GCC Regenerative Medicine Symposium

April 21, 2017

BioScience Research Collaborative Auditorium
6500 Main St.
Houston, TX
The Gulf Coast Consortia (GCC), located in Houston, Texas, is a dynamic, multi-institution collaboration of basic and translational scientists, researchers, clinicians and students in the quantitative biomedical sciences, who benefit from joint training programs, topic-focused research consortia, shared facilities and equipment, and exchange of 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, antimicrobial resistance, neuroengineering, addiction sciences, and regenerative medicine. Current members include Baylor College of Medicine, Rice University, University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, The University of Texas M. D. Anderson Cancer Center, and the Institute of Biosciences and Technology of Texas A&M Health Science Center.
8:00-8:30 Light breakfast and poster setup

8:30 Welcome

8:40 Keynote: *Cellular Therapies in Reconstructive Transplantation*
Maria Siemionow, University of Illinois in Chicago

**Session 1: New Technologies**

9:30  *3D printing of Vascularized Tissues for in Vitro and in Vivo Studies*
Jordan Miller, Rice University

9:45  *Natural Matrix Model for Isolating Circulating Tumor Cells in Cancer Metastasis*
Min Kim, Houston Methodist Research Institute

10:00 Break

**Session 2: Gastrointestinal**

10:15  *Human Intestinal Enteroids: New Models of GI Epithelium for Research and Regenerative Medicine*
Sarah Blutt, Baylor College of Medicine

10:30  *Salivary Stem Cell-Based Solutions for Relief of Xerostomia (Dry Mouth)*
Cindy Farach-Carson, UTHealth

**Session 3: Military Applications**

10:45 Panel: Military Applications
Mark Wong, UTHealth
Charles Cox, UTHealth

11:45-1:30 Lunch and Poster session

12:30 Poster judging

**Session 4: NeuroRegenerative Medicine**

1:30  *Stem Cell Therapy for Spinal Cord Injury Using Patient-specific iPSCs*
Ying Liu, UTHealth

1:45  *MSC for TBI: Attempting to Relate in Vitro Activity with in Vivo Efficacy*
Scott Olson, UT Health
2:00  

**Rapid Fire Trainee Presentations**

**Predocs**

*Preparation of Chondroitin Sulfate Crosslinked, Thermally Responsive Hydrogel for Osteochondral Tissue Regeneration*

Yuseon Kim, Rice University

*Human iPSC-based Modeling of Argininosuccinic Aciduria Reveals Roles of ASL in Endothelial Differentiation and Angiogenesis*

Jordan Kho, BCM

*Pancreatic Cell Fate Determination Relies on Notch Ligand Trafficking by NFIA*

Marissa A. Scavuzzo, BCM

*Tunable Delivery of Bone Morphogenetic Protein-2 (BMP-2) Via a Light-Activatable Adeno-Associated Virus (AAV) Platform*

Esther Lee, Rice University

*Using A Hybrid Enzyme to Promote Neuroregeneration—A Role of Prostacyclin on Neurodegenerative Diseases*

Qinglan Ling, UH

*Multi-layered Vascular Grafts with Improved Compliance Matching and Resistance to Suture Damage for Long Term Patency*

Allison Post, TAMU IBT

**Postdocs**

*MiR-590 Promotes Transdifferentiation of Porcine and Human Fibroblasts towards a Cardiomyocyte-like Fate by Directly Repressing Specificity Protein 1*

Vivek Singh, BCM

*Improvement in Neurological Dysfunction and Tissue Injury following Progenitor Cell Therapies in Traumatic Brain Injury: a Meta-Analysis*

Margaret Jackson, UT Health

*Sox11 and Sox4 Regulate Epidermal Differentiation and Re-epithelization after Wounding*

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*Hyaluronan Mediated Extracellular Matrix and Angiogenesis Results in Attenuated Renal Fibrosis*

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*β1 Integrin-mediated Coordinated Motility in Reorganizing Human Salivary Stem/Progenitor Cells*

Danielle Wu, Rice University

*Treating Spinal Cord Injury with Mesenchymal Stem Cell Extracellular Vesicles Improves Locomotor Recovery, Mechanical Sensitivity and Neuroinflammation*

Katherine Ruppert, UT Health
3:30  Center for Biomaterials: Introduction and Overview  
      Anthony Melchiorri, Rice University

3:45  Break

4:00  Keck Seminar: Mesenchymal Stem Cells as a Discovery Platform for Novel Therapies in Traumatic Brain Injury  
      Shibani Pati, Blood Systems Research Institute

5:00  Awards followed by Reception
Special thanks to our sponsors:
Maria Siemionow, MD, PhD, DSc
Professor, Orthopedics
Director, Microsurgery Research
University of Illinois, Chicago

Cellular Therapies in Reconstructive Transplantation

About Dr. Siemionow:
Maria Siemionow, MD, PhD, DSc, is a world renowned scientist and microsurgeon. She earned her medical degree from the Poznan Medical Academy in 1974 and completed her residency in orthopedics. She earned a PhD, in 1985, for her thesis Evaluation of different microsurgical techniques of vessel anastomosis of diameter less than one millimeter and also completed a hand surgery fellowship at the Christine Kleinert Institute for Hand and Microsurgery in Louisville, KY. In 1990, Dr. Siemionow was appointed Associate Professor of Surgery at the University of Utah, and in 1992, she earned a PhD, DSc for her thesis Hemodynamics of the microcirculation of the free muscle flap, direct in vivo study. From 1995 - 2013, Dr. Siemionow was the Director of Plastic Surgery Research and Head of Microsurgical Training for Cleveland Clinic's Department of Plastic Surgery. In 2005, she was appointed Professor of Surgery in the Department of Surgery at Cleveland Clinic Lerner College of Medicine of Case Western Reserve University. In 2008 she led the team of surgeons that performed the first near-total face transplantation in the United States in 2008. In 2014, she was appointed as Professor of Orthopaedics and Director of Microsurgery Research at The University of Illinois at Chicago. She specializes in microsurgery, hand surgery, peripheral nerve surgery, transplantation, and microsurgery research. Dr. Siemionow is also leading the way in developing new technology for minimal immunosuppression in transplantation, and enhancement of nerve regeneration. Dr. Siemionow has over 330 scientific publications. She has edited three plastic surgery textbooks, two popular science books, and has contributed to 98 published book chapters. Additionally, she serves on the editorial board of nine professional society journals and is an ad hoc reviewer for six professional society journals.

Abstract:
There is a need for development of new anti-rejection therapies for solid organ and vascularized composite allografts (VCA) such as face and limb transplants to eliminate side effects of currently available immunosuppressive protocols. We have successfully induced tolerance in the limb allograft transplants using short-term protocol of alpha-beta TCR/CsA therapy. This was accompanied by development of stable chimerism of donor origin. Based on this encouraging results, we have developed a novel tolerance inducing therapy by fusion of bone marrow cells of Donor and Recipient origin and created Donor-Recipient Chimeric Cells. These cells are recognized by transplant recipient as “Self” and support organ survival. We will introduce Chimeric Cells as supportive therapy for face and limb transplants and will reduce the side effects, the cost and the need for lifelong immunosuppression.

The other approach we are currently taking as a continuation of Chimeric Cell therapy application is to create the universal chimera which will serve as the universal bone marrow transplantation with direct application to the wounded warriors in case of a nuclear bomb attack. We are currently developing a second and third generation of Chimeric Cells leading to ultimate goal of creating universal Chimeric Cells. We also are testing cryopreservation methods for Chimeric Cell storage and propose to introduce a Universal Chimera Bank for civilians and military programs.
Abstract:
Tremendous strides have been achieved over the past several decades in the field of tissue engineering to construct implantable thin tissue constructs such as skin, cornea, and bladder. However, obtaining functional, physiologically relevant solid organ constructs is still a major challenge due to the necessity of a vascular system to supply nutrients and remove waste in thick constructs. Additionally, tissue engineers have historically focused on fabricating tissue constructs containing a single vascular network. However, a key feature of complex biological systems is the presence of interpenetrating networks, such as the respiratory tree with its entangled blood vessel network. These architectures are central to mammalian physiology yet they have remained elusive to fabricate in vitro. To address this challenge, we are developing 3D printing systems to fabricate tissue constructs on the order of several centimeters containing complex interpenetrating vascular networks. Fundamental control of photochemistry during stereolithographic 3D printing is now allowing us to pattern complex interpenetrating vascular networks within soft hydrogels composed primarily of water. Complex mathematical topologies serve as our vascular blueprints, and mammalian cells can also be entrapped in the patterned hydrogel during fabrication. We expect the performed studies will provide insight to the field by demonstrating some of the architectural features which are thought to be required to build living tissues the size of human organs.
Min Kim, MD
Assistant Professor, Surgery
Houston Methodist Research Institute

Natural Matrix Model for Isolating Circulating Tumor Cells in Cancer Metastasis

About Dr. Kim:
Dr. Min Kim has an undergraduate degree from Cornell University and his medical degree from the Johns Hopkins School of Medicine. During medical school, he received the prestigious Howard Hughes Fellowship to perform research at the National Institutes of Health. During medical school, he received the Harold Lamport Research Award, the Paul Ehrlich Research Award and the Henry Strong Denison Scholarship for his research. In addition, he was awarded the Recognition for Outstanding Research at the National Institutes of Health.

After completing medical school, Dr. Kim attended Beth Israel Deaconess Medical Center, a teaching hospital of Harvard Medical School, where he completed his general surgery residency. After his general surgery training, he received a thoracic surgery fellowship at The University of Texas M.D. Anderson Cancer Center where he completed his training in cardiothoracic surgery.

Dr. Kim is currently a Chief of Thoracic Surgery at Houston Methodist Hospital with expertise in minimally invasive thoracic surgery and thoracic surgical oncology. In addition, he has been appointed an Assistant Professor of Surgery at Weill Cornell Medical College and Assistant Member of the Methodist Hospital Research Institute.

Dr. Kim has an active translational lab researching the mechanisms involved in lung cancer metastasis. His clinical interests include treatment of benign and malignant diseases of the esophagus, lung, mediastinum, diaphragm and chest wall. He specializes in minimally invasive procedures, such as VATS and robot assisted lobectomy and minimally invasive esophagectomy, to treat patients with disease of the thoracic cavity.

Abstract:
Cancer metastasis is the stage of tumor development that most often leads to patient mortality. Tumor microenvironment plays a critical role in tumor growth and metastasis. Interaction between the tumor cells and the associated stroma modulate the tumor progression and patient prognosis. We have been successful in mimicking the process of metastasis using the acellular or natural matrix 4D lung model.

The acellular 4D lung model was created by taking a rat heart and lung block and removing all of the cells. This natural lung matrix maintains its three-dimensional architecture, including perfusable vascular beds with a preserved airway. The matrix is composed of collagen, proteoglycans, and elastic fibers that preserve the architecture of airways and capillaries. A unique feature of the matrix is that this composition is preserved among species in the distal airways. Thus, human, rat, or mouse cells can proliferate on this matrix. Furthermore, the basement membranes of the alveolar septa are preserved after decellularization in this model. We have customized the decellularization chamber and bioreactor.

We have found that when tumor cells are placed through the trachea, they form nodules in the natural matrix when it is placed in a bioreactor. Moreover, the model allows tumor cells to secrete proteins that are more similar to patients with lung cancer than the same tumor cells grown on a petri dish. We named it a 4D model since it has perfusion of the tumor nodules that allows it to change over time in addition to growth in the 3D space.
Sarah Blutt, PhD  
Associate Professor, Molecular Virology  
Baylor College of Medicine  

*Human Intestinal Enteroids: New Models of GI Epithelium for Research and Regenerative Medicine*

**About Dr. Blutt:**
Dr. Blutt’s research interest centers on the gastrointestinal tract and understanding how the epithelium repairs itself following damage induced by pathogenic viral infections and in response to the local populations of commensal bacteria. Her projects focus on using molecular and cellular techniques in both in vitro and in vivo model systems to address the overall hypothesis that microbes are a significant component of gastrointestinal health. She trained as a cell biologist and immunologist and currently use her expertise to study stem cell activation. Dr. Blutt participated in the Texas Medical Center Digestive Diseases Center and is a member of the Pediatric Gastroenterology Training grant at Baylor College of Medicine. Some of her work has centered on understanding pathogenesis and immune response to rotavirus infection, a common viral pathogen that infects the small intestine. She is currently using the human enteroids as a model of the gastrointestinal epithelium focusing on the response of stem cells to pathogenic infection and establishing in vitro models to study both small intestinal and liver physiology.

**Abstract:**
The intestinal stem cell niche is influenced by signals from the immune system, the mesenchyme that underlies the crypt, the smooth muscle that exerts mechanical forces, and by the epithelium itself. Much has been learned from models in which the niche itself is damaged and the ensuing regeneration, such as in radiation or chemotherapeutic context, but there is little known about how damage to the villus epithelium influences crypt homeostasis and regeneration. A limitation in defining epithelial factors that regulate the niche has been the absence of in vitro models that recapitulate the diverse nature of the intestinal epithelium. Recently, human intestinal enteroids (HIEs) have been established and contain the normal complement of epithelial cell types (stem, enterocyte, goblet, enteroendocrine, and Paneth cells). We have utilized HIE cultures as pre-clinical models to study the response of the epithelium to common enteric viral pathogens such as human rotavirus (HRV) and human norovirus (HNV). Studies on these enteric viruses have been limited because they are difficult to culture in transformed cell lines and do not infect small animals. Both HRVs and HNVs are thought to predominate in the mature enterocytes in the small intestine. HIEs derived from small intestine biopsies or surgical tissues were inoculated with HRV or HNV. Infection and replication was confirmed by assessing the amount of viral RNA and yield of virus per HIE culture as well as the number of infected cells. Undifferentiated HIEs, consisting primarily of immature enterocyte, Paneth, and stem cells, were not susceptible to infection compared to fully differentiated HIEs that consist predominately of mature enterocytes confirming in vivo findings that the villus enterocyte is the primary target of HRV and HNV infection and replication. Infected enteroids were characterized by increased proliferation and increased LGR5 expression suggesting HRV and HNV activate stem or transitional cell populations. Further confirmation of putative stem cell activation was obtained from transcriptional analysis of WNT signaling family members that were increased following both viral infections. Studies in the murine model of rotavirus infection indicated the upregulation of intestinal stem cell responses in vivo in infected mice validating the HIE as a model to investigate epithelial regeneration. To begin to identify signaling pathways that result in stem cell activation, RNAseq analysis was performed on HRV-infected HIEs. These findings establish HIEs as a model to understand the how the epithelium regenerates in response to epithelial damage in the villi resulting from gastrointestinal infections such as HRV and HNV. In addition, they allow us to ask new questions about human host-pathogen interactions such as innate immune responses, cell-cell communication, and identification of new drug therapies to prevent/treat diarrheal disease.
Cindy Farach-Carson, PhD
Professor; Director of Clinical & Translational Research
UT Health
Salivary Stem Cell-Based Solutions for Relief of Xerostmia (Dry Mouth)

About Dr. Farach-Carson:
Dr. Farach-Carson is a native of Galveston, Texas. She is an active researcher and has a federally and industry funded laboratory focused on tissue engineering, extracellular matrix and cancer biology. She is a pioneer in the use of complex 3D systems for cell and microtissue culture of both normal and cancerous tissues. She is the author of over 200 publications and frequently serves as a reviewer for both grant applications and journal articles. After eleven years as a faculty member at the University of Texas Health Science Center (UTHSC) Dental Branch, she left Houston in 1998 to join the faculty at the University of Delaware where she was a professor of Biological and Materials Sciences from 1998-2009. She was the founding director of the Center for Translational Cancer Research, a role she began in 2005 that brought together four institutions and hospitals with a focus on accelerating translation of cancer research findings to the clinic. She came to Rice in 2009 to provide scientific leadership and vision for the BioScience Research Collaborative and to foster a climate of interdisciplinary and translational research and innovation. She joined the Texas Medical Center as a strategic advisor in 2014. In fall of 2016, Cindy returned as a Professor of Diagnostic and Biomedical Sciences to the UTHSC, now UTHealth, in the School of Dentistry. She is passionate about bringing research from bench discovery through the marketplace and finally to the clinic. She hopes to one day bring the salivary gland her team is building to the clinic for patients who suffer from xerostomia due to hyposalivation (dry mouth) after having radiation for head/neck cancer.

