell populations on three-dimensional scaffolds toward the development of biologically active hybrid scaffold/extracellular matrix constructs for regenerative medicine applications as well as testing of anticancer drugs. This talk will present recent examples of biomaterial-based approaches for the development of tissue engineering technologies to meet clinical needs.

Antonios G. Mikos is the Louis Calder Professor of Bioengineering and Chemical and Biomolecular Engineering at Rice University. He is the Director of the J.W. Cox Laboratory for Biomedical Engineering and the Director of the Center for Excellence in Tissue Engineering at Rice University. He received his Dipl.Eng. (1983) from the Aristotle University of Thessaloniki, Greece, and his Ph.D. (1988) in chemical engineering from Purdue University. He was a postdoctoral researcher at the Massachusetts Institute of Technology and the Harvard Medical School before joining the Rice Faculty in 1992 as an assistant professor.

Mikos' research focuses on the synthesis, processing, and evaluation of new biomaterials for use as scaffolds for tissue engineering, as carriers for controlled drug delivery, and as non-viral vectors for gene therapy. His work has led to the development of novel orthopaedic, dental, cardiovascular, neurologic, and ophthalmologic biomaterials. He is the author of over 500 publications and 25 patents. He is the editor of 15 books and the author of one textbook (Biomaterials: The Intersection of Biology and Materials Science, Pearson Prentice Hall, 2008). He has been cited over 44,000 times and has an h-index of 115.

Faculty Speakers

Laura Smith Callahan, Ph.D.

Assistant Professor
Department of Neurosurgery, Center for Stem Cell and Regenerative Medicine,
Department of Nanomedicine and Biomedical Engineering
University of Texas Health Science Center at Houston

Emulating the differentiation signals provided by the extracellular matrix during development within tissue engineering matrices can promote stem cell differentiation and tissue formation toward the desired lineage. To quickly identify the optimal bioactive signaling concentrations, modulus, and architecture within tissue engineering matrices to promote this "directed" differentiation and tissue formation from stem cells, combinatorial methods, which have long been applied to drug discovery in the pharmaceutical industry and only recently applied to biomaterial development, will be utilized. Gradient hydrogel samples are one combinatorial method strategy that can be used to examine cellular response to materials over a wide range of conditions in both two and three dimensional culture. This talk will focus on development of two and three dimensional polyethylene glycol gradient systems for the study of tethered bioactive factors and Young's Modulus on stem cell differentiation.

Dr. Smith Callahan received her B.S. in Bioengineering from the University of Toledo, M.S in Biomedical Engineering with a concentration in biotechnology and tissue engineering from the University of Michigan and Ph.D. in Biomedical Engineering from the University of Michigan. Her thesis work, under the direction of Peter X. Ma, focused on the effects of nanofibrous scaffolding on the osteogenic differentiation of embryonic stem cells. After completing her thesis, Dr. Smith Callahan was awarded a 1 year post-doctoral fellowship on the Regenerative Science T90 training grant which allowed her to study further the effects of nanofibrous scaffolding on the neural differentiation of embryonic stem cells. Desiring additional training in peptide and polymer chemistry and soft material characterization, Dr. Smith Callahan transitioned to a post-doctoral position at the Institute of Polymer Science at the University of Akron with Matthew L. Becker. At the University of Akron, her work focused on the effects of bioactive peptides and gradient hydrogels on stem cell differentiation to mesenchymal and neuronal lineages.

Dr. Smith Callahan brings a combination of expertise in polymeric synthesis and processing, tissue engineering and stem cell biology. Her research interests include tissue engineering, the effect of cell-material interactions on cellular differentiation and tissue formation, cell sources for tissue engineering applications and scaffold development focusing on applications relevant to the repair of the central nervous and musculoskeletal systems after injury.

Anshu Mathur, Ph.D.

Associate Professor
Department of Plastic Surgery
The University of Texas MD Anderson Cancer Center

A relationship between nano/micro and macro structural and mechanical characteristics of *Bombyx mori* silk fibroin (SF) solution and scaffolds is investigated for developing biomaterials for biomedical applications. The effects of SF solution concentration (0.91% to 3.33% (w/v)), filter size (0.22, 0.45, 100 μm), pH (4, 5.5, 7.4), and ion concentration (no PBS, 0.1× PBS, 1× PBS), on the SF solution and scaffold properties are assessed. Under these various conditions, the mean SF particle size in solution varies from 42 nm to 547 nm. Scaffold pore size remains above 100 μm and porosity around 90 %. SF fibril size ranges from 2.8 to 5.0 μm, SF fibril density 8 to 461 fibrils/mm^2, ultimate tensile strength (UTS) 95 to 254 kPa, and elastic

modulus (E) 256 to 885 kPa. All scaffolds exhibit higher amounts of β sheet than random coil conformation. A decrease in pH leads to a decrease in particle size and fibril size but an increase in fibril density and UTS, whereas a slight increase in ionic content results in an increase in particle size and random coil conformation but a decrease in UTS, E, and β-sheet content. In summary, a system has been established to tune the nano, micro, and macro properties of SF solution and scaffolds for fabricating clinically translatable reconstructive biomaterials.

Dr. Mathur's research encompasses a new and upcoming area of Engineered Biologics and their applications in Regenerative Medicine and Nanomedicine. Her doctoral training in Biomedical Engineering from Duke University with Master's training in the area of Polymer Chemistry at NC State University provided her an opportunity to develop unique expertise and apply them in the area of reconstructive therapy for cancer patients. Her current work at MD Anderson Cancer Center is highly applied and translational for repair and reconstruction of cancer patients. The laboratory that she heads is called Tissue Regeneration and Molecular Cell Engineering Labs (TRAMCEL). The work in the laboratory is focused in many areas of regenerative medicine such as musculofascia, bone, trachea, and microvascular guidance. There are various groups in the laboratory that comprise of a clinical trainee and a bioengineering trainee who study and develop Engineered Scaffold Design per clinical application, Characterize the physical and mechanical properties of cells and scaffolds at the nanoscale and macro scale, Investigate Stem Cell-scaffold interactions, Study tissue remodeling in various animal models (rats, sheep, guinea pig), and Fabricate and Characterize Nanotherapeutics for Delivery via a Reconstructive Tissue Flap.

James Dennis, Ph.D.

Associate Professor
Department of Orthopedic Surgery
Baylor College of Medicine

Dr. Dennis's lab focuses on the cell biology of mesenchymal stem cells and chondrocytes and the application of these cells to tissue engineering, particularly in the areas of cartilage regeneration for joint repair and for the tissue engineering of autologous trachea replacements. In addition, the Dennis laboratory is investigating the relationship between complement signaling and osteoporosis. Dr. Dennis earned his Ph.D. in biology at Case Western Reserve University in 1995, and received post-doctoral training in the laboratory of Dr. Arnold Caplan. In 2005, Dr. Dennis became an assistant professor in the Department of Orthopaedics at Case Western Reserve University where he stayed until 2010 when he accepted a position as a Research Associate Member at the Benaroya Research Institute in Seattle, Washington.

Human CD34+ cells deficient in miRNA-377 show improved cardiac regenerative ability after myocardial ischemia in SCID mice

[1]Joladarashi D, [1]Thandavarayan RA, [1]Suresh Babu S, [1]Bovshik EI, [2]Srikanth GV, [2]Verma SK, [3]Mackie AR, [2]Khan M, [3]Vaughan EE, [2]Kishore R, [1]Krishnamurthy P.
[1]Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston, TX
[2]Department of Pharmacology, Stem Cell Therapy Program, Center for Translational Medicine, School of Medicine, Temple University, Philadelphia, PA
[3]Feinberg Cardiovascular Research Institute, Northwestern University, Chicago IL

Corresponding author: Dr. Prasanna Krishnamurthy, DVM, MVSc, PhD. Scientist, Department of Cardiovascular Sciences, Houston Methodist Research Institute, Center for Cardiovascular Regeneration Houston Methodist DeBakey Heart and Vascular Center, 6670 Bertner Ave, MS: F8-046, Houston, TX-77030, E-mail: pkrishnamurthy@houstonmethodist.org

microRNAs (miRNA/miR) dysregulation has been implicated in cardiac remodeling after injury or stress, however its effects on endothelial progenitor cells (EPC) biology and function, particularly in the context of cell-based therapy for cardiomyopathy is not fully understood. The purpose of this study was to determine the miRNA profile of EPCs in response to chronic inflammatory stimuli and evaluate if miRNA-377 impairs differentiation and function of bone marrow-derived EPCs, after transplantation in myocardial ischemic conditions. Furthermore, to demonstrate the translational relevance of these studies to the on-going clinical trials with human CD34+ cells, we investigated the effect of miRNA-377-deficiency in human CD34+ cells on cardiac repair in immunodeficient mice. miRNA array profile of EPCs in response to inflammatory stimuli (LPS treatment for 12 hrs) was analyzed using a PCR-based miRNA microarray platform covering a total of 352 mouse miRNAs. The effect of pre-miRNA (pre-miR-377, stimulates miRNA) on EPC migration was assessed using modified Boyden chamber and *invitro* vascular tube formation was examined using matrigel assay. GFP-labeled EPC (transfected with either anti-miR-377 or control-anti-miR) were transplanted intramyocardially after induction of MI. EPC-mediated neovascularization, left ventricular function and myocardial repair was evaluated at 14 and 28 days, post-MI. miRNA array data from EPCs in response to inflammatory stimuli has indicated up-regulation of number of miRNAs related to cell survival/death and angiogenesis with a robust increase in miR-377. pre-miR-377 (miRNA mimics) transfection in EPCs inhibits their migration (modified Boyden chamber, $P<0.05$) and vascular tube formation ability (matrigel assay, $P<0.05$). Furthermore, EPCs treated with miR-377 mimic showed decrease in expression of STK35 (a novel serine/threonine kinase localized in the nucleus). Moreover, STK35 is predicted as a potential target gene of miR-377 by computational analysis. Interestingly, in a relevant mouse model of myocardial infarction (MI), intramyocardial transplantation of miR-377-silenced (anti-miR-377 transfected, GFP-labeled) EPCs promotes neovascularization (at 28 days, post-MI) leading to improvement in myocardial repair (reduced fibrosis and infarct size). Echocardiography showed LV function was significantly improved in mice receiving miR-377-silenced EPCs compared to control-miR-transfected EPCs. Studies involving human CD34+ cell (deficient in miR-377) and intramyocardial transplantation in NOD-SCID mice after myocardial ischemia showed reduction in cardiac fibrosis and cell death, associated with increased neovascularization and improvements in LV function ($P<0.05$). Taken together, these data suggest that inhibiting miR-377 in EPCs might promote their engraftment and angiogenic ability after transplantation into ischemic myocardial tissue, possibly through activation of STK35 signaling axes.

Funding sources: This work was supported, in part, by National Institutes of Health (NIH) grant 1R01HL116729 (to P.K.) and American Heart Association National-The Davee Foundation SDG Grant 0530350N (to P.K.).

Development of CRISPR/Cas9 Lineage Reporters for Differentiation and Purification of Transcription Factor-Defined Neural Progenitors from Human Pluripotent Stem Cells

Li S[1], Xue H[1], Wu J-B[1], Liu Y[1,2]
1. Department of Neurosurgery, Center for Stem Cells and Regenerative Medicine, the Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, Texas, USA
2. The Senator Lloyd & B.A. Bentsen Center for Stroke Research, the Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, Texas, USA
Corresponding author: Liu Y, Department of Neurosurgery, University of Texas Health Science Center at Houston. 1825 Pressler Street, SRB-630G, Houston, TX 77030. Tel: (713)500-3567, Fax: (713)500-2424, Email: ying.liu.1@uth.tmc.edu

Objectives: Pluripotent stem cell technologies are powerful tools for modeling development and disease, and for drug screening and regenerative medicine. Faithful gene targeting in pluripotent stem cells greatly facilitates these applications. We have developed a fast and precise CRISPR(Clustered Regularly Interspaced Short Palindromic Repeats) /Cas9 (CRISPR-associated) technology-based method and obtained a collection of reporter constructs of neural lineage specific markers, including Neurogenin2 and Olig2, and generated neural lineage reporters. This platform allows us to directly purify transcription factor (TF)-defined specific progenitor populations by fluorescence activated cell sorting (FACS) for subsequent molecular characterization and high-throughput drug screening.

Methods: Knockin vectors were constructed by recombineering and multisite gateway. Single guide RNAs (sgRNAs) were designed with ZiFiT software and subcloned into sgRNA-expression vector using Golden-Gate assembly. Multiple sgRNAs were constructed and Surveyor assays were performed to identify suitable sgRNAs for subsequent transfection. Next, human iPSC line ND2.0 was electroporated with sgRNA, Cas9 and the TF targeting vector. Correctly targeted clones were verified by Southern blot analysis. Off-target prediction and analysis were carried out using CasOT, a Perl-based software, and PCR. Positive TF reporter cells were induced by dual SMAD inhibitor dorsomorphin and SB431542, followed by retinoid acid and purmorphamine (Shh agonist) in N2B27 medium. mCherry-expressing (Neurog2+) or GFP-expressing (Olig2+) cells were FACS-purified. A detailed differentiation time course of a specific TF-defined population was recorded and subsequent differentiation toward neurons and glia was performed. Furthermore, global gene expression profile of FACS-purified reporter cells was compared by bead based cDNA microarray.

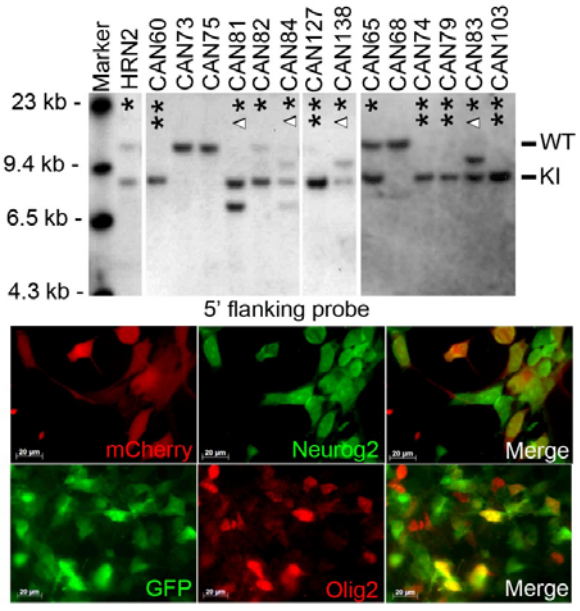


Fig 1. Generation of Neurog2-mCherry and Olig2-GFP knockin reporters in hiPSC ND2.0. (*Top*) Southern blot analysis for CRISPR-assisted Neurog2 targeting (designated as CAN clones) yielded a significantly improved targeting efficiency compared to conventional targeting (clone HRN2). * and **, targeting at one and two alleles respectively. Δ, deletion at one allele. (*Middle*) After differentiation, cells were purified by mCherry+ sort. Knockin mCherry expression recapitulated the endogenous Neurog2 expression (green). (*Bottom*) Similarly, Olig2-GFP cells were induced to express robust native GFP which recapitulated Olig2 (red) expression.

Results: Gene targeting efficiency was greatly improved in CRISPR/Cas9 mediated targeting (~36% correctly targeted clones) compared to conventional targeting protocol (~3%) at the same locus. Interestingly, ~33% of positive clones were targeted at both alleles (*Fig 1, top panel*). No off-target events were detected. Neurog2 and Olig2 reporter lines were induced and FACS-purified for mCherry (Neurog2+) or GFP (Olig2+) cells for further differentiation (*Fig 1, middle and bottom panels*) and gene expression analysis.

Conclusions: The characterization of purified Neurog2 and Olig2 cells provides novel insights on the complex and dynamic transcriptional regulation of human neural development, and provides a tool for future study on gene functions and neural circuit regulation. Overall, CRISPR editing coupled with FACS-sorting progenitor cells in a lineage reporter platform during stem cell differentiation should be broadly applicable in any stem cell derivatives and subpopulations of any lineages.

Funding sources: Staman Ogilvie Fund-Memorial Hermann Foundation, the Bentsen Stroke Center at the University of Texas Health Science center at Houston and the National Institutes of Health (HHSN268201200154P).

Transdifferentiation of Fibroblasts to Endothelial Cells In Vivo

Shu Meng, Frank Ospino, Wing Tak Wong, Nazish Sayed, John P. Cooke
Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston, TX

Background: Our lab discovered that activation of TLR3 signaling using small molecules triggers epigenetic plasticity and permits transdifferentiation of fibroblasts into endothelial cells (iECs) *in vitro*. However, whether transdifferentiation could happen *in situ* is unknown. Here we hypothesized that activation of TLR3, together with activators of key pathways involved in EC development (VEGF, BMP4, bFGF) that drive endothelial cell (EC) specification, may induce transdifferentiation of fibroblasts into ECs ("iECs") *in vivo*.

Methods: A transdifferentiation cocktail, based on the *in vitro* studies, contained polyI:C (TLR3 agonist), endothelial growth factors (VEGF, BMP4, bFGF) and PKA agonist 8-Br-cAMP. A matrigel plug assay was used to test the *in vivo* transdifferentiation process. Growth factor reduced matrigel (500ul) was mixed with 10^6 GFP-human neonatal dermal fibroblasts (HNDF) and together with the transdifferentiation cocktail or single component of the cocktail. The matrigel combinations were injected subcutaneously into NOD/SCID/IL2r mice. Matrigel plugs were removed after 14 or 28 days, sectioned and immunostained for GFP and human CD31. Matrigel plugs were also digested to isolate cells to perform FACS analysis using GFP and human CD144 antibody. Digested cells were adherent to gelatin coated dishes for 24hr, and adherent cells were collected for RNA and RT-PCR analysis of human CD31 gene.

Results: At day 14, RNA and protein expression of human CD31 was only detected in cells from matrigel to which the transdifferentiation cocktail was added, but not in cells added with single component of the cocktail. At day 28, human CD31+ endothelial cells could still be detected in the lumen of capillary-like structures in the matrigel plug. Human CD31 and GFP signal was localized to luminal structures within the matrigel. Similarly, GFP+CD144+ cells were detected in cells isolated from matrigel mixed with full transdifferentiation cocktail, and only rarely from matrigel containing components of the cocktail. When the NFκB inhibitor Bay117082 was added to the transdifferentiation cocktail, the gene expression of human CD31 by RT-PCR was significantly reduced.

Conclusion: We have successfully developed a small molecule cocktail to transdifferentiate fibroblasts into ECs *in vivo*. Activation of TLR3 signaling and correct environmental cues are both essential for transdifferentiation *in vivo*. Inhibition of NFκB signaling dramatically reduced transdifferentiation. Thus innate immune signaling appears to be required for efficient transdifferentiation *in vivo*. This study provides proof-of-concept for the feasibility of a small molecule approach for therapeutic transdifferentiation *in vivo*.

Reprogrammed Cardiac Fibroblasts to Rescue Heart Failure

Singh VP[1], Mathison M[1], Chiuchiolo MJ[2], Wang H[1], Reineke EC[1], Xu X[1], Patel VK[1], Belaguli NS[1], Kaminsky SM[2], Crystal RG[2], Cooney AJ[1], Rosengart TK[1]
1. Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX
2. Department of Genetic Medicine, Weill Cornell Medical College, New York, NY

Corresponding author: Singh VP. DeBakey Department of Surgery, Baylor College of Medicine, 1 Baylor Plaza, R431J, Houston, TX 77030, E-mail: viveks@bcm.edu

Objectives: There is an urgent clinical need to develop new therapeutic approaches to treat heart failure, but the biology of cardiovascular regeneration is complex. Adult mammalian hearts have very limited capacity to repair as cardiomyocytes are regarded as terminally differentiated cells. Hence, the renewal of injured hearts is a fundamental goal of regenerative cardiovascular medicine. Replacing cardiomyocytes lost during a heart attack (myocardial infarction) results in less scar tissue formation, preventing the ensuing contractile dysfunction that can lead to chronic heart failure. A new generation of reprogramming technology involves trans-differentiating one adult somatic cell type directly into another. We reported previously that administration of gene transfer vectors encoding Lenti-Gata4 (G), Mef 2c (M) and Tbx5 (T), reprograms rat cardiac fibroblasts into induced cardiomyocytes (iCMs) *in vitro* and improves cardiac function in myocardial infarction models. The efficacy of this strategy could, however, be limited by the need for fibroblast targets to be infected 3 times-once by each of the 3 transgene vectors. We hypothesized that a polycistronic "triplet" vector encoding all 3 transgenes would enhance post-infarct ventricular function compared with use of "singlet" vectors. Secondly, the clinical utility of this cardiac regeneration strategy would be facilitated by the use of a non-integrating, short-term expression viral vector such as adenovirus. In the present study, we also sought to determine whether rat cardiac fibroblasts could be converted to iCMs by adenoviral expression of Gata4 (G), Mef2c (M), and Tbx5 (T) and compare the efficiencies of two viral reprogramming strategies.
Methods: *In vivo*, adult male Fischer 344 rats (n = 6) underwent coronary ligation with or without intramyocardial administration of an adenovirus encoding all 3 major vascular endothelial growth factor (VEGF) isoforms (AdVEGF-All6A positive), followed 3 weeks later by the administration to AdVEGF-All6A-positive treated rats of singlet lentivirus encoding G, M, or T (1 × 105 transducing units each) or the same total dose of a GMT "triplet" lentivirus vector. *In vivo*, human adenovirus serotype 5 (Ad5) or lentivirus expressing rat GMT or corresponding null viruses were transduced into cultures of rat primary cardiac fibroblasts. Transdifferentiation was determined 2 weeks post-transduction by FACS and immunofluorescence (IF) analysis for the activation of endogenous cardiac troponin T (cTnT) and other cardiac markers, as well as detection of *in vitro* contractile activity.
Results: Western blots demonstrated that triplet and singlet vectors yielded equivalent GMT transgene expression, and fluorescence activated cell sorting demonstrated that triplet vectors were nearly twice as efficacious as singlet vectors in generating induced cardiomyocytes from cardiac fibroblasts. Echocardiography demonstrated that GMT triplet vectors were more effective than the 3 combined singlet vectors in enhancing ventricular function from post-infarct baselines (triplet, 37% ± 10%; singlet, 13% ± 7%; negative control, 9% ± 5%; $P < .05$). FACS analysis demonstrated that the Ad encoding for GMT induced 7% of adult cardiac fibroblasts to express cTnT, compared with 6% cells treated with lentivirus encoding GMT, and 0.3% of cells treated with a null virus. Importantly, only AdGMT-treated cells demonstrated *in vitro* contractile activity, occurring 14 days after GMT administration.
Conclusions: These data have confirmed that the *in situ* administration of G, M, and T induces post-infarct ventricular functional improvement and that GMT polycistronic vectors enhance the efficacy of this strategy. Additionally, adenoviral vectors encoding GMT effectively reprogram cardiac fibroblasts into cardiomyocyte-like cells possessing *in vitro* contractile activity. The ability to directly reprogram fibroblasts to replace lost or malfunctioning cardiomyocytes opens a gateway for new therapeutics capable of regenerating cardiac muscle and repairing injured hearts.

Funding Source: Grant from James and Lisa Cohen to TKR and RGC.

The Hippo Signaling and Mechanical Tension Coordinately Regulates Epicardial Progenitor Cell Fate

Xiao Y[1,2,3], Zhang M [1,3], Morikawa Y, [1,2], Martin J.F.[1,2,3*]
[1]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX
[2]Texas Heart Institute, Houston, TX
[3]Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX

[*]Corresponding author: Department of Molecular Physiology and Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA, E-mail: jfmartin@bcm.edu

Epicardium is the outmost layer of heart and epicardial cells mainly becomes cardiac fibroblasts and smooth muscle cells during embryogenesis. In addition, epicardial cells and epicardial derived cells, EPDC, recently have emerged as a group of multi-potent cells making cellular and paracrine contribution to adult heart regeneration through reactivating embryonic gene program. Therefore, understanding the molecular mechanisms regulating epicardium development would be expected to offer new therapeutic targets in cardiac repair post injury.

Hippo signaling pathway is well-known for its role in organ growth control through regulating cell proliferation. One of the downstream effector of the pathway is the co-transcription factor Yap and its activity and nuclear-cytoplasmic shuffling are regulated by upstream kinase Lats1/2. In addition to Hippo dependent regulation, Yap activity is also influenced by mechanical tension and mediates mechanical cues dependent cell behavior alteration. Our recent studies revealed that knocking out Hippo signaling in adult cardiomyocyte promotes cardiomyocyte to re-enter cell cycle and enhance heart regeneration. However, the function of Hippo signaling and crosstalk with mechanical tension in EPDC is completely unknown.