Abstract:
The salivary gland is a complex glandular tissue built upon the core synthetic and secretory function of polarized salivary acinar cells, the directional pipeline established by the assembled ductal cells and the stimulated myoepithelial cells. Regulated saliva transport into the oral cavity absolutely depends upon glandular integration with vascular and autonomic nervous tissue. The biological principles by which this assembly occurs during salivary gland organogenesis are increasingly well understood, although it remains difficult to achieve functional restoration of an replacement salivary gland for patients suffering from xerostomia (dry mouth) after radiation for head and neck cancer or other dry mouth syndromes. A successful strategy to build an artificial salivary gland suitable for implantation minimally must incorporate the following 7 features: 1) instill cellular growth potential without overgrowth; 2) trigger and retain the differentiated, properly assembled branched state involving multiple cell types; 3) build and retain tight junctions among polarized acini; 4) create and retain proper luminal structures for accumulation and transport of salivary components (fluid and protein); 5) support long term synthesis and regulated, directional secretion of salivary components; 6) establish and retain proper assembly of ECM components, cell and tissue barriers; and 7) recruit vascular and nervous tissue to maintain the gland and ensure tissue integration. While such strategy is de rigueur for the successful restoration of a functional gland, accomplishing this is best done using a systematic modular approach. Cues from matrix, growth factors and neighboring cells provide essential information to initiate and maintain stable long term differentiation of gland structures arising through processes of branching morphogenesis, clefting, and continued tissue integration to fully restore salivary flow.
Mark Wong, DDS  
Chairman and Program Director, Department of Oral and Maxillofacial Surgery  
UT Health  
*Space Maintenance, Wound Optimization, and Osseous Reconstruction; Technologies to assist in the management of the Wounded Warrior*

**About Dr. Wong:**  
Dr. Mark Wong is Professor and Bernard and Gloria P. Katz Chairman of the Department of Oral and Maxillofacial Surgery at The University of Texas School of Dentistry at Houston, where he also serves as the Director of Residency Training. He is also an Adjunct Professor of Bioengineering in the Department of Bioengineering, Rice University. His clinical and research interests are focused on reconstructive surgery, tissue engineering of bone and the biomechanical characterization and regeneration of the temporomandibular joint. His research is funded by the National Institutes of Health and the Department of Defense. Dr. Wong has authored over 70 publications and has served on a number of educational and research committees for various professional organizations. He is a Past President of the American Board of Oral and Maxillofacial Surgery, Past President of the American Academy of Craniomaxillofacial Surgeons, and Past President of the International Board for the Certification of Specialists in Oral and Maxillofacial Surgery.

**Abstract:**  
One of the most challenging types of injuries created by high velocity missiles or blast injuries is the contaminated composite wound missing hard and soft tissue and special structures like the teeth. A multi-phase treatment protocol has been developed to address several challenges faced by the facial reconstructive surgeon. Techniques to preserve the soft tissue envelope surrounding missing bone, release of antibiotics to optimize wound healing, and the regeneration of vascularized and non-vascularized bone flaps using an in vivo “bioreactor” will be described.
Charles Cox, MD  
Professor, Pediatric Surgery; George & Cynthia Mitchell Distinguished Chair, Neuroscience  
UT Health  

**Stem Cell Therapies for Neurological Injuries**

**About Dr. Cox:**
Dr. Charles S. Cox, Jr., is Professor of Pediatric Surgery, and the George and Cynthia Mitchell Distinguished Chair in Neuroscience, directing the Pediatric Surgical Translational Laboratories and Pediatric Program in Regenerative Medicine at the University of Texas Medical School at Houston. He is co-director of the Texas Trauma Institute and directs the Pediatric Trauma Program at the University of Texas-Houston/Children’s Memorial Hermann Hospital in the Texas Medical Center. A Texas native, Dr. Cox received his undergraduate degree from the University of Texas at Austin in the Plan II Liberal Arts Honors Program. Upon graduating from the University of Texas Medical Branch, he completed his Surgery residency at the University of Texas-Houston/Children’s Memorial Hermann Hospital in the Texas Medical Center. Further post-graduate fellowships were completed in Pediatric Surgery at the University of Michigan, a NIH T32 sponsored clinical and research fellowship in cardiopulmonary support/circulatory support devices/bio-hybrid organs at the Shriner’s Burns Institute, and Surgical Critical Care/Trauma at the University of Texas Medical School at Houston. He is certified by the American Board of Surgery in Surgery, with added qualifications in Pediatric Surgery and Surgical Critical Care. He served in Afghanistan with the 82nd Airborne in the 909th Forward Surgical Team in 2002.

The Pediatric Translational Laboratories (https://med.uth.edu/pediatricsurgery/research/research-centers-and-programs/translational-laboratories/) and Pediatric Program in Regenerative Medicine (https://med.uth.edu/pediatricsurgery/research/research-centers-and-programs/program-in-childrens-regenerative-medicine/) represent a multi-disciplinary effort that addresses problems that originate with traumatic injury and the consequences of resuscitation and critical care. The Program focuses on progenitor cell based therapy (stem cells) for traumatic brain injury, and related neurological injuries (hypoxic-ischemic encephalopathy, stroke, spinal cord injury), recently completing the first acute, autologous cell therapy treatment Phase I study for traumatic brain injury in children, as well as a DOD funded Phase 1/2a trial for severe TBI in adults(2015). Recently, the NIH funded the first Phase Ib clinical trial for cellular therapies in children with severe TBI, and the DOD Joint Warfighter Program has funded a Phase 2b trial in adults (2016-2020). The program has been continuously funded since 1998 through the National Institutes of Health, Department of Defense/MRMC, Texas Higher Education Coordinating Board/Emerging Technology Funds, Industry Collaboration (Athersys, Inc.; Celgene Cellular Therapeutics; CBR, Inc), and philanthropic contributions. The Program is housed in state-of-the-art laboratory facilities (4500 sf), and includes two cGMP, Class 10,000 facilities for the production of clinical grade cell and tissue products: Hoffberger Cellular Therapeutics Laboratory and the Griffin Stem Cell Therapeutics Research Laboratory. Other major areas of interest include: (1) resuscitation induced organ edema and dysfunction, (2) the neuroinflammatory reflex, (3) mesenchymal stromal cell exosomes as anti-inflammatory agents, and (4) mechanotransduction of stem cells to enhance their anti-inflammatory properties. Three biotechnology start-ups have arisen out of this work: EMIT Corporation, Coagulex, Inc., and Cellvation, Inc., and funded via various venture groups.


**Abstract:**
The Department of Defense funds many research programs and activities in the field of Regenerative Medicine, arguably launching the field to prominence with the creation of AFIRM I and II (Armed Forces Institute of Regenerative Medicine). Other funding has occurred in the DOD via the Combat Casualty Care Programs as well as the Joint Warfighter Programs, TATRC (Telemedicine and Advanced Technology Research Center), DARPA, and the CDMRP (Congressionally Directed Medical Research Program). This session will discuss a sequence of CCC/JWF funded research clinical trials in traumatic brain injury as well as experience gained in elements of DOD funding that distinguish it from other traditional National Institutes of Health or National Science Foundation funding. The importance of linking research to Technology Readiness Levels, Operational Medicine, and unique aspects of the Broad Agency Announcements will be discussed.
Ying Liu, MD, PhD
Assistant Professor, Neurosurgery
McGovern Medical School and Center for Stem Cell and Regenerative Medicine of the Brown Foundation Institute of Molecular Medicine, UT Health Science Center

*Stem Cell Therapy for Spinal Cord Injury Using Patient-specific iPSCs*

**About Dr. Liu:**
Dr. Liu is a fellowship-trained investigator. She earned her medical degree at Peking University Health Science Center in China, received her Ph.D. from the University of Utah. Before joining UT Health, she was an assistant project scientist at the University of California, San Diego and the Scripps Research Institute. Dr. Liu’s research has been presented and published widely. She has co-authored articles that have appeared in Nature Communications, Nature Protocols, Proceedings of the National Academy of Sciences (PNAS), Stem Cells, among other journals. Her long-term goal as an investigator is to elucidate the fundamental mechanisms of neural differentiation with the aim of translating this knowledge into novel therapeutic strategies for neural repair and protection.

**Abstract:**
As a potentially unlimited autologous cell source, patient induced pluripotent stem cells (iPSCs) provide great capability for tissue regeneration, particularly in spinal cord injury (SCI). Despite significant progress made in translation of iPSC-derived neural progenitor cells (NPCs) to clinical settings, a few hurdles remain. Among them, non-invasive approach to obtain source cells in a timely manner, safer integration-free delivery of reprogramming factors, and purification of NPCs before transplantation are top priorities to overcome. Here, we developed a safe and cost-effective pipeline to generate clinically relevant NPCs. We first isolated cells from patients’ urine and reprogrammed them into iPSCs by non-integrating Sendai viral vectors, and carried out experiments on neural differentiation. NPCs were purified by A2B5, an antibody specifically recognizing a glycoconjugate on the cell surface of neural lineage cells, via fluorescence activated cell sorting. To test the functionality of the A2B5+ NPCs, we grafted them into the contused mouse thoracic spinal cord. Eight weeks after transplantation, the grafted cells survived, integrated into the injured spinal cord, and differentiated into neurons and glia. Our specific focus on cell source, reprogramming, differentiation and purification method purposely addresses timing and safety issues of transplantation to SCI models.
Scott Olson, PhD  
Assistant Professor, Program in Regenerative Medicine  
UT Health Science Center  

**MSC for TBI: Attempting to Relate in Vitro Activity with in Vivo Efficacy**

**About Dr. Olson:**  
Scott Olson is an MSC Biologist working in the Children’s Program in Regenerative Medicine in the Department of Pediatric Surgery at McGovern School of Medicine. Dr. Olson completed his doctorate in the lab of Dr. Darwin Prockop at Tulane University’s Center for Gene Therapy studying novel methods by which mesenchymal stem cells (MSC) can contribute to tissue repair. At University of California at Davis’s Health Sciences Institute for Regenerative Cures, Dr. Olson worked to apply MSC as a platform to develop new treatments for Huntington’s Disease. Dr. Olson joined UTHealth in September 2011.  
Dr. Olson is involved in developing and transitioning studies with direct translational applications. At UT Health, his primary focus is bringing his expertise in the field of adult stem cells, specifically MSC, to explore their potential in the treatment of Traumatic Brain Injury (TBI) and in trauma-associated neuroinflammation in general. MSC have been used in a number of completed, ongoing, and proposed clinical trials with reported therapeutic benefits. Dr. Olson strives to better describe the role of MSC in CNS injuries, highlighting their innate therapeutic abilities in an effort to create an improved treatment for TBI.

**Abstract:**  
Mesenchymal stem cells (MSC) are currently being used to treat a huge variety of diseases and conditions across the world. Likewise, there are a wide range of culture conditions, priming steps, proprietary media, cell phenotypes, that culminate in contradictory reports of efficacy attributed to a number of different mechanisms. We recognize that one of the most difficult parts of working with MSC is in the lack of discrete markers that can be conclusively attributed to a therapeutic process, meaning that there is currently no way to predict whether one cell population may be better than another without using them.  
In regards to traumatic brain injury (TBI), it has become clear in animal models that a major mechanism of MSC efficacy involves immunomodulation whereby MSC are capable of reducing systemic and neuroinflammation that can result in decreased blood-brain barrier permeability and lead to an increase in neurogenesis and improvement in neurocognitive function.  
We propose to re-frame the analysis of MSC in vitro to evaluate cellular potency as a function of cellular activity, rather than surface marker. We found two different populations of cells that had very different results in treating TBI in vivo. We then used a mixed-cell cytokine inhibition assay to measure the capability of different MSC to reduce TNF-α and IFN-γ in response to antigen stimulated primary splenocytes. This activity was then cross-correlated with MSC expression of a short list of frequently published anti-inflammatory genes in response to TNF-α and IFN-γ. This resulted in a positive correlation between COX-2 and immunomodulatory potential, a result that was then confirmed with in vivo loss-of-function and gain-of-function assays to treat TBI.  
We are now applying this strategy to analyze additional therapeutic cells, treatment strategies, and MSC-derived microvesicles internally and in a number of collaborations, both academic and industry, resulting in a number of interesting observations.
Rapid Fire Trainee Talks

Predocs:

Yuseon Kim, Rice University
*Preparation of Chondroitin Sulfate Crosslinked, Thermally Responsive Hydrogel for Osteochondral Tissue Regeneration*

Jordan Kho, BCM
*Human iPSC-based Modeling of Argininosuccinic Aciduria Reveals Roles of ASL in Endothelial Differentiation and Angiogenesis*

Marissa A. Scavuzzo, BCM
*Pancreatic Cell Fate Determination Relies on Notch Ligand Trafficking by NFIA*

Esther Lee, Rice University
*Tunable Delivery of Bone Morphogenetic Protein-2 (BMP-2) Via a Light-Activatable Adeno-Associated Virus (AAV) Platform*

Qinglan Ling, UH
*Using A Hybrid Enzyme to Promote Neuroregeneration—A Role of Prostacyclin on Neurodegenerative Diseases*

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*Multi-layered Vascular Grafts with Improved Compliance Matching and Resistance to Suture Damage for Long Term Patency*

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*MiR-590 Promotes Transdifferentiation of Porcine and Human Fibroblasts towards a Cardiomyocyte-like Fate by Directly Repressing Specificity Protein 1*

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Qi Miao, BCM
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Danielle Wu, Rice University
*β1 Integrin-mediated Coordinated Motility in Reorganizing Human Salivary Stem/Progenitor Cells*

Katherine Ruppert, UT Health
*Treating Spinal Cord Injury with Mesenchymal Stem Cell Extracellular Vesicles Improves Locomotor Recovery, Mechanical Sensitivity and Neuroinflammation*
Anthony Melchiorri, PhD  
Rice University  
*Center for Biomaterials: Introduction and Overview*

**About Dr. Melchiorri:**  
Anthony Melchiorri is the Associate Director of the Biomaterials Center at Rice University. His primary interests are in cultivating widespread use of and education on biomaterials and biofabrication tools such as 3-D printing, electrospinning, and bioplotting. He has implemented and employed these techniques in a variety of environments, ranging from startups to larger biotech companies and academic research institutions.

**Abstract:**  
The Biomaterials Lab is dedicated to improving patient health and overcoming medical challenges through the development and support of biomaterials research, education, and entrepreneurship. Tools, training, and support are provided to students at all levels, faculty, staff, and collaborators at Rice University and the surrounding community. The Lab houses the education and equipment needs to fabricate and characterize materials, enhancing biomaterials-related activities including developing regenerative medicine techniques, designing devices, and building prototypes.
Shibani Pati, MD, PhD  
Scientific Director, Cellular Therapeutics  
Blood Systems Research Institute  

Mesenchymal Stem Cells as a Discovery Platform for Novel Therapies in Traumatic Brain Injury

About Dr. Pati:
Dr. Pati is currently employed at Blood Systems Research Institute (BSRI) in San Francisco California. She is the Scientific Director of Cellular Therapies for BSRI and Blood Systems Inc. and an Associate Professor at BSRI and the University of California San Francisco (UCSF) - Department of Laboratory Medicine. She is by training a cancer vascular biologist with an interest in the role of endothelial dysfunction and vascular compromise in the pathogenesis of human disease- specifically trauma and traumatic brain injury (TBI). Dr. Pati received her MD. PhD. from the University of Maryland and completed a post-doctoral fellowship in Physical Medicine and Rehabilitation at the Baylor College of Medicine and the University of Texas in Houston. Following her fellowship, Dr. Pati worked at the Center for Translational Injury Research (CeTIR) at the University of Texas Houston. Dr. Pati’s specific areas of investigation involve the use of stem cells, blood products and novel resuscitative modalities that can mitigate endothelial dysfunction, inflammation and coagulation disturbances found in traumatic injury. Her lab aims to specifically understand the mechanisms of vascular compromise in injury and novel methods by which to modulate it. Recent studies by Dr. Pati have involved the mechanisms of action of mesenchymal stem cells in TBI.

Abstract:
Traumatic brain injury (TBI) is the leading cause of death and disability in children and adults under 44 and is considered a “silent epidemic” in the United States in both civilian and military populations. TBI can trigger a number of pathological processes that contribute to the acute and chronic morbidity and mortality associated with the disease. Mesenchymal stem cells (MSCs) have been shown to have therapeutic potential in multiple conditions characterized by neuronal injury and neurocognitive dysfunction including TBI. Although there are over 300 trials involving MSCs listed on ClinicalTrials.Gov for the treatment of various diseases, the mechanisms of how they work are largely unknown. Many groups including our own have shown that the beneficial effects of MSCs are mediated by multiple soluble factors including MSC derived microvesicles. It is our goal to define the mechanisms of action of MSCs in TBI, with specific focus on our identified MSC derived proteins- TIMP3 and Wnt3a. This talk will discuss how these proteins were identified and what is known about their therapeutic mechanisms of action in TBI. This talk will discuss how mechanistic studies on MSCs can be utilized as a discovery platform for novel therapies for neurotrauma and other applications.
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Intrinsic Phenotypic Differences between Adult And Fetal Dermal Fibroblasts In Response To Mechanical Tension

M. Fahrenholtz¹, H. Li¹, M. A. Chandramouli¹, X. Wang¹, M. Rae¹, W. Short¹, P. Duann¹, K. Grande-Allen², P. L. Bollyky³, S. G. Keswani¹, S. Balaji¹

1. Department of Surgery, Baylor College Of Medicine, Houston, TX, USA; 2. Department of Bioengineering, Rice University, Houston, TX, USA; 3. Medicine-Infectious Disease, Stanford University, Palo Alto, CA, USA

Scarless fetal wounds are characterized by negligible resting tension and elevated levels of fibroblast-generated hyaluronan(HA). In contrast, wounds with increased tension or critically large fetal wounds heal with increased scar. We hypothesize that higher mechanical tension will differentially alter fibroblast-mediated HA metabolism in adult and fetal fibroblasts. C57BL/6J fetal (fetalFB,E14.5) and adult (adultFB,8 wk) dermal fibroblasts were cultured on silicone membranes +/-10% static strain (1, 3, 6, and 12h). Monolayers were analyzed via PCR for HA-synthesis(HAS1-3), HA-remodeling(HYAL1-2, KIAA1199), and HA-receptor(CD44) genes, normalized to static fetalFB. Total HA was analyzed by ELISA at 12h. Data is reported as mean+/-SD (n=3), with p-values calculated by two-way ANOVA (post-hoc Tukey’s test). Under static conditions, fetalFB had increased expression of HAS2 (2.08+/-0.91 vs 1.68+/-0.30, p<0.01) with no difference in HAS1 or HAS3, but decreased KIAA1199 (1.42+/-0.15 vs 8.15+/-5.68, p<0.001) and HYAL2 (1.24+/-0.17 vs 1.46+/-0.13, p<0.05) compared to adultFB. Tension significantly decreased HAS2 in adultFB (1.68+/-0.30 vs 0.75+/-0.42, p<0.001), but had no effect on fetalFB HAS2. However, in both fibroblasts, tension resulted in an increase in HAS3 expression (fetalFB 1.59+/-0.84 vs 4.67+/-2.07, p<0.001; adultFB 1.77+/-0.93 vs 3.81+/-2.17, p<0.01). Additionally, in fetalFB and adultFB, tension increased HYAL1 (p<0.001,12h versus all other time points); but only decreased KIAA1199 expression in adultFB(2.39+/-2.25 vs 8.15+/-5.68, p<0.001). AdultFB had more HA than fetalFB(0.476+/-0.294 vs 0.180+/-0.029, p<0.05). Tension decreased HA content in adultFB(0.233+/-0.062, p=0.059) with no effect in fetalFB. Tension downregulated CD44 gene expression in adultFB compared to fetalFB(2.66+/-0.08 vs 0.33+/-0.08, p<0.001). Biomechanical tension has a differential effect on HA metabolism in adultFB and fetalFB. The adultFB have an HA synthase and degradation profile that suggests higher production of low molecular weight HA that is pro-inflammatory and may be inducible by tension. These insights into the intrinsic differences between regenerative fetalFB and fibrotic adultFB may yield targets to attenuate fibrosis.

Funding Sources: This work is funded by Departmental startup funds and Wound Healing Foundation – FLASH award to S.B.
Fabrication of Biomimetic Bone Grafts with Multimaterial 3D Printing

Dhavalikar P1, Sears N1, Whitely M1 Cosgriff-Hernandez E1
1. Department of Biomedical Engineering, Texas A&M University

Corresponding author: Prachi Dhavalikar, Department of Biomedical Engineering, Texas A&M University, 2121 West Holcombe Blvd, Houston, TX, email: prachisdhavalikar@gmail.com

Introduction: Each year, millions of people are hospitalized due to bone injuries. A subset of these injuries are critical size defects, which are a result of trauma, tumor resection surgeries, and congenital defects. For treatment of critical size defects, an ideal bone graft should be biodegradable, porous, and has similar mechanical properties to natural bone. Furthermore, bone defects are non-uniform in size and shape and treatments must be custom fit to the injury. To this end, our lab has developed a tunable bone graft system that is biodegradable, highly porous, and has compressive properties comparable to cancellous bone. These materials are fabricated via polymerization of high internal phase emulsions (HIPEs), and are capable of microscale integration with native bone. Using the HIPEs as emulsion inks for 3D printing allows for creation of complex geometries and architectures which can enable fabrication of highly customized bone grafts. These prints have hierarchical porosity resulting in increased permeability of the scaffolds. This unique property of 3D printed constructs can be critical for regenerative processes like vascularization. Consequently, we have observed that increasing the porosity leads to decreases in the mechanical properties of our scaffolds. Therefore, the goal of the current study was to modify our first generation printer to be able to print multi-materials to improve scaffold mechanical properties. Specifically we proposed to print reinforced scaffolds using thermoplastic polymer poly(lactic acid) (PLA) for a hard outer shell and emulsion inks for a porous interior to replicate bone’s natural structure.

Materials and Methods: To prepare emulsion inks, pre-polymer was combined with 10 wt% surfactant (polyglycerol polyricinoleate) and 1 wt% initiator (phenylbis (2,4, 6-trimethylbenzoyl)-phosphine oxide, and mixed for 2.5 minutes are 2500 rpm using a FlacTek Speedmixer DAC 150 FVZ-K. An aqueous solution of calcium chloride (5 wt%) was then added to the organic phase (w:o 75:25) in three additions and mixed at 500 rpm for 2.5 minutes after each addition. A last mix for 2.5 minutes at 2500 rpm was used to improve homogeneity and increase viscosity for printing. Prepared inks were loaded into one cartridge; PLA filament was fed through to a separate cartridge. Emulsion inks and melted PLA were extruded alternatively layer-by-layer through respective syringes and motor actuated plungers. The emulsions inks rapidly cured after deposition by UV exposure, while the PLA cooled via exposure to ambient temperature. Printed scaffolds were evaluated using scanning electron microscopy for integration of two phases. Additional characterization included mechanical properties and permeability testing.

Results and Discussion: We were able to successfully demonstrate fabrication of multi-material constructs of emulsion inks and PLA. Constructs maintained good integration of the two phases. The combination of paste extrusion with thermoplastic printing produced scaffolds with superior mechanical properties (Figure 1), while maintaining their permeability.

Conclusions: This study demonstrates a way to custom fabricate multi-material scaffolds that maintain high level of permeability and strong mechanical properties. Due to their biomimetic nature, these scaffolds have great potential to improve clinical outcomes for treatment of critical size bone defects.

Funding Sources: Texas A&M University Graduate Diversity Fellowship
Altered Shear Forces Precipitate Fibrotic Remodeling in Discrete Subaortic Stenosis


1. Department of Surgery, Congenital Heart Surgery Service, Texas Children’s Hospital
2. Department of Surgery, Pediatric Surgery, Texas Children’s Hospital/Baylor College of Medicine
3. Department of Bioengineering, Rice University

Corresponding author: Sundeep G. Keswani, Department of Surgery, Texas Children’s Hospital, 6621 Fannin St., Houston, TX, E-mail: sgkeswan@texaschildrens.org

Discrete subaortic stenosis (DSS) is a congenital anomaly characterized by abnormal fibrous tissue formation that causes left ventricular outflow tract (LVOT) obstruction. Treatment is usually surgical intervention but is associated with significant recurrence. The pathogenesis of DSS is unknown because of a lack of appropriate models, but current literature points to altered shear stresses in the LVOT due to altered geometry. Therefore, we have created a novel system to mimic intra-cardiac shear-force and hypothesized that altered shear stress is sensed by endocardial endothelial cells (EEC) resulting in cytokine-mediated fibrotic tissue formation.

A parallel plate flow chamber was developed to apply variable shear to EEC monolayers and spatially-organized hydrogel co-cultures of EEC and cardiac fibroblasts (CF). EEC and CF were isolated from porcine LVOT and subjected to static, low, and high shear conditions based on echocardiographic data then analyzed by ICC (CD31 and VE-Cadherin) and PCR array. To determine the role of CD31 signaling in EEC shear response, a Src inhibitor was applied to the high shear condition. Human DSS tissues obtained from the TCH Tissue Biorepository (n=7) were compared to dermal scar specimens by immunohistochemistry and histology.

EEC and CF have been successfully isolated and used in mono- and co-culture. High shear conditions caused the de-localization of mechanosensitive CD31 from the VE-Cadherin-rich cellular junctions. PCR array revealed up-regulation of pro-inflammatory CSF1 and CSF3 in the high shear condition (4.19 and 4.71 fold regulation versus low shear) and up-regulation of chemoattractants CCL3, CCL4 and CCL5 (15.34, 15.58 and 7.59 fold regulation). Treatment of the cells with Src family kinase inhibitor resulted in a ~3.5-fold decrease in CCL3 and CCL4 and 4-fold decrease in CCL5 compared to high shear with no treatment. Src inhibition also decreased CSF3 expression by 2-fold. Histological staining revealed phenotypic similarities between DSS tissues and other fibrotic tissues (such as dermal scar), including increased collagen deposition (Herovici), disorganized collagen bundles (Trichrome), and presence of activated fibroblasts (25% of resident cells, ASMA).

Our data suggests altered shear forces result in changes to the expression profile of mechanosensitive proteins in EEC, particularly CD31, and CD31 signaling inhibition results in a partial reversal of the pro-inflammatory profile. Additionally, histologic characteristics of DSS mirror fibrotic tissues in other organs. Understanding the pathogenesis of DSS through mechanosensing may elucidate therapeutic targets and implications for other conditions characterized by fibrosis associated with altered mechanical forces.

The authors would like to acknowledge funding from the Virginia and L.E. Simmons Family Foundation Mini Collaborative Research Grant.
Thiolated Poly(Lactic-co-Glycolic Acid) Macromers for Biomolecule Conjugation by Alkyne-Azide Click Chemistry

Guo JL¹, Kim YS¹, Engel PS², Mikos AG¹
1. Department of Bioengineering, Rice University
2. Department of Chemistry, Rice University

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

Objective: Synthetic polymer hydrogels offer high tunability and many other favorable properties for tissue engineering applications. However, these hydrogels usually require the conjugation of additional biomolecules to induce biological activity and produce effective tissue repair in vivo. To meet this need, we are designing a thiol-terminated poly(lactic-co-glycolic acid) (PLGA) macromer with alkyne moieties for the conjugation of biomolecules via alkyne-azide “click” chemistry. By exploiting the high selectivity and yield of the alkyne-azide cycloaddition reaction, we aim to achieve highly selective binding of tissue-relevant, azide-linked biomolecules to our polymer. Ultimately, this PLGA-based polymer will provide nucleophilic thiol termini for crosslinking with other macromer systems, while enabling the conjugation of azide-linked biomolecules to these polymer systems.

Methods: In the process of synthesizing a thiolated, alkyne-presenting PLGA macromer, we first generate a dithiol terminated alkyne – known as butyne dithiol (BDT) – and a di-mesylate terminated PLGA polymer – known as PLGA-mesylate. BDT is generated by the thiolation of 1,4-dichloro-2-butyne – via potassium thioacetate attack and subsequent hydrolysis of the thioester bond. PLGA-mesylate, on the other hand, is generated by the triethylamine-catalyzed mesylation of PLGA-diol. In the final reaction, PLGA-mesylate is thiolated by the nucleophilic attack of excess BDT, effectively displacing both mesylate groups with BDT to generate the final PLGA-BDT macromer.

Results: NMR characterization of the PLGA-mesylate and BDT syntheses has revealed high yield and highly pure products corresponding to the expected structures, with near 100% conversion in the case of PLGA-mesylate. The final product, PLGA-BDT, has been shown by NMR to have a 60% conversion of terminal mesylates to BDT. GPC characterization of PLGA-diol, PLGA-mesylate, and PLGA-BDT has shown minor increases in average molecular weight – under 50% – along the entire synthetic pathway, indicating that runaway polymerization has been effectively avoided.

Conclusions: We have successfully synthesized PLGA-BDT – a thiolated, alkyne-presenting polymer. Future studies will provide proof-of-concept for the alkyne-azide “click” reaction between PLGA-BDT and azide-linked biomolecules such as chondroitin sulfate, and demonstrate the nucleophilic capacity of the thiol termini to react with epoxy rings and other electrophilic groups.

The authors would like to acknowledge support by the National Institutes of Health (R01 AR068073).
Observing the Effect of Mechanobiology by Lung Cancer Cell during Metastasis

Han RI¹, Wanna CA¹, Mehta SM¹, Gibbons DL², Grande-Allen KJ¹

1. Department of Bioengineering, Rice University, Houston, TX 77005
2. Department of Thoracic/Head and Neck Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030

Corresponding author: Richard I. Han, Bioengineering, Rice University, Houston TX, rih2@rice.edu

A mouse model of human lung adenocarcinoma driven by mutations in K-ras and p-53 genes was adapted to investigate roles of mechanics and cellular epithelial to mesenchymal transition (EMT) during metastasis. Tumor cells were grown in collagen gels under static tension, so that the mechanobiological properties and the cell-matrix interactions, in particular the collagen structural changes could be elucidated.

Metastatic (344SQ) and non-metastatic (393P) tumor cells were encapsulated in rat tail collagen-I, and studied under static tension (anchored) for 7 days. Collagen gels were processed for light microscopy (picrosirius red for collagen), confocal microscopy (ZO-1, DAPI and collagen), two photon microscopy, cell macerated SEM and TEM. The elastic modulus were determined via mechanical testing and the alignment of collagen fibrils were analyzed with MATLAB.