To study the role of Hippo signaling in the development of epicardium and EPDC, we used epicardial specific line, *Wt1*^{CreERT2}, to conditionally delete *Lats1/2*. Cre activity was induced at embryonic day (E)11.5 and *Lats1/2* CKO hearts exhibited disorganized coronary vasculature at E14.5. We used genetic lineage tracing approach to track EPDC and revealed increased endothelial cells and decreased fibroblasts and smooth muscle cells, which suggested that deletion of *Lats1/2* in EPDC changed cell fate. To gain insight into molecular pathways involved in EPDC fate switching, we performed RNA-seq analysis. We found that signals regulating cytoskeleton organization and extracellular matrix were disrupted, which suggested the alteration of cellular mechanical force. Therefore, we further examined the shape and cytoskeleton organization of epicardial cells using super-resolution microscopy. We found these cells altered cell morphology from being flat and compact cells to spherical shaped cells by deleting *Lats1/2*. F-actin stained by phalloidin suggested disorganized cytoskeleton in *Lats1/2* CKO epicardial cells. To examine the alteration of mechanical properties of epicardial cell, we measured epicardial cell stiffness by atomic force microscopy in situ. Lats1/2 deficient epicardial cells became stiffer than control cells. Consistent with Hippo deficiency and enhanced cell stiffness, increased nuclear Yap were observed in mutant epicardial cells.

Since mechanical properties of cellular microenvironment are known to regulate cell shape and influence cell fate determination, we hypothesize that Hippo pathway involved in mechanical property regulation to affect EPDC fate. To recapitulate cell stiffness alteration, we plated epciardial cells and EPDC onto tunable stiffness substrate. On the hard substrate (10kpa), cells spread out in a flat shape and Yap translocated into nucleus. Majority of cells derived into endothelial cells. On the soft substrate (0.2kpa), cells compacted in a round shape and Yap was sequestered in the cytoplasm. None of cells gave rise to endothelial cells.

Taken to together, we concluded that Hippo signaling in EPDC modulates mechanical tension, and mechanical tension dependent cell shape alteration, which is critical for cell fate determination during embryogenesis.

Funding sources: Texas Heart Institute Cardiomyocyte Renewal Investigation Startup

Development of a Model Surface for Bioprosthetic Heart Valves

Fahrenholtz M[1], Wen S[1], Grande-Allen KJ[1]
1. Department of Bioengineering, Rice University, Houston, TX

Corresponding author:
Grande-Allen KJ, Department of Bioengineering, Rice University
6500 Main St. MS-142, Houston, TX, 77030 Email: grande@rice.edu

Congenital heart defects affect thousands of children annually, and in many cases, require replacement of the heart valves. Currently available bioprosthetic heart valve replacements are, unfortunately, still subject to complications such as thrombosis, inflammation, and calcification. To decrease the incidence of these complications, the surface of these bioprosthetics could be modified to encourage integration with patient tissues through endothelialization. However, tissue valves are inhomogeneous and difficult to analyze by standard methods, thus, we have constructed a layered hydrogel as a model surface which mimics the mechanical properties and reactive group availability of tissue-based valves.

The model surface was constructed by first entrapping collagen, a common component of the heart valve matrix, in a polyethylene glycol diacrylate (PEGDA) hydrogel. By including collagen, we were able to study the effect of glutaraldehyde, the fixative that acts on collagen to produce bioprosthetic valves, on reactive group availability, mechanics, and cell response. Next, an amine-rich peptide was covalently linked to the surface to provide additional reactive groups to better mimic the tissue surface for the optimization of surface modification. Tissue samples were obtained from porcine aortic valves, and fixed in glutaraldehyde, according to common methods in industry and the literature. All samples were analyzed by micropipette aspiration for surface mechanics and XPS and confocal microscopy for reactive group availability. Human endothelial cells were cultured in contact with tissue samples and the model surface to determine the effects of glutaraldehyde fixation on cell metabolic activity via the MTT assay.

As expected, significant increases in the stiffness of the surface were measured after fixation with glutaraldehyde in tissue samples; however, the effect was less pronounced for the model surface due to the lower protein density. The 4% PEGDA formulation of the model surface was successful in mimicking the mechanical properties of fixed tissue (effective modulus = 17.5 ± 2.7 and 17.5 ± 3.4, respectively), while 3.5% PEGDA corresponded to unfixed tissue (effective modulus = 4.39 ± 0.65 and 3.37 ± 1.00, respectively). XPS and confocal imaging revealed slightly lower reactive amine group density on the surface of the hydrogel as compared to fresh and fixed tissue. The results of the MTT assay revealed a significant decrease in cell metabolic activity when cultured in contact with fixed tissues as compared to control conditions. Treating the tissues with the common detoxification agents urazole and l-glutamic acid did not significantly recover metabolic activity levels. By contrast, regardless of treatment, gels did not show any significant toxicity towards cells.

In conclusion, we have successfully constructed a model surface which mimics important surface characteristics of bioprosthetic valve tissue. We show that by altering the hydrogel formulation, we are able to closely mimic the mechanical properties of both fixed (bioprosthetic) and fresh (control) tissues. Additionally, the reactive group availability on the model surface, while lower than tissue, will provide insight into how well we can expect surface modification strategies to adhere to the bioprosthetic valve. Finally, it is clear from the MTT assay results that glutaraldehyde has a significant detrimental effect on cells that cannot completely be overcome by the treatment methods tested. The model surface is not significantly cytotoxic, thus we may expect to see somewhat lower initial cell density when finally testing cell-surface interactions on our modified tissues. This model will enable facile testing and screening of various surface modification methods, and will be an important tool in translating emerging technologies for tissue integration to heart valve replacement devices.

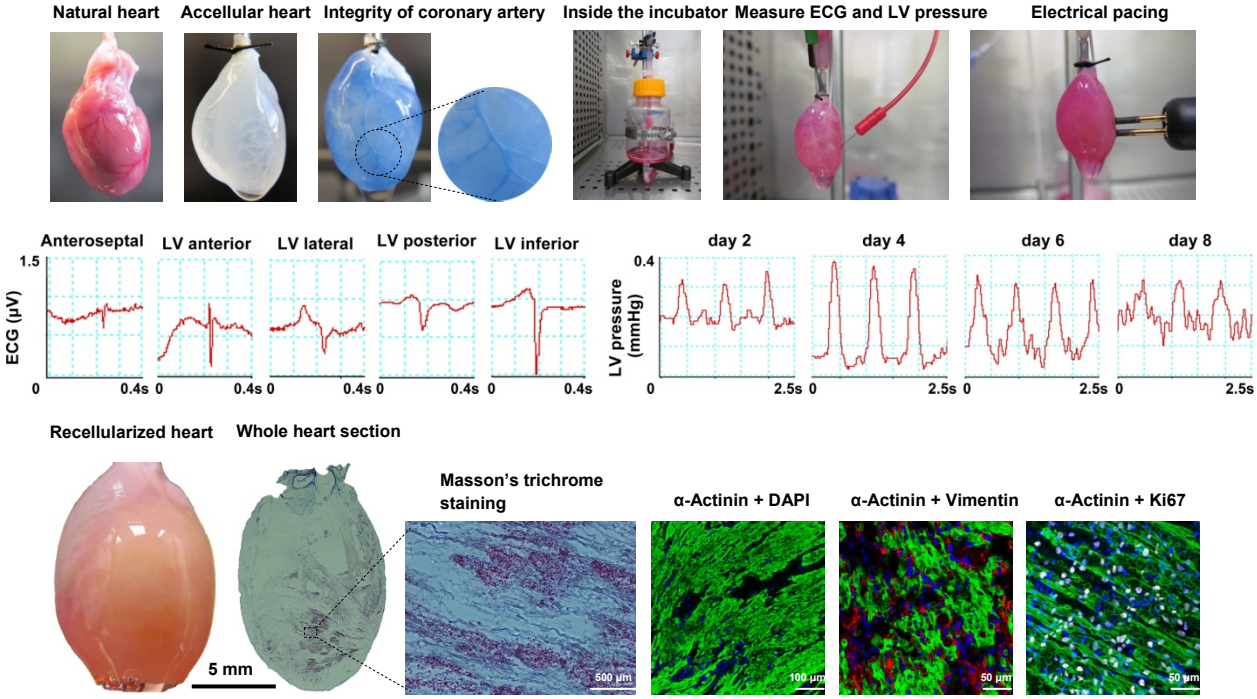
The authors would like to acknowledge funding from the March of Dimes and P.E.O. International.

Fabricating a Bioartificial Heart by Seeding a Rat Heart Scaffold with Neonatal Cardiac Cells

Tao ZW, Mohamed M, Hogan M, Salazar B, Patel NM, Birla RK
Department of Biomedical Engineering, Cullen College of Engineering, University of Houston, Houston, Texas

Corresponding author: Birla RK, Department of Biomedical Engineering, Cullen College of Engineering, University of Houston, 3605 Cullen Blvd, Rm 2005, Houston, TX 77204, Email: rkbirla@central.uh.edu

Objectives There is a chronic shortage of donor hearts. The ability to fabricate complete bioartificial hearts (BAHs) may be an alternative solution.
Methods Rat hearts were isolated and subjected to a detergent based decellularization protocol to remove all cellular components, leaving behind an intact extracellular matrix. One hundred million cardiac cells, isolated from neonatal rat hearts, were directly injected to populate the acellullar scaffolds. BAHs were maintained in a custom fabrication gravity fed perfusion culture system.
Results BAHs replicated a partial subset of properties of natural rat hearts. The highest spontaneous heart rate (116 ± 5 bpm) was detected on day 2 and the greatest LV pressure (0.35 ± 0.05 mmHg) was detected on day 4 after cell seeding; then both of them gradually decreased throughout culture. ECG QRS patterns from anteroseptal wall was Qr, LV anterior wall was rSr', LV lateral wall was QS, LV posterior wall was QS and LV inferior wall was rS or QS. The highest paced response frequency of the recellularized hearts was 5Hz. Addition of isoprenaline 1 nM, 10 nM and 100 nM sequentially caused dramatic heart rate increases; but premature ventricular contractions occurred when isoprenaline was at 100 nM. The recellularized hearts (0.42 g ± 0.07) are 36% of the mass of natural adult hearts (1.16 ± 0.13g). Masson's trichrome and immunofluorescence staining on whole heart sections showed a higher concentration of cardiac tissues were in the LV anterior, inferior and LV lateral walls than in the RV anterior wall.



Condition Media From Human Lung Fibroblast Grown on 4D Model Inhibit Cancer Metastasis

Mishra DK, Kim MP.
Department of Surgery, Houston Methodist Hospital, Houston, USA

Corresponding Author: Dr Min P Kim, MD, FACS, 6550 Fannin St, Suite 1661, Houston, TX-77030, Email:mpkim@houstonmethodist.org

Objective: Tumor microenvironment plays an important role in cancer development, progression and metastasis. Fibroblasts are important constituent of tumor microenvironment, which modulate the malignant progression by paracrine signaling. In current study, we plan to explore the role of normal fibroblast in tumor metastasis using our *ex vivo* 4D model.

Methods: Fibroblasts were isolated from tumor (CAF) and adjacent normal lung (LF) from a patient with adenocarcinoma of the lung using Thy1+ flow sorting. *Ex vivo* 4D model is created by decellularing a rat lung and heart block using SDS and Triton-X. The acellular lung is connected to a bioreactor that has flow through the pulmonary artery of media. The tumor is placed in the left side of the lung and the metastatic lesions are found on the right side. We seeded 4D lung cancer model with H460 lung cancer cells. They were treated with condition media from 4D model seeded with CAF or LF. We measured the primary tumor size, number of circulating tumor cells and number of metastatic lesions per HPF. We used student's t-test and used $p<0.05$ as significant value.

Results: Both CAF and LF grew in the acellular lung. The 4D model seeded with H460 treated with condition media from LF had significantly less metastatic lesion formation compared to the 4D model seeded with H460 treated with condition media from CAF. On day 9, we observed 1.8 cells vs 41.8 cells with LF vs CAF. Similarly, it was 22.9 vs 92.9 and 21 vs 200.1 cells on day 12 and day 15 respectively. However, there was no gross difference in primary tumor nodule size with either of conditioned media on *ex vivo* 4D model with H460 cancer cells.

Conclusions: Overall, this study suggests an inhibitory effect on metastasis due to presence of normal fibroblast conditioned media. Further comparison of conditioned media from normal fibroblast and CAF along with gene expression profile can provide an important insight, which may help in developing novel therapeutic regimens.

Funding:

MPK was funded by American Association for Thoracic Surgery

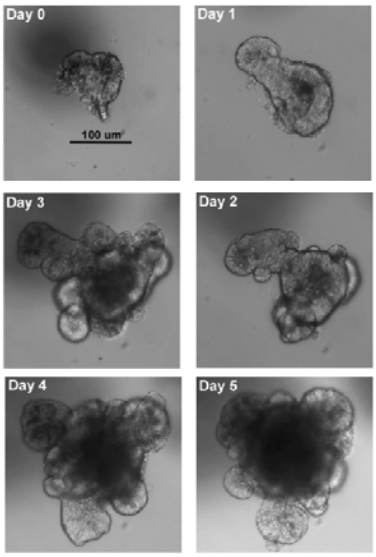
Three-Dimensional Enteroids As Novel Systems For The Study of Enteric Infections

Vela, L.E.[1], Zeng, X.L.[1], Estes, M.K.[1] and Maresso, A.[1]
1. Department of Molecular Virology & Microbiology, Baylor College of Medicine, Houston, Texas, USA

Corresponding Author:
Maresso A. Department of Molecular Virology and Microbiology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. Email: Maresso@BCM.edu

According to a 1999 World Health Organization report, six infectious agents are responsible for 90% of *infectious* disease deaths, with one death occurring every three seconds. Recent data demonstrates the number one bacterium responsible for diarrhea in children between 0-11 months is ***E. coli*** and it is in the top three in children 12-23 months old. Infections of the intestinal tract are the leading cause of mortality worldwide in all age groups. Knowledge of how ***E. coli*** interacts with the host intestinal epithelium, and the changes that result from this interaction, will lead to a better understanding of the pathophysiology of disease. Progress in understanding such infections is hampered by poor model systems.

Current infectious disease models are limited in that they rely on single, homogenous tissue culture of certain cell types to observe the host-pathogen interaction. Recognizing that the future of biomedicine lies in the development of versatile and competent models systems, we developed the first 3-dimensional intestinal organoid model system of bacterial infection to study the pathogenesis of infections. Enteroids represent the precursors to the growth of tissues *in situ*, and, as such, contain mucin, a lumen, crypts, villi, and the five different cell types prominent in the real mammalian intestine. The intestine of mammals contains a rich ecosystem of hundreds of different species of bacteria, with the occasional infection by an invading pathogen. We have use these mini-guts as infection surrogates for ***E. coli*** invasion and have successfully delivered bacteria into the lumen of these structures using two very different techniques: mechanical disruption and micro-injection. Hypothesizing that such structures will allow the study of ***E. coli ex vivo***, we demonstrate the successful colonization of enteroids of mouse and human origin with two ***E. coli*** pathotypes. We also demonstrate the functional characterization of these enteroids using immunofluorescence microscopy and Western blotting, and show we can control the dose, timing, and delivery of bacteria into such structures. Additional, we show that these enteroids have distinct biological responses to the introduction of mutated pathogen in comparison to wild-type ***E. coli***. By expanding the knowledge of how diarreahgenic ***E. coli*** cause disease in the intestine it will lead to better treatments against this global killer.



These models (murine and human) will facilitate research to further understand the mechanisms and interactions occurring in the intestinal system during an infection. We believe the future of biomedical science pivots on innovative and diverse experimental procedures. Growing, controlling, and experimentally manipulating 3D cultures is one way. These precursors of *ex vivo* organ growth have many benefits over current single-type cell cultures. The explosion of interest in the human microbiome, and the numerous reports linking it to human disease, are the force behind this objective. This is the first step towards using such models to understand the molecular basis of infection.

Polyethylene Glycol Hydrogels Functionalized with a Continuous Ile-Lys-Val-Ala-Val Concentration Gradient for Optimizing Neural Differentiation of Murine Embryonic Stem Cells in 2D

Yang Y-H[1], Ma C[2], Smith Callahan LA[1]

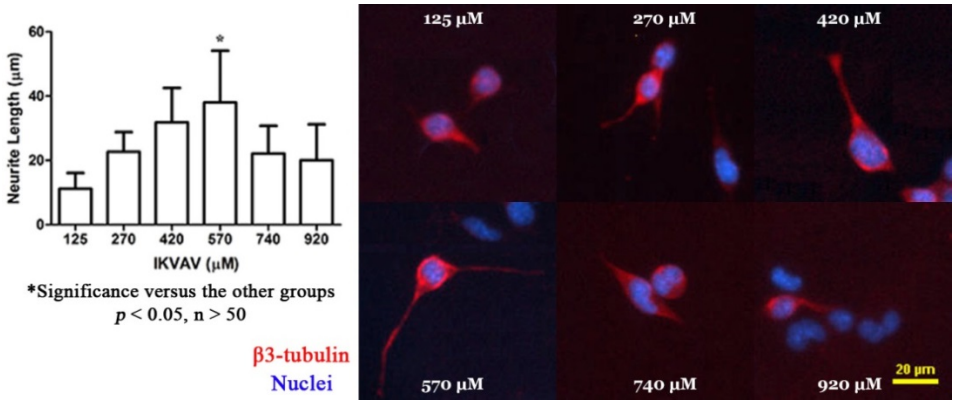
1. Department of Neurosurgery and Center for Stem Cell and Regenerative Medicine, University of Texas Health Science Center at Houston, Houston, Texas, U.S.A.
2. Department of Clinical Medicine, Shanghai Jiao Tong University, Shanghai, China

Corresponding author: Smith Callahan LA, Dept. of Neurosurgery, University of Texas Health Science Center at Houston, 1825 Pressler Street Suite 630F, Houston, TX 77030, Email: Laura.A.SmithCallahan@uth.tmc.edu

Spinal cord injuries (SCI) which can result in neurological impairments affect approximately 270,000 persons in the U.S. and cost the health care system over $10 billion per year in direct medical costs and disability support. Pluripotent stem cells (PSCs) capable of giving rise to all cell types in the body represent a potential therapeutic candidate for SCI repair. Due to inconsistency amongst currently available protocols for directed differentiation of PSCs, however, confounding outcomes have been identified. Therefore, there is a need to standardize culture parameters to maximize phenotype of differentiating cells derived from PSCs. The objective of this study was to develop a novel polyethylene glycol dimethacrylate (PEGDM) hydrogel system that possesses continuous chemically defined gradient properties in order to determine an optimal range of peptide concentrations for two-dimensional (2D) induction of neural differentiation of murine embryonic stem cells (mESCs). Ile-Lys-Val-Ala-Val (IKVAV) from laminin, a major component in the basement membrane that regulates cell adhesion, survival and migration, was used as the model bioactive peptide.

50 mm × 10 mm × 1 mm hydrogel gradients were assembled by dispensing 12% 10 kDa PEGDM solutions with and without 1.9 mM IKVAV through two syringe pumps running in inverse linear ramping profiles ranging from 0 mL/h to 52 mL/h, respectively, over 75s into a custom mold, followed by photopolymerization with 2.3 mJ/cm^2 for 6 min. After swelling in media, one 9.6-mm disc was punched out every 10 mm along each gradient, resulting in six discs per gradient. Discs were then evaluated for IKVAV concentration (C_{IKVAV}), swelling ratio, mesh size and mechanical properties. For cellular experiments, the cored discs were placed in the wells of 48-well plates, seeded with D3 mESCs and cultured with serum-free neural media for 6 days with medium exchanges every other day. Cell-seeded constructs were stained for neuron-specific β3-tubulin at day 3 and length of neurite extension was measured. Cultured cells were harvested at days 3 and 6 for analyses of gene expression, alkaline phosphatase (ALP) and apoptotic activities. Statistical significance was determined using one-way or two-way ANOVA in conjunction with the Bonferroni post-test with a p-value of < 0.05.

Assembled PEGDM hydrogels presented a linear C_{IKVAV} gradient ranging from 125 μM to 920 μM with an R squared value of .998 while other material properties remained similar throughout the gradient. Compared with the starting mESC population, cells cultivated with IKVAV at any levels exhibited inferior ES-related mRNA expression and secreted a lower content of ALP, a molecule highly expressed in ESCs, suggesting that the cells were undergoing differentiation. TUJ1 mRNA expression, an immature neuron marker, increased under all of the C_{IKVAV} conditions except for 920 μM at day 3 while the peak appeared in 420 μM and 570 μM groups at day 6. Similarly, the highest mRNA expression of MAP2, a mature neuron marker, was detected in both 420 μM and 570 μM samples at day 6. A significantly longer neurite extension was identified in the cells cultured with 570 μM IKVAV as shown in the figure. ESCs in the 920 μM group experienced a greater level of apoptosis during the cultivation which may account for their compromised neural differentiation.



Through development of a hydrogel gradient system to maximize neural differentiation of mESCs in 2D, our results suggest that better differentiation is achieved at a C_{IKVAV} between 420 μM and 570 μM whereas an excessive C_{IKVAV} can induce apoptosis in differentiating ESCs. Collectively, the present work demonstrates that hydrogels functionalized with bioactive peptides provide a defined and tunable platform and thus can be utilized to characterize culture conditions suitable for cell growth and differentiation.