Under static tension, the 344SQ contracted 15.3% more and were 14.7kPa stiffer than 393P (at collagen=2mg/ml). Ultra-structurally, the 393P were rounded and clustered together, whereas the 344SQ were elongated and closely associated with collagen. The tight junction protein (zonula occludens-1 ZO-1) were overt and widely expressed in 393P, whereas ZO-1 in 344SQ were scarcely and limited to edges. Under two-photon microscope, the 344SQ cells were closely associated with collagen and clustered to form a ribbons-like structures. The collagen fibril alignment in 344SQ was much more aligned and was significantly different to 393P (p<0.001).

The metastatic cells were closely associated with collagen and exerted more contractile force than the non-metastatic hence the stiffer gels. EMT during metastasis enhanced the cell-matrix interactions; the re-modulated metastatic cell distribution in collagen gel contained a higher tolerance to tension compares to clustered non-metastatic cells. Further study is required to understand the interaction of mechanical, cytokine and ECM signaling.
Perlecan Domain I and Growth Factor Gradients in Hydrogels for Salivary Gland Engineering

Hubka KM1, Wu D2, Grindel BJ5, Pradhan-Bhatt S3,4, Witt RL3,4, Carson DD2, Harrington DA6, Farach-Carson MC2,3,6

1. Department of Bioengineering, Rice University
2. Department of BioSciences, Rice University
3. Department of Biological Sciences, University of Delaware
4. Helen F. Graham Cancer Center & Research Institute, Christiana Care Health Systems
5. Department of Cancer Systems Imaging, University of Texas MD Anderson Cancer Center
6. Department of Diagnostic & Biomedical Sciences, School of Dentistry, UTHealth

Corresponding author: Mary C. Farach-Carson, Department of Diagnostic & Biomedical Sciences, UTHealth, School of Dentistry, 1941 East Road BBS-4220, Houston, Texas, E-mail: Mary.C.FarachCarson@uth.tmc.edu

Introduction: Morphogen gradients direct tissue formation during embryogenesis. Heparan sulfate proteoglycan 2, perlecan, mediates the formation of heparin-binding growth factor (HBGF) gradients via its N-terminal heparan sulfate (HS) chains decorating domain I (PlnD1). PlnD1 serves as an HBGF-delivery vehicle in salivary tissue engineering strategies. Because primary human salivary stem/progenitor cells (hS/PCs) can sense morphogen gradients, we engineered a 3D hydrogel containing PlnD1-based growth factor gradients to direct hS/PC differentiation into salivary ductal and acinar cell phenotypes for gland replacement. Materials and Methods: A stereolithographic 3D printer generated molds of multichannel gradient maker devices (MGMDs). A modular hyaluronic acid (HA)-based hydrogel consisting of a thiol-modified HA backbone and PEG-diacylate crosslinker was used as a biocompatible matrix to support hS/PC expansion and self-organization. Free thiols distributed along the HA backbone permitted covalent incorporation of acrylated PlnD1 (PlnD1-PEG-Ac). First, fluorescent microparticles were added to hydrogel precursor solutions and flowed through the MGMD to form gradients of microparticles at flow rates of 0.1, 0.3, and 0.9 mL/min. Fluorescent microparticle gradient formation was measured as fluorescence intensity across the hydrogels by confocal microscopy. Second, PlnD1 was PEGylated using acrylate-PEG-succinimidyl valerate (Ac-PEG-SVA) by reacting the SVA with the free amines via surface lysines on the PlnD1 core protein. Western blotting confirmed the size increase of the acrylated PlnD1. Using a flow rate of 0.9 mL/min determined from the steepest microparticle gradient, PlnD1-PEG-Ac gradient hydrogels were formed. To localize PlnD1, FGF-2 was labeled with amine-reactive tetrafluorophenyl (TFP)-Alexa Fluor 488 (AF488). To protect the heparin-binding sites, FGF-2 was pre-bound to a heparin column during labeling, after which FGF2-AF488 was salt eluted from the heparin column and labeling efficiency quantified. FGF-2 gradients were established by incubating PlnD1-gradient hydrogels in FGF2-AF488-containing solutions and fluorescence intensity across the hydrogel width was quantified by confocal microscopy. Third, hS/PCs were encapsulated in gradient hydrogels and viability was assessed by live/dead assay. Last, migratory positive control MC3T3-E1 cells were encapsulated in PlnD1-gradient hydrogels, incubated with or without 100 ng/mL FGF-2 and PDGF, and imaged at multiple time points to determine cell migration in response to morphogen gradients. Results and Discussion: Flowing hydrogel precursor solutions containing microparticles or PlnD1 through the MGMDs yielded gradients across the width of the hydrogel. FGF-2 gradients were established in the PlnD1 hydrogel after initial loading and were stable >30 days. Encapsulated salivary hS/PCs were viable in PlnD1 gradient hydrogels. MC3T3-E1 cells sensed and migrated in response to the gradient release of HBGFs by moving in the direction of increasing growth factor concentration. Conclusions: Highly stable HBGF gradients were formed from PlnD1 gradient templates in 3D HA hydrogels. Encapsulated salivary hS/PCs were viable in gradient matrices in vitro. Cell migration in response to various HBGFs could be quantified, thus this approach is suitable to support
branching of hS/PCs used in salivary tissue engineering applications that require physiologically relevant morphogen gradients of HBGFs. **Acknowledgements:** Supported by the NIDCR Grant R01 DE022969 and the NCI Grant P01 CA098912. KMH acknowledges funding from the NIDCR Ruth L. Kirchstein NRSA Individual Predoctoral Fellowship F31 DE025179.
Improvement in Neurological Dysfunction and Tissue Injury following Progenitor Cell Therapies in Traumatic Brain Injury: a Meta-Analysis

Jackson M, Srivastava A, Cox C.

1. Department of Pediatric Surgery, UTHealth McGovern Medical School, Houston, Texas.

Corresponding author: Margaret L. Jackson, Department of Pediatric Surgery, UTHealth McGovern Medical School, 6431 Fannin Street, Houston, Texas 77030.
Margaret.L.Jackson@uth.tmc.edu

Background: No treatment is available to reverse injury associated with traumatic brain injury (TBI). Progenitor cell therapies show promise in both pre-clinical and clinical studies. We conducted a meta-analysis of pre-clinical studies using progenitor cells to treat TBI.

Methods: EMBASE, MEDLINE, Cochrane Review, Biosis, and Google Scholar were searched for articles using pre-specified search strategies. Studies meeting inclusion criteria underwent data extraction. Analysis was performed using Review Manager 5.3 according to a fixed-effects model, and all studies underwent quality scoring.

Results: Of 430 abstracts identified, 38 met inclusion criteria and underwent analysis. Average quality score was 4.32 out of 8 possible points. No study achieved a perfect score. Lesion volume (LV) and Neurologic Severity Score (NSS) outcomes favored cell treatment with standard mean difference (SMD) of 0.86 (95%CI 0.64, 1.09) and 1.36 (95%CI 1.11, 1.60) respectively. Rotarod (RR) and Morris Water Maze (MWM) outcomes also favored treatment with improvements in SMD of 0.34 (95%CI 0.02, 0.65) and 0.46 (95%CI 0.17, 0.74) respectively. While LV and NSS were robust to publication bias assessments, RR and MWM were not. Heterogeneity ($I^2$) ranged from 74% to 85% among the analyses, indicating a high amount of heterogeneity among studies. Precision as a function of quality score showed a statistically significant increase in the size of the confidence interval as quality improved.

Conclusions: Our meta-analysis study reveals an overall positive effect of progenitor cell therapies on LV and NSS with a trend towards improved motor function and spatial learning in different TBI animal models.

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Human iPSC-based Modeling of Argininosuccinic Aciduria Reveals Roles of ASL in Endothelial Differentiation and Angiogenesis

Kho J1,2, Tian XY4, Wong JWT4, Bertin T2, Jiang MM2, Kim J3, Bissig KD1,3, Nagamani SCS2, Lee B1,2

1Program in Developmental Biology, Baylor College of Medicine, Houston, TX
2Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX
3Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX
4Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston, TX

Corresponding author: Jordan Kho, Program in Developmental Biology, Baylor College of Medicine, 1 Baylor Plaza R830, Houston, TX, E-mail: kho@bcm.edu

The urea cycle functions to detoxify ammonia from protein breakdown into urea. Catalyzing the fourth reaction of urea cycle in the liver, argininosuccinate lyase (ASL) is the only enzyme in the body able to synthesize endogenous L-arginine, a substrate for the synthesis of urea and other biologically important metabolites including nitric oxide (NO). Deficiency of ASL in human leads to argininosuccinic aciduria (ASA). In addition to hyperammonemia, subjects with ASA can develop hypertension. Our recent studies suggest that beyond ureagenesis, ASL has a cell-autonomous role in maintaining NO homeostasis in other non-hepatic tissues. We hypothesized that loss of ASL in endothelial cells (ECs) alone is sufficient to cause defects in vascular functions and structures, leading to hypertension. To test this hypothesis, we generated a mouse model with endothelial-specific deletion of Asl (Asl cKO). We found that Asl cKO mice develop hypertension. This hypertension was secondary to endothelial-specific NO deficiency as demonstrated by abnormal relaxation of aortic rings and correction with treatment with an NOS-independent NO supplement. To further evaluate the consequences of ASL deficiency on human vasculature, we utilized the cell reprogramming technology to generate ECs with patient-specific mutations. Human induced pluripotent stem cells (hiPSC) were derived from fibroblasts of both ASA and healthy subjects, and further differentiated into ECs. Interestingly, we discovered that ASA hiPSC differentiated less efficiently into ECs as compared to control hiPSC. Furthermore, ASA hiPSC-derived ECs have reduced NO production, increased oxidative stress, and impaired angiogenesis as shown by their reduced capacity to form capillary-like structures on Matrigel in vitro and blood capillaries in vivo upon subcutaneous injection within Matrigel into immunodeficient mice. Our study using a novel mouse model and hiPSCs-derived endothelial cells from patients with a rare Mendelian form of hypertension shows that loss of ASL-mediated NO synthesis in endothelial cells alone causes structural and functional abnormalities in endothelial cells, and contributes to pathogenesis of hypertension. Lastly, our study provides the first proof-of-principle that hiPSC-derived ECs can be used as a model system to study genetic forms of systemic hypertension and highlights the utility of this technology in exploring the pathogenesis of other vascular diseases.

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Preparation of Chondroitin Sulfate Crosslinked, Thermally Responsive Hydrogel for Osteochondral Tissue Regeneration

Kim Y1, Guo JL1, Lam J1, Mikos AG1

1. Department of Bioengineering, Rice University

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

In situ gelling hydrogel constructs have shown great promise for the repair of bone, cartilage, and other tissue types. Our research group has developed in situ thermally gelling hydrogels for bone repair, as well as bi-layered hydrogels that affect the repair of osteochondral tissue containing bone and cartilage. Because hydrogels have inherent issues with syneresis, or shrinking, chemical crosslinkers have been widely utilized to prevent such behavior. In this study, we aimed to study the feasibility of using chondroitin sulfate (CS), a sulfated glycosaminoglycan, as the biological crosslinking agent. CS is a major component of cartilage ECM, and it has also been shown to enhance chondrogenesis of mesenchymal stem cells. Once modified with adipic acid dihydrazide (ADH), it can undergo crosslinking reaction with (N-isopropylacrylamide) (PNiPAAm)-based thermogelling macromer. Pendant epoxy group was chosen as the site of crosslinking, since amine-epoxy reaction does not require exogenous catalyst, and can occur in mild conditions without generating harmful by-products.

Carboxylate groups on glucuronic acid repeat units of CS were modified with ADH using carbodiimide crosslinking chemistry, and the degree of modification was quantified via 1H-NMR. The results show that CS can be modified with ADH at about 50% modification efficiency. The chemical crosslinking reaction between modified CS and PNiPAAm-based hydrogel was also verified via simple inverted tube method, in which the hydrogel underwent, along with thermal gelation at 37°C, irreversible chemical crosslinking. These preliminary results show that CS can indeed be utilized as the chemical crosslinker for PNiPAAm-based hydrogel. Further experiments will be conducted in the near future to quantify the effect of CS composition on the swelling behavior of hydrogel, and how it also affects the encapsulated mesenchymal stem cells.

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Laser Sintered Carbohydrates for Sacrificial Templating of Vascular Networks

Kinstlinger IS,1 Yalacki, DR1, Vasquez K1, and Miller JS1

1Department of Bioengineering, Rice University, Houston, TX, USA

Corresponding author: Jordan S. Miller, Department of Bioengineering, Rice University, 6100 Main St. MS-420, Houston, TX, 77005. Email: jmil@rice.edu

Engineered tissues densely populated with cells rapidly develop a necrotic core in the absence of convective transport of oxygen and nutrients through vascular networks. Here, we introduce laser sintered carbohydrate materials and their use as biocompatible templates for vascular networks. 3D fluidic networks can be created by encasing sintered carbohydrate templates in diverse hard and soft materials, then dissolving away the templates. This approach provides 3D geometric control, speed, reproducibility, and flexibility with respect to materials.

Based on our experience using carbohydrates as sacrificial vascular templates, we hypothesized that sintered carbohydrates would provide improved control over the architecture of 3D vascular templates. Indeed, we have demonstrated that carbohydrate materials can be sintered into 3D filament networks which are self-supporting, water-soluble, and cylindrical and smooth after post-processing. Laser sintered carbohydrate filaments were amenable to sacrificial templating in a variety of bulk matrices to yield open, perfusable channels. Specifically, 3D perfusable networks were formed in cell-laden fibrin, polycaprolactone foams, and PDMS. Thus, this approach is expected to be useful for patterning vascular networks in diverse soft tissues, bone, and microfluidic devices. Already, we have observed that cell activity in thick hydrogels is improved with flow perfusion through sacrificially templated channels.

This novel approach has the potential to meet a major need in the field for reproducible perfusable vasculature within engineered tissue. We expect this technique to be useful for sustaining high cell densities inside large-scale engineered tissues. Independent control of vascular architecture, ECM material, and cell populations also makes this an ideal experimental platform to study angiogenesis, vasculogenesis, interaction between vessels and parenchymal cells, and the effects of flow on these phenomena.
Fabrication of Aligned Fibrous Scaffolds with Continuous Gradients for Interfacial Tissue Engineering

Kishan A1, Robbins A2, Mohiuddin S1, Jiang M2, Moreno M2, Cosgriff-Hernandez E1
1. Department of Biomedical Engineering, Texas A&M University
2. Department of Mechanical Engineering, Texas A&M University
Corresponding author: Alysha Kishan, Department of Biomedical Engineering, Texas A&M University, 2121 West Holcombe Blvd, Houston, TX, email: alyshakishan@gmail.com.

Introduction: Electrospinning has gained popularity in recent years as a technique to generate fibrous scaffolds for various applications. Several researchers have utilized gradient electrospinning setups to produce scaffolds that mimic the mechanical and biochemical transitions of native tissue interfaces. However, current techniques do not allow for these gradients to be in the direction of fiber alignment, as displayed when native tendon transitions to bone. To address this limitation, we have developed an electrospinning method with fine control over gradient dimensions that also allows for gradients in the direction of fiber alignment. This technique provides a novel method to produce highly aligned scaffolds with linear gradient properties that better mimic the structure of the tendon to bone interface, as well as other transitions. These unique scaffolds also have the potential to enable spatial control over cellular behavior.

Materials and Methods: Biodegradable poly(ether ester urethane)ureas (B PURs) were synthesized using a previously established protocol. 10 wt% BPUR 10 and BPUR 50 + hydroxyapatite in 1,1,1,3,3,3-hexafluoro-2-propanol were connected in parallel and flow rates were altered to produce a gradient. 9 kV was applied to the needle tip and -8 kV was applied to the collector. The collector consisted of a wheel of parallel copper wires that rotated 90° in 6 hours (Figure 1). Gradient formation was verified through fluorescence. Fiber alignment was quantified from SEM images and biochemical properties were evaluated pre- and post-mineralization in simulated body fluid. Human mesenchymal stem cell adhesion was analyzed. Tensile testing was performed in both parallel and transverse directions to determine the degree of anisotropy and to identify a gradient in stiffness. A finite element model was created to determine the gradient in strain.

Results and Discussion: The compositional gradient was characterized using a fluorescent dye and the results indicated a continuous transition from the BPUR 50 to the BPUR 10. As expected, the fiber alignment of the gradient meshes induced a corresponding alignment of adherent cells in static culture. Tensile testing of the sectioned meshes confirmed a graded transition in mechanical properties and an increase in anisotropy with fiber alignment. Mechanical and biochemical gradients were also confirmed via FTIR, with mineralization occurring only in the designated bone-like region. Finite element modeling was utilized to illustrate the gradient mechanical properties across the full length of the mesh and lay the foundation for future computational development work. Current studies are characterizing the individual and synergistic effects of mineralization, tensile properties, and cyclic loading on cellular behavior.

Conclusion: This study demonstrates a novel method to produce highly aligned, gradient scaffolds that can be utilized to direct cellular behavior through a combination of topographical, biomechanical, and biochemical cues. Overall, this unique method provides additional capabilities to the researcher in mimicking the complexity of native tissue transitions.

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Tunable Delivery of Bone Morphogenetic Protein-2 (BMP-2) Via a Light-Activatable Adeno-Associated Virus (AAV) Platform

Lee EJ¹, Piepergerdes TC¹, Vasquez SM¹, Gomez EJ¹, Suh J¹, Tabor JJ¹, Mikos AG¹

¹. Department of Bioengineering, Rice University

Corresponding author: Antonios G. Mikos, Department of Bioengineering, Rice University, 6500 Main St., Houston, TX, Email: mikos@rice.edu

Tissue regeneration in critical-size bone defects often requires supraphysiological dosages of recombinant bone morphogenetic protein-2 (BMP-2) protein, potentially eliciting bone overgrowth and other undesirable consequences. Alternative to delivering BMP-2 protein as well as to improve control of BMP-2 levels in target cells, we hypothesize that a light-activatable adeno-associated virus (AAV) gene delivery platform may provide a facile, robust approach for externally fine-tuning BMP-2 administration. We leveraged a previously engineered vector to deliver the BMP-2 transgene to cells in vitro. Briefly, a transgene cassette (BMP2 fused to green fluorescent protein (GFP)) generated by standard molecular cloning was packaged into AAV serotype 2 wild-type and light-responsive variants. Quantitative PCR (qPCR) was used to determine virus titers, while Western Blots confirmed virus capsid formation. After introducing BMP2-GFP into human embryonic kidney (HEK) 293T cells, flow cytometry revealed up to 90% transduction efficiencies, and qPCR detected increased BMP-2 expression levels in transduced cells versus untransduced controls. Light induction experiments employed custom light-emitting diode plate apparatuses. We evaluated the tunability of BMP2-GFP. This AAV platform is minimally invasive compared to UV light/chemical inducer-controlled systems, and utilizes wavelengths within tissue penetration range, holding promise for in vivo applications.

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Using A Hybrid Enzyme to Promote Neuroregeneration—A Role of Prostacyclin on Neurodegenerative Diseases

Ling Q, Murdoch E, and Ruan K-H*

The Center for Experimental Therapeutics and Pharmacoinformatics, Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston

Corresponding author: Ke-He Ruan, Ph.D., M.D., The Center for Experimental Therapeutics and Pharmacoinformatics, Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Room 521 Science and Research 2 Building, Houston, TX 77204-5037, United States. Email: kruan@central.uh.edu

Neuroinflammation is the hallmark of neurodegenerative diseases, such as Alzheimer’s disease. Upon the occurrence of neuroinflammation, both pro-inflammatory and anti-inflammatory factors are secreted, which may further lead to either neurodegeneration or neuroregeneration. In our study, we found that prostacyclin, a downstream metabolite of cyclooxygenase (COX) pathway, could promote neuroregeneration. We created three novel hybrid enzymes, COX-2-10aa-mPGES-1, COX-1-10aa-PGIS, and COX-1-10aa-TXAS to redirect the metabolism of arachidonic acid (AA) specific to prostaglandin E2 (PGE2), prostacyclin (PGI2), and thromboxane (TXA2), respectively. The cDNA of these three hybrid enzymes were transfected to HT-22 cell, a well-established hippocampal neuronal cell line. The transfected HT-22 cells were challenged with amyloid β (Aβ) peptide (25-35). It was observed that COX-1-10aa-PGIS transfected neuronal cells could regenerate against Aβ-induced neurotoxicity, while the control cells and the HT-22 cells transfected with other genes showed limited regeneration. One-step further, we cultured the primary hippocampal neurons isolated from postnatal day 0-1 transgenic mice (over expressing COX-1-10aa-PGIS), and then challenged the cells with Aβ peptide (amino acid sequence 25-35). We found that the cultured primary prostacyclin-producing neurons showed resistant to neurodegeneration under Aβ 25-35 treatment, and these effects were blocked by aspirin, which inhibits COX-1-10aa-PGIS activity. In order to verify the protective role of prostacyclin, we treated WT primary hippocampal neurons with Iloprost, a synthetic analogue of prostacyclin, together with Aβ peptide (1-42). We found that without Iloprost, neurons showed a delayed regeneration, which was reversed by the Iloprost treatment. Therefore, the data suggests that prostacyclin could redirect neuroinflammation to neuroregeneration. Based on these results, our study could also provide some preliminary steps for determining the use of using nonsteroidal anti-inflammatory drugs (NSAIDs) to prevent neurodegenerative diseases.

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Sox11 and Sox4 Regulate Epidermal Differentiation and Re-epithelization after Wounding

Miao Q1,2, Chen F5, Ku AT1,2, Ramos C1,2, Lefebvre V6, Mo Q5, and Nguyen H1-4,*

1Stem Cells and Regenerative Medicine Center  
2Center for Cell and Gene Therapy  
3Department of Molecular and Cellular Biology  
4Department of Dermatology  
5Division of Biostatistics, Dan L. Duncan Cancer Center  
Baylor College of Medicine  
6Lerner Research Institute, Cleveland Clinic

*Corresponding author: Hoang Nguyen, Center for Cell and Gene Therapy, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX, E-mail: hoangn@bcm.edu

A highly dynamic transcriptional network of genes control differentiation and migration of epidermal cells during skin morphogenesis and regeneration. SOX4 and SOX11, two SoxC class members, have been shown to act redundantly to regulate the survival and properties of progenitor cells during embryogenesis of several tissue types. SOX4 has been found to exert a role in epidermal stem cell activation. Here, we found that SOX11 is expressed in embryonic epidermal progenitor cells and silenced postnatally. Using gain- and loss-of-function studies and transcriptional analyses, we discover that SOX11, together with SOX4, regulates downstream target genes that are implicated in differentiation and cell adhesion/migration. Noticeably, genes involved in cytoskeleton organization are significantly altered in Sox11- and Sox4-deficient cells. We found that SOX11 is induced in keratinocytes at the wound edge, and ablation of Sox11 and Sox4 impairs cell migration and re-epithelization. We identified that fascin actin-bundling protein (FSCN1), is directly activated by SOX11 and SOX4, and functions to promote the migration of keratinocytes.

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Electrospun Three-dimensional Models of Osteosarcoma Tumor Microenvironments

Molina, ER¹; Satish, T¹; Menegaz, BA²; Lamhamedi-Cherradi, SE²; Ludwig, JA²; Mikos, AG¹

¹Department of Bioengineering, Rice University, Houston, TX, USA
²Department of Sarcoma Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA

Corresponding Author: Antonios G. Mikos, Department of Bioengineering, Rice University, 6500 Main St., Houston, TX, USA, E-mail: mikos@rice.edu

Abstract:
Osteosarcoma (OS) is the most common primary tumor of the bone comprising 56% of all bone cancers and is the third most common tumor of adolescence. While modern chemotherapy and surgical resection have increased 5-year survival to 70%, patients that present with metastatic disease remain at around 30% 5-year survival, a number that has stagnated in recent decades. Therapy failure is due in large part to OS heterogeneity where individual tumors and metastatic lesions comprise variably differentiated cell populations which harbor therapy-resistant populations. This problem is compounded by the lack of accurate pre-clinical models of OS that rely heavily on standard 2D monolayer culture on hard plastic surfaces. Recent attempts at the identification of tumor initiating cells (TICs) in osteosarcoma involve hydrogels and suspension culture in to generate populations of a more aggressive phenotype characterized by increases in drug resistance, plasticity, and tumorigenic potential. However, there currently exists no reliable platform for the study of selected OS populations nor are there in vitro systems that adequately assess tumor differentiation heterogeneity. This works aim to develop three-dimensional microenvironments in electrospun poly-caprolactone (PCL) suitable for assessing cancer biology, including critical pathological pathways, and drug resistance mechanisms. Osteosarcoma cells in our systems have been shown to have differential activation of the IGF-1 and Wnt pathways which are critical to OS progression, differential growth patterns and phenotypes, and reduced responsiveness to chemotherapy that may more accurately represent the behavior of tumors in vivo.

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Inhibiting Sphingosine Kinase 2 Mitigates Mutant Huntingtin-Induced Neurodegeneration in Neuron Models of Huntington’s Disease

Jose F. Moruno-Manchon¹, Ndidi-Ese Uzor¹,², Maria P. Blasco¹, Sishira Mannuru³, Nagireddy Putluri⁴, Erin E. Furr-Stimming⁵ and Andrey S. Tsvetkov¹,²
¹Department of Neurobiology and Anatomy, the University of Texas McGovern Medical School
²The University of Texas Graduate School of Biomedical Sciences, Houston, TX, 77030
³The University of Texas Medical Training Program, Houston, TX, 77030
⁴Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, 77030
⁵Department of Neurology, University of Texas Medical School, Houston, TX 77030

Presenting author: Jose Felix Moruno-Manchon, Department of Neurobiology and Anatomy, the University of Texas McGovern Medical School, 6341 Fannin street, Houston, Texas, E-mail: jose.felix.morunomanchon@uth.tmc.edu

Huntington disease (HD) is the most common inherited neurodegenerative disorder. It has no cure. The protein huntingtin causes HD, and mutations to it confer toxic functions to the protein that lead to neurodegeneration. Thus, identifying modifiers of mutant huntingtin-mediated neurotoxicity might be a therapeutic strategy for HD. Sphingosine kinases 1 (SK1) and 2 (SK2) synthesize sphingosine-1-phosphate (S1P), a bioactive lipid messenger critically involved in many vital cellular processes, such as cell survival. In the nucleus, SK2 binds to and inhibits histone deacetylases 1 and 2 (HDAC1/2). Inhibiting both HDACs has been suggested as a potential therapy in HD. Here, we found that SK2 is nuclear in primary neurons and, unexpectedly, overexpressed SK2 is neurotoxic in a dose-dependent manner. SK2 promotes DNA double-strand breaks in cultured primary neurons. We also found that SK2 is hyperphosphorylated in the brain samples from a model of HD, the BACHD mice. These data suggest that the SK2 pathway may be a part of a pathogenic pathway in HD. ABC294640, an inhibitor of SK2, reduces DNA damage in neurons and increases survival in two neuron models of HD. Our results identify a novel regulator of mutant huntingtin-mediated neurotoxicity and provide a new target for developing therapies for HD.

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In Vivo Implantation and Perfusion of a Gel Containing 3D printed Internal Microchannel Networks

Paulsen SJ¹, Chen C, Renganaden Sooppan M.D.², Jason Han B.A.², Anderson H. Ta B.A.¹, Patrick Dinh ¹, Ann C. Gaffey M.D. ², Chantel Venkataraman B.A.², Alen Trubelja B.S.², George Hung B.S.², Jordan S. Miller Ph.D. ¹, Pavan Atluri M.D.²

1 - Department of Bioengineering, George R. Brown School of Engineering, Rice University, Houston, TX.
2 - Division of Cardiovascular Surgery, Department of Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Corresponding Author: Jordan Miller; Department of Bioengineering, Rice University; 6100 Main St.; Houston, TX 77005 Email: jmil@rice.edu

Recent advances in 3D bioprinting have enabled the development of tissues containing micro-channel networks for rapid perfusion of engineered tissues. However, little focus has been placed on developing new surgical implantation techniques allowing for immediate perfusion of the engineered channels, which is essential for maintaining cell viability within the implanted tissue. To address these concerns, we have developed a proof-of-concept technique for implanting vascularized constructs in-line with a rat femoral artery.

We first produced polydimethylsiloxane (PDMS) gels containing branched micro-channel networks using sacrificial carbohydrate glass-extrusion printing. Gels were implanted in-line with the femoral arteries of 10 male Wistar rats. First, an incision was made to expose the femoral sheath and isolate the femoral artery, and a silk suture was used to obtain control of the artery. The proximal and distal arteries were then cannulated using separate angiocatheters, which were secured by the silk sutures. The proximal and distal ends of the artery were then temporarily clamped before transecting the artery and trimming the angiocatheters to fit the inlet and outlet channels of the PDMS gel. Next, the catheters were mounted in the inlet and outlet of the gel before removing the clamp and allowing blood to flow. Flow through the channels was then monitored through the gel using Doppler imaging technology over 3 hours.

After implantation of the gels, laser Doppler imaging results showed that there was noticeably more flow through the PDMS gel and paw at 1 and 3 hours post implantation compared with the negative control group, which had a ligated femoral artery. However, after 3 hours little flow was observed through the gel due to clotting (Fig. 2). Our study outlines a method for in vivo implantation of vascularized gels allowing for immediate perfusion of the channel network. Furthermore, by using computational modeling to assess flow prior to implantation we can better understand the mass transport within an implanted gels and predict cell viability. In future work we will focus on improving the hemocompatibility of the implanted gels to prevent clotting and improve patency of the implanted channel networks over time.

References

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Multi-layered Vascular Grafts with Improved Compliance Matching and Resistance to Suture Damage for Long Term Patency

Allison Post¹, Alysha Kishan¹, Elizabeth Cosgriff-Hernandez¹
1. Department of Biomedical Engineering, Texas A&M University

Corresponding author: Allison Post, Biomedical Engineering, Texas A&M University, 2121 West Holcombe Blvd, Houston, Texas, E-mail: apost10103@gmail.com

Statement of Purpose: Coronary artery bypass graft surgery is one of the most common procedures performed in the U.S. Autologous vessels are the gold standard but are unavailable in up to 85,000 patients. Synthetic grafts are used in these cases despite high rates of thrombosis or intimal hyperplasia attributed to a compliance mismatch between the graft and native vessel. We address the clinical need for an off-the-shelf, small diameter vascular graft with sustained thromboresistance and long-term patency by improving compliance matching in a multi-layer graft. An electrospun polyurethane outer layer provides the requisite mechanical properties, and the non-fouling poly(ethylene glycol) hydrogel coating provides initial thromboresistance and collagen-mimetic proteins promote endothelialization post-implantation for sustained thromboresistance. We report advances of two critical aspects for clinical translation: 1) increased compliance-matching and burst pressure (BP) retention; 2) enhanced hydrogel resistance to suture damage without detriment to bioactivity and thromboresistance.