Funding Sources: Start-up funds from Department of Neurosurgery and William Stamps Farish Fund

POSTER SESSION A

(in order by poster number)	Poster Session	Poster No.
Rapid and Efficient Preparation of Equine and Canine Regenerative Cells from Umbilical Cord Matrix using a Semi- Automated Process Almario MP, Martinez RF, Nazari-Shafti T, Bruno IG, Alt E, McClure SR, and Coleman ME.	A	2
The Misunderstood Cell: A Study of Valvular Endothelial Cell Phenotype Blancas AA, Balaoing LR, Acosta FM, and Grande-Allen KJ.	A	4
Arhgef4 Influences Oligodendrocyte Precursor Cells Recruitment At Lesion Site In A White Matter Injury Model Chaboub LS, Lee HK, and Deneen B.	A	6
Structural Comparison of 3D Printed Photosensitive Resin and Poly(ethylene glycol) Polymer Hydrogels by Digital Light Projection Photolithography Grigoryan B, Greenfield P, Ta A, and Miller JS.	A	8
Modular Hydrogel Design to Promote Salivary Gland Regeneration Harrington DA, Chapela PJ, Hubka KM, Kothari R, Martinez M, Wu D, Pradhan-Bhatt S, and Farach-Carson MC.	A	10
Three-Dimensional Artificial Heart Muscle to Supplement the Framework of a Bioartificial Heart Hogan MK, Mohamed MA, Tao ZW, Gutierrez L, and Birla RK.	A	12
Re-Differentiation of Culture Expanded Human Chondrocytes is Significantly Enhanced by Low Oxygen Tension Kean TJ, Whitney GA, Traeger G, Fernandes RJ, and Dennis JE.	A	14
Dysregulation of Hyaluronan Homeostasis and Metabolism is Mediated by Exogenous TGFβ and its Inhibitors in Aortic Valve Interstitial Cells Krishnamurthy VK, Stout A, and Grande-Allen KJ.	A	16
Hippo and Wnt Signaling in Cardiac Regeneration Leach JP, Heallen T, Morikawa Y, Tao G, and Martin JF.	A	18
Daam2-PIP5K is a novel regulatory pathway for Wnt signaling and therapeutic target for remyelination in the CNS Lee HK, Chaboub LS, Zhu W, Zollinger D, Rasband MN, Fancy SP, and Deneen B.	A	20
"Smart" Bone Cement: Synthesis and Characterization of Metronidazole Acrylate Livingston CE, Tatara AM, and Mikos AG.	A	22
Drug-Drug Interaction between Irinotecan and Taxol: Implication for Combination Therapy Mallick P and Ghose R.	A	24
Intra-Femoral Artery Perfusion in Mice; a Practical Method for Cell Delivery into Skeletal Muscles Matthias N, Hunt SD, Wu J, Robinson HA, Azhdarinia A, and Darabi R.	A	26
Effect of Gelatin Incorporation on the Mineralization and Osteogenesis of Stem Cell Laden Hydrogels Roh TT, Vo TN, and Mikos AG.	A	28
Effect of Variable Flow Perfusion on an In Vitro Tumor Model for Ewing Sarcoma Santoro M, Lamhamedi-Cherradi SE, Menegaz BA, Ramamoorthy V, Kasper FK, Ludwig JA, and Mikos AG.	A	30

POSTER SESSION B

Poster Abstracts

(in alphabetical order by presenting author's last name)	Poster Session	Poster No.
Microwell Fabrication by Laser Ablation of PDMS for Cancer Spheroid Generation Albritton JL, Flores JA, and Miller JS.	B	1
Rapid and Efficient Preparation of Equine and Canine Regenerative Cells from Umbilical Cord Matrix using a Semi- Automated Process Almario MP, Martinez RF, Nazari-Shafti T, Bruno IG, Alt E, McClure SR, and Coleman ME.	A	2
Valve Interstitial Cells Act in a Pericyte Manner Promoting Angiogensis and Transdifferentiation by Valve Endothelial Cells Arevalos CA, Walborn A, Nyguen J, Berg J, and Grande-Allen KJ.	B	3
Acute Myeloid Leukemia Cells Induce Osteogenic Differentiation in Mesenchymal Stem Cells through Up-regulation of RUNX2 Battula VL, Le PM, Sun J, Mc. Queen T, Somanchi A1, Wang R, Strunk D, Shpall EJ, Ruvolo P, Kantarjian H, Konopleva M, and Andreeff M.	B	47
The Misunderstood Cell: A Study of Valvular Endothelial Cell Phenotype Blancas AA, Balaoing LR, Acosta FM, and Grande-Allen KJ.	A	4
Human Intestinal Enteroid Cultures: A New Functional Model of Gastrointestinal Virus Infection Blutt SE, Saxena K, Ettayebi K, Zeng X, Broughman J, Crawford SE, Karandikar U, Conner ME, Foulke-Abel J, In J, Kovbasnjuk O, Zachos NC, Donowitz M, and Estes MK.	B	5
Arhgef4 Influences Oligodendrocyte Precursor Cells Recruitment At Lesion Site In A White Matter Injury Model Chaboub LS, Lee HK, and Deneen B.	A	6
Hydrogel-Based 3D Model of Patient-Derived Prostate Xenograft Tumors Suitable for Drug Screening Fong EL, Martinez M, Yang J, Mikos AG, Navone NM, Harrington DA, and Farach-Carson MC.	A	48
Differentiation of Human Progenitor Cells on Decellularized Cardiac Tissue Thibault RA, Chau E, Gordon AA, Gobin AS, Resende MM, Schwartz RJ, and Taylor DA.	B	7
Structural Comparison of 3D Printed Photosensitive Resin and Poly(ethylene glycol) Polymer Hydrogels by Digital Light Projection Photolithography Grigoryan B, Greenfield P, Ta A, and Miller JS.	A	8
Enhancing Bone Regeneration with Composite Microspheres that Reflect the Osteogenic Niche Haase C, Dodson C, Gregory C, and Kaunas R.	B	9
Modular Hydrogel Design to Promote Salivary Gland Regeneration Harrington DA, Chapela PJ, Hubka KM, Kothari R, Martinez M, Wu D, Pradhan-Bhatt S, and Farach-Carson MC.	A	10
Hippo Signaling and Mitophagy in Cardiomyocyte Renewal Heallen T, Zhang M, Leach JM, and Martin JF.	B	11
Three-Dimensional Artificial Heart Muscle to Supplement the Framework of a Bioartificial Heart Hogan MK, Mohamed MA, Tao ZW, Gutierrez L, and Birla RK.	A	12
Efficacy Of Platelet Rich Plasma For Refractory Lateral Epicondylosis Jayaram P and Malanga G.	B	13
Re-Differentiation of Culture Expanded Human Chondrocytes is Significantly Enhanced by Low Oxygen Tension Kean TJ, Whitney GA, Traeger G, Fernandes RJ, and Dennis JE.	A	14

Microwell Fabrication by Laser Ablation of PDMS for Cancer Spheroid Generation

Albritton JL[1], Flores JA[1], Miller JS[1]
1. Department of Bioengineering, George R. Brown School of Engineering, Rice University, Houston, TX

Corresponding author:
Albritton JL, Dept. of Bioengineering, Rice University
6100 Main St, MS-142, Houston, TX 77030. E-mail: jacob.albritton@rice.edu

Abstract:

Tumor invasion of the surrounding extracellular matrix leads to metastasis. Multicellular cancer spheroids are commonly used to study invasion; however, spheroids are difficult to manufacture uniformly in large quantities. Fabricated microwells are a good solution, but commercial sources are expensive and photolithographic methods for generating new well molds are costly and time-consuming. Using a commercially available CO2 laser cutter and the control afforded by open-source firmware and software, our lab has developed a rapid prototyping method for generating microwells by laser ablation of poly(dimethylsiloxane) (PDMS). Previous work has shown that ablation of PDMS typically yields a conical well geometry, but with absolute control over the x, y, and z-axes, laser power, and residence time, we show other geometries are possible through multiple iteration and out-of-focus ablation. Furthermore, dense packing of microwells increases efficiency of spheroid generation from potentially limited cell sources. The facile generation of uniform and inexpensive microwells for cell culture will allow our group and others to effectively generate cancer spheroids for 3D matrix invasion studies.

Rapid and Efficient Preparation of Equine and Canine Regenerative Cells from Umbilical Cord Matrix using a Semi- Automated Process

Almario MP[1], Martinez RF[1], Nazari-Shafti T[2], Bruno IG[1], Alt E[2], McClure SR[3], Coleman ME[1]
1. InGeneron, Inc. 8205 El Rio St., Houston, Texas, USA
2. Tulane University, Department of Surgery, 1430 Tulane Ave., New Orleans, Louisiana, USA
3. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA.

Corresponding author:
Coleman ME, InGeneron, Inc 8205 El Rio St., Houston, Texas, USA, Email: mcoleman@ingeneron.com

Mesenchymal stem cells (SCs), primarily from bone marrow (BM) and adipose tissue (AT), have proven to be effective in reducing inflammation and promoting tissue regeneration and healing. The matrix of the umbilical cord (UCM/Wharton's Jelly) contains a high concentration of viable, rapidly dividing umbilical cord stem cells (UC-SC) that may be used to treat the newborn or banked for future use. To date, protocols that have described the isolation of SCs from UCM require up to 24 hours to process and rely on expansion in culture prior to use. Acquiring large numbers of UC-SC at the time of isolation may eliminate the need for expansion in cell culture and associated costs and potential changes in cell phenotype and bioactivity.

This protocol describes processing, filtration, and centrifugation of canine and equine UCM tissue using a semi-automated commercial system (ARC® system) that enables reproducible and efficient isolation of regenerative cells including UC-SCs within 2 hours. Hence, the protocol overcomes the need for culture expansion typically employed to obtain 1×10^7 or more cells required for many clinical applications.

Processing using the ARC® system resulted in $1.65 \pm 0.45 \times 10^6$ UC-SC/g equine UCM with viability of $93\% \pm 4\%$ (out of 5 biological replicates) and $1.08 \pm 0.18 \times 10^6$ UC-SC/g canine UCM with viability of $70\% \pm 9\%$ (biological triplicate). Equine umbilical cords were obtained shipped overnight at 4^oC with collaboration of Dr. Scott McClure at Iowa State University. Canine umbilical cords were collected with owner consent by local veterinarians and shipped overnight at 4^oC. Cells were all processed within 24 hours of collection at birth. Colony forming fibroblast (CFU-F) assay revealed high regenerative potential (colony forming units: $13\% \pm 2\%$). Gene expression profile and proliferation potential of UC-SCs was consistent with that of fetal and neonatal SCs. Differentiation assays confirmed the ability of UC-SCs to differentiate into cells of the ectodermal (neuronal) and mesodermal (adipogenic, chondrogenic, osteogenic) lineage as previously reported. (*SM Hoynowski et al. 2007. Biochem. Biophys. Res. Commun. 362:347*).

Valve Interstitial Cells Act in a Pericyte Manner Promoting Angiogensis and Transdifferentiation by Valve Endothelial Cells

Arevalos CA, Walborn A, Nyguen J, Berg J,Grande-Allen KJ

K. Jane Grande-Allen, Ph.D. , Dept. of Bioengineering, Rice University
6100 Main St., MS 142, Houston, TX 77005, 713-348-3704, grande@rice.edu

Introduction: The most common aortic valve disease is calcific aortic valve disease (CAVD), but there are no non-surgical treatments for this condition due to a lack of knowledge about valve biology. There is growing interest in the neovascularization of the aortic valve, which is normally avascular, during CAVD progression. Alpha smooth muscle actin (aSMA) positive cells, markers of pericytes and valve interstitial cells (VIC), have been localized histologically along the exterior of these neovessels, suggesting that VICs can differentiate into a proangiogenic pericyte-like cell type. Due to the potential benefits of an anti-angiogenic therapy for the treatment of CAVD, this study investigated the perivascular potential of VICs to stabilize valve endothelial cell (VEC) angiogenesis and transdifferentiation.

Methods: In the present study, VICs were co-cultured short and long term with VECs in a matrigel model in comparison to the vascular derived 10 T1/2 fibroblast pericyte precursors (10 T1/2) and Mouse microcirculatory endothelial cells (MCEC) over a 7 day co-culture. The two cell types were tracked using a PKH dye and live confocal fluorescence microscopy in order to identify their spatial relationships with one another with time. A leader-follower inspired tracking algorithm was used to track VEC/VIC interactions with time to establish what proportion of each cell type was leading the other. Immunocytochemistry was used to stain for angiogenic marker expression in VEC/VIC cocultures. VEC/VIC dynamics were altered using small molecule inhibitors to find important pathways in VEC/VIC angiogenic pathways.

Results: All VEC/VIC co-cultures displayed higher lacunarity vascular networks at 7 hours compared to VEC and a vascular endothelial cell control. Surprisingly, all valve cell conditions spontaneously formed spheroids by 24 hours except the ROCK inhibited condition, which maintained a flat vascular network formation as reported previously. Untreated VEC/VIC co-cultures invaded into the Matrigel significantly more than in all other conditions. ROCK inhibition prevented VEC/VIC network contraction, PMA treated valve co-cultures formed spheroids but did not sprout, Angiopoietin 1 treatment inhibited VEC network contraction, and inhibition of the Angiopoietin1-Tie2 pathway inhibited VEC/VIC spheroid formation and network formation.

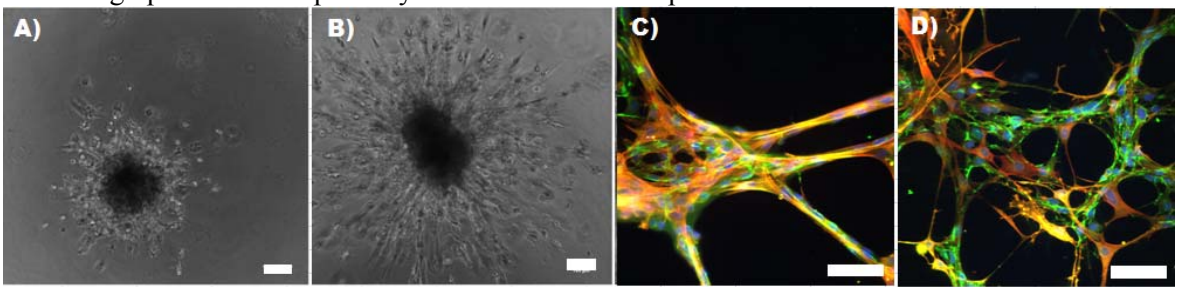


Figure 1. Representative images of A) VEC controls and B)VEC/VIC co-cultures after 7 days. Representative ICC images of C) VEC/VIC and D) Rock inhibitor treated VEC/VIC co-cultures after 7 hours. (Green: CD31, Red: F-actin, Yellow: aSMA, Blue: Dapi)

Discussion: These results align with current hypothesises of the pathology of CAVD and offer new insights into the VEC/VIC dynamics during its progression. The change from a vasculogenic network formation to an invasive spontaneous spheroid formation by VECs grown in long term culture suggests the VECs are undergoing transdifferentiation. Interestingly, VICs promoted this sprouting into the matrix and were found wrapped around the VECs at each stage suggesting a pericyte-like phenotype. VICs were found on the tips of most sprouts when co-cultured with VECs, mimicking tip cells, a traditional endothelial cell phenotype found on the leading edge of an invading angiogenic sprout. This study provides evidence for the importance of ROCK , Angiopoietin, and PKCα pathways in VEC/VIC related pathologies. Better understanding of VEC/VIC biology, these interactions could be used to develop therapies for CAVD.

This work was funded by American Heart Association grant 11IRG5550052 and a National Science Foundation Graduate Research Fellowship (C.A.A).

The Misunderstood Cell: A Study of Valvular Endothelial Cell Phenotype

Blancas AA, Balaoing LR, Acosta FM, Grande-Allen KJ
Department of Bioengineering, Rice University, Houston, Texas

Corresponding Author:
Grande-Allen KJ, MS 142, Rice University
6100 Main, Houston, Texas 77005

The endothelial cell population that comprises the outer layer of heart valves is unique in comparison to vascular populations. Although there has been some characterization of valve endothelial cells (VEC), more thorough analysis is necessary in order to generate an appropriate seeding endothelial population for valve replacement therapies. In this study, porcine VEC are compared to porcine aortic endothelial cells (PAEC) using several assays to determine similarities and differences between both populations. Understanding how the phenotype of VEC compares to vascular endothelial cells (EC) could also further elucidate mechanisms of valve dysfunction and disease.

Funding sources: NIH R01HL107765 and March of Dimes 1-FY08-409

Human Intestinal Enteroid Cultures: A New Functional Model of Gastrointestinal Virus Infection

Blutt SE[1], Saxena K[1], Ettayebi K[1], Zeng X[1], Broughman J[1], Crawford SE[1], Karandikar U[1], Conner ME[1],Foulke-Abel J[2], In J[2], Kovbasnjuk O[2], Zachos NC[2], Donowitz M[2], and Estes MK[1]
1. Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX
2. Department of Medicine, Gastroenterology Division, Johns Hopkins University School of Medicine, Baltimore, MD

Corresponding Author: Estes MK, Dept. of MolViro and Micro, Baylor College of Medicine
One Baylor Plaza, Houston TX 77030, E-mail: mestes@bcm.edu

A significant limitation in translational research in the gastrointestinal tract is the absence of reliable pre-clinical models that recapitulate human physiology and disease pathology. We have established models of the small intestinal epithelium using advancements in stem cell biology pioneered by the Clever's Lab (Hubrecht Institute). Human intestinal enteroids (HIEs) self-organize into villus-crypt structures, which contain the normal complement of intestinal epithelial cell types (enterocytes, goblet, enteroendocrine, and Paneth cells) and are physiologically active based on responses to agonists. We have characterized these new, non-transformed, 3D HIE cultures as pre-clinical models of human rotavirus (HRV) infection, which kills nearly 500,000 children annually. Studies on HRVs have been limited as they are difficult to culture in transformed cell lines and do not infect small animals. RVs generally exhibit host range restriction in replication. Thus, HRV does not replicate in mice whereas mouse RV and some other animal RVs (ARV) exhibit a broader host range and do replicate in mice.

In order to determine whether RVs exhibit host range restriction in HIEs, we inoculated differentiated and undifferentiated HIEs from several patients with either HRVs or ARVs from a bank of HIEs (N=52) derived from patient small intestine biopsies or surgical tissues. We quantitated the (1) amount of viral RNA by qRT-PCR, (2) number of infected cells using flow cytometry and immunofluorescence confocal microscopy, and (3) yield of virus by fluorescent focus assay. HRVs infected more cells within HIEs than ARV (50% compared to 13%). Undifferentiated HIEs, consisting primarily of immature enterocytes and stem cells, were less susceptible to infection compared to fully differentiated HIEs that consist predominately of mature enterocytes (12.6% compared to 37.6%). Like HRV, infection with ARV was also higher in differentiated HIEs compared to undifferentiated HIEs (13% compared to 8%). Electron microscopy and immunofluorescence studies validated that HRV-infected HIEs show classical features of RV-infected cells, including presence of virus factories (viroplasms) and induction of lipid droplets. HRV causes diarrhea by several mechanisms including modulating fluid flow through increased apical chloride channel activity. We investigated whether HRV or the RV enterotoxin, NSP4, cause physiological changes in HIEs. Through time-lapse microscopy, we observed that both HRV infection and NSP4 treatment of HIEs induced luminal swelling indicative of increased fluid secretion.

These findings establish HIEs as new models to understand host physiology, disease pathophysiology and the intestinal epithelial response, including host restriction to gastrointestinal infections such as HRV infection. HIEs allow us to address new questions about human host-pathogen interactions such innate immune responses, stem cell activity, cell-cell communication within the epithelium in response to infection as well as to identify and test new drug therapies to prevent/treat diarrheal disease. In addition, HIEs provide a physiologically relevant model system to examine the role of the microbiota in altering infection or drug treatment.

Funding: National Institutes of Health Grants U18-TR000552, R01-AI080656, P30-DK056338 (Baylor), and P30-DK089502 (Hopkins) and Howard Hughes Medical Research Fellowship 57007689.

Arhgef4 Influences Oligodendrocyte Precursor Cells Recruitment At Lesion Site In A White Matter Injury Model

Chaboub, LS[1,2], Lee, HK[2], Deneen, B[1,2,3].
1. Program in Developmental Biology, Baylor College of Medicine, Houston, USA
2. Center For Cell And Gene Therapy, Baylor College of Medicine, Houston, USA
3. Department of Neuroscience, Baylor College of Medicine, Houston, USA

Corresponding author:
Chaboub, LS, Program in Developmental Biology and Center For Cell And Gene Therapy, Baylor College of Medicine, Houston, TX, 77030, USA. E-mail: chaboub@bcm.edu

While very diverse in their origins and presentations, demyelination disorders of the central nervous system (CNS) share common cellular events. Recruitment of oligodendrocyte precursor cells (OPCs) at the lesions site, which normally function to remyelinate axons, is an especially important step in proper remyelination, and has been shown to be defective in at least two disorders; multiple sclerosis and hypoxic ischemic encephalopathy (HIE). In order to understand how these cellular events occur during the disease state, it is imperative to understand how these same processes are regulated during normal development. Therefore, identification of novel genes involved in glial cell development might shed light into demyelination disorders, especially white matter injury (WMI).

In a developmental screen performed in the spinal cord, we recently identified Arhgef4 as a novel gene expressed in both glial precursor cells and mature astrocytes. Arhgef4 is a guanine exchange factor for the Rho GTPase family of protein and has been shown to regulate migration in other systems. During spinal cord development, we showed that Arhgef4 influences glial precursor cells migration. We hypothesize that Arhgef4 would also influence migration of precursor cells in disease state. Lysolecithin injection into the ventral white matter region of the spinal cord is a well-recognized model of WMI where the kinetics and cellular events of remyelination are well established. Using this model, we showed that Arhgef4 is expressed in the lesion area, validating the use of this technique to further our understanding the role of Arhgef4 in WMI. Lesions performed on Arhgef4 germline knock-out (KO) animals appear to have a delayed remyelination kinetics when compared to heterozygous littermate animals. Furthermore, Arhgef4 KO lesions present with a significant decrease in the number of OPCs present inside the lesion area, suggesting that the recruitment of OPCs at lesion sites is inhibited in the absence of Arhgef4.

We will utilize in vitro OPC cultures to differentiate whether Arhgef4 influences the migration of OPCs into the lesion site or their proliferation. Ongoing studies will focus on further characterizing Arhgef4 KO lesions as well as performing misexpression of Arhgef4 into the lesion to assess whether it can accelerate remyelination. In addition we will assess the effect of Arhgef4 expression in a mouse WMI hypoxic model that closely mimics HIE.

This study identified a novel player in OPCs recruitment at the lesion site during remyelination events. Guanine exchange factors, such as Arhgef4, are potentially good target for therapies since they affect critical cellular pathways while being tissue specific.

Funding source provided by CPRIT RP101499

Differentiation of Human Progenitor Cells on Decellularized Cardiac Tissue

Thibault RA[1], Chau E[1], Gordon AA[1], Gobin AS[1], Resende MM[1], Schwartz RJ[1], Taylor DA[1]
1. Department of Regenerative Medicine, Texas Heart Institute, Houston, TX

Corresponding Author: Taylor DA, Department of Regenerative Medicine, Texas Heart Institute, MC 3-116, PO Box 20345, Houston, TX 77225 Email: dtaylor@texasheart.org

The generation of a contractile human cell-based cardiac patch for clinical use will likely require the differentiation of human stem or progenitor cells into the major cardiac components: cardiomyocytes, fibroblasts, neurons, and vascular cells. The extracellular matrice (ECM) is known to influence stem cell differentiation as it is comprised of structural and instructional proteins. Of importance are heparan sulfated proteoglycans (HSPGs), which interact with growth factors that can affect lineage differentiation. Growth factors, such as bFGF and VEGF, can bind to HSPGs and control the differentiation of endothelial progenitor cells and mesenchymal stem cells into mature endothelial cells and smooth muscle cells, respectively. It has also been demonstrated that cardiac progenitor cells cultured on decellularized pericardium increased expression of early cardiac markers. Previous work in this laboratory established the retention of the major ECM constituents including glycosaminoglycans (GAGs), collagen types I and III, laminins, and fibronectin after sodium dodecylsulfate (SDS) perfusion decellularization of cardiac tissue. We hypothesize that the decellularized left ventricular tissue (dLV) combined with growth factor containing culture media directs progenitor cells towards mature cell lineages. In this study, gene expression levels of endothelial, smooth muscle, cardiac, and stem cell markers were analyzed in human adipose-derived stem cells (hADSCs), human blood derived endothelial colony-forming cells (hECFCs), and MESP1/ ets2 reprogrammed hADSCs (rhADSCs) cultured on dLVs.

Hearts were isolated from rats and decellularized via SDS perfusion. dLVs were removed and a 10 mm circular patch obtained. SDS and DNA assays were performed on the acellular patches. Immunofluorescence for VEGF, bFGF, or TGF-β, and Alcian Blue staining for mucosubstances were performed on both acellular dLV and dLV incubated in each media type. Confluent cell densities of hADSCs, hECFCs, or rhADSCs were cultured on the endocardial surface of the dLV for 4 or 21 days under static conditions in 1) MesenPRO plus RS growth supplement, 2) Endothelial Growth Media-2 containing VEGF, IGF-1, bFGF, and EGF, or 3) α-MEM containing heat-inactivated horse serum and dexamethasone, respectively. Cells cultured on rat collagen type I gels were used as controls. Expression levels of endothelial, cardiac, smooth muscle, and stem cell markers were quantified by using real time PCR and normalized to cells cultured on tissue culture plastic. Masson's trichrome staining was used to visualize cellular distribution throughout the cell-seeded patches. Care of the rats and hECFCs were obtained in accordance with protocols approved by the Texas Heart Institute IACUC and IRB.

Acellular dLV and media soaked dLV retained VEGF, bFGF, TGF-β, and GAGs. Negligible amounts of DNA remained and no detectable SDS was present in the acellular dLV. The hECFCs cultured on dLV downregulated expression of CD31 at day 21. At 4 days, rhADSCs upregulated connexin 43, dHAND, and NKX2.5, while at day 21 connexin 43, dHAND, troponin T type 2, and myosin heavy chain 7 were upregulated and nkx2.5 was downregulated when compared to the cells cultured on collagen gels. The hADSCs did not upregulate any cardiac, vascular, or stem cell genes; however these cells proliferated to a high degree and infiltrated the patch.

Human progenitors of several types survived and upregulated gene expression consistent with increased lineage-specific maturation on dLV. hECFCs and rhADSCs showed enhanced expression of endothelial and cardiac genes, respectively. The upregulation of these markers may be due to the presence of GAG-bound growth factors within the decellularized tissue or simply reflect an enhanced matrix based response in conjunction with supplements added to the media. Future studies will explore the co-culture of hECFCs, hADSCs, and rhADSCs on dLVs for synergistically enhanced differentiation.

This work was supported in part by the American Heart Association Jon Holden DeHaan Foundation and by Houston Endowment, Inc. We would also like to thank Dr. D. Timms for use of the 3D printer.

Structural Comparison of 3D Printed Photosensitive Resin and Poly(ethylene glycol) Polymer Hydrogels by Digital Light Projection Photolithography

Grigoryan B[1], Greenfield P[1], Ta A[1], Miller JS[1]
1. Bioengineering Department, Rice University, Houston, Texas

Corresponding author:
Grigoryan B, Bioengineering Dept., Rice University
6100 Main St, MS-142, Houston, TX 77030, Email: bagrat.grigoryan@rice.edu

Motivated by the lack of available organs for transplantation, the field of tissue engineering offers the potential to develop patient-specific 3D tissue constructs with defined spatial control. However, our field generally lacks the ability to recreate, *in vitro*, the heterogeneous patterns of cells and matrix which are hallmarks of native tissue. Three-dimensional (3D) printing techniques are now offering researchers the ability to control the shape and architecture of constructs with high precision. Our lab has developed an open-source digital light projection (DLP) photolithography system to 3D print tissue constructs made from photoactive materials. We printed structures made from commercially available acrylate resins as well as synthetic hydrogels based on poly(ethylene glycol) diacrylate. Quantification of print fidelity and reproducibility were determined for both resins and polymer hydrogels. In addition, time sweeps examined from photorheological experiments were used to optimize exposure settings to obtain constructs with appropriate "green strength" between layers. The controlled exploration of the wide parameter space available with DLP photolithography will allow us to engineer 3D matrices for future studies assessing cell behavior in heterogeneous and physiologically relevant settings.

This work was supported in part by NSF Graduate Research Fellowship (BG).

Enhancing Bone Regeneration with Composite Microspheres that Reflect the Osteogenic Niche

Haase C[1], Dodson C[1], Gregory C[2], and Kaunas R[1]

1. Department of Biomedical Engineering, Texas A&M University, College Station, TX
2. Department of Molecular & Cellular Medicine and Institute for Regenerative Medicine, Texas A&M Health Science Center, Temple, TX

Corresponding author: Haase C, Department of Biomedical Engineering, Texas A&M University, 5045 Emerging Technologies Building MS 3120 College Station, TX 77843, E-mail: candyhaase@gmail.com

Introduction: Of the 5.6 million bone fractures that occur annually in the United States, about 10% fail to repair[1]. As a result there has been interest in using human mesenchymal stem cells (hMSCs) in bone regeneration because of their ability to assist damaged tissues to heal themselves. Systemic injection of hMSCs results in dilution and tends to form lethal pulmonary emboli in lungs, suggesting that direct implantation or injection is a more favorable approach[2]. We have generated cell-laden collagen microspheres[3] that can be locally implanted or injected into the bone defect. There is therefore a need for an engineered composite microsphere that provides adequate cellular responses for rapid and reliable healing of bone fractures to avoid surgeries. The canonical Wingless (cWnt) signaling pathway is imperative during healing of bone fractures and embryonic bone development[4]. We have demonstrated that inhibiting peroxisome proliferator-activating receptor gamma with GW9662 reduces negative cross-talk on the cWnt pathway resulting in the establishment of a pro-osteogenic hMSC phenotype (hereafter referred to as *osteogenically enhanced hMSCs*, OEhMSCs). OEhMSCs secrete an extracellular matrix that mimics the composition of anabolic bone tissue (hMatrix). We evaluated the effect of delivering OEhMSCs with hMatrix on osteorepair and are developing osteogenic microspheres for co-administration of hMatrix and OEhMSCs that will facilitate cell retention *in vivo*.

Materials and Methods: Polyethylene glycol diacrylate (PEGDA) microspheres are produced using a continuous-flow emulsion and photocrosslinking technique. The continuous phase is light mineral oil with 0.5% Span 80. The dispersed phase is a solution of 30% PEGDA and 4% Irgacure 2959 in PBS. The platform is able to produce monodisperse microspheres ranging from 200 to 600 µm by changing the flow rate of PEGDA solution. The hMatrix is then functionalized (Acrylate-PEG-hMatrix) and crosslinked to the sphere surface to promote cell adhesion. Calcein-AM and Propidium Iodide staining are used to assess cell viability. Alkaline phosphatase kinetics, alizarin red staining, qRT-PCR, and ELISA for osteogenic markers are used to assess osteogenesis.

Results and Discussion: OEhMSC administration into murine calvarial defects resulted in 60% healing in 3 weeks[5]. However, the hMSCs didn't remain at the lesion during the remodeling phase of bone repair. To increase stem cell retention, OEhMSCs were administered with hMatrix and the results demonstrated 80-100% healing after 3 weeks[5]. Additionally, the hMSCs remained at the lesion after the remodeling phase of bone repair. An osteogenic microsphere was developed to deliver hMatrix to hMSCs in order to promote stem cell retention. Initial feasibility of this process was demonstrated using collagen type I since this is the major component of hMatrix (Figure 1A). We have also assessed functionalized collagen crosslinking to PEGDA sphere surface by immunostaining for collagen type I (Figure 1B).

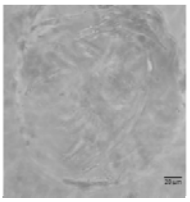
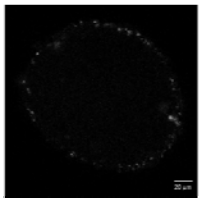
A  B 

Figure 1. A. Phase contrast image of hMSCs spreading on composite microspheres consisting of functionalized collagen (Acrylate-PEG-Collagen) crosslinked to the surface of a PEGDA microsphere. Scale bar, 20 µm. **B.** A confocal slice of a PEGDA microsphere immunostained for collagen type I to assess functionalized collagen crosslinking to the surface. Scale bar, 20 µm.

Conclusions: Conventional hMSC-based therapies have had limited success in bone regeneration. hMatrix increased OEhMSC retention at the bone defect, resulting in dramatically improved bone repair. Injectable microspheres that incorporate GW9662 and hMatrix will facilitate translation of this strategy to the clinic. The composite microsphere approach for stem cell delivery holds great potential for regenerative medicine in other tissues as well.

Acknowledgements: This material is based upon work supported by the National Science Foundation Graduate Research Fellowship DGE-1252521 and project grant CBET-1264848.

References: [1]Mathew, G. et al. *Indian J Orthop* 2009; [2]Lee, R. et al. *Cell Stem Cell* 2009; [3]Hong, S. et al. *Lab Chip* 2012; [4]Silkstone, D. et al. *Nature* 2008; [5]Zeitouni, S. et al. *Sci Transl Med* 2012

Modular Hydrogel Design to Promote Salivary Gland Regeneration

Harrington D A,[1] Chapela P J,[1] Hubka K M,[2] Kothari R,[1] Martinez M,[1] Wu D,[1] Pradhan-Bhatt S,[3] Farach-Carson M C[1,2]

2. Department of BioSciences, Rice University
3. Department of Bioengineering, Rice University
4. Helen F. Graham Cancer Center & Research Institute, Center for Translational Cancer Research (CTCR), University of Delaware, Newark, DE

Corresponding author:
Farach-Carson, M C, Department of BioSciences, Rice University
6100 Main St., MS-601, Houston, TX 77005

The salivary glands (parotid, submandibular, sublingual) collectively provide a unique target for regenerative medicine from many perspectives: their elegant branched structure enables secretory products to be produced, concentrated, and delivered to the oral cavity. Unlike other exocrine organs, the salivary glands are largely accessible. Attention to their regeneration is unique in dental tissue engineering as compared to other targets, which are often mineralized tissues or their high modulus replacements. From an engineering perspective, salivary gland regeneration requires and enables the use of low modulus hydrogels, which are among the most amenable substrates to modification with biologically-derived peptides, in an effort to mimic the function, composition, and structure of native ECM. From a biological perspective, salivary regeneration from a single-cell population challenges our ability to understand and control cell phenotype, polarity, and communication.

Clinically, dysfunctions of the salivary gland remain only partially understood and palliatively treated. Xerostomia, or "dry mouth," is a symptom of salivary disease; it may be due to a systemic autoimmune response, as is observed in Sjögren's syndrome. A primary focus of our work is the relief of *induced* xerostomia, which may occur in patients with head and neck cancers (HNC). Xerostomia arises in these patients not due to effects from the cancer (which is successfully resolved by surgical removal of the primary tumor and targeted radiation treatment to the surrounding area), but because of selective damage to the secretory salivary acinar cells as a result of radiotherapy. The unexpected consequence of rendering patients cancer-free is a lifetime of dental issues that result from poor or nonexistent saliva output.

Our team of cell biologists, materials engineers, and clinical experts has devised a modular hydrogel system with features designed to promote the expansion of healthy salivary biopsies *in vitro*, and their re-assembly into multicellular aggregates. Our system incorporates relevant elements from the basement membrane and surrounding ECM in an effort to promote proper cell organization and polarity. Growth factors are delivered from synthesized depots to encourage salivary cell branching and maturation, while other depots deliver factors to promote angiogenesis and/or neurogenesis. By employing classic tissue engineering paradigms and incorporating known cues from the native tissue, we are attempting to create neoglands that can be reintegrated into the preserved ductwork in these HNC patients, and restore salivary function.

Funding Acknowledgement: This work is supported by NIH/NIDCR R01 DE022969, and philanthropic contributions to Christiana Care Health System. DW is supported by NIH/NIDCR F32 DE024967. MM is supported by NIH/NIDCR R01 DE022969-S1, and NSF GRFP 1450681.

Hippo Signaling and Mitophagy in Cardiomyocyte Renewal

Heallen T[1, 2], Zhang M[2], Leach JM[2], Martin JF[1, 2]
5. Cardiomyocyte Renewal Lab, Texas Heart Institute, Houston, TX
6. Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX

Corresponding author:
Martin JF, Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine
One Baylor Plaza, Room 509E, Mail Stop 335, Houston, TX, 77030. E-mail: jfmartin@bcm.edu

Background: Defects in the clearance of damaged mitochondria (mitophagy) lead to cardiomyopathy and heart failure. Recent work from our lab revealed that the Hippo-signaling pathway inhibits postnatal cardiomyocyte renewal and regeneration. Our preliminary data strongly suggest that Hippo signaling inhibits mitophagy in damaged or stressed myocardium. Moreover, these data suggest that the Hippo effector Yap regulates cardiac expression of mitochondrial quality control genes, including Parkin (Park2). Altogether, we hypothesize that Yap promotes mitophagy and cardiomyocyte survival.

Objective: Hippo-signaling is a key negative regulator of postnatal cardiomyocyte regeneration. To investigate the hypothesis that the Hippo effector Yap positively regulates mitophagy to promote cardiomyocyte survival, we are evaluating mitophagy and Parkin activity in Hippo deficient cardiac models. We inactivated core pathway gene Salvador in a cardiac apex resection model to determine the influence on the cardiomyocyte cell cycle and survival.

Methods: To disrupt Hippo signaling in all studies, Salvador was conditionally deleted in mouse cardiomyocytes using the inducible Cre line *Myh6-Cre/Esr* via tamoxifen injection. We performed genetic suppression using *Park2* loss of function to determine whether Park2 is required for the Hippo mutant cardiomyocyte regeneration phenotype. $Park2^{-/-}$ mutants were obtained from Jackson Laboratory and are homozygous viable. To assay regeneration, apexes of P8-stage mouse hearts were surgically resected and examined after 4 and 21-day recovery phases via histology, immunocytochemistry and expression profiling. Mitophagy and Park2 activity were assayed in these hearts via biochemical and immunofluorescent approaches. Expression profiling and ChIP sequencing were performed to determine whether Yap directly regulates mitophagy genes.

Results: Transmission electron microscopy of control and Hippo mutants after apex resection revealed that control border zone cardiomyocytes accumulate severely swollen mitochondria whereas Hippo-deficient mitochondria are significantly better protected from the stress of resection. ChIP and luciferase reporter assays indicate Yap binding at the Park2 locus, suggesting that Park2 levels are Hippo-regulated. Immunofluorescence and Western blot analyses indicates that cytosolic and mitochondrial Park2 protein levels are significantly upregulated in Hippo-deficient cardiomyocytes. Notably, Park2 deletion strongly suppressed regeneration of Hippo-deficient cardiomyocytes.

Conclusions: Our cardiomyocyte ultrastucture studies strongly suggest that Hippo signaling inhibits mitophagy in damaged or stressed myocardium. Consistently, our data indicate that the Yap-regulated mitophagy gene Park2 is upregulated in regenerating Hippo-deficient myocardium and is essential to this regeneration phenotype. Collectively, these data suggest that Hippo signaling inhibits mitophagy and cardiomyocyte survival following cardiac injury.

Three-Dimensional Artificial Heart Muscle to Supplement the Framework of a Bioartificial Heart

Hogan MK[1], Mohamed MA[1], Tao ZW[1], Gutierrez L[1], Birla RK[1]
1. Department of Biomedical Engineering, University of Houston, Houston, TX

Corresponding Author: Hogan MK, Dept. of Biomedical Engineering, University of Houston
3605 Cullen Blvd, SERC 2021, Houston TX 77204, E-mail:mkh388@gmail.com

Objectives: The only current long-term solution heart failure is organ transplantation. Many devices can serve as a bridge to transplant, but the common restricting factor is a lack of donor organs. Given the limitations of mechanical devices, a biological alternative therapy for heart disease represents a significant focus in the field of regenerative medicine. Along these lines, our group has begun work to combine a structurally complex decellularized heart scaffold with a fibrin based artificial heart muscle (AHM) in order to establish the basic framework of a bioartifical heart (BAH).

Materials and Methods: Thrombin and fibrinogen were combined to form a fibrin hydrogel scaffold for the artificial heart muscle. Primary rat neonatal cardiac myocytes were isolated via an established method from 2-3 day old rats and 4 million (M) cells were seeded onto the fibrin gel. Spontaneous contraction of the cardiac cells caused compaction and delamination of the cell containing fibrin in to the geometry defined by the minutien pins. An adult rat heart was surgically removed and passively decellularized using a series of detergent solutions and confirmed with Masson's trichrome and confocal stains. (Fig. 1.1.1, 1.1.2, 1.2.1, 1.2.2 and 1.2.5) AHM was wrapped around the outside of the decellularized heart using polypropylene sutures. (Fig. 1.3) BAH constructs were incubated for 3-4 days prior to functional and histological assessment. Sections of native heart muscle, decellularized heart scaffold, and BAH constructs were histologically assessed using Masson's Trichrome and immunohistochmical (IHC) staining techniques. Antibodies α-actinin and collagen were probed on AHM and BAH constructs. Electrical properties of beating BAHs were assessed using 8 evenly spaced electrodes in a grid at different locations on the BAH. Bioelectric signals were measured using an AD instruments Octal bioamp and LabChart software. Signals were processed using a moving average smoothing function and assessed for frequency and biopotential amplitude.

Results and Discussion: Decellularization techniques often involve the use of perfused detergents in order to remove cellular components from tissues. We were able to passively decellularize hundreds of organs to the point of visual and histological equivalence of similarly perfused organs. Our 4M-cell concentration AHM presented with contractile forces of 2602±638 μN (n=9) and frequencies in the range of 3.45±1.78 Hz (n=23). (Fig. 1.4.2) While the AHM contractile forces are on the order of 10 times lower than ventricular adult rat heart muscle twitch forces, spontaneous frequency measurements are of similar magnitude to the resting heart rate of neonatal rats. After wrapping, contraction was observable under a light microscope. A sample reading of 8 independent biopotentials indicated a contractile rate of 4.5 Hz with biopotential amplitudes in the range of 58±45 μV (n=8). Contractions were synchronous for all eight measured points. (Fig. 1.4.1) Histological assessment of BAH constructs confirms an organized and acellular matrix for the decellularized scaffold. (Fig. 1.2.2, 1.2.5) The AHM portion presented as a cell dense layer with distinct z-band patterns of α-actinin around the perimeter of the construct. (Fig. 1.2.3, 1.2.4, 1.2.5, 1.2.6)

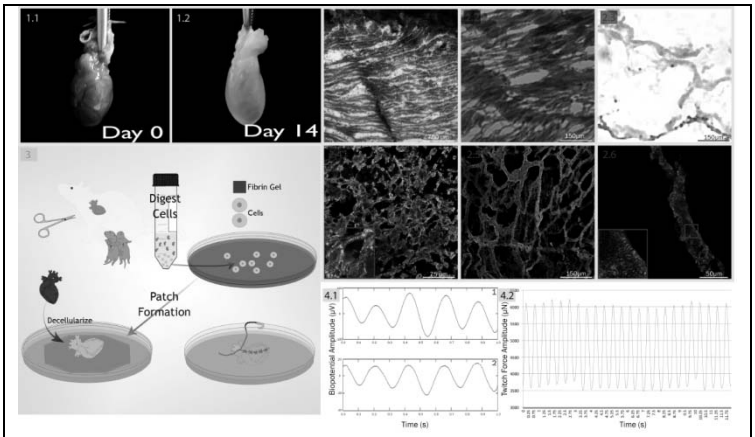


Figure 1 – Bioartificial Heart Framework: *1.1* Natural heart, *1.2* Decellularized heart, *2.1* Masson's of a natural heart, *2.2* Masson's of a decellularized heart, *2.3* H&E of a BAH, *2.4* IHC of AHM (α-actinin: green), *2.5* IHC of decellularized heart (collagen: yellow), *2.6* IHC of BAH (α-actinin: green), *3* Formation schematic, *4.1* Two sample measured BAH biopotentials, *4.2* Sample AHM twitch force measurement

Conclusions: The methods described herein allow for the assembly of a BAH framework using AHM as the function delivery mechanism onto decellularized heart scaffolds.

Acknowledgements: The authors would like to thank NIH for financial contribution to our research.

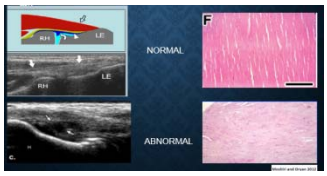
Efficacy Of Platelet Rich Plasma For Refractory Lateral Epicondylosis

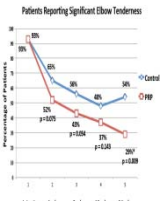
Jayaram P[1], Malanga, G[2]
7. Department of Physical Medicine and Rehabilitation, Baylor College of Medicine, Houston, TX
8. New Jersey Sports and Regenerative Institute, Cedar Knolls, NJ
Corresponding author:
Jayaram P, Dept. Department of Physical Medicine and Rehabilitation, Baylor College of Medicine, Houston, TX
E-mail: Jayaram@bcm.edu

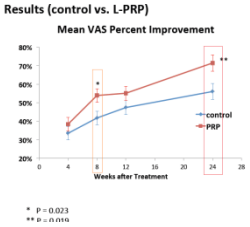
OBJECTIVE: Platelet Rich Plasma is efficacious for refractory lateral epicondylitis
Lateral Epicondylosis is an enthesopathy of the common extensor origin at the elbow. This pathology typically affects those with a mean age of 35 to 54 years. Primary treatments include physiotherapy, orthotic splints, steroid injections and surgery. However first line treatments are not always effective, leading to a chronic degeneration of the common extensor tendon. Refractory epicondylitis can be treated with platelet rich plasma producing favorable and safe outcomes compared to the previously mentioned treatments. Platelet Rich Plasma is an autologous concentration of human platelets in a small volume of plasma. These Platelets are an abundant source of alpha granules that store growth factors and lead to downstream tissue regeneration.

METHOD: Eligibility Criteria: Needed a clinical diagnosis of chronic lateral epicondylitis, RCTs, human subjects, English, adults. Quality evidence indicators include representative population, relevant baseline characteristics, dropout rate less than 80%, objective outcome assessments, concealed allocation, outcome clearly defined. Exclusion: Cohort studies, Case Studies, Review Articles, Non-English, Animal Studies. Data Extraction: All titles assessed by at least 2 physicians and or 1 PhD, 2 researchers read independent articles to see if it met inclusion criteria. Data extracted included number of participants, study design, inclusion criteria, intervention, control group, primary outcome measures pain and function, time of follow up, outcomes from intervention and control group (percentage of improvement)

RESULTS: 4 STUDIES
1. Peerboom's et al 2010: PRP group had better outcomes on VAS Pain Scale
Compared to CSG group (P<.001). Similar DASH (functional outcome) between both
Groups (P=.005) Positive effect of PRP on Pain Limitations: No ultrasound guidance and
Steroid a poor control.2. Gosens et al 2012: PRP Reduces Pain and Increases Function Significantly at
Two Year Follow Up. PRP Superior to Corticosteroids. 3. PRP (type 1 A) is superior to autologous whole blood in short term regarding pain. PRP safe for lateral epicondylitis. Small sample size N=28
4. Mishra et al 2014: Injection with PRP (type 1 A) superior to needling with bupivacaine (active control) at 24 weeks. PRP is safe for lateral epicondylitis Clinical recommendations: Should offer PRP for refractory epicondylitis, Costs for treatments (Surgery $10,000 + US), Excellent decade long safety profile, >300 patients treated with PRP in published studies







CONCLUSION

Authors	Randomized	Blind Assessment	Power Calculation	Level of Evidence	Follow up	PRP group	Control Group
Peerbooms et al	YES	YES	YES	I	12 mon	46.8	47.3
Gosenset al	YES	YES	YES	1	24 mon	46.8	47.3
Thanasas et al	YES	YES	YES	1	12 mon	36.6	35.9

Re-Differentiation of Culture Expanded Human Chondrocytes is Significantly Enhanced by Low Oxygen Tension

Kean, TJ[1], Whitney, GA[2], Traeger, G[3], Fernandes, RJ[3], Dennis, JE[1]
1) Orthopedic Surgery, Baylor College of Medicine, Houston
2) Matrix Biology Program, Benaroya Research Institute, Seattle, WA
3) Dept of Orthopaedics, University of Washington, Seattle, WA

Kean, TJ, Orthopedic Surgery, Baylor College of Medicine, 1 Baylor Plaza, ABBR R730C, Houston 77030
E-mail: tjkean@bcm.edu

Objectives

Cartilage tissue has poor intrinsic repair capacity that could be addressed by *in vitro* tissue engineering of functional replacement tissue. This is not achievable using current culture methods. It was hypothesized that low (5%) oxygen tension and increased culture duration would improve the mechanical properties of scaffold-free tissue engineered human cartilage.

Methods

Human chondrocytes were expanded under low (5%) or atmospheric (21%) oxygen tension on devitalized porcine synoviocyte matrix. Cells were trypsinized and seeded at high density ($4.4x10^6$ cells/cm^2) in a custom biochamber and switched to differentiation medium at either low or atmospheric oxygen tension for 21 days and 46-56 days. After re-differentiation, samples were taken for biochemical (glycosaminoglycan (GAG), collagen, and collagen cross-link content), histological, and mechanical assessment. Conditions were compared using the paired t-test.

Results

Low oxygen tension supported solid, scaffold-free, tissue engineered cartilage sheet formation from all donors (6 of 6) at both time points. Whereas, atmospheric oxygen tension supported solid sheet formation in only half of the donors at week 3 and ⅔ of the donors (4 of 6) at week 7. Re-differentiation at low oxygen tension 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 oxygen tension at week 3 and 4.4-fold at week 7 (Table 1). Collagen content was also increased at week 3 and at week 7 (Table 1). Collagen crosslink density was significantly increased with longer culture duration (~7 Weeks vs. 3 Weeks) at low oxygen tension (+0.90 hydroxylysyl- and lysyl-pyridinoline / collagen [mole/mole] ± 0.30) but the increase was not significant at atmospheric O_2 (+0.45 ± 0.48).

Conclusions

For a functional tissue engineered cartilage replacement, sufficient compressive strength must be achieved. These studies indicated that expansion and re-differentiation of human-derived chondrocytes at 5% O_2 showed increased compressive stiffness compared to atmospheric controls. The increase in stiffness is predominantly a result of increased glycosaminoglycan accumulation.

Acknowledgements

Funding was provided through NIH grants AR053622 and DE015322 to J.E.D. and AR057025 to R.J.F.

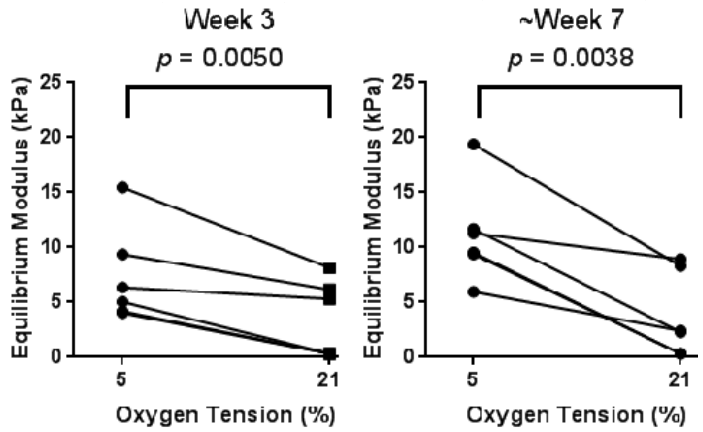


Figure 1 - Equilibrium moduli of cartilage sheets grown at low (5%) and atmospheric (21%) oxygen tension
Each symbol represents the average of sheets from each donor. lines connect sheets from the same donor at each oxygen tension. Sheets that were too thin to test were set at the lowest tested value.