Methods: Outer Layer: A 25wt% solution of Bionate® in dimethylacetamide was electrospun with a flow rate of 0.5ml/hr through a blunted 20G needle with an applied 15kV. The rotating mandrel collector with an applied -5kV was placed 50 cm from the needle tip. Mesh thickness was varied from 0.1 – 0.4 mm by adjusting collection time. Compliance was measured by connecting graft segments to a syringe pump. The graft diameter was measured using a laser micrometer at 80 and 120 mmHg. Compliance was calculated by $C = \Delta D/D_0 \cdot \Delta P = (D_{120} - D_{80})/D_{80} \cdot 40$. Flow rate was increased with obstructed flow until the tube burst to determine burst pressure. Inner Layer: Hydrogels were made by mixing 10% PEG(3.4k) diacrylate (PEGDA) or 20% PEG(3.4k) diacrylamide and n-vinylpyrrolidone (PEGDAA-NVP) at a ratio of 1:54 in H2O and UV cured with 1% Irgacure. Hydrogel suture damage was determined by counting the number of particles after a needle pass. To confirm thromboresistance, gels were incubated with stained platelets, washed with PBS, the attached platelets lysed, and solution absorbance measured. Bioactivity retention was confirmed by adding collagen to the hydrogel precursor solution and seeding bovine aortic endothelial cells for 3 hrs, washed with PBS, and cell adhesion quantified.

Results: A compliance increase was observed with decreasing thickness of electrospun Bionate®. A graft composition was identified that doubled compliance while retaining a high BP. Sutured hydrogel particle reduction from PEGDA to PEGDAA-NVP improves resistance to suture damage dramatically with comparable cell and platelet attachment.

Conclusions: This work shows our electrospun polyurethane graft can be tuned to achieve BP comparable to the saphenous vein while increasing graft compliance with the expectation of improving patency by reducing intimal hyperplasia. We have enhanced the hydrogel coating with improved resistance to suture damage that maintains the desired thromboresistance and cell behavior. These studies represent important milestones in development of our vascular graft.

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Figure 1. Improving compliance and suture damage resistance without affecting burst pressure or thromboresistance. (A) Compliance of decreasing thickness of electrospun Bionate®. (B) Maintenence of burst pressure across thicknesses of Bionate®. (C) Suturing of PEGDA and PEGDAA-NVP hydrogels. (D) Average particles of hydrogel created during suturing.
Teriflunomide to Treat Traumatic Brain Injury

Prabhakara KS1, Kota DJ1, Jones G1, and Olson SD1.
1. Children’s Program in Regenerative Medicine, Department of Pediatric Surgery, University of Texas Medical School at Houston

Corresponding author: Scott D. Olson, Department of Pediatric Surgery, University of Texas Medical School at Houston, 6431 Fannin, Houston, Texas. E-mail: Scott.D.Olson@uth.tmc.edu

Traumatic brain injury (TBI) remains a serious public health problem in the United States. According to the World Health Organization (WHO), TBI is a permanent, chronic disease process, caused by non-reversible pathological alterations, requiring special rehabilitation, and/or a long period of observation, supervision, or care. However, despite intensive investigative endeavors, there are no FDA-approved drugs designed to reduce morbidity and mortality associated with TBI.

Neuroinflammation, which been detected as lasting in humans for up to 17 years, plays a pivotal role in the pathogenesis of TBI. In the acute stage of TBI, inflammation activates and mobilizes immune cells toward the site of injury that interfere with the endogenous capacity of the brain to repair itself, exacerbating neuronal death. In the chronic stage, an inflammatory microenvironment caused by an excessive activation of immune cells further contributes to secondary neuronal death and a subsequent positive feedback inflammatory loop.

Teriflunomide (Aubagio) has been approved by the US Food and Drug Administration (FDA) for use in the treatment of multiple sclerosis since 2012. Teriflunomide has been shown to act as an inhibitor of dihydroorotate-dehydrogenase (DHODH), a key mitochondrial enzyme involved in the de novo synthesis of pyrimidines in rapidly proliferating T lymphocytes and B lymphocytes. Moreover, teriflunomide has been shown to affect immunological responses outside of its ability to inhibit pyrimidine synthesis in rapidly proliferating cells, suggesting Teriflunomide may have additional therapeutic mechanisms.

In here, we studied the treatment effect of teriflunomide in severe/moderate and mild injury models of rats. Our results show that in both the rat models the drug could recover the blood-brain barrier integrity and ameliorate inflammation in brain both short term and long term. Consequently, we saw improvement in memory and special learning abilities after treatment in severe/moderate injury. However, our mild injury model didn’t have any behavioral deficits.

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Mesenchymal Stems Cells to Treat Neuropathic Pain in Spinal Cord Injury

Ruppert KA1, Kota DJ1, Prabhakara KS1, Grill RJ2, Olson SD1
1 Department of Pediatric Surgery, School of Medicine, University of Texas Health Science Center, Houston, TX
2 Department of Neurobiology & Anatomical Science, School of Medicine, University of Mississippi Medical Center, Jackson, MS

Ruppert KA, Dept. of Pediatric Surgery, School of Medicine, University of Texas Health Science Center, 6431 Fannin St., Houston, TX 77030, E-mail: katherine.a.ruppert@uth.tmc.edu

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

The objective is to determine whether early delivery of MSC attenuates the development of neuropathic pain and the associated inflammatory response and whether delayed delivery of MSC improves chronic neuropathic pain and inflammation. To accomplish this, adult, male, Sprague-Dawley rats will receive a moderate, spinal contusion injury at thoracic level 10. All injured subjects and age-matched controls are subjected to neurosensory assessments of mechanical allodynia (von Frey filaments) and thermal hyperalgesia (Hargreave’s test) in hind paws. All neurosensory assessments are performed prior to injury and again at 7 days, 14 days, 21 days and 28 days post-injury for the acute study and again monthly for the chronic study. MSC are suspended in sterile PBS at a concentration of 50 x 10^6 cells/ml for intravenous and intrathecal delivery at 24 hrs (acute) or at 7 months (chronic) post-injury. Spinal cord tissue is harvested at 28 days or 8 months post-injury, sectioned and stained for immunohistochemical detection of inflammation, indicated by activated microglia.

MSC treatment after CNS injury is appealing due to the immune-regulatory and anti-inflammatory characteristics of MSC. The data generated in this study suggests that the administration of MSC after spinal contusion injury partially prevents neuropathic pain associated with mechanical allodynia in the hind paws. The data produced in our chronic study reveal MSC treatment in the chronic phase of injury effectively improve pain and function scores, and indicate a decrease in neuroinflammation. These results support our hypothesis that MSC are an effective cellular therapy approach to treat SCI-dependent neuropathic pain.

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Co-encapsulation of Mesenchymal Stem Cells and Cord Blood Improve Locomotor Recovery and Neuroinflammation in Spinal Cord Injury

Ruppert KA¹, Kota DJ¹, Prabhakara KS¹, Olson SD¹
1 Department of Pediatric Surgery, School of Medicine, University of Texas Health Science Center, Houston, TX

Ruppert KA, Dept. of Pediatric Surgery, School of Medicine, University of Texas Health Science Center, 6431 Fannin St., Houston, TX 77030, E-mail: katherine.a.ruppert@uth.tmc.edu

Over 17,000 new spinal cord injuries (SCI) occur each year in the United States, contributing to approximately 282,000 persons currently living with SCI. Currently, treatment consists of surgical stabilization, physical rehabilitation, and analgesia. There is an unmet need for new treatments to mitigate additional damage that occurs in the hours, days, and weeks after SCI. In the acute phase of SCI, a neuroinflammatory reaction occurs, launching inflammatory cascades, secondary neurodegeneration and alterations to motor and sensory function. Pro-inflammatory cytokines and leukocyte infiltration play a significant role in the development of secondary injuries associated with SCI and present a specific target for cellular therapy. The use of bone marrow-derived mesenchymal stem cells (MSC) has been shown to modulate inflammation, reducing secondary injuries in the field of spinal cord injury (SCI). MSC utilize effector leukocytes, such as macrophages and monocytes, to exhibit systemic effects. The objective of this study is to evaluate the efficacy of MSC when directly co-encapsulated with umbilical cord blood effector cells.

Our objective is to demonstrate that local application of co-encapsulated MSC and leukocyte effector cells will have a synergistic effect on neuroinflammation in the injured microenvironment and improve behavioral outcomes. MSCs are isolated and grown based on a previously established protocol. The cells are encapsulated in alginate hydrogel beads that are implanted adjacent to the contused T10 spinal cord 24hrs after injury. Animals are randomly assigned to one of five groups: Naïve, Sham + encapsulated MSC, Sham + co-encapsulated MSC + CB, SCI + encapsulated MSC, SCI + co-encapsulated MSC + CB, alginate beads alone, encapsulated MSC cells, encapsulated cord blood cells or MSC + cord blood encapsulated. The effects of the alginate encapsulated cells are measured by locomotor recovery, mechanical and thermal sensitivity, as well as immunohistochemical staining for leukocyte infiltration, demyelination, inflammatory mediators and blood-spinal cord barrier permeability. Animals are sacrificed 14 days after injury and spinal cords are harvested, sectioned and stained for immunoreactivity.

Preliminary data indicates that local application of co-encapsulated MSC and cord blood cells significantly improves locomotor recovery 14 days post-injury when compared to MSC introduced systemically (*, p value<0.05). Interestingly, encapsulated MSC animals showed significantly improved locomotor recovery scores on days 2, 3, 5, 7, 10 and 14 when compared to all other groups. Observed histological changes indicate that encapsulation of MSC and co-encapsulation of MSC+CB cells provides increased protection against secondary injury. Immune cell modulation supports our hypothesis that encapsulation and co-encapsulation with effector cells reduce inflammatory mediators involved in SCI-induced neuroinflammation.

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Treating Spinal Cord Injury with Mesenchymal Stem Cell Extracellular Vesicles Improves Locomotor Recovery, Mechanical Sensitivity and Neuroinflammation

Ruppert KA1, Nguyen TT1, Prabhakara KS1, Olson SD1
1 Department of Pediatric Surgery, School of Medicine, University of Texas Health Science Center, Houston, TX

Ruppert KA, Dept. of Pediatric Surgery, School of Medicine, University of Texas Health Science Center, 6431 Fannin St., Houston, TX 77030, E-mail: katherine.a.ruppert@uth.tmc.edu

Acute spinal cord injury continues to be a devastating problem worldwide with high morbidity and mortality. Mortality after hospital admission following acute spinal cord injury is between 4.4% and 16.7% globally. Long term morbidities include sensory, motor, and autonomic dysfunction. Despite high morbidity and mortality, currently no consensus on pharmacological intervention exists to alter the clinical course of acute spinal cord injury and improve neurological outcome. Therapeutic strategies have focused on regeneration to reverse the primary direct injury or immune modulation to limit the secondary injury. Mesenchymal stem cells (MSC) harvested from bone marrow, cord blood, peripheral blood, adipose, and other organs show promising therapeutic potential. The proportion of MSC that do survive transplantation in acute spinal cord injury is implicated in inflammatory modulation, angiogenesis, decreased blood spinal cord barrier leakage, and improved functional recovery in animal studies.

Extracellular vesicles (EVs) are heterogeneous particles with lipid bilayer, containing growth factors, lipids, microRNAs, mRNAs, and proteins. They are secreted by numerous cell types including MSC. MSC-derived extracellular vesicles (MSCEv) can contain stem cell-like self-regenerative activity, potentially have low malignancy, less immunogenic activity, and less pulmonary first pass effect compared to MSC. Animal studies demonstrate potential therapeutic benefit of MSCEv following neurologic injury. Recent reports indicate improved cognitive function using MSCEv in traumatic brain injury (TBI) mouse model. Angiogenesis, neurogenesis, and reduction of neuroinflammation contribute to the functional recovery of TBI in rats following MSCEv administration. Despite the wide therapeutic potential, MSCEv have not been previously studied in spinal cord injury (SCI) animal model.

Our hypothesis is the intravenous administration of MSCEv following SCI will attenuate neuroinflammation and improve functional recovery using Basso, Beattie, Bresnahan (BBB) Locomotor Rating and mechanical sensitivity using the Von Frey Dixon Up-Down protocol. Animals will be randomly assigned to three groups: sham laminectomy, or T10 contusion + vehicle or T10 contusion + MSCEv. At 14 days post-injury, animals will be sacrificed, spleen will be harvested for neurotransmitter assays, and blood and spinal cord will be harvested for flow cytometry and immunohistochemistry.

Preliminary data indicate significantly higher locomotor recovery scores in SCI + MSCEv animals when compared to SCI + vehicle animals on days 5, 7 and 14 following injury (p<0.0001, p<0.001 and p<0.001, respectively). Animals treated with MSCEv also demonstrate significantly higher force thresholds in the mechanical sensitivity test performed 14 days post-injury when compared to those that received vehicle (p<0.05). Flow cytometry analysis of spinal cord reveals increased activation of M1 and M2 microglia in MSCEv treated animals compared to vehicle. Spleen analysis indicates increased myeloid cells and MDSC with decreased NK cells and leukocytes in MSCEv treated animals.

These results support our hypothesis that MSC-secreted extracellular vesicles are an effective therapeutic after spinal cord injury in a rodent model. Additional time points for administration, as well as investigation of the effect MSCEv may have on chronic SCI will continue in an effort to develop this cellular therapy for potential clinical application.

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Pancreatic Cell Fate Determination Relies on Notch Ligand Trafficking by NFIA

Scavuzzo MA1, Chmielowiec J2, Yang D3, Wamble K2, Teaw J2, Chaboub LS1, Duraine L4, Sharp R2, Glasgow SM2, Brou C6, Deneen B1,2,5, and Borowiak M1,2,3,5,7

1Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030, USA
2Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children’s Hospital, and Houston Methodist Hospital, Houston, TX 77030, USA; Stem Cell and Regenerative Medicine Center, Baylor College of Medicine, Houston, TX 77030, USA
3Molecular and Cellular Biology Department, Baylor College of Medicine, Houston, TX 77030, USA
4Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, USA
5Department of Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA
6Department of Cell Biology and Infection, Institute Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, FR
7McNair Medical Institute, Baylor College of Medicine, Houston, TX 77030

Corresponding author: Malgorzata Borowiak, Center for Cell and Gene Therapy, Baylor College of Medicine, One Baylor Plaza, Houston, TX borowiak@bcm.edu

The Notch pathway is known to dictate the fate of all three main pancreatic cell types. Our findings for the first time uncover the mechanisms regulating Notch signaling in the pancreas, with vesicle trafficking affecting the ratio of active vs. non-active ligand on the membrane. This mechanism of Notch regulation is in stark contrast to the current model, in which stochastic changes in gene expression allow pancreatic progenitors to modulate Notch activation. Interestingly, we identified a genetic model in which Notch trafficking is disturbed. We found that pancreatic deletion of the transcription factor NFIA led to increased expression of Mib1, Chmp4c and thus endocytosis of the Notch ligand Delta-like 1 (Dll1). The rate of Dll1 recycling, indicating ligand activation, was unaffected in our mutant mice. Thus the increased endocytosis of Dll1 led to a shift in the ratio of membrane bound Dll1/Notch1, allowing for increased Notch1 activation. As a result, NFIA-deficient mice formed more ducts and fewer endocrine cells, a Notch gain-of-function phenotype. This shows that in the normal context pancreatic endocrine cells form by inhibiting Notch through cis-inhibition.

Epistatic analysis of Rbpjk with NFIA confirmed that the influence of NFIA on endocrine cells was through trafficking. Further, loss of NFIA together with Rbpjk rescued the acinar defect observed from Rbpjk deletion, indicating that NFIA plays a deterministic role in acinar cell formation.

The generation of human endocrine cells in vitro has the potential to alleviate diabetes, however, while directing stem cells towards the pancreatic progenitor stage is robust, the differentiation efficiency plummeted when progenitors are directed to the endocrine stage. We found that ectopic expression of NFIA in mouse and human development promoted endocrine formation, with as much as a 222-fold increase in the expression of the beta cell marker INS in human embryonic stem cell derived pancreatic progenitors. The discovery of NFIA as a novel endocrine fate regulator could very well provide the mechanism that would improve derivation of human beta cells for regenerative therapy and drug screening.