Table 1 Data Expressed as % of Atmospheric Oxygen Control

Donor (passage)	GAG		Collagen	
	Week 3	~Week 7	Week 3	~Week 7
A(1)	214	258	107	182
A(2)	218	926	118	303
B(1)	NA	407	NA	147
B(2)	170	243	101	110
C(1)	261	115	149	151
D(1)	697	720	114	207
Average	**312**	**445**	**118**	**183**

NA = no atmospheric oxygen sheet data

Prostaglandin E2 Predicts Therapeutic Efficacy of Mesenchymal Stem Cells in Experimental Traumatic Brain Injury

Kota DJ, Prabhakara KS, DiCarlo B, Liao GP, Smith P, Cox CS, Olson SD

Corresponding author:
Olson SD, Dept of Pediatric Surgery, University of Texas Medical School at Houston, 6431 Fannin St. MSB 5.233, Houston, TX 77030, 713-500-7259, Scott.D.Olson@uth.tmc.edu

Traumatic brain injury (TBI) while being a serious public health problem, it is soon predicted to become the third leading cause of death and disability worldwide. In the US, more than 6.5 million patients suffer from TBI, accounting for $60 billion in estimated costs. Apart from the obvious financial loss, TBI patients often face significant physical, cognitive and psychological disorders, including major depressive disorder. Following the primary trauma, complex and chronic secondary injuries extend the neurological loss caused by the primary mechanical trauma, and constitute the leading cause of in-hospital deaths following TBI. During secondary injuries of TBI, neuroinflammation plays a key role.

TBI and other neurological injuries and conditions are currently being treated in pre-clinical and clinical trials by a number of cellular therapies. Commonly used are Mesenchymal stem cells (MSC) due to their widespread usage, safety, and relative ease to isolate and culture. There has been a wide range in reported efficacy from using these cells, which have been prepared in a variety of conditions with a known significant amount of donor variability. In this study, we seek to find a correlation between in vitro performance and in vivo efficacy. We specifically designed assays seeking to explore the responsiveness of MSC to immunological cues to address one of their primary modes of therapeutic activity in TBI.

Our results indicate there are intrinsic differences in the immunomodulatory capacity of MSCs from bone marrow and amniotic fluid. This difference mirrored the effects of MSCs in an experimental model of TBI. Among the immunomodulatory factors assessed here, the therapeutic benefit directly correlated with the expression of PGE_2 by MSCs in culture, suggesting that measurement of PGE_2 could be a very useful potency marker for the prediction of therapeutic efficacy of MSCs in TBI.

The authors were supported in part by the Clare A. Glassell Family Pediatric Stem Cell Research Fund, Mission Connect Grant #013-110, and Genzyme ISS #GZ-2013-11010.

Dysregulation of Hyaluronan Homeostasis and Metabolism is Mediated by Exogenous TGFβ and its Inhibitors in Aortic Valve Interstitial Cells

Krishnamurthy VK[1], Stout A[2], Grande-Allen KJ[1]
1. Department of Bioengineering, Rice University, Houston TX 77005
2. Department of Materials Science and Nanoengineering, Rice University, Houston TX 77005

Corresponding Author: Dr. K. Jane Grande-Allen, Department of Bioengineering, Rice University, MS-142, 6100 Main Street, Houston, TX 77005. Email: grande@rice.edu.

Aortic valve disease (AVD) is one of the leading causes of cardiovascular mortality. Increased valve interstitial cell (VIC) activation, misexpression of glycosaminoglycans (GAGs) and biomechanical dysfunction are hallmarks of AVD, and are frequently associated with TGFβ pathway abnormalities. Metabolic defects remain unexplored in AVD. We investigated the effects of exogenous TGFβ, pathway inhibition (Smad/canonical and ERK1/2/non-canonical), and mechanical stretch, on aortic VICs regulation of metabolic content and GAGs, specifically, homeostasis of GAG hyaluronan (HA). Confluent VIC cultures from suckling (1-3 month old) pigs were serum-starved and treated with exogenous TGFβ ± canonical (SB431542) and non-canonical (U0126) pathway inhibitors for 24 hrs. Static, 5% and 10% cyclic uniaxial strain conditions were induced using a FlexCell system. Expression of aSMA (VIC activation marker), hyaluronan synthase (HAS) - 1, 2 and 3 (involved in HA synthesis), hyaluronidase (Hyal) - 1 (involved in HA degradation), CD44 (HA receptor), PGC-1a (promotion of fatty acid oxidation) and UCP-3 (uncoupling protein) were assessed in the cDNA from harvested cells using qRT-PCR. Glucose and lactic acid were quantified from harvested conditioned media using Glu-Lac assay. Our results show increased VIC activation and HAS-2 synthesis in presence of TGFβ; the inhibitors rescued HAS-2 but not aSMA expression. HAS-1 and 3 expression levels were modest in all treatment and strain conditions. Hyal-1 was upregulated in VICs exposed to TGFβ, notably at high strain levels. CD44 expression was enhanced by the inhibitors; VICs demonstrated up to 50-fold increase in CD44 expression with the combined inhibitor treatment. PGC-1a and UCP-3 show a trend for decreased expression with 5% strain in all treatments. Glucose in the media was reduced in presence of TGFβ under both static and 5% strain; however, the inhibitors down regulated glucose uptake by cells. Lactate production was not significantly impacted due to TGFβ or the inhibitors under static conditions, however, 5% strain caused TGFβ to increase lactate secretion by cells. Overall, our study suggests differential HA and metabolic regulation due to TGFβ and its inhibitors in a dynamic mechanical environment. The findings of this study has significant implications in elucidating AVD pathogenesis, and in valve tissue engineering and bioprostheses.

Funding sources: This study was supported by AHA awards: 13POST16350003 (VKK), 14UFEL20380152 (AS), and 12EIA9300065 (JGA).

Osteochondral Defect Repair Using Bilayered Hydrogels Encapsulating Both Chondrogenically And Osteogenically Pre-differentiated Mesenchymal Stem Cells In A Rabbit Model

Lam J[1], Lu S[1], Lee EJ[1], Trachtenberg JE[1], Meretoja VV[1], Dahlin RL[1], van den Beucken JJJP[2], Tabata Y[3], Wong ME[4], Jansen JA[2], Kasper FK[1], Mikos AG[1]
1. Department of Bioengineering, Rice University, Houston, TX
2. Department of Biomaterials, Radboud umc, Nijmegen, The Netherlands
3. Department of Biomaterials, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan
4. Department of Surgery, Division of Oral and Maxillofacial Surgery, The University of Texas School of Dentistry, Houston, TX
Corresponding Author: Mikos AG, Department of Bioengineering, Rice University, P.O. Box 1892, MS-142, Houston, TX 77251-1892, Email: mikos@rice.edu

While cell-based therapies for osteochondral defect repair are gaining traction, inconsistencies in therapeutic efficacy between various strategies indicate that ideal conditions for unlocking the full healing potential of mesenchymal stem cells (MSCs) still remain largely unknown. The present study investigates the ability of MSC-laden bilayered hydrogels encapsulating chondrogenically and osteogenically (OS) pre-differentiated MSCs to effect osteochondral defect repair in a rabbit model. By varying the period of chondrogenic pre-differentiation from 7 (CG7) to 14 days (CG14), the effect of chondrogenic differentiation stage on osteochondral tissue repair was also investigated.

Rabbit MSCs were subjected to either chondrogenic or osteogenic pre-differentiation, encap- sulated within respective chondral/subchondral layers of a bilayered hydrogel construct, and then implanted into femoral condyle osteochondral defects. Rabbits were randomized into one of four groups (MSC/MSC, MSC/OS, CG7/OS, and CG14/OS; chondral/subchondral) and received two similar constructs bilaterally. Defects were evaluated after 12 weeks.

All groups exhibited similar overall neo-tissue filling. The delivery of OS cells when compared to undifferentiated MSCs via the subchondral construct layer resulted in improvements in neo-cartilage thickness and regularity. The addition of CG cells in the chondral layer with OS cells in the subchondral layer, however, did not augment tissue repair as influenced by OS cells alone when compared to the control. Instead, CG7/OS implants resulted in more irregular neo-tissue surfaces when compared to MSC/OS implants. Notably, the delivery of CG7 cells, when compared to CG14 cells, with OS cells stim- ulated morphologically superior cartilage repair. However, neither osteogenic nor chondrogenic pre- differentiation affected detectable changes in subchondral tissue repair.

Cartilage regeneration in osteochondral defects can be enhanced by MSCs that are chondrogenically and osteogenically pre-differentiated prior to implantation. Longer chondrogenic pre-differentiation periods, however, lead to diminished cartilage repair.

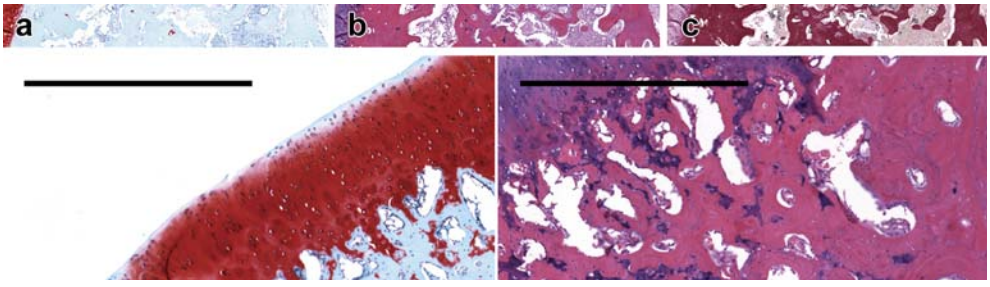


Figure 1: Histological sections stained with (a) Safranin-O/Fast Green, (b) Hematoxylin/Eosin, and (c) van Gieson's Picrofuchsin (scale bars: 1000 μm) showing osteochondral tissue repair at 12 weeks for the CG7/OS group.

This work was supported by the National Institutes of Health Grant R01 AR048756.

Hippo and Wnt Signaling in Cardiac Regeneration

Leach J P[2], Heallen T[1], Morikawa Y[1], Tao G[2], Martin J F[1,2,3,4]
[1] Texas Heart Institute, Houston, Texas, 77030
[2] Department of Molecular Physiology and Biophysics,
[3] Program in Developmental Biology, Baylor College of Medicine,
[4] Cardiovascular Research Institute, Baylor College of Medicine, Houston, Texas, 77030

Corresponding author: Leach JP, Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, e-mail: leach@bcm.edu

The leading cause of death in the United States is heart failure brought on by heart disease, and a loss of functional cardiac muscle. Because heart muscle regenerates poorly, loss of cardiomyocytes leads to a weakening of the heart culminating in heart failure. There is still paucity in understanding endogenous mechanisms regulating heart regeneration. Therefore our objective is to clarify the mechanisms that prevent meaningful cardiomyocyte renewal.

The Hippo signaling pathway and its down stream effector Yap are known regulators of intrinsic organ size by modulating proliferation during development. Our lab has demonstrated Hippo signaling inhibits cardiomyocyte proliferation during development to restrain heart size. Furthermore, the Wnt signaling effector, β-catenin is required for this Hippo knockout-mediated cardiac-overgrowth. Wnt/β catenin signaling has been well chraracterized in stem cells, and modulation of Wnt signaling like Hippo signaling presents an interesting potneital for treating cardiac disease. Additionally, multiple studies have established a link between Hippo and Wnt signaling through activation and interaction of the down-stream transcription factors Yap and β-catenin. However the interaction of these two pathways during cardiac regeneration is poorly understood. Further confusion exists when considering in a rat model of myocardial infarction; adenoviral expression of β-catenin reduces apoptosis of cardiomyocytes and fibroblasts resulting in increased cardiac functional recovery. However, in mouse models of myocardial infarction using constitutive-knockout or -GOF of β-catenin throughout development; knockout is beneficial while GOF is detrimental to cardiac function. Thus, the specific effect of β-catenin activity in cardiomyocytes following myocardial infarction has yet to be determined. Thus, we hypothesize Hippo/Wnt signaling effectors Yap/β-catenin regulate cardiomyocyte regeneration.

We have used two methods of cardiac damage in the mouse: Apex resection (AR) and LAD-ligation (MI). In a cardiomyocyte-specific inducible Salvador-knockout we have demonstrated Hippo signaling inhibits cardiac regeneration. Indeed, knockout of the Hippo pathway genes Salvador (Salv) and Lats 1/2 promotes adult cardiomyocyte renewal. Adult cardiomyocytes re-enter the cell cycle as indicated by EDU assays and proliferation markers; pHH3, AurkB, and Ki67. Furthermore, inducible knockout of Salv enhances functional recovery and reduces fibrosis severity in both the post-natal AR and MI models as well as in the adult mouse model of MI. Others have now shown similar results looking at over-expression and GOF for Yap. Our current studies aim to clarify the role of beta-catenin during this cardiac regeneration. Similar to Salvador knockout, an inducible gain-of-function beta-catenin completely regenerates after AR. While the same inducable GOF beta-catenin after MI is still being evaluated and prelimiary data indicates a failure to regnerate. Thus far, uur findings have uncovered Hippo signaling as an endogenous repressor of adult cardiomyocyte renewal and regeneration. Hence, targeting core members of the Hippo pathway may serve as a powerful therapeutic approach for treating cardiac disease, while careful consideration of Wnt signaling still needs to be evaluated.

Funding support: NIH Cardiovascular Training Grant, 5T32HL007676-24

An Optogenetic Approach to Bone Tissue Engineering

Lee EJ[1], Zimak J[1], Diehl MR[1,2], Tabor JJ[1,3], Mikos AG[1,4]
1. Department of Bioengineering, Rice University, Houston, Texas
2. Department of Chemistry, Rice University, Houston, Texas
3. Department of Biochemistry and Cell Biology, Rice University, Houston, Texas
4. Department of Chemical and Biomolecular Engineering, Rice University, Houston, Texas

Corresponding author:
Mikos AG, Dept. of Bioengineering, Rice University
MS 142, P.O. Box 1892, Houston, TX 77251-1892, Email: mikos@rice.edu

Optogenetic tools employ light-responsive modules to control gene expression with robust spatiotemporal resolution and minimal invasiveness. These attractive properties may be leveraged to increase precision in therapeutic molecule administration. We are particularly interested in adapting a system derived from *Arabidopsis thaliana*, in which red light triggers complex formation between chromophore-bound Phytochrome B (PhyB) and Phytochrome Interacting Factor 6 (PIF6), while subsequent exposure to far red light leads to their dissociation. This study outlines the design of a red/far red light switchable promoter system to control expression of the potent osteoinductive factor bone morphogenetic protein 2 (BMP2).

A hierarchical assembly strategy based on work by Guye et al. (Nuc Acids Res, 2013) was pursued to integrate the various gene cassettes (red/far red light-responsive domains, BMP2, chromophore biosynthesis) since it facilitates construction of large, multi-gene mammalian circuits in a systematic and standardized fashion. Preliminary characterization and validation of individual components was also performed in human embryonic kidney (HEK) 293T cells. These studies relied on an exogenous source of chromophore, phycocyanobilin (PCB), purified from *Spirulina* via methanolysis.

As confirmed by colony PCR and DNA sequencing, gene cassettes were inserted into their respective destination vectors. Carrier vector backbones were constructed following several design iterations to introduce components associated with mammalian cell entry and genomic integration. Exposing cells transfected with a PhyB mutant Y276H plasmid confirmed the quality of extracted PCB, as constitutive fluorescence was observed independent of light induction. Flow cytometry revealed that cells co-transfected with PhyB-PIF6 and mCherry fluorescent reporter or PhyB-PIF6 and BMP2-mCherry responded accordingly to red and far red light illumination.

Summarily, a light-controlled gene regulatory circuit can be constructed using a hierarchical approach. Red light in the presence of PCB activates gene expression, while far red light exposure in the presence of PCB also exhibits some residual expression.

Funding Sources: EJL acknowledges support from a National Science Foundation Graduate Research Fellowship.

Daam2-PIP5K is a novel regulatory pathway for Wnt signaling and therapeutic target for remyelination in the CNS

Lee HK[1], Chaboub LS[1,2], Zhu W[1,3], Zollinger D[4], Rasband MN[4], Fancy SP[5], and Deneen B[1-4,6]

1. Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, USA
2. Program in Developmental Biology, Baylor College of Medicine, Houston, USA
3. Integrative Molecular and Biomedical Sciences Graduate Program, Baylor College of Medicine, Houston, USA
4. Department of Neuroscience, Baylor College of Medicine
5. Department of Neurology, University of California, San Francisco, USA.
6. Correspondence: Deneen B, deneen@bcm.edu

Wnt signaling plays an essential role in developmental and regenerative myelination of the CNS, however contributions of proximal regulators of the Wnt receptor complex to these processes remain undefined. To identify components of the Wnt pathway that regulate these processes, we applied a multifaceted discovery platform and found that Daam2-PIP5K comprise a novel pathway regulating Wnt signaling and myelination. Using dorsal patterning of the chick spinal cord we found that Daam2 promotes Wnt signaling and receptor complex formation through PIP5K-PIP_2. Analysis of Daam2 function in oligodendrocytes (OLs) revealed that it suppresses OL differentiation during development, after white matter injury (WMI), and is expressed in human white matter lesions. These findings suggest a pharmacological strategy to inhibit Daam2-PIP5K function, application of which stimulates remyelination after WMI. Put together, our studies integrate information from multiple systems to identify a novel regulatory pathway for Wnt signaling and new therapeutic target for WMI.

The Mesp1-lineage of Cardiac Progenitor Cells Differentiate into Cardiomyocyte and Vascular Cells and Repair Post-MI hearts

Liu Y[1], Chen L[1], Diaz AD[2], Xu X[3], Cooney A[3], McConnell B[2] and Schwartz RJ[1,4]
1 Department of Biology and Biochemistry, 2 Department of Pharmacological and Pharmaceutical Sciences, University of Houston; 3 Department of Molecular and Cellular Biology, Baylor College of Medicine; 4 Stem Cell Engineering, Texas Heart Institute

Corresponding author:
Liu Y, Dept. of Biology and Biochemistry, University of Houston
4800 Calhoun Rd., 369 SR2, Houston, TX 77204 E-mail: yliu54@uh.edu

Mesp1 directs multipotential cardiovascular cell fates, even though it's only transiently expressed prior to the appearance of the cardiac progenitor program. Because of Mesp1's transitory nature, Mesp1-cardiac progenitor cell (CPC) lineages were traced by following EYFP expression in murine $Mesp1^{Cre/+}$; $Rosa26^{EYFP/+}$ ES cells. These earliest CPCs strongly expressed cardiac mesoderm markers (Flk1, PDGFRa, Hand2), cardiac transcription factors (Nkx2-5, Tbx5, Mef2c), but not pluripotent markers (Oct4, Sox2, Nanog). These CPCs expressed miRNAs involved in early cell fate decisions but not those in terminal cardiomyocytes. The early EYFP+ cells represented a sub-population of the Flk1+/PDGFRa+ cells, which were widely used to isolate CPCs. BMPs, canonical Wnts and Activin were required for induction and accumulation of Mesp1-CPCs, but extended BMP4 was not compatible with cardiomyocyte formation. Meantime, canonical Wnt inhibitor IWR1 was sufficient to drive CPCs toward cardiomyocyte, suggesting drastic change of extracellular cues is essential for the switch from CPC specification to differentiation. In a post-myocardium infarction mouse model, injected Mesp1-CPCs contributed to cardiac muscle cells, vascular smooth muscle cells and endothelial cells, with evident neovascularization in both infarct and border zones. Mesp1-CPC proliferation remained evident 12 weeks after injection into the post-MI murine hearts. Remarkably, the Mesp1-EYFP+ CPC-injected animals showed improved ventricular pump function and better rate of survival. Our results have laid a useful platform for studying CPCs and their progeny in repairing injured hearts.

This work is funded by American Heart Association (11SDG5260033) and a University of Houston startup fund.

"Smart" Bone Cement: Synthesis and Characterization of Metronidazole Acrylate

Livingston CE[1], Tatara AM[1], Mikos AG[1]
1. Department of Bioengineering, Rice University, Houston, Texas

Corresponding author:
Mikos AG, Dept. of Bioengineering, Rice University
6500 Main Street, Houston, Texas, E-mail: mikos@rice.edu

Traditional methods of antibiotic delivery, such as intravenous or oral delivery, are disadvantageous because systemic delivery results in a low concentration of antibiotic distributed non-specifically throughout the body. An alternative approach is designing a local delivery vehicle capable of releasing antibiotic only upon exposure to bacteria. Our goal is to create a polymer that can be used to deliver metronidazole (MTZ), an antibiotic commonly used to treat anaerobic bacterial infections such as periodontitis or osteomyelitis. We have developed a one-pot reaction to create an acrylated antibiotic, metronidazole acrylate (MTZA), capable of being copolymerized with the bone cement polymethylmethacrylate (PMMA). The presence of extracellular esterases, such as those produced by anaerobic bacteria, can cleave the MTZA from the bone cement, resulting a "smart" bone cement that releases metronidazole locally only upon infection. The objectives of this study were to confirm synthesis and purification MTZA as well as examine the mechanical properties of PMMA-MTZA constructs.

To achieve these objectives, MTZA was first synthesized through a one-pot acrylation reaction, in which MTZ, acryloyl chloride, and triethylamine were gradually brought to room temperature while being stirred overnight in a chloroform solvent. Results were confirmed via Proton Nuclear Magnetic Resonance (H^1NMR) spectroscopy. MTZA and PMMA were co-polymerized in polytetrafluoroethylene molds to fabricate constructs (4 mm in diameter and 8 mm in height) with MTZA incorporation ranging from 0-5 wt%. These constructs were evaluated for mechanical properties (compressive strength and modulus) and compared to PMMA-MTZ constructs.

Successful synthesis of MTZA was demonstrated through the changes in H^1NMR spectrum between MTZ and MTZA. Increased amount of MTZA in PMMA-MTZA resulted in a decrease in construct mechanical properties; however, the mechanical properties were similar to those observed in PMMA-MTZ blends. In conclusion, metronidazole can be acrylated and co-polymerized with bone cement.

We would like to acknowledge support from the Louis Calder Endowed Professorship.

Dual growth factor delivery from bilayered, biodegradable hydrogel composites for spatially-guided osteochondral tissue repair

Lu S[1], Lam J[1], Trachtenberg JE[1], Lee EJ[1], Seyednejad H[1], van den Beucken JJJP[2], Tabata Y[3], Wong ME[4], Jansen JA[2], Mikos AG[1,*], Kasper FK[1,*]

1. Department of Bioengineering, Rice University, Houston, USA
2. Department of Biomaterials, Radboud University, Nijmegen Medical Center, Nijmegen, The Netherlands
3. Department of Biomaterials, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan
4. Department of Surgery, Division of Oral and Maxilofacial Surgery, The University of Texas School of Dentistry at Houston, Houston, USA

[*]Corresponding Authors: Antonios G. Mikos, Ph.D.
Louis Calder Professor, Department of Bioengineering, Rice University
P.O. Box 1892, MS-142, Houston, Texas, 77005-1892
w: 713-348-5355, e: mikos@rice.edu

F. Kurtis Kasper, Ph.D.
Senior Faculty Fellow, Department of Bioengineering, Rice University
P.O. Box 1892, MS-142, Houston, Texas, 77005-1892
w: 713-348-3027, e: kasper@rice.edu

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. Overall, bilayered OPF hydrogel composites demonstrate potential as spatially-guided, multiple growth factor release vehicles for osteochondral tissue repair.

Funding Sources: This work was supported by the National Institutes of Health (R01-AR048756)

Drug-Drug Interaction between Irinotecan and Taxol: Implication for Combination Therapy

Mallick P, Ghose R
University of Houston, Department of Pharmacological and Pharmaceutical Sciences (PPS), Houston, TX – 77030

Ghose R, Dept. of PPS, University of Houston
1441, Moursund Street, Texas Medical Centre, 77030, E-mail: rghose@central.uh.edu

Background: There are about 14 million new cancer cases globally each year. Despite advances in cancer research, the success in cancer therapy is hampered by complexity of molecular pathways that foster drug resistance. Combination therapies offer the potential for improved effectiveness and decrease resistance. Irinotecan based combination therapy have shown significant response rate (25-35%) in downsizing the liver tumor. Taxol shows superior outcome, in terms of response rates, progression-free survival (PFS), and overall survival (OS). Therefore, combination therapy of irinotecan and taxol are under clinical evaluation for metastatic or recurrent adenocarcinoma of the esophagus and gastric cardia, advanced small and non-small cell lung cancer (NSCLC). In Phase I study of this combination in advanced non-small cell lung cancer, preceding taxol treatment increased the AUCs of both irinotecan and its active metabolite, SN-38. Interestingly, we found that induction of uridine diphosphate-glucuronosyltransferase (Ugt1a1) and Cytochrome P450 (Cyp) 3a11 by Taxol is Toll-like receptor (TLR) 4-dependent, *in vitro*.
Hypothesis: We hypothesize that pretreatment of taxol will induce expression of Cyp3a11 and Ugt1a1 leading to increase metabolism of Irinotecan and glucuronidation of SN38, respectively, thus alter the levels of SN38.
Objective: To determine drug-drug interaction between Taxol and Irinotecan involves Ugt1a1 and Cyp3a11 in mouse hepatocytes.
Method: Primary hepatocytes isolated from adult TLR4 WT and TLR4 Mutant male mice were treated with 20 μM of taxol. RNA was extracted from the cells, cDNA was prepared and gene expression was analyzed by real-time PCR analysis. In another experiment, primary hepatocytes isolated from adult TLR4 WT were treated with 20 and 100 μM of Irinotecan and 5 μM of SN38 in presence and absence of 20 μM of taxol. Supernatant was collected at different time points over a period of 24hr. Supernatants were analyzed for SN-38 and SN38G using LC-MS/MS.
Result: Taxol significantly induced expression of Cyp3a11 (~600fold) and Ugt1a1 (~4 fold) in TLR4WT mice whereas 2-3 folds lower in TLR4 Mutant, Cyp3a11 (~230 fold), and Ugt1a1 (~1.5 fold). The levels of SN38G were ~2 fold higher from 0.25 to 2.5hrs in presence of taxol with SN38 treatment. Taxol induced levels of SN38G by 2 and 3 folds at 24hrs when pretreated with 20 and 100μM of Irinotecan, respectively.
Conclusion: The finding indicates that taxol may affect the therapeutic fidelity of co-administered anti-cancer medication by inducing drug metabolizing enzymes and thus limiting combination therapy options in cancer treatments.

POU3F2 Regulates Endothelial Cell Differentiation And Vascular Development

Matrone G[1], Tian XY[1], [2]Blau H, Wong Wing T[1*], Cooke John P[1*]
[1]Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston TX, USA; and
[2]Baxter Laboratory, Stanford University
*Co-corresponding authors

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. In particular, 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 the endothelial cell differentiation and in the zebrafish vascular development.
Methods and Results: 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 analyse 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. Real Time PCR and Western blotting were used to analyse gene and protein expression respectively. POU3F2 knockdown in mESCs reduced $Flk1^{+}CD144^{+}$ cell population during differentiation of mESCs. POU3F2 knockdown also reduced endothelial cell markers in mESC derived ECs, 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 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 mESC to EC lineage. In addition, we show that POU3F2 is required for normal vascular development in the zebrafish.

Intra-Femoral Artery Perfusion in Mice; a Practical Method for Cell Delivery into Skeletal Muscles

Matthias N[1], Hunt S.D.[1], Wu J[1], Robinson H.A.[2], Azhdarinia A.[2] and Darabi R.[1]

1. Center for Stem Cell and Regenerative Medicine, The University of Texas Health Science Center at Houston, Houston, TX
2. Center for Molecular Imaging ,The University of Texas Health Science Center at Houston, Houston, TX

Corresponding author: Radbod Darabi, M.D., Ph.D., Center for Stem Cell and Regenerative Medicine, The University of Texas Health Science Center at Houston, 1825 Pressler Street, SRB 630A, Houston, TX 77030, Email: radbod.darabi@uth.tmc.edu

Stem cell therapies have been suggested as promising approaches for treating muscle disorders such as muscular dystrophies. In order to test the efficiency of these new therapies in mice models of muscular dystrophies, the arterial route of delivery is advantageous as provides uniform muscle exposure to the therapeutic agents and avoids lung barrier. However there is no in depth methodological description of this technique and its downstream muscle perfusion efficiency. This lack of clarity makes it hard for other investigators to use this technique. Therefore this study is designed to develop a reproducible and practical method for intra-femoral artery perfusion in the mouse. Furthermore, we have compared two common sites of femoral artery canulation for their efficacies in downstream muscle perfusion and tested human derived cell delivery into muscle.

Ten adult (3-4 months old) NSG-mdx^{4cv} mice were divided into 2 groups (5 mice each group). The femoral artery was canulated in two anatomical locations (the proximal and distal to superficial caudal epigastric artery- SCEA). Briefly, after a 1 cm incision was made at the inguinal region on the right hindlimb parallel to the femoral vascular bundle, the artery was separated from the vein with a 6-0 silk suture for the temporary ligation of the artery. After making a partial incision in the artery wall, the 32-gauge intrathecal catheter was guided into the artery and advanced followed by the catheter. The hind limb was perfused using a perfusion pump at a rate of 50 µl/ minute. For muscle perfusion visualization, fluorescent dye was injected and perfused hindlimbs were evaluated using near infrared fluorescent imaging. Different muscle compartments were also dissected and analyzed for individual muscle evaluation. Moreover, to test the transplantation efficacy of this route for cell therapy, human iPS derived myogenic progenitors were perfused through femoral artery with the same perfusion rate and analyzed one month later by Immunohistochemistry.

Our results describe a detailed anatomical description of the femoral artery and its branches as well as a step by step technical guide to perform this delicate method in mice. Moreover, it also indicates huge differences in skeletal muscle perfusion among these anatomical sites. While the proximal canulation site provides robust and uniform perfusion of different muscle compartments including tibialis anterior (TA), gastrocnemius (GC), extensor digitorum longus (EDL), medial and lateral thigh muscle groups in the hindlimb, the distal site fails to perfuse the major muscle groups. Furthermore the vessels of the cell perfused muscles were filled with human iPS derived cells and the cells were able to engraft into muscles one month post perfusion.

Taking together, this data describes a step by step guide for femoral artery perfusion in mice and confirms its efficacy for cell or gene delivery into skeletal muscles of small rodents.

Acknowledgement: *This study has been funded by a research grant from MDA USA.*

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

Corresponding author:
Paulsen SJ, Dept. of Bioengineering, Rice University
6100 Main St. MS-142, Houston 70030, USA, E-mail: sam.paulsen@rice.edu

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.

Effect of Gelatin Incorporation on the Mineralization and Osteogenesis of Stem Cell Laden Hydrogels

Roh, Terrence T*[1], Vo, Tiffany N*[1], Mikos, Antonios G[1]
9. Department of Bioengineering, Rice University, Houston, TX, USA
*Both authors contributed equally to this work

Corresponding author: Mikos, Antonios G, Dept. of Bioengineering, Rice University, Houston, TX, USA 6100 Main St. MS-142, Houston, TX 77005, USA E-mail: mikos@rice.edu

Reconstruction of craniofacial bone defects remains a clinical challenge due to limitations of current treatments, such as allografts and autografts, and associated risks. Alternatives are synthetic hydrogel systems capable of delivering cells and biomolecules, thereby promoting localized bone regeneration. Of particular interest are thermoresponsive hydrogels that are liquid at room temperature but gel at physiological temperatures, allowing for minimally invasive injection in a defect site. Experiments investigating an injectable thermoresponsive hydrogel system composed of poly(*N*-isopropylacrylamide) (PNiPAAm) based thermogelling macromers (TGMs) and polyamidoamine (PAMAM) crosslinkers have shown rapid chemical and physical gelation without shrinking, 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 whether the incorporation of a protein-based polymer, gelatin, in the form of microparticles (GMPs) or nanoparticles (GNPs), could enhance hydrogel mineralization in this system, and second, to evaluate whether gelatin incorporation and size could improve encapsulated MSC viability and osteogenic differentiation.

Methods: TGMs and PAMAM crosslinkers were synthesized from established polymerization protocols [1]. 50-100 μm diameter GMPs or 130 nm diameter GNPs crosslinked with 10mM glutaraldehyde were made as previously described [2-3]. 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 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. To evaluate the effect of gelatin particle size and surface area, 15 polymer wt% (w/v) cellular hydrogels with a 20 wt% (w/w) loading of GMP or 0.5 wt% GNP were investigated. Hydrogels were cultured in dexamethasone-containing complete osteogenic media for 0, 7, 14, and 28 days (n=4) following established protocols [2]. At each timepoint, samples were analyzed by Live/Dead confocal imaging, calcium biochemical assay and histology.

Results: Injectable, physically and chemically crosslinking hydrogels were successfully fabricated from the mixing of TGMs, PAMAM crosslinkers, GMP or GNP, and MSCs. Viable encapsulated MSCs were confirmed with Live/Dead confocal imaging over a period of 28 days. Calcium content was significantly increased across 28 days in GMP-containing hydrogels, which was reflected in the von Kossa histological stains. MSC encapsulation was shown to further increase the calcium content. However, despite the increased surface area of the nanoparticles, mineralization of the hydrogels was not significantly affected by the gelatin particle size.

Conclusions: The results show that incorporation of MSCs and gelatin particles in injectable, dual crosslinking hydrogels can be successfully created and support cell viability over time. Incorporation of gelatin particles was shown to increase hydrogel mineralization and cell viability by promoting ion binding and cell attachment, respectively, improving the regenerative potential of this injectable thermoresponsive hydrogel.

References: [1]Vo et al. *Biomacromolecules*, 15(1):132-142 (2014) [2]Tzouanas et al. *J Biomed Mater Res A*, 102(5):1222-1230 (2014) [3]Ishikawa et al. *Biomaterials*, 33:9097-9104 (2012)

Acknowledgements: We acknowledge support by the National Institutes of Health (R01 DE017441). T.N.V. acknowledges support from a Ruth L. Kirschstein fellowship from the National Institute of Dental and Craniofacial Research (F31 DE023999).

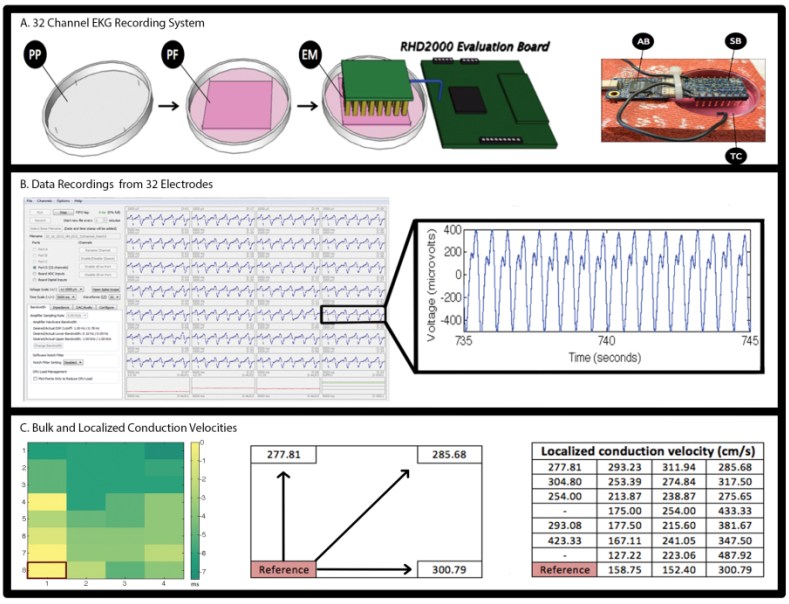
System for the Measurement of Electrophysiological Properties of 3D Artificial Heart Muscle.

Salazar BH[1], Reddy AK[2,3], Tao Z[1], Madala S[3], Birla RK[1]
10. Department of Biomedical Engineering, University of Houston, Houston, TX, US
11. Department of Medicine, Baylor College of Medicine, Houston, TX, US
12. Indus Instruments, Webster, TX, US.

Corresponding author:
Birla RK, Dept. of Biomedical Engineering, Cullen College of Engineering, University of Houston
3605 Cullen Blvd., Rm. 2005, Houston, TX 77004. E-mail: rkbirla@uh.edu

Cardiac tissue engineering offers new and exciting possibilities for repairing or replacing damaged portions of the heart. In order to achieve a viable and permanent solution, the bioengineered heart muscle must closely resemble native heart tissue and mimic its electrophysiological properties for proper contractile function. The purpose of this study was to develop, test, and validate a novel 32-electrode system that allowed us to measure the electrical activity of 3D artificial heart muscle (3D-AHM) and compare it to native tissue. This will enable us to investigate different electrophysiological aspects of the constructed tissue to move forward toward *in vivo* implantation in the future. In our present study, neonatal rat cardiac cells were cultured in a fibrin gel to drive the formation of 3D-AHM. Histological evaluation shows extensive cellularization and cardiac tissue formation present in the constructs. These cardiac constructs were then evaluated with a customized electrocardiogram (EKG) sensing system to acquire the metrics associated with their electrophysiological properties. Time delays between the signals, we found, were in the range of 0 to 7ms. As well, optical maps showed a slight trend in impulse propagation throughout the tissue. Conduction velocities were calculated longitudinally at 277.81cm/s, transversely at 300.79cm/s, and diagonally at 285.68cm/s. After evaluation of the QRS complex we found the amplitude of the R-wave to be 438.42 ± 36.96μV and the average duration was found to be 317.5 ± 16.5ms. Additionally, this system was tested along side native rat hearts to not only compare to our fabricated tissues, but also to compare our findings to values found in literature. The data collected in this study provides a distinct picture about the intrinsic properties of the 3D-AHM while proving our system's efficacy for EKG data procurement.

Funding sources:
Funding provided by NIH (Grant number: R01-EB011516) as well as the Department of Biomedical Engineering and the Cullen College of Engineering at the University of Houston.



Effect of Variable Flow Perfusion on an In Vitro Tumor Model for Ewing Sarcoma

Santoro M[1,2], Lamhamedi-Cherradi SE[2], Menegaz BA[2], Ramamoorthy V[2], Kasper FK[3], Ludwig JA[2], Mikos AG[1,3]

13. Department of Chemical and Biomolecular Engineering, Rice University, Houston, USA
14. Department of Sarcoma Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, USA
15. Department of Bioengineering, Rice University, Houston, USA

Corresponding author:
Mikos AG, Department of Bioengineering, Rice University
6500 Main Street, BioScience Research Collaborative, MS-142, Houston, TX 77030, USA,
E-mail: mikos@rice.edu;

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. Along this direction, our laboratory has investigated the culture of Ewing sarcoma (ES) cell lines on an electrospun poly(ε-caprolactone) 3D scaffold that recapitulates key aspects of the bone tumor microenvironment. A drawback of this 3D culture system, however, is its limited capacity to supply nutrients to the construct's interior under static conditions, which may bias preclinical drug testing results.

To overcome this challenge, we hypothesized that a flow perfusion bioreactor would promote mass transfer within the scaffold, and therefore improve cell culture conditions. In this study, we examined how flow perfusion affects ES culture with respect to cell morphology, proliferation, and spatial distribution of distinctive phenotypic markers throughout the construct.

Electrospinning was used to fabricate cylindrical scaffolds of poly(ε-caprolactone) 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 (3D-S), or into a flow perfusion bioreactor, using to three different flow rates: 0.04 (3D-B1), 0.08 (3D-B2), and 0.4 mL/min (3D-B3). Samples were analyzed for DNA yield quantification, proliferative and apoptotic biomarker expression using flow cytometry or immunofluorescence microscopy, and cellular morphology using Scanning Electron Microscopy (SEM).

Comparison of DNA levels showed no statistical difference among all groups except for group 3D-B3, which exhibited a significantly higher DNA content at each time point. Expression of apoptotic markers was also similar among all groups, with a range of 56-66% viable ES cells. The group 3D-B3 displayed a higher percentage of viable cells at day 5. 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. All bioreactor groups had more than 50% of ES cells positive for both CD99 and Ki67 for the entire duration of the experiment, indicating healthy and proliferative culture conditions. Conversely, the percentage of $CD99^{+}/Ki67^{+}$ cells was lower in group 3D-S, and decreased substantially between 5 and 10 days. SEM imaging of representative scaffolds visualized a small-rounded cell morphology in all groups, as well as an increased colonization of scaffold surface with time. 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 in group 3D-B1 promoted cell infiltration after 10 days of culture, while cells in group 3D-S were confined to scaffold edges. Groups 3D-B2 and 3D-B3 showed a significant cell migration after only 5 days of culture. By day 10, ES cells colonized most of the scaffold thickness, particularly in group 3D-B3**.**

In conclusion, our results showed increased ES cell growth and survival within bioreactor with high flow rates as compared to static conditions. ES cells maintained a small-rounded morphology, similar to human specimens. In addition, as visualized by fluorescent microscopy, flow perfusion promoted a homogeneous ES cell distribution throughout the PCL scaffold. Our data suggest that flow perfusion with controlled rate is superior to static culture when culturing ES cells within an engineered bone tumor niche. This methodology will be further advanced to enable preclinical drug screening and mechanistic studies of sarcoma biology.

Acknowledgments:
This research is supported in part by The University of Texas M. D. Anderson Cancer Center's Support Grant CA016672 and the National Institutes of Health Grant R01 AR057083.

Multilayer 3D Paper Constructs for the Culture and Analysis of Aortic Valvular Interstitial Cells

Matthew C. Sapp[1], Hannelle J. Fares[1], K. Jane Grande-Allen[1]

[1]Department of Bioengineering, Rice University, Houston, TX, 77005

Corresponding author:
Matthew Sapp, Rice University, Dept of Bioengineering, MS 142 6100 Main St. Houston, TX 77005
ms42@rice.edu

Calcific aortic valve disease is the progressive hardening and calcification of the aortic valve in a process that is currently irreversible. With valve replacement as the only viable option, recent research has focused on more robust tissue valve replacements and noninvasive methods for treating or preventing valve disease. Aortic valvular interstitial cells (VICs) have been targeted in studies on valve calcification because of their role in the production and turnover of leaflet extracellular matrix. Valve cells have been studied extensively *in vitro* in 2D culture systems, and there has been an increasing focus on developing 3D culture systems that better model the in vivo leaflet environment. Culturing VICs in an environment that models the aortic valve is an essential step towards understanding the progression of calcific aortic valve disease. Here the adaption of a 3D stacked paper-based culture system is presented for analyzing VICs in a thick collagen gel matrix.

Filter paper layers, modeled after a 96-well plate design, were printed with a wax well-plate template and then seeded with valve cell and collagen mixtures that quickly gelled into 3D cultures. Stacking these layers permitted extensive customization of culture thickness and cell density profiles to model the full thickness of native valve tissue. Valve cells were cultured in single layers of filter paper to monitor the behavior of the cells over time in the filter paper system. Multilayer cultures were used to explore cell activation and migration throughout each layer in the system.

Aortic valvular interstitial cells seeded into the paper-based constructs consistently demonstrated high survival up to 14 days of culture with significant increases in cell number through the first 3 days of culture. After 4 days following seeding, valve cells in single layer cultures showed reduced smooth muscle α-actin expression with a stabilized cell density, suggesting a transition from an activated phenotype to a more quiescent state. These results establish the filter paper-based method as a viable culture system for analyzing valve cells in an *in vitro* 3D model of the aortic valve.

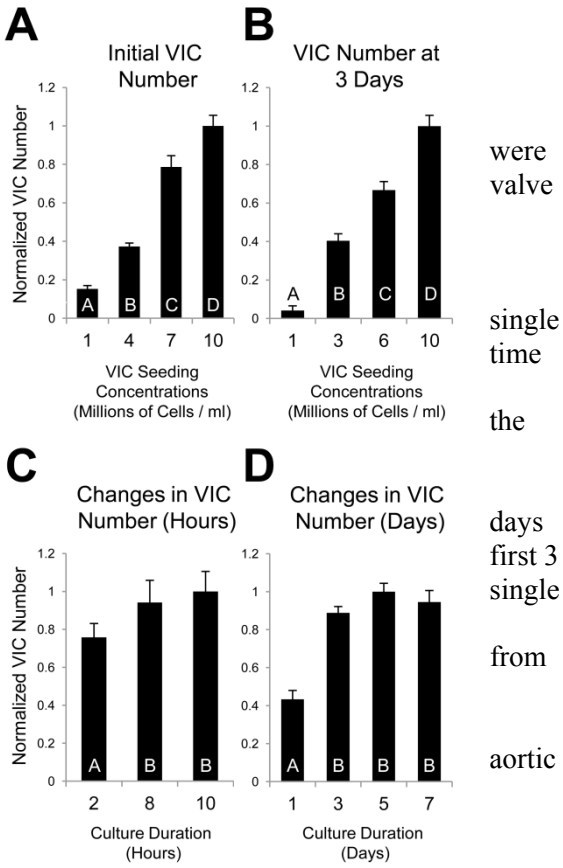


Figure 1. VIC number was studied in single layer sheets. Letters on each bar represent significant differences in cell number between initial seeding concentrations (Fig. 2A-B) and culture durations (Fig. 2C-D). Significance was determined using an ANOVA with a post-hoc Tukey's test. (A) Calcein fluorescence was proportional to the number of VICs seeded into a well. (B) Wells initially seeded with more VICs continued to have a greater number of VICs at 3 days. (C) VIC number in each well slightly increased over 10 hours following seeding. (D) VIC number per well stabilized within 3 days following seeding, suggesting a slowing in proliferation.

Funding Sources: This work was funded by AHA Innovative Research Grant number 11IRG5550052.

Isl1 is Alternative Spliced into Distinct Protein Isoforms During Pancreatic Development

Scavuzzo, M.A.[1], Sharp, R.[2,3,4], Wamble, K.[2,3,4], Borowiak, M.[1,2,3,4,5*]

1. Program in Developmental Biology, Baylor College of Medicine
2. Department of Molecular Biology
3. Center for Cell and Gene Therapy
4. Stem Cell and Regenerative Medicine Center
5. McNair Medical Institute Scholar

*Corresponding author: Malgorzata Borowiak, Baylor College of Medicine, One Baylor Plaza, N1020, Houston, TX 77030

Gene processing is highly regulated and allows for specificity of diverse developmental programs. The post-transcriptional regulation of pre-mRNAs allows for one single gene to potentially code multiple protein isoforms with different functions, greatly expanding the functional capacity of the genome and providing modularity of gene products in different tissues and during periods of physiological change. Isl1, a LIM homeobox transcription factor expressed during pancreatic development, is necessary for the formation of functional endocrine cells. During development the expression of Isl1 is dynamic; first expressed in pancreatic mesenchyme and epithelial cells while later only expressed in the endocrine producing epithelial tissue of the pancreas. However, mechanisms that regulate the expression of Isl1 and the functional significance of variants of Isl1 are unknown. 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 regulatory mechanisms that govern this switch in early development and its impact on the maturation of progenitors into functional beta cells, an *in vitro* approach using human and mouse ES cells was utilized. Isl1a and Isl1b-dependent transcriptional targets were analyzed through shRNA knockdown and overexpression using the pINDUCER system during the differentiation of human and mouse ES cells into beta cells at various stages. Understanding the transcriptional changes associated with the shift in Isl1 splice variant expression and the consequences of precociously changing the variant population during development will reveal the significance of Isl1 and Isl1 processing. By analyzing these processes during the development of ES cells into beta cells, not only will novel information about the underlying mechanisms and functional significance of this splice transition be revealed; insights into the maturation process of beta cells from ES cells will have direct clinical relevance, as advances in the maturation of beta cells *in vitro* provide a window of hope for patients affected with type I diabetes. The pleitropic factor Isl1 is involved in the differentiation of many tissues, such as motor neurons, and steps towards understanding the role of Isl1 in the differentiation of ES cells to target tissues will improve regenerative therapy and have broad translational impacts.

Clindamycin as an Osteogenic and Antimicrobial Agent

Shah S, Santoro M, Mikos AG
Department of Bioengineering, Rice University, Houston, TX, USA

Corresponding author:
Mikos AG, Dept. of Bioengineering, Rice University, Houston, TX
6100 Main Street, MS-142, Houston, TX 77005, mikos@rice.edu

Osseous regeneration in the context of infection remains a significant clinical challenge to patients and orthopedic surgeons. Infection is the most common complication of traumatic lower extremity bone injury. The objective of this study is to evaluate the osteogenic potential of clindamycin hydrochloride, a commonly used lincosamide antibiotic. In order to evaluate the effects of clindamycin dose on toxicity and synthetic activity, rabbit MSCs were cultured in monolayer with an initial seeding density of 10,500 cells/cm^2. Cells were cultured in either general medium (GM) (high glucose Dulbecco's Modified Eagle Medium without ascorbic acid, β-glycerol phosphate or dexamethasone); or GM with varying concentrations of clindamycin (10, 25, 50, 100, or 500 μg/mL); or complete osteogenic medium (OM) (GM with ascorbic acid, β-glycerol phosphate, and dexamethasone). Cytotoxicity and markers of osteogenesis were quantified using resazurin assay, DNA assay, and alkaline phosphatase (ALP) assay. Quantification of DNA normalized to DNA content in the GM group shows a trend of clindamycin-induced dose- dependent toxicity on MSCs, which is an expected trend based on previous literature. In this study, only the 500 μg/mL group shows statistical significance from GM and OM groups at days 8, 16, and 24. Quantification of ALP normalized to DNA shows increased synthetic activity by cells incubated with clindamycin compared to GM alone and even compared to OM. The resazurin assay revealed that exposure to 10-100 μg/mL clindamycin results in increased mitochondrial metabolic activity, but 500 μg/mL clindamycin results in inhibition of mitochondrial metabolic activity. While the 500 μg/mL group underwent markedly reduced mitochondrial metabolic activity and increased cell death, the synthetic activity of these cells remained relatively high compared to GM and OM groups. The results of this experiment indicate that clindamycin may potentially stimulate the differentiation of MSCs, and further studies into this phenomenon are warranted.