This study reveals mechanisms regulating Notch activity in the pancreas, with a novel pancreatic gene, NFIA, controlling trafficking and cis-inhibition, in turn governing cell fate.

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MiR-590 Promotes Transdifferentiation of Porcine and Human Fibroblasts towards a Cardiomyocyte-like Fate by Directly Repressing Specificity Protein 1

Singh VP1, Mathison M1, Sanagasetti D1, Pinnamaneni JP1, Gibson BW2, Yang J1, Rosengart TK1
1. Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX, USA
2. Center for Comparative Medicine, Baylor College of Medicine, Houston, Texas, USA

Correspondence to:
Corresponding author: Rosengart TK, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, 1 Baylor Plaza, MS 390, Houston, TX 77030, E-mail: todd.rosengart@bcm.edu

Background: Reprogramming of cardiac fibroblasts into induced cardiomyocyte-like cells (iCMs) represents a promising potential new therapy for treating heart disease, inducing significant improvements in post-infarct ventricular function in rodent models. Because reprogramming factors effective in transdifferentiating rodent cells are not sufficient to reprogram human cells, we sought to identify reprogramming factors potentially applicable to human studies.

Methods and Results: Lentivirus vectors expressing Gata4, Mef2c, and Tbx5 (GMT); Hand2 (H), Myocardin (My), or microRNA-590 were administered to rat, porcine and human cardiac fibroblasts in vitro. iCM production was then evaluated by assessing expression of the cardiomyocyte marker cardiac troponin T (cTnT), while signaling pathway studies were performed to identify reprogramming factor targets.

GMT administration induced cTnT expression in ≈ 6% of rat fibroblasts but failed to induce cTnT expression in porcine or human cardiac fibroblasts. Addition of H/My and/or miR-590 to GMT administration resulted in cTNT expression in ≈ 5% of porcine and human fibroblasts, and also upregulated the expression of the cardiac genes MYH6 and TNNT2. When co-cultured with murine cardiomyocytes, cTnT-expressing porcine cardiac fibroblasts exhibited spontaneous contractions. Administration of GMT plus either H/My or miR-590 alone also downregulated fibroblast genes COL1A1 and COL3A1. miR-590 was shown to directly suppress the zinc finger protein Sp1, which was able to substitute for miR-590 in inducing cellular reprogramming.

Conclusions: These data support porcine studies as a surrogate for testing human cardiac reprogramming, and suggest that miR-590-mediated repression of Sp1 represents an alternative pathway for enhancing human cardiac cellular reprogramming.

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Incorporation of Synthetic Polymers and Carbohydrates for the Development of Injectable Calcium Phosphate Cements

Smith BT, Lu A, Watson E, and Mikos AG
1. Department of Bioengineering, Rice University, Houston, TX 77030

Corresponding Author: Antonios G. Mikos, Department of Bioengineering, Rice University, Houston, TX, 77030, email: mikos@rice.edu

Introduction: Alpha-tricalcium phosphate (αTCP) has been extensively used to fabricate injectable calcium phosphate cements (CPCs) for craniofacial bone regeneration because of its osteoconductive properties. In order to enhance the rate of CPC degradation and simultaneously favor bone tissue ingrowth, the addition of several porogens has been investigated. However, several of these porogens have major drawbacks such as interference with cement setting, and/or degrade over months, and hence the ability for bone ingrowth is limited. Objective: To characterize CPCs with varied weight ratios of glucose microparticles (GMPs) and poly(lactic-co-glycolic acid) (PLGA) microparticles and evaluate the effects of concentration of GMPs on the degradation rate.

Methods: GMPs were fabricated by mixing 50 g of glucose powder (D-(+)-glucose, Sigma-Aldrich, St. Louis, MO) with 10 mL deionized water (ddH2O) and flash freezing. Following lyophilization, glucose microparticles (GMPs) of 150-300 μm in diameter were obtained through sieving. Sieved fractions of GMPs were then lyophilized overnight, purged with nitrogen, and stored at -20°C. PLGA MPs were generously provided by Corbion Purac B.V. (Gorinchem, the Netherlands) and had a copolymer ratio of 50:50 and a mean particle size of 60 μm. To fabricate the CPC constructs, the αTCP phase was first mixed with the PLGA MPs and GMPs until evenly distributed, then a 24 wt % Na2HPO4 solution was be added and the mixture was rapidly stirred. The mixtures were injected into Teflon molds 6 mm in diameter and 12 mm high, allowed to cure, and vacuum dried overnight. Samples (n=6 per time point) were placed in 3 mL of phosphate-buffered saline (PBS, pH 7.4) and incubated at 37 °C on a shaker table (70 rpm) for 8 weeks. Every 7 days sample buffer was refreshed. At days 3, 28 and 64 specimens were dried for 24 hours prior to calculating a final dry weight. Samples were then evaluated by microcomputed tomography (open and closed porosity, and pore interconnectivity).

Results: All GMP/PLGA/CPCs formulations tested showed a setting time less than 15 min, as measured according to ASTM 622. Degradation studies showed that scaffolds with higher concentrations of GMPs had a greater initial mass loss over the course of 3 days (p< .001); however, the concentration of PLGA MPs had no affect on the mass loss over the same time period (p=.985). At week 8, constructs with higher concentrations of PLGA MPs had a greater mass loss. Furthermore, GPC showed that increased concentrations of GMPs resulted in PLGA with shorter chain lengths, supporting the hypothesis that GMPs increase the degradation of PLGA.

Conclusion: By altering the concentration of GMPs and PLGA MPs present within the constructs, the CPC degradation rate can be controlled, influencing properties such as porosity available for bone ingrowth. Future work will investigate, the compressive moduli of constructs, surface structure, crystalline structure of the ceramic phase, and their cytocompatibility.

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Maximizing human induced pluripotent stem cell derived neural stem cell survival, axon extension and gene expression of neural differentiation markers using polyethylene glycol hydrogels containing a continuous concentration gradient in n-cadherin derived peptide His-Ala-Val-Asp-Lle

Lim HJ1, Khan Z1, Perera TH1, Wilems TS1, Kurosu Y1, Lu X1, Ravivarapu KT1, Mosley MC1 and Smith Callahan LA1

1Department of Neurosurgery and Center for Stem Cell and Regenerative Medicine, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas

Corresponding Author: Laura A. Smith Callahan, Department of Neurosurgery, McGovern Medical School at the University of Texas Health Science Center at Houston. 1825 Pressler, Houston, Texas, E-mail: laura.a.smithcallahan@uth.tmc.edu

Statement of Purpose: Preclinical models of stem cell therapy in spinal cord injury (SCI) have demonstrated improved efficacy with matrix inclusion. Optimization of matrices for clinically relevant cell types will likely lead to further increases in neurological recovery in clinical settings. Human induced pluripotent stem cell derived neural stem cells (hNSC) are a desirable cell source for SCI therapy development. However, the culture of hNSC for material optimization studies is time consuming and costly. Use of combinatorial strategies to maximize the number of tested conditions, while reducing the number of cells required will expedite the optimization process and transition to clinical use. As n-cadherin (NCAD) plays a role in the structure and function of the central nervous system, emulation of its signaling in matrices has been investigated, but conflicting reports of its ability to promote cellular adhesion, neurite extension, and neural differentiation exist. The objective of this study is to determine the effect of NCAD derived peptide HAVDI on hNSC survival, gene expression of neural maturation markers, and neurite extension using polyethylene glycol hydrogels containing a continuous gradient in HAVDI concentration.

Methods: 50 mm × 10 mm × 1 mm hydrogel gradients were fabricated by dispensing 10% 8 kDa PEGDM solutions with and without 1 mM HAVDI through two syringe pumps running in inverse linear ramping profiles over 75s into a custom mold, followed by photopolymerization with 2.3 mJ/cm2 for 10 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 HAVDI concentration (C_{NCAD}), swelling ratio, mesh size and mechanical properties. For cellular experiments, the discs were seeded with hNCS and cultured with serum-free neural media for 10 days with media exchanges every other day. For survival studies, hydrogen peroxide was added the day after seeding and a MTS assay conducted 24hrs later. Neurite length from β3-tubulin staining and gene expression analysis by real time PCR were conducted.

Results: Gradient hydrogels presented a linear C_{NCAD} gradient ranging from 158 µM to 760 µM, while other material properties remained similar throughout the gradient. Compared with the starting hNSC seeding population plated on Matrigel, cells cultivated with NCAD at any level exhibited greater tolerance of oxidative stress. Exposure to 310µM hydrogen peroxide, demonstrated a NCAD peptide concentration dependent effect on hNSC survival. Peak gene expression of neuronal markers, TUJ1 and MAP2, occurred in hNSC cultured on hydrogels containing between 435µM and 577µM NCAD peptide concentration at day 10. Maximal Olig2 expression and minimal Sox2 expression also occur in cells cultured on hydrogels containing 577 µM concentration of NCAD peptide. Significantly longer neurites were observed in hNSC cultured on hydrogels contacting 577µM or less NCAD peptide compared to cells cultured on hydrogels containing high NCAD peptide concentrations.

Conclusions: Use of gradient material samples, quickly identified maximal expression of our neural lineage targets in hNSC cultured hydrogels containing between 435 µM and 577 µM of HAVDI. Greater
utilization of such combinatory approaches will facilitate material optimization for human cell types and speed progressing of combination therapies utilizing multiple components such as materials and cells to the clinical.

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Stimulated MSC Releases Extracellular Vesicles that Improve Inflammatory Inhibition Process In Vitro

Srivastava A.K. 1, Siqinzhaorigetu1, Bair H1, Prabhakara KS1, Toledano Furman NE1, Vykovakal JY2, Ruppert R1, Cox CS1, Harting MT1, Olson SD1

1. Department of Pediatric Surgery, University of Texas McGovern Medical School, Houston, TX, USA
2. McCombs Institute for the Early Detection and Treatment of Cancer, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
3. Corresponding author: Amit K. Srivastava, Ph.D., Department of Pediatric Surgery, Program in Children's Regenerative Medicine, University of Texas McGovern Medical School, 6431 Fannin Street, Houston, TX. E.mail: Amit.K.Srivastava@uth.tmc.edu

Abstract

Extracellular vesicles (EVs) secreted by mesenchymal stem cells (MSCs) have been proposed to be a key mechanistic link in the therapeutic efficacy of cells in response to cellular injuries through paracrine effects. We hypothesize that inflammatory stimulation of MSCs results in the release of EVs that have enhanced anti-inflammatory effects. The present study evaluates the anti-inflammatory properties of EVs derived from inflammation-stimulated and naïve MSCs (MSCEv+ and MSCEv respectively) using a current good manufacturing practice (cGMP)-compliant tangential flow filtration (TFF) system. We extensively characterized the EVs and found a number of differences in protein and cytokine expression and RNA content, despite consistency in size and presentation of common antigens. MSCEv+ further attenuated inflammatory cytokine release compared to MSCEv when co-cultured with activated primary leukocytes, with a distinctly different pattern of cellular uptake. The efficacy of MSCEv+ was attributed to COX2/PGE2 expression, as a COX2 specific inhibitor reduced the potency of MSCEv+ in vitro. The present study demonstrates that EVs can be isolated in a cGMP-compliant manner and characterized using a number of assays that may determine a relevant measure of potency. Inflammatory stimulation of MSCs renders release of EVs that have enhanced anti-inflammatory properties that are due to COX2 expression and PGE2 production.

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Silica Nanoparticle Delivery of Small Molecules for Cardiac Differentiation of Pluripotent Stem Cells

Tsao C¹, Taraballi F², Pandolfi L², Velazques-Mao A¹, Tasciotti E², Jacot J⁴,⁵

¹. Department of Bioengineering, Rice University, Houston, TX, USA
². Department of Regenerative Medicine, Houston Methodist Research Institute, Houston, TX, USA
³. Congenital Heart Surgery Service, Texas Children’s Hospital, Houston, TX, USA
⁴. Department of Bioengineering. University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA

Corresponding author: Christopher Tsao, Bioengineering, Rice University, Houston, TX, cjmtsao@rice.edu

Abstract: Induced pluripotent stem cells have shown great potential in cardiac differentiation. Through inhibition of the GSK3/Wnt signaling pathways, iPSC can be differentiated into functional cardiac cells. In this study we developed a technique to temporally release GSK3/Wnt small molecule inhibitors by encapsulation in porous silica particles. Porous silica particles were loaded with GSK3 inhibitor CHIR99021 or Wnt inhibitor IWP2. The particles containing IWP2 were coated with 5wt% PLGA 50:50 to delay release 72 hours. Induced pluripotent stem cells reprogrammed through mRNA transfection were cultured on transwell membranes to aid in analysis. HPLC suggests a burst release of CHIR99021 within the first 24 hours and a delayed release of IWP2 after 72 hours. Cultured cells upregulated both early (Nkx 2.5, Isl-1) and late (cTnT, MHC, Cx43) cardiac markers, assayed with qRT-PCR, and began spontaneous contraction at 3.0±0.6Hz at 15-21 days after the start of differentiation. Calcium labeling dye showed transients typical of immature cardiomyocytes. This study showed that the cardiac differentiation of pluripotent stem cells can be directed by porous silica vectors with temporally controlled release of small molecules inhibitors. These results suggest methods for automating and eliminating variability in manual maintenance of inhibitor concentrations in the differentiation of pluripotent stem cells to cardiomyocytes.

Figures: (Left) Dual release profile of inhibitors from porous silica particles (Right) Beating frequency measured by Ca2+ sensitive dye, Indo-1.
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The Development of a Multi-functional Wrap for Allograft Revitalization

Taneidra Walker¹, Alysha Kishan¹, and Elizabeth Cosgriff-Hernandez¹

1. Department of Biomedical Engineering, Texas A&M University

Corresponding author: Taneidra Walker, Department of Biomedical Engineering, Texas A&M University, 2121 West Holcombe Blvd, Houston, TX, email: taneidra.walker@gmail.com.

Introduction: Allograft failure in bone regeneration is caused by factors such as post-operative infections and loss of bioactivity due to processing leading to non-unions. In this work, we sought to develop an adjuvant multi-functional wrap for allografts to reduce bacterial infections and promote revitalization through the controlled release of multiple bioactive factors. In particular, gallium maltolate (GaM) is released rapidly as an anti-microbial agent, while stromal cell-derived factor1 (SDF1) and vascular endothelial growth factor (VEGF) are released to promote tissue integration through endogenous cell recruitment and neovascularization. To facilitate the independent, temporal release of these multiple factors, a co-electrospinning setup was used to fabricate a multi-modal crosslinked gelatin and poly(lactide-co-glycolide) (PLGA) wrap.

Materials and Methods: Two distinct gelatin crosslinking mechanisms were used to tune gelatin degradation rate, and thus, growth factor release kinetics from each gelatin release system in the co-electrospun wrap. A reactive gelatin crosslinking mechanism consisted of in-line blending of gelatin with a hexamethylene diisocyanate (HDI) crosslinker. HDI facilitated both crosslinking of the gelatin and anchoring of the growth factors directly to the gelatin through reaction with the lysine residues, allowing for sustained release of the VEGF. Additionally, photocrosslinking gelatin consisted of methacrylating gelatin with 2-isocyanatoethyl methacrylate (IEMA) prior to electrospinning and photocuring with UV light during electrospinning. In contrast to the reactive crosslinking, growth factors are not anchored to the gelatin, and release from the photocrosslinked gelatin-methacrylate occurs upon fiber swelling, allowing for moderate release of the SDF1.