Organizers in adult growth control and regeneration

Shang C.
Department of Medicine. Baylor College of Medicine. Houston.

Corresponding author: Shang C. Department of Medicine. Baylor College of Medicine. 1504 Taub Loop. 2Rm81-001. Houston, TX 77030. Email: cshang9@gmail.com

Objective: review the model of adult growth control system and related evidence

Method: literature review

Results: A model of growth control system published in 1980s suggests that the organizers in embryogenesis continue to exist and partially retain their function after embryogenesis. The organizers are the macroscopic singular points of the morphogen gradient and bioelectric fields as predicted. They have higher metabolic rate, higher density of gap junctions and stem cells than the surrounding tissue. The growth control model predicts that the organizers are likely to exist at the extreme points of surface or interface curvature of the body. Changes in bioelectric field at organizers precede the morphological and anatomical changes in morphogenesis and pathogenesis. Subtle perturbations at organizers can cause long lasting systemic effects. These features of organizers can be used to control growth and regeneration in adult tissue by methods such as exerting electric field of physiological strength at extreme points of surface curvature. There is increasing evidence that acupuncture points are likely to have originated from organizers in embryogenesis. These points can be detected by using magnetic source imaging. The following predictions and corollaries of the model have also been independently confirmed: Morphogens continue to exist and function after embryogenesis. High density of gap junctions become concentrated at discrete boundaries and organizers during development. Acupuncture has extensive growth control effects and its efficacy is inversely related to age and disease chronicity.

Conclusions: Many corollaries and predictions of the growth control model have been independently confirmed and have important implications in regenerative medicine. The growth control system is embedded in various physiological systems and is part of the foundation of physiology and pathophysiology.

A Novel Elastomer/Gelatin Microparticle Composite Material

Tatara AM[1], Lam J[1], Mikos AG[1]
16. Department of Bioengineering, Rice University, Houston, Texas

Corresponding author:
Mikos AG, Dept. of Bioengineering, Rice University
6500 Main Street, Houston, Texas, E-mail: mikos@rice.edu

Poly(glycerol sebacate) acrylate (PGSA) is a biodegradable elastomer that can be injected and crosslinked by free radical polymerization. We have encapsulated gelatin microparticles (GMPs) within a PGSA construct in order to facilitate the delivery of proteins and cells in addition to providing an elastomeric substrate for tissue growth. The objective of this study was to demonstrate the feasibility of composite fabrication, as well as compare the degradation and swelling of constructs as a function of degree of acrylation and incorporation of GMPs.

Composite constructs were created by blending swollen GMPs in uncrosslinked PGSA, adding a photoinitiator, and injecting the uncrosslinked composite material into molds. Blue light from a standard dental photocuring system was applied to initiate crosslinking. Crosslinked constructs were placed in phosphate buffered saline (pH 7.4) at 37^{o}C under mild agitation and recovered after 1 day, 7 days, and 15 days for gravimetric analysis. These composite constructs had tunable swelling properties based on both degree of PGSA acrylation and incorporation of GMPs. Fabrication parameters did not have a significant effect on material degradation.

In conclusion, we have demonstrated the feasibility of combining gelatin microparticles into an elastomeric substrate. By controlling elastomer crosslinking density by degree of acrylation as well as GMP incorporation, composite swelling can be tuned. These constructs retain ~80% mass after 15 days in media, regardless of fabrication parameters.

We would like to acknowledge support from the American Academy of Implant Dentistry Foundation as well as the Louis Calder Endowed Professorship.

Superior Methods to Examine Bone Tumor and Host Tissue Interactions Using Micro-Gravity Bioreactors

Tondon A[1], Reese R[1], Clough B[2], Gregory C[2] and Kaunas R[1]

1. Department of Biomedical Engineering, Texas A&M University, College Station, TX
2. Institute of Regenerative Medicine, Texas A&M Health Science Center, Temple, TX

Corresponding author: Tondon A, Department of Biomedical Engineering, Texas A&M University
5045 Emerging Technologies Building, College Station, TX 77843-3120, E-mail: a.tondon@tamu.edu

Introduction: When tumors establish in bone, catastrophic tissue damage occurs as a result of accelerated bone destruction and inhibition of repair. The resultant osteolytic lesions (OL) cause pain and fractures and provide an ideal niche for tumor propagation. Our current inability to kill resident tumor cells and repair OLs renders malignant bone disease (MBD) terminal in most cases. The key to repairing OLs, and thus treating MBD, lies in understanding the mechanisms by which tumors inhibit osteoblastogenesis. *We are developing a novel cell-based disease model of MBD in which human mesenchymal stem cells (hMSCs) and bone tumor cells are co-cultured in a rotating wall vessel (RWV) to promote the growth of 3D tissue constructs*. We have previously demonstrated that inhibiting peroxisome proliferator activated receptor-γ (PPARγ) with the small molecule GW9662 promotes an osteo-enhanced phenotype (OEhMSCs)[1]. OEhMSCs secrete extracellular matrix that mimics the composition of anabolic bone tissue (hMatrix)[1]. hMatrix co-administered with OEhMSCs has a unique capacity for rapid repair of bone defects in mouse calvaria[1]. Tumor-derived secretion of the cWnt-antagonist Dickkopf-1 (Dkk-1) is known to cause bone destruction, inhibition of repair and metastasis.

Materials and Methods: OEhMSCs and MOSJ cells (MOSJ-Dkk1 and MOSJ-pLenti controls) are seeded separately onto hMatrix-coated spheres and co-cultured in the RWV. The ability of OEhMSCs to differentiate into osteoblasts and generate bone in the presence of MOSJ-Dkk1 and MOSJ-pLenti cells will be assessed using a variety of established techniques (alkaline phosphatase (ALP) assay, ELISA and qRT-PCR for osteogenic markers).

Results and Discussion: Culture conditions for OEhMSCs in the RWV have been optimized; good viability and osteogenic differentiation were achieved after 8 days (Fig 1A&C). Interestingly the RWV facilitates the formation of large cell aggregates (>2 mm in diameter in most cases) while still maintaining cell viability (Fig 1D). ALP assays performed on OEhMSCs in the RWV under osteogenic culture conditions showed increased ALP activity indicating osteogenic differentiation (Fig 1E). Successful culture of both MOSJ-Dkk1 and MOSJ-pLenti in the RWV were also achieved (Fig 1B). ALP and ARS assays of these cultures showed that control MOSJ-pLenti cells had a relatively high level of ALP activity both in the presence and absence of osteogenic factors. Whereas, MOSJ-Dkk1 cells in the absence of osteogenic factors was only 20% of the controls and increased 50% under osteogenic conditions. Optimal parameters for co-culture of OEhMSC and MOSJ in RWVs are being established. Subsequently, the RWV culture protocols will be translated to equipment used for culture under true microgravity on the International Space Station.

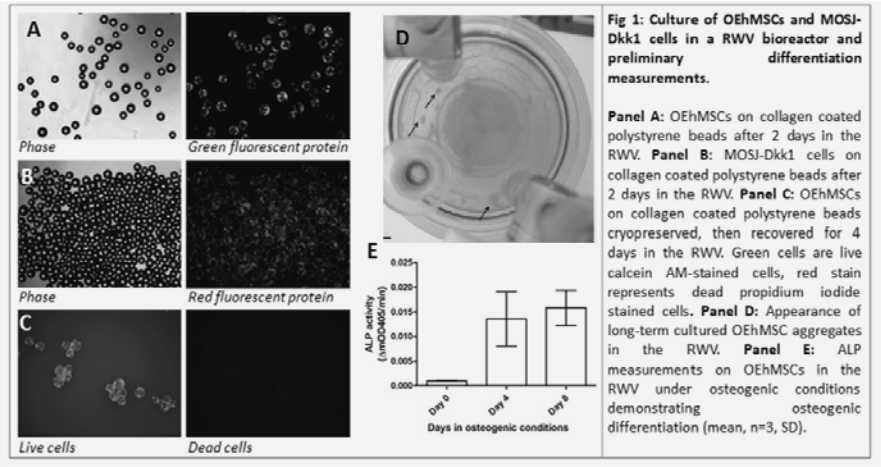


Fig 1: Culture of OEhMSCs and MOSJ-Dkk1 cells in a RWV bioreactor and preliminary differentiation measurements.

Panel A: OEhMSCs on collagen coated polystyrene beads after 2 days in the RWV. **Panel B:** MOSJ-Dkk1 cells on collagen coated polystyrene beads after 2 days in the RWV. **Panel C:** OEhMSCs on collagen coated polystyrene beads cryopreserved, then recovered for 4 days in the RWV. Green cells are live calcein AM-stained cells, red stain represents dead propidium iodide stained cells. **Panel D:** Appearance of long-term cultured OEhMSC aggregates in the RWV. **Panel E:** ALP measurements on OEhMSCs in the RWV under osteogenic conditions demonstrating osteogenic differentiation (mean, n=3, SD).

Conclusions: Osteogenic differentiation of OEhMSCs was achieved in a RWV. Culture of MOSJ-Dkk1 cells indicates that they are inhibitory to osteogenesis. Development of a co-culture system involving OEhMSCs and MOSJ-Dkk1 cells will bring about new methods for the investigation of tumor-osteoprogenitor interactions in a 3D culture environment. We anticipated that this approach will accelerate the screening of drugs promoting bone regeneration, and thus negating the osteolytic effects of certain bone tumors. The results of these studies will substantially improve our understanding of tumor expansion and bone interactions during long term exposure to microgravity.

Acknowledgements: This work is funded by the Center for Advancement of Science in Space (CASIS).

References: [1]Zeitouni, S. et al. *Sci Transl Med* 2012; 4:132–155. [2]Krause, U. et al. *Cell Death Dis* 2014; 5:e1093.

Sirtuin 6 reprograms macrophage phenotype and prevents cardiac dysfunction in diabetes

[1]Thandavarayan RA, [1]Joladarashi D, [1]Suresh Babu S, [1]Bovshik EI, [2]Kishore R, [1]Krishnamurthy P
[1]Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston, TX
[2]Department of Pharmacology, Stem Cell Therapy Program, Center for Translational Medicine, School of Medicine, Temple University, Philadelphia, PA

Corresponding author: Dr. Prasanna Krishnamurthy, DVM, MVSc, PhD. Scientist, Department of Cardiovascular Sciences, Houston Methodist Research Institute, Center for Cardiovascular Regeneration, Houston Methodist DeBakey Heart and Vascular Center, 6670 Bertner Ave, MS: F8-046, Houston, TX-77030, E-mail: pkrishnamurthy@houstonmethodist.org

Clinical and experimental studies provide evidence that metabolic and inflammatory pathways are functionally interconnected to cardiovascular diseases. Dynamic changes in macrophage activation [classical M1 activation (promote inflammation) or alternative M2 activation (promote wound healing)], in response to various stress signals, modulate cardiac physiopathology in diabetes. Sirtuin 6 (SIRT6), a NAD-dependent nuclear deacetylase plays an important role in genomic stability, cellular metabolism, stress response and aging. However, the mechanism by which SIRT6 activity affects macrophage phenotype and cardiac function in diabetes is still unexplored. Mouse bone marrow-derived macrophages (BMM) exposed to high glucose (HG, 25mM D-glucose) showed reduced expression of SIRT6 as compared to low glucose (LG, 5Mm D-glucose)- and osmotic control (OC, 5mM D-glucose+20mM D-mannitol)-treated cells, associated with increased expression of pro-inflammatory cytokine and transcription factors (NFkb, c-JUN, FOXO, SP1 and STAT1). In addition, SIRT6 level was reduced in peritoneal macrophages of both diabetic models (streptozotocin induced and db/db mice) as compared to non-diabetic mice. SIRT6 knockdown in RAW 264.7 cells exaggerated inflammatory response when exposed to HG. In contrast, IL-4-induced increase in mRNA expression of macrophage M2 phenotype markers like Arg1, Chi4l4, Retnla and IRS-2, but not IRS-1 expression was repressed suggesting that alternative macrophage (M2) phenotype was defective in SIRT6 deficient BM-macrophages under HG condition. SIRT6 protein expression was low in myocardial infarction-induced (MI) and diabetes-affected hearts. Interestingly, mice receiving intramyocardial injection of SIRT6-deficient macrophages showed further deterioration in left ventricular function, post-MI. Taken together, these data highlight a role for SIRT6 in regulating the balance of M1/M2 polarization, therefore, modulate macrophage mediated cardiac repair and regeneration in numerous inflammatory disease states including diabetes.

Funding sources: This work was supported, in part, by National Institutes of Health (NIH) grant 1R01HL116729 (to P.K.) and American Heart Association National-The Davee Foundation SDG Grant 0530350N (to P.K.).

Silencing 14-3-3 Protein Exacerbates Cardiac Dysfunction By Suppressing Cardiac Regeneration After Coronary Artery Ligation In Mice

[1,2]Thandavarayan RA**,** [2]Arumugam S, [3]Giridharan VV, [2]Pitchaimani V, [2]Karuppa Gounder V, [2]Sreedhar R, [2]Harima M, [1]Joladarashi D, [1]Suresh Babu S, [4]Suzuki K, [2]Watanabe K, [1]Krishnamurthy P
[1]Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston, TX.
[2]Niigata Univ of Pharmacy and Applied Life Sciences, Niigata, Japan.
[3]Department of Pharmacology, JKK college of Pharmacy, Komarapalayam, India.
[4]Niigata University Graduate School of Medicine, Niigata, Japan.

Corresponding author: Dr. Prasanna Krishnamurthy, DVM, MVSc, PhD. Scientist, Department of Cardiovascular Sciences, Houston Methodist Research Institute, Center for Cardiovascular Regeneration, Houston Methodist DeBakey Heart and Vascular Center, 6670 Bertner Ave, MS: F8-046, Houston, TX-77030, E-mail: pkrishnamurthy@houstonmethodist.org

14-3-3 family members are dimeric phosphoserine-binding proteins participating in signal transduction and checkpoint control pathways. We have previously reported that 14-3-3 protein protects against pressure-overload induced heart failure in mice. However, the role of 14-3-3 protein in myocardial infarction (MI)-induced cardiac dysfunction still remains to be determined. MI was induced by ligating the left anterior descending (LAD) coronary artery in wild type (WT) and cardiac-specific expression of dominantnegative 14-3-3 protein mutant (DN14-3-3) mice. One week later sham surgery; there was no significant difference in cardiac function in WT and DN14-3-3 mice. DN14-3-3 mice with MI showed increased mortality and reduced left ventricular ejection fraction and fractional shortening as compared to WT mice. DN14-3-3 mice showed increased markers of maladaptive cardiac remodeling, cardiac hypertrophy, inflammation, fibrosis and cardiac cell apoptosis as compared to their WT counterparts. Mechanistically, DN14-3-3 mice exhibited increased activation of endoplasmic reticulum (ER) stress. Interestingly, Stem cells marker (C-Kit, ISL-1) expressions were significantly reduced in DN14-3-3 mice with MI when compared to WT mice. In conclusion, depletion of 14-3-3 protein leads to increased mortality associated with increased cardiac dysfunction and adverse cardiac remodeling in mice subjected to MI, possibly, via exacerbation of ER stress and activation of death signaling pathways and suppression of cardiac regeneration. Thus, identification of drugs that can modulate cardiac 14-3-3 protein levels might provide a novel protective therapy for heart failure.

Funding sources: This research was supported by Yujin Memorial Grant, Ministry of Education, Culture, Sports, Science and Technology, Japan (WK) and by a grant from the promotion and Mutual Aid Corporation for Private Schools, Japan (WK).

Open-Source Three-Dimensional Printing of Biodegradable Polymer Scaffolds for Tissue Engineering

Trachtenberg JE[1], Mountziaris PM[1], Miller JS[1], Wettergreen[1] M, Kasper FK[1], Mikos AG[1]
1. Department of Bioengineering, Rice University, P.O. Box 1892, MS 142, Houston, Texas, 77251-1892, USA

Corresponding Author:
Professor Antonios G. Mikos, Department of Bioengineering, Rice University, P.O. Box 1892, MS 142, Houston, Texas, 77251-1892, USA. Tel.: +001-713-348-5355, Fax: +001-713-348-4244, Email: mikos@rice.edu

Objective: The fabrication of scaffolds for tissue engineering requires elements of customization depending on the application and is often limited due to the flexibility of the processing technique. This investigation seeks to address this obstacle by utilizing an open-source three-dimensional printing (3DP) system that allows vast customizability and facilitates reproduction of experiments. The effects of processing parameters on printed poly(ε-caprolactone) (PCL) scaffolds with uniform and gradient pore architectures have been characterized with respect to fiber and pore morphology and mechanical properties.

Methods: PCL scaffolds of uniform pore architecture (n = 4 per group) were fabricated following a full factorial design at varying pressures (P), printing speeds (F), and fiber spacing (s) using a 3D printer with open-source electronics. Both scaffolds with uniform and gradient pore architectures were then investigated for mechanical testing (n = 3 per group). The porosity of the 3DP scaffolds was measured using gravimetry. Fiber diameter, pore size, and fiber spacing were measured using optical microscopy. Scaffold mechanical properties were measured to evaluate the influence of pore size and organization (gradient vs. uniform) on compressive modulus and yield strength. Samples were compressed using a mechanical testing system. All measurements are represented as the mean + standard deviation. For statistical analysis, the means were compared using a one-way analysis of variance for each F/s combination. Data were tested on a normal distribution and a p-value < 0.05 was considered to indicate significance, in which statistical differences were determined using Tukey's Honestly Significant Differences test. For mechanical testing, a linear regression was fit to the data.

Results and conclusions: In general, faster printing speeds required a higher operating pressure to achieve repeatable fabrication, and at some pressures, it was not possible to print certain F/s combinations. Printing was possible for all combinations at the mid-range pressure of 14psi. At F = 300 and 400mm/min, it was possible to fabricate scaffolds with a minimum porosity of 19+3% and 20+1% and a maximum of 60+0% and 55+1%, respectively. Average porosities for F = 300 and 400 were 40+13% and 36+12%, respectively. Porosity generally decreased with increasing pressure. For scaffolds printed at speeds of 300 and 400mm/min, a minimum fiber diameter of 0.69+0.13mm and 0.74+0.08mm and a maximum of 1.22+0.10mm and 1.47+0.14mm were respectively achieved. Average fiber diameters for F = 300 and 400 were 0.97+0.17mm and 1.02+0.22mm, respectively. A minimum pore size of 0.60+0.10mm and 0.49+0.15mm and a maximum of 1.66+0.14mm and 1.77+0.14mm were measured for scaffolds printed at speeds of 300 and 400mm/min, respectively. Average pore sizes for F = 300 and 400 were 1.13+0.34mm and 1.09+0.39mm, respectively.

This study demonstrates the ability to tailor the fiber diameter, pore size, and porosity through modification of pressure, printing speed, and programmed fiber spacing. A model was also used to predict the compressive mechanical properties of uniform and gradient scaffolds, and it was found that modulus and yield strength declined with increasing porosity. The use of open-source 3DP technologies for printing tissue engineering scaffolds provides a flexible system that can be readily modified at a low cost and is supported by community documentation. In this manner, the 3DP system is more accessible to the scientific community, which further facilitates the translation of these technologies toward successful tissue engineering strategies.

Acknowledgments: We acknowledge support by the National Institutes of Health (R01 AR048756 and R01 AR057083) and the Armed Forces Institute of Regenerative Medicine (W81XWH-08-2-0032) for work in the areas of bone and cartilage tissue engineering. J.E.T. acknowledges funding from the National Science Foundation Graduate Research Fellowship Program and the Howard Hughes Medical Institute.

High-throughput toxicity screening using magnetic 3D bioprinting

Tseng H, Gage JA, Haisler WL, and Souza GR
Nano3D Biosciences, Houston, TX

Corresponding author: Souza GR, Nano3D Biosciences, 7000 Fannin St., Ste. 2140, Houston, TX 77030, email: gsouza@n3dbio.com

A growing demand exists for 3D cell culture models for drug screening that predict *in vivo* response more accurately than traditional 2D and animal models. To meet this demand, we developed a real-time and dynamic 3D assay for drug screening using magnetically bioprinted 3D cultures. This methodology uses magnetized cells to rapidly assemble and print cells into 3D cellularized spheroids or rings that contract over time, and at rates that can vary with drug concentration.

This assay was validated using vascular smooth muscle cells and 3T3 murine embryonic fibroblasts. Cells were first incubated overnight with the magnetic nanoparticle assembly and bioprinted the next day either into 3D dots or rings, and allowed to contract or dilate over few hours. Contraction or dilation due to cell-cell interaction and migration was captured using a mobile device programmed to image whole plates of 3D cultures at regular intervals, eliminating the need to image rings individually under a microscope. This assay is label-free, allowing for post-assay experimentation to explore mechanisms of action and yield more content per experiment.

Here we validate drug responses in 3D by fluorescent staining for viability and cytoskeletal organization post-assay, and a 2D viability assay. With increasingly toxic drug concentrations, printed spheroids and rings responded at different rates and with expected drug interaction response and/or toxicity. This assay was validated using a wide range of compounds showing distinct drug sensitivities compared to cells in 2D. Some of the compounds evaluated include: all-trans retinoic acid, doxorubicin, forskolin, pseudoephedrine, phenylephrine, U46619, blebbistatin, verapamil, rofecoxib, and ibuprofen. Other cell types have been tested, including cancer cell lines, such as MCF-10A, and primary cell lines, like vascular smooth muscle cells.

Tissue-Engineered Human Lungs

Vega SP [1,2], Niles JA [2], Cortiella J [3], Nichols JE [1,2]
1. Department of Microbiology and Immunology, Graduate School of Biomedical Sciences, University of Texas Medical Branch, Galveston, Texas
2. Department of Internal Medicine, Division of Infectious Diseases, University of Texas Medical Branch, Galveston, Texas
3. Department of Anesthesiology, John Sealy Hospital, University of Texas Medical Branch, Galveston, Texas

Corresponding author : Vega SP, Dept. of Microbiology and Immunology, Graduate School of Biomedical Sciences, University of Texas Medical Branch, Mail Route 1070, 301 University Boulevard, Galveston, Texas, 77555, E-mail : spvega@utmb.edu

Tissue-engineered (TE) human lung models serve as experimental tools to study initiation and progression of disease caused by respiratory pathogens or xenobiotic agents, for toxicology studies or even for the screening of drug therapies with the potential for clinical applications and for regenerative medicine. Natural acellular (AC) human lung scaffolds retain the three-dimensional (3D) extracellular matrix (ECM) of natural lung tissue and contain structural and mechanical properties necessary to support tissue development. AC human lung scaffolds are composed of regions with varying elasticity and stiffness that provide site-specific cues for organized cell attachment and differentiation. The goal of this project was to produce human lung tissue using AC lung scaffolds and human lungs cells to create experimental model systems and to develop bioengineered human lung tissue that may be used for regenerative medicine.

Human lung tissue was obtained as discarded human materials following Institutional Review Board (IRB) approved protocols at UTMB and Methodist Hospital Research Institute. Scaffolds were produced by decellularization of lung tissue as previously published (Nichols, 2013) We have developed specialized cell installation procedures for the numbers of cells used and culture conditions for recellularization of AC lung scaffolds Recellularization of scaffolds is done using primary lung cells that include various cell phenotypes including type I and II alveolar epithelial cells (AEC), fibroblasts and endothelial cells. Human lung cell lines of immortalized AEC, fibroblasts, smooth muscle cells and endothelial cells developed in our laboratory are also used for recellularization of the AC scaffolds. Cell attachment, cell viability and cell apoptosis within the lung constructs were assessed using immunohistochemistry and vital fluorescent staining. By using fluorescence microscopy, we were able to examine expression of lung specific markers in the TE lung models. We have done fluorescent staining to observe the presence of pro-surfactant protein C (pro-SPC) positive type II AEC and aquaporin 5 (AQP 5) positive type I AEC. Immunoprecipitation for pro-SPC, SPC, SPD and AQP was also done to further verify the presence of these lung specific proteins. Multi-photon microscopy coupled with second harmonic generation imaging was used to examine the collagen and elastin content of AC lung scaffolds before the addition of cells and after cells are added and cultured on the scaffolds. Once methods of construct production are established we intend to use the model to examine progression of disease caused by respiratory pathogens such as influenza A and B.

Funding Sources: UTMB John Sealy Grant, DARPA-Engineered Tissue Systems (BAA-01-42), NIH U18

Electrophysiologically Functional Cardiomyocytes Derived From Mouse Induced Pluripotent Stem Cells

Hongran Wang[1], Yutao Xi[2], Yi Zheng[3], Xiaohong wang[1,3], Austin J. Cooney[1]
1. Department of Molecular and Cellular Biology and Surgery, Baylor College of Medicine, Houston, Texas, 77030;
2. Stem Cell Center, Texas Heart institute, Houston, Texas, 77030;
3. Electrophysiology Research Lab, Texas Heart institute, Houston, Texas, 77030.

Corresponding author:
Austin J. Cooney, Department of Molecular and Cellular Biology and Surgery, Baylor College of Medicine, Houston, Texas, 77030;

Induced pluripotent cells (iPSCs) have been shown to efficiently differentiate into the three germ layers similar to embryonic stem cells (ESCs). It provides an opportunity to use iPSCs to establish preclinical allogeneic transplantation models. iPSCs were generated from mouse embryonic fibroblasts (MEFs) with transfection of four retrovirally encoded transcription factors: Oct4, Sox2, Klf4 and c-Myc, without antibiotic selection. iPSCs in which the transfected genes were silenced were used to differentiate into cardiac cells. After embryoid body formation, iPSCs differentiate into Flk1 positive cardiac progenitor, and cardiac cells that express typical cardiac cell markers including alpha sarcomeric actinin (α-actinin), cardiac alpha myosin heavy chain (α-MHC), cardiac troponin T (cTnT), connexin 43 (CX43), as well as the cardiac transcription factors: nk2 homebox 5 (nkx2.5), gata binding protein 4 (gata4). Transplantation of incompletely differentiated iPSCs into myocardial infarcted SCID mouse model give rise to teratomas. Electrophysiology studies indicated that iPSCs have a capacity like ES cells to differentiate into functional cardiac cell phenotypes based on action potential characteristics measured through patch-clamp detection of single contracting cells. Electrophysiological activity was also recorded by optical mapping with the RH237 voltage-sensitive dye. These results showed that functional cardiac cells could be obtained from somatic cells via reprogramming, thus acquiring the potential to repair acute myocardial infarction.

Novel Determinants of Differentiation to Endothelial Lineage

Wong WT[1], Tian XY[1], Matrone G[1], Blau HM[2], Cooke JP[1]
1. Center for Cardiovascular Regeneration, Houston Methodist Research Institute, Houston, TX
2. Baxter Laboratory for Stem Cell Biology, Stanford University, Stanford, CA

Abstract

Objectives: We aim to use induced pluripotent stem cells (iPSCs) to generate endothelial cells (iPSCS-EC) for angiogenic therapy in patients with coronary or peripheral arterial disease. Currently, there is insufficient information to efficiently or reliably create EC by directed differentiation from pluripotent cells. Using our extensive expertise in the generation of heterokaryons and in characterization of EC phenotype, we will identify novel determinants of endothelial phenotype for the purpose of directed differentiation. Our specific aims are to identify novel determinants of differentiation to endothelial lineage and confirm the role of these novel determinants in endothelial differentiation and development. ***Methods and Results:*** We are using heterokaryons as a tool to discover novel genes required for differentiation of pluripotent cells to EC, and to determine the temporal order of gene activation or suppression. Mouse embryonic stem cells were fused with human endothelial cells in stable non-dividing heterokaryons. Using RNA-seq we determined the temporal pattern of gene expression in pluripotent stem cells and identified early acting candidate factors underlying reprogramming to an EC fate. The role of candidate factors were then confirmed via loss-of-function studies by siRNA-mediated gene knockdown and gain-of-function by retroviral vector mediated over-expression. The novel transcription and/or epigenetic factors identified from the RNA-seq analyses of the heterokaryon studies were then validated in studies of pluripotent stem cell differentiation to endothelial lineage *in vitro*. Gain- or loss-of- function studies were performed to assess the importance of candidate factors in differentiation to an endothelial phenotype. The endothelial phenotype of human iPSC-ECs were confirmed *in vitro* via assays (e.g. tubulogenesis and nitric oxide production), and *in vivo* (using murine models of matrigel plug implantation, hindlimb ischemia). The importance of candidate factors in endothelial development was assessed using the zebrafish model and morpholino technology. ***Conclusions:*** These studies provide a systematic, mechanistic approach to identifying key regulators of endothelial cell development, and will provide insights that will be useful in formulating strategies of directed differentiation of pluripotent stem cells to the endothelial lineage.
Funding Sources: This work was supported by grants to Dr. Cooke from National Institutes of Health (U01HL100397). Dr. Wong was supported by a NIH PCBC Jump Start Award (PCBC_JS_2012/1_02) and American Heart Association Scientist Development Grant (AHASDG) (13SDG15800004).

Study of Skeletal Muscle Differentiation in hES/ iPS Cells Using Knock-in Reporters for PAX7 and MYF5

Wu J[1], Hunt S.D.[1], Matthias N[1] and Darabi R.[1]
1. Center for Stem Cell and Regenerative Medicine, University of Texas Health Science Center at Houston, Houston, TX, 77030, USA.

Corresponding author:
Darabi R, Center for Stem Cell and Regenerative Medicine, University of Texas Health Science Center at Houston, 1825 Pressler Street, Houston, TX, 77030, USA, Email: radbod.darabi@uth.tmc.edu

Stem cell therapy is one of the most potential therapies for degenerative disorders including muscular dystrophies (MD). With the availability of cell reprogramming and generation of patient specific induced pluripotent stem cells (iPS cells) from somatic cell, a new era of stem cell therapy has emerged and many research groups are now focused on differentiation of ES/ iPS cells to different tissues and progenitors. However, in the case of skeletal muscle differentiation, myogenic differentiation from human hES/ iPS Cells and isolation of homogeneous myogenic progenitors are quiet inefficient and so far there is no clinically applicable efficient protocol for it.

In order to solve this problem, we hypothesize that by generation of knock-in reporter human ES/ iPS cells for early myogenic genes (PAX7 and MYF5), myogenic progenitors can be differentiated and isolated from differentiating ES/ iPS cells. The paired-box transcription factor Pax7 is one of the key regulators of early myogenesis during embryo development and an important factor for specification and function of adult muscle stem cells (satellite cell). MYF5 is also another upstream gene in myogenic specification of somitic cells during embryogenesis and following the activation of PAX genes (PAX3/7).

To do this, we have designed a homology recombination (HR) vector for targeting the genes and incorporated a polycystronic 2A-GFP reporter before the stop codon for each gene. Since the efficiency of traditional homologous recombination is very low, we have used a pair of RNA guided cas9 nickases to generate a double strand break (DSB) near the stop codon to facilitate HR with the targeting construct.

By using this technique and after electroporation of cas9 pairs along with targeting constructs, several HR targeted human iPS colonies for both PAX7 and MYF5 were identified which survived during positive and negative selections. Moreover, PCR sequencing results verified HR targeted clones with no indels. These results indicated CRISPR/Cas9 mediated HR efficiency as a powerful genome editing tool for generation of knock-in reporters in human ES/ iPS cells.

Moreover, our preliminary data demonstrated that early treatment with CHIR99021, a small-molecule GSK-3β inhibitor, was sufficient to initiate the expression of GFP and PAX7 in hiPSC derived embryoid bodies (EBs). We plan to screen different chemical compounds and identify the best differentiation protocol (monolayer cells vs EBs with different small molecules or cytokines inductions). We also plan to characterize the GFP^+ cells *in vitro* (differentiation potential, gene expression, surface markers) and test regeneration potential of these cells *in vivo* in mice models of muscle disorders.

Our final goal is to develop an efficient protocol for myogenic differentiation and 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.

TSPO as an Indicator of Microglia Activation and Neuroinflammation in Traumatic Brain Injury

Zelnick PJ, Caplan HW, Pavuluri Y, Mandy FB, Mandy FJ, Mitchell M, Cox CS, Bedi SS
17. Department of Pediatric Surgery, University of Texas Medical School at Houston, Houston, TX, USA

Corresponding author:
Zelnick PJ, Department of Pediatric Surgery, University of Texas Medical School at Houston
7000 Fannin St, Houston, TX 77030, USA, E-mail: pamela.zelnick@uth.tmc.edu

Traumatic brain injuries are characterized by an inflammatory response in the injured brain, resulting in the infiltration of leukocytes, activation of glial cells, microglia and astrocytes and ultimately, the release of pro- and anti- inflammatory cytokines. This neuroinflammation occurs rapidly and is believed to be the cause of a number of both beneficial and detrimental effects (Woodcock et. al. 2013). Microglia are resident macrophages that sense pathological tissue alterations and act primarily to protect and repair the brain when damaged. These cells have a low threshold of activation and will activate in response to even the most minor pathological changes in the CNS (Graeber 2010). Most TBI studies indicate that extended microglial activation becomes damaging over time, with autophagy possibly contributing to pathology through phagocytosis of healthy cells in addition to damaged tissues (Ramlackhansingh et. al. 2011; Hernandez-Ontiveros et. al. 2013). The Peripheral Type Benzodiazepine Receptor (PTBR), also known as translocator protein (TSPO) is localized on the outer mitochondrial membranes of reactive astrocytes, microglia and macrophages and its primary role is the transport of cholesterol across mitochondrial membranes (V. Papadopoulos, 1998). In patients with TBI, PET ligand binding to PTBR is significantly raised in various areas of the brain even after several months to years post injury (A. F. Ramlackhansingh et al.). Given that TBI results in prolonged inflammation and increased proliferation and activation of microglia we hypothesize that *TBI will result in a similar upregulation for PTBR and that PTBR/TSPO may serve as a viable marker to indicate inflammation in patients as well as in rodents.* A controlled cortical impact device (Leica.) was used to administer a unilateral brain injury in adult mice, and brains were harvested at either 24h, 72h or 28 days. Brains were then sliced with a Leica Vibratome into 30 micron slices and sections were stained using a standard free floating staining protocol (Bedi et al., 2013) with IBA1 and TSPO primary antibodies used for identifying microglia and PTBRs (respectively). Quantification of active/resting microglia was conducted by analyzing morphology of microglia in photomicrographs. Qualification of PTBR expression was conducted by visual identification of presence or absence of TSPO+ staining in the thalamus. Injured mice showed an increase in the total microglia/macrophage population at 72 hours ($p< 0.05$) and 28 days ($p<0.001$) post-injury (but not at 24 hours), and a significant increase in activated microglia/macrophages bilaterally in the hippocampus at 72 hours and 28 days post-TBI. Qualitative assessments of photomicrographs indicated marked microglial/ macrophage activation in the ipsilateral thalamus of the injured brains as well as marked increase in TSPO+ cells in the thalamus at 28 days in all injured slices (4/4). There was no TSPO+ staining present in the uninjured slices at 28 days (0/5). The data supports the notion that TBI and resulting prolonged inflammation are linked to an upregulation of the PTBR. Thus, changes in PTBR expression (monitored in vivo using PET ligand [11C](R)PK11195/PBR28) could potentially be used to monitor treatment progress over long periods of time, in correlation with changes in cognitive behavior.

Funding for this project has been provided by both a University of Texas Medical School Dean's Office Stipend as well as additional funding from Department of Pediatric Surgery.

Protection and maintenance of the Cochlear Blood-Labyrinth Barrier

Wright, AM[1,6], Seymour, ML[1,2], Labbate C[1,2], Samuel, EL[6,7], Kent TA[4,5], Tour, JM[6,7] and Pereira, FA[1,2,3].
1. Huffington Center on Aging, Baylor College of Medicine, Houston Texas
2. Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas
3. Bobby R. Alford Department of Otolaryngology – Head and Neck Surgery, Baylor College of Medicine, Houston, Texas
4. Department of Neurology, Baylor College of Medicine, Houston, Texas
5. Michael E. DeBakey VA Medical Center, Houston, Texas
6. Department of Chemistry, Rice University, Houston, Texas
7. The Smalley Institute for Nanoscale Science and Technology, Rice University, Houston, Texas

Corresponding Author:
Pereira, FA, Huffington Center on Aging, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA, Email: fpereira@bcm.edu

Platinum-based drugs are highly ototoxic agents for the treatment of solid tumors such as prostate, head and neck, ovarian, and leukemia cancers, and can cause chronic peripheral vascular damage and hearing loss in up to 60% of pediatric patients. In the cochlea, cisplatin alkylation in mitochondria leads to excess generation of reactive oxygen species (ROS) and eventual death of cells and vasculature that maintain the cochlear labyrinth-blood-barrier (CBLB) in the *stria vascularis*. The compromised barrier enables cisplatin to infiltrate the *scala media* and cause similar ROS-induced death of sensory hair cells in the organ of Corti and permanent hearing loss. Various drugs and antioxidants have proved ineffective to protect and maintain auditory function in the long term; and there are no Federal Drug Administration approved drugs to treat cisplatin-induced ototoxicity. We have been investigating the effect of poly(ethylene glycol)-functionalized hydrophilic carbon clusters (PEG-HCCs) to maintain and potentially regenerate the CBLB and vasculature. PEG-HCCs are highly effective antioxidant nanoparticles with possibly catalytic activities to neutralize superoxide free radicals.

Cisplatin damage within the inner ear can be quantified by examining the integrity of the vasculature and the number of perivascular macrophage-like melanocytes (PVM/Ms) that participate to maintain and potentially regenerate the CBLB in the *stria*. We have performed preliminary studies that PEG-HCC enhances the function of the cochlear for hearing abilities. To determine a mechanism of how this occurs, we have characterized various parameters: the timing of PVM/M and vascular losses after cisplatin treatments, oxidative stress in the stria, localization and enrichment of PEG-HCCs and a derivative Adamantine-PEG-HCC at the stria. We isolate the cochlear stria vascularis to characterize the protection or damage of the vasculature and the cochlear stria-blood-barrier by immunofluorescence staining of endothelial and PVM/M markers followed by confocal microscopy imaging. Our studies demonstrate that PEG-HCCs prevent the loss of specialized melanocytes (PVM/Ms) that participate in the CBLB. This could mean that when cisplatin and PEG-HCCs are given concurrently, the PVM/Ms comprising the CBLB would be reinforced by the presence of PEG-HCCs and thus more resistant to cisplatin-induced damage and from daily metabolic functions.

Acute Myeloid Leukemia Cells Induce Osteogenic Differentiation in Mesenchymal Stem Cells through Up-regulation of RUNX2

Battula VL[1], Le PM[1], Sun J[1], Mc. Queen T[1], Somanchi A[1], Wang R, Strunk D[3], Shpall EJ[2], Ruvolo P[1], Kantarjian H[1], Konopleva M[1], Andreeff M[1,2],

1. Section of Molecular Hematology and Therapy, Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.
2. Department of Stem Cell transplantation and Cellular Therapy, MDACC
3. Institute of Experimental and Clinical Cell therapy, Spinal Cord and Tissue Regeneration Center, Paracelsus Medical University, Salzburg, Austria.

Corresponding Authors: V. Lokesh Battula, Ph.D., Assistant Professor, Molecular Hematology and Therapy, Leukemia Department, UT MD Anderson Cancer Center, Unit 425. E vbattula@mdanderson.org,

The leukemia bone marrow micro-environment (BME) is comprised of the endosteal and vascular niches, provides vital support for cellular growth and conveys drug resistance to leukemia cells. Mesenchymal stem/stromal cells (MSCs) present in the bone marrow niche induce cell survival and anti-apoptotic proteins in acute myeloid leukemia (AML) cells and protect them from chemotherapy. The mechanisms underlying BME-mediated chemo-resistance however have not been fully elucidated. Here, we hypothesize that AML cells induce functional changes and prime MSCs to protect leukemia cells from chemotherapy. To test our hypothesis, we have compared age matched (between 40-60 years) bone marrow derived MSCs from AML patients (AML-MSC, n=10) and normal (N-MSC, n=10) individuals and analyzed their proliferation, cell surface phenotype, multi-lineage differentiation and chemo-protection potential. AML-MSCs are phenotypically different, with their polygonal morphology and larger cell size compared to N-MSCs which are elongated and spindle shaped appearance. The average cell doubling time of AML-MSCs is 52±8hrs compared to 34±6hours for N-MSCs during their exponential growth phase ($p<0.01$). Cell surface phenotyping by flow cytometry revealed that most of the markers known to be expressed on N-MSCs including CD105, CD90, CD73, CD51, CD44, SUSD2, CD106, CD140b, CD140a, CD106 and CD271 were also expressed on AML-MSCs at similar levels. Interestingly, tissue non-specific alkaline phosphatase (TNAP, clone W8B2), a cell surface protein highly expressed in naïve-MSCs and osteoblast progenitors was 10-14 fold higher in AML- as compared to N-MSCs. Since TNAP is also a osteoblast specific marker, we compared osteoblast differentiation potential of N- vs AML-MSCs. Surprisingly, a dramatic increase in alkaline phosphatase activity was observed in AML-MSCs even without induction of osteoblast differentiation. mRNA analysis by qRT-PCR revealed that osteoblast specific genes including osteopontin, TNAP, osteocalcin, and osterix were 5-10 fold up-regulated in AML-MSCs compared to N-MSCs before induction. In N-MSCs, the expression of these markers was induced only under osteoblast differentiation conditions. These data indicate that AML-MSCs are primed to differentiate into-osteoblasts. Adipocyte differentiation was assessed by Oil-Red O staining for lipid droplets and revealed a > 95% reduction ($p<0.0001$) in the number mature adipocytes in AML-MSCs compared to N-MSCs suggesting that AML-MSCs lack the ability to differentiate into adipocytes. To understand the mechanism inducing osteogenic specific differentiation of AML-MSCs, we performed mRNA expression analysis of genes that regulate this process. We found RUNX2, a transcription factor that induces osteogenic but inhibits adipogenic differentiation, was 4-5 fold increased in AML-MSCs compared to N-MSCs. To validate these observations, we co-cultured N-MSCs in the presence or absence of OCI-AML3 cells for 3-5 days and FACS sorted the MSCs for gene expression analysis. We observed a 3-4 fold up-regulation of TNAP protein expression by flow cytometry and 4-6 fold up-regulation of osteoblast specific markers including osteopontin, alkaline phosphatase and osterix in MSCs co-cultured with OCI-AML3 cells. In addition, RUNX2 was up-regulated and localized to nucleus in MSCs when co-cultured with OCI-AML3 cells. These data suggest that AML cells induce osteogenic differentiation in BM-MSCs by up-regulation of RUNX2. To identify the clinical significance of these observations, we examined the ability of AML- and N-MSCs to protect AML cells from chemotherapy. Co-culture of OCI-AML3 cells with either AML- or N-MSCs and treatment with Cytarabine revealed a 15±4.5% increase in the number of live leukemia cells when co-cultured with AML-MSCs compared to N-MSCs. These data indicate that AML-MSCs protect leukemia cells better from chemotherapy than normal MSCs. In conclusion, AML cells induce osteogenic differentiation in MSCs through up-regulation of the RUNX2 transcription factor. Increased chemo-protection of AML cells by AML-MSCs suggests a prominent role of these cells in AML relapse. Targeting RUNX2 and thereby inhibition of osteoblast differentiation of MSCs may provide enhanced treatment options for AML therapy.

Funding source:
NCI, Leukemia SPORE career development award to Battula V.L.NIH, P01 to Andreeff M.

Hydrogel-Based 3D Model of Patient-Derived Prostate Xenograft Tumors Suitable for Drug Screening

Fong EL[1], Martinez M[2], Yang J[3], Mikos AG[1], Navone NM[3], Harrington DA[2], Farach-Carson MC[1,2,3]

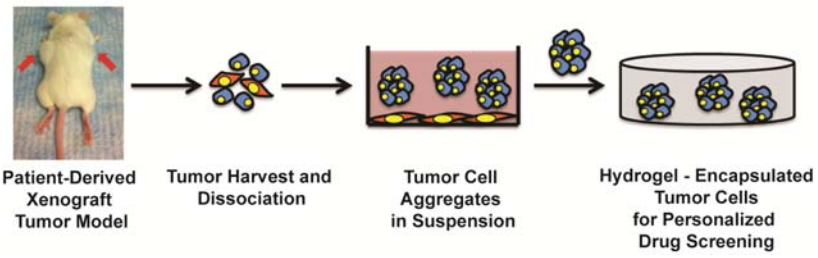
18. Department of Bioengineering, Rice University, Houston, Texas, USA
19. Department of BioSciences, Rice University, Houston, Texas, USA
20. Department of Genitourinary Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Corresponding Author:
Farach-Carson MC, Departments of BioSciences and Bioengineering, BioScience Research Collaborative, Rice University, 6500 Main Street, Houston, Texas 77030, USA, Email: farachca@rice.edu

Despite a decreasing overall mortality rate for prostate cancer (PCa) patients, survival time remains significantly poor with bone metastasis. The development of effective treatments has been hindered, in part, by the lack of cell lines and/or xenograft models that accurately recapitulate the complex metastatic microenvironment. Without appropriate models to reflect the disease, mechanistic studies to accurately elucidate the players involved in PCa progression in bone have been difficult to implement, impeding the development of clinically effective therapeutics targeted to bone metastases.

To overcome this problem, the patient-derived xenograft (PDX) PCa model using immunocompromised mice was established previously to model the disease with greater fidelity than is possible with currently employed cell lines grown on tissue culture plastic. However, poorly adherent PDX tumor cells exhibit low viability in standard culture, making it difficult to manipulate these cells for subsequent controlled mechanistic studies.

Based on the suitability of hyaluronan (HA)-based hydrogel systems for the culture of PCa cell lines, we hypothesized that these systems would similarly support the viability of PDX PCa cells *in vitro*. Hence, in the present study, we developed a novel protocol to encapsulate PDX PCa cells within 3D HA-based hydrogels and examined tumor cell morphology, viability, proliferative capacity and phenotype. We demonstrate that the hydrogel maintains PDX cell viability over several weeks with continued native androgen receptor expression. Furthermore, a differential sensitivity to docetaxel, a chemotherapeutic drug, was observed as compared to a traditional PCa cell line. These findings underscore the potential impact of this novel 3D PDX PCa model as a diagnostic platform for rapid drug evaluation and ultimately push personalized medicine towards clinical reality.



This research is supported by the National Institutes of Health Grants P01 CA98912, Prostate SPORE grant 5P50 CA140388, the Cancer Prevention & Research Institute of Texas grant RP11055 and the David H. Koch Center for Applied Research in Genitourinary Cancers at The University of Texas MD Anderson Cancer Center. E.L.S.F. acknowledges funding support from the National University of Singapore-Overseas Graduate Scholarship.

MiR-322/503 Cluster Plays an Essential Role in Cardiac Specification

Shen X[1], Soibam B[1], Yu W[1], Schwartz RJ[1], and Liu Y[1]
1. Department of Biology and Biochemistry, University of Houston

Corresponding author:
Liu Y, Dept. of Biology and Biochemistry, University of Houston
4800 Calhoun Rd., Houston, Texas, 77004, USA, E-mail: yliu51@central.uh.edu

Understanding the mechanisms of early cardiac fate determination may lead to better approaches in promoting heart regeneration after injury. MicroRNAs (miRNAs) involved in the process are particularly interesting due to their small profile and relatively shorter path to clinic. With Mesp1 as the marker, we used a Mesp1-Cre/Rosa-EYFP reporter system to track the earliest cardiac progenitors, and identified the miRNAs enriched in these cells. Among them, the miR-322/503 cluster is found to be a powerful regulator of the cardiac program: (1) in a screening of more than 20 CPC-enriched miRNAs, miR-322/503 was the most powerful in driving calcium flux activity in mouse embryonic stem cells (mESCs) differentiation; (2) induced ectopic expression of miR-322/503 to mimic the natural course in mESCs led to α-actinin expression and significant increases of cardiac transcription factors (Tbx5, Mef2C, Nkx2-5 and α-MHC); (3) inhibitors of miR-322 and miR-503 significantly reduced expression of α-actinin and the above cardiac TFs. Remarkably, miR-322/503 regulates the cardiac program by inhibiting an RNA-alternative splicing/decay factor, CUG-binding protein 1 (Celf1), which is also known for a role in myotonic dystrophy pathogenesis. The evidences include (i) miR-322 and miR-503 had a shared target site at 3'UTR of Celf1; (ii) expression patterns of miR-322/503 and Celf1 were mutually exclusive, with the highest Celf1 expression in the brain; (iii) miR-322/503 repressed Celf1 protein expression in a dose-dependent manner; (iv) Celf1-shRNA induced up-regulation of cardiac transcription factors and α-actinin, mimicking the function of miR-322/503; (v) ectopic Celf1 expression repressed expression of cardiac transcription factors, while promoted expressions of early neural markers, including Sox1, Zic1, Nestin and Pax6. In summary, we have identified a miR-322/503-Celf1 pathway that promotes cardiac differentiation by preventing activation of other lineages. This new regulatory mechanism may be used to direct cardiac regeneration after heart injury, and treat myotonic dystrophy where Celf1 up-regulation is responsible for skeletal muscle wasting and other symptoms.

We acknowledge the following funding sources: American Heart Association (11SDG526003 3) and a University of Houston startup fund.