Results and Discussion: SEM analysis confirmed that fiber diameter was unchanged in both of these gelatin release systems. Fiber diameter and sol-gel analysis indicated comparable fiber diameters and gel-fraction allowing for direct comparison of release kinetics. Initial results demonstrated that reactive crosslinked gelatin resulted in 58% release of model growth factor, FITC-albumin, over 7 days whereas the photocrosslinked gelatin resulted in 69% release over 4 hours. We have also demonstrated successful co-electrospinning to generate two distinct fiber populations of both gelatin release systems in a single mesh without affecting fiber morphology. Current studies are focused on developing a PLGA release system for the rapid release of GaM, evaluating bioactivity retention of growth factors release from wrap, and conducting release analysis on co-electrospun wrap.

Conclusion: Overall, this research highlights the potential of a multi-functional biomaterial platform harnessing the complexity to generate independent, temporal control of multiple bioactive factors to improve allograft efficacy in bone regeneration.
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Hyaluronan Mediated Extracellular Matrix and Angiogenesis Results in Attenuated Renal Fibrosis

Xinyi Wang¹, Meredith Rae¹, Pu Duann¹, Mathangi Chandramouli¹, Hui Li¹, Monica Fahrenholtz¹, Jizhong Cheng², Swathi Balaji¹, Sundeep Keswani¹

1. Laboratory for Regenerative Tissue Repair, Texas Children's Hospital, Baylor College of Medicine. Houston TX 77030
2. Department of Medicine, Division of Nephrology, Baylor College of Medicine. Houston TX 77030

Corresponding author: Sundeep Keswani, Department of Surgery, Division of Pediatric Surgery, Texas Children’s Hospital/Baylor College of Medicine, One Baylor Plaza, Houston, TX
E-mail: sundeep.keswani@bcm.edu

Renal fibrosis is a pathological characteristic of chronic kidney disease (CKD), and is a product of aberrant inflammation, extracellular matrix (ECM) deposition and peritubular capillary loss. We have demonstrated a novel role for interleukin-10 (IL-10) in abrogating dermal fibrosis by regulating hyaluronan (HA) metabolism and angiogenesis. We therefore hypothesized that hyaluronan attenuates renal fibrosis via its molecular weight variation to influence extracellular matrix remodeling, promoting angiogenesis and reducing inflammation.

Primary renal fibroblasts (FB) were isolated from 8-10 week male C57BL/6J (WT) mice. IL-10(200ng/ml) +/- hyaluronidase (HYAL, 1.5unit/ml), HA matrices were analyzed by particle-exclusion assay at 24h. Gene expression of HA synthases (HAS1-3), HYAL1-2, TGFβ-1 and VEGF were assessed by qPCR at various time points. 8 weeks WT and IL-10 null male mice were injected with lenti-IL-10/lenti-GFP (1x10¹⁰ IU) under the kidney capsule. 3d after the injection, unilateral ureteral obstruction (UUO) was performed. UUO/sham kidneys and serum were collected at 14d for RNA, ELISA, and immunohistochemical (IHC) analysis. n=3/treatment group; p-value by ANOVA.

In vitro, HAS1-3 gene expression is up-regulated at 2h after IL-10 treatment, and HYAL 1-2 was shown significantly down-regulated. A 1.88-fold increase in HA-rich matrix formation was shown with 24h IL-10 stimulation, and the effect was abrogated by HYAL (p<0.05). In vivo, the up-regulation of HAS1 expression in both WT and IL-10 null mice; Moreover, HA expression is less in the IL-10 null mice than WT mice, which also demonstrated more fibrosis than WT mice. In both null and WT mice, lenti-IL-10 treatment resulted in less dilated tubules, decreased kidney fibrosis, and preserved tubular integrity in kidneys, compared to control treated mice. The serum HA level was 1.72-fold higher in lenti-IL-10 treated mice as compared to lenti-GFP treated (p<0.05).

Our data suggests that hyaluronan has a fundamental role in obstruction-related renal fibrosis. Further, the upregulated angiogenesis and reduced inflammatory response are associated with HA metabolism changes, with an end result of attenuated fibrosis. Taken together, this is a previously unreported mechanism for HA in the kidney, which may lead to innovative future therapies.

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Regulatory and Effector T-Cells Potentiate Wound Repair By Regulating Inflammation And Extracellular Matrix

Xinyi Wang¹, Swathi Balaji¹, Mathangi Chandramouli¹, Meredith Rae¹, Pu Duann¹, Hui Li¹, Monica Fahrenholtz¹, Paul Bollyky², Manish Butte³, Sundeep Keswani¹

1. Laboratory for Regenerative Tissue Repair, Texas Children's Hospital, Baylor College of Medicine. Houston TX 77030
2. Department of Medicine, Stanford University School of Medicine, Stanford CA 94035
3. Department of Pediatrics, David Geffen School of Medicine at University of California, Los Angeles 90095

Corresponding Author: Sundeep Keswani, Department of Surgery, Division of Pediatric Surgery, Texas Children’s Hospital/Baylor College of Medicine, One Baylor Plaza, Houston, TX
E-mail: sundeep.keswani@bcm.edu

Lymphocytes have been shown to play a role in tissue fibrosis in several models, but their role in dermal wound repair is unclear. We previously demonstrated that hyaluronan (HA) can stimulate CD4+CD25+FoxP3+ T cells to produce an anti-inflammatory milieu, which is permissive of decreased fibrosis. We hypothesize that lymphocytes influence dermal healing and fibrosis.

In a loss-of-function experiment, make excisional 6mm stented wounds in SCID mice that lack of T and B cells, and compared to WT mice. Next, gain-of-function of different lymphocytes subsets by adoptive transfer of 1)total lymphocytes; 2)CD4+CD25-T cells; 3)CD4+CD25+T cells.(1x10⁶ FACS-characterized syngeneic cells injected into SCID mice). To investigate role of Tregs, wounds were created after selective depletion of CD4+CD25+FoxP3+Tregs in FoxP3DTR mice(FACS) analysis. Wounds harvested at day 7,14,30 and analyzed for wound closure(imaging), healing outcome(H&E), inflammation(CD45+ and F4/80+ cells/40X-HPF), and fibrosis(Trichrome; α-SMA). Data mean±SD, p values by ANOVA and t-test.

SCID mice demonstrated increased dermal fibrosis (increased trichrome staining day14&30, α-SMA IHC D7) and an exaggerated inflammatory infiltrate compared to WT mice (CD45+,SCID34.50% vs WT11.00%;p<0.05: Macrophages scores increased in SCID). Adoptive transfer of total lymphocytes (TL), CD4+CD25-Tcells and CD4+CD25+Tcells were successfully engrafted at day 7 by FACS; and by qPCR FoxP3 and CD25 locally. Transfer of all three cell types reduced inflammatory infiltrate (neutrophils: TL11.70%;CD4+CD25-Tcells 6.4%; CD4+CD25+T cells 20.10%; p<0.05; macrophage scores decrease compared to control SCID). Treg depletion in FoxP3DTR mice significantly increased collagen content and fibrosis at D14(131077.2±12948.34 vs.96515.63±23828.77, p<0.001), and increased CD45+ cells compared to the controls(37±18.1% vs.16.33±7.8%, p=0.017).

Our data suggests that CD4+CD25+ T cells have a physiologic role in the regulation of wound fibrosis and repair, which is in part mediated by the attenuation of the inflammatory response and effects on the
ECM. Understanding the individual contributions of lymphocyte subsets will permit an improved understanding of physiologic and pathologic wound repair.

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A Tunable Polymer System for Tissue Engineering

Watson E¹, Kim C¹, Tatara AM¹, Yang S¹, Mikos AG¹
1. Department of Bioengineering, Rice University, Houston, TX 77030

Corresponding Author: Emma Watson, Department of Bioengineering, Rice University, Houston, TX, 77030, email: ew20@rice.edu

Introduction: An easily tunable system is of great utility to the tissue engineering field as scaffold mechanical properties have been shown to affect cell differentiation. Terminal diols (or carbon chains with terminal hydroxyl groups) can be reacted with diacids (short chains with terminal carboxylic acids) via condensation to form a polyester. By altering the length of the diol and the saturation of the diacid, a polymer system with varied properties can be created. The ratio of fumaric acid (an unsaturated diacid) to succinic acid (a saturated diacid) can be altered to tune properties of the resulting polyester. Objective: To characterize polyesters with varied ratios of fumaric and succinic acid in the initial monomer feed and to evaluate network properties when crosslinked using a crosslinking molecule.

Methods: Polymers were synthesized in 20g batches using decanediol (Sigma-Aldrich) in a 1:1 molar ratio with a diacid and 0.1mol% p-toluene sulfonic acid (PTSA) at 120°C for 48 hours. The diacids fumaric acid or succinic acid (Sigma-Aldrich) were added in molar ratios of 1:0 (100% fumaric acid), 1:1 (50% fumaric, 50% succinic acid), 1:9 (10% fumaric, 90% succinic acid), and 1:19 (5% fumaric acid, 95% succinic acid). The polymer was purified in 5g batches by dissolving in 80mL chloroform and adding to 160mL of water in a separatory funnel. The chloroform phase then underwent rotary evaporation to total volume of approximately 10mL. This was then added to chilled diethyl ether and allowed to stir for 1 hour. The cloudy solution was then filtered. After drying, the polymer was weighed and evaluated via nuclear magnetic resonance spectroscopy (NMR) and gel permeation chromatography. The polymer was added in a 1:1 wt ratio to n-vinylpyrrolidone (Sigma-Aldrich) using 0.1 wt% Irgacure 819 to form matrices. This mixture was packed into disc-shaped Teflon molds (6mm in diameter, 1mm in height) and exposed to blue light to crosslink. These constructs were dried, placed in 20mL of chloroform for 48 hours, weighed swollen, and then dried for 24 hours prior to calculating a final dry weight. From these values, the swelling and sol fraction of the scaffolds was calculated.

Results: With all 4 fumaric acid to succinic ratios, polyester chains formed, as evidenced by NMR and GPC. However, the ratio of fumaric acid to succinic acid that was incorporated into the polymer was lower than the initial monomer feed. GPC showed all polymers had similar chain lengths. The 4 molar ratio of fumaric to succinic acid tested were capable of forming crosslinked networks; however, the handling properties of these networks were different. Networks with more fumaric acid swelled less and had lower sol fraction than those with higher amounts of succinic acid. The highest swelling in chloroform occurred in the 1:19 group, with the scaffold undergoing observable change in both transparency and dimensions.

Conclusion: By altering the ratio of fumaric to succinic acid in the initial monomer feed, the crosslinking density of the polymer can be controlled, impacting properties such as swelling and sol fraction. Future work will investigate the swelling and sol fraction of networks in an aqueous solvent, the network compressive moduli, the network degradation, and cell studies.

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Modeling Intestinal Epithelial Homeostasis Ex Vivo

Wilson R1, Estes M2, Grande-Allen KJ1
1. Department of Bioengineering, Rice University
2. Department of Molecular Virology and Microbiology, Baylor College of Medicine

Corresponding author: K. Jane Grande-Allen, Department of Bioengineering, Rice University, 6500 Main St., Houston, TX. Email: grande@rice.edu

Objectives: The recent development of organoid technologies that recapitulate the native structure and function of human tissues in vitro have the potential to revolutionize the fields of regenerative medicine and tissue engineering. Advancements in intestinal stem cell biology have led to the in vitro cultivation of polarized functional units of human intestinal epithelium. In contrast to traditional, transformed cell lines, these cultures, known as enteroids, contain all the major intestinal epithelial cell types and perform many functions of the epithelium in vivo. Despite these advantages, enteroids struggle to adequately replicate intestinal epithelial homeostasis – the complex process by which intestinal stem cells renew the epithelium. The objective of this research is to apply bioengineering technology to these organoid models to improve their ability to mimic the small intestinal environment. In vivo, homeostasis is maintained by morphogen gradients that are generated by the micro-topography of the small intestine. Undifferentiated cells are located in pit-like crypts, where high levels of niche factors produced by the surrounding stroma maintain them. As cells migrate up the crypts, they experience progressively lower concentrations of niche factors, ultimately forming a differentiated niche on the protruding intestinal villi. Herein, we describe our efforts to create reproduce these features of the small intestinal architecture in a synthetic hydrogel scaffold that will ultimately permit the formation of an ex vivo intestinal stem cell niche.

Methods: We developed a hydrogel from eight-arm poly(ethylene glycol) norbornene that can support a co-culture of human intestinal enteroids and the stromal niche cells. The bio-inert nature of the PEG hydrogel allows it to have separate cellular compartments: a stromal region in the bulk, and an epithelial region on the surface. Anatomically sized villi (height: 1 mm, diameter = 500 µm) were 3D printed in plastic and then transferred to the hydrogel via a sacrificial alginate mold. The surface of the hydrogel was modified, both covalently and non-covalently, to support the attachment of epithelial cells with a variety of cell-adhesive peptides and proteins. Finally, epithelial monolayers were formed on the villous gels.

Results: Hydrogels with villous topography were successfully formed (Fig 1), and the dimensions of the villi corresponded closely to the dimensions of the alginate mold. Enteroids were able to successfully form monolayers and migrate up the hydrogel villi. However, successful monolayer formation required the non-covalent attachment of full-length collagen IV. Monolayers would not form on covalently modified hydrogels.

Conclusions: We have formed hydrogel scaffolds with villous topography that support the growth of monolayers of human intestinal epithelial cells. This technology has excellent potential as a platform for recreating the intestinal stem cell niche ex vivo, and illustrates the synergy between organoid development and bioengineering techniques.
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β₁ Integrin-mediated Coordinated Motility in Reorganizing Human Salivary Stem/Progenitor Cells

Wu D¹, Pradhan-Bhatt S²,³, Witt RL²,⁴, Harrington DA⁵, Farach-Carson MC¹,³,⁵,⁶

1. Department of BioSciences at Rice, Rice University
2. Center for Translational Cancer Research, Helen F. Graham Cancer Center & Research Institute
3. Department of Biological Sciences and Biomedical Engineering Program, University of Delaware
4. Department of Otolaryngology and Head and Neck Surgery, Thomas Jefferson University
5. School of Dentistry, University of Texas Health Science Center
6. Department of Bioengineering, Rice University

Corresponding author: Danielle Wu, Department of BioSciences, Rice University, 6100 Main St. Houston, TX 77005, E-mail: daniellewu@rice.edu

Objectives: Our aim is to develop an autologous 3D model for reimplantation of functional salivary components to relieve xerostomia. The growth and organization of primary human salivary cells into multicellular secretory units prior to implantation is of key importance. Primary human salivary stem/progenitor cells (hS/PCs) grow and reorganize in bioactive and biocompatible 3D hydrogel systems rich in hyaluronic-acid (HA). We hypothesize that mechanotransduction mechanism activation by specific ligands mediates hS/PC growth and organization that precedes polarization and vectorial secretion.

Methods: Basement membrane proteins secreted by hS/PCs in 3D and β₁ integrin expression were determined by immunocytochemistry. Angular velocities ω (rad/hr) of hS/PC clusters (diameters of 20-52 µm) were calculated from 15 hr time-lapse imaging videos acquisitioned every 5 min to describe coordinated cellular motility. Perturbation of the expression of β₁ integrin (ITGB1 siRNA, n=37) and the interference of β₁ integrin with its ligands (CD49α blocking antibody, n=32) were performed with proper controls (n=25 and n=32, respectively), and ω of clusters were measured in each group and analyzed.

Results: Clusters with coordinated rotation dynamics had smooth edges and organized basement membranes composed of laminin, collagen IV, and perlecan/HSPG2. As expected, early β₁ integrin siRNA treatment prevented hS/PC clusters from forming. β₁ integrin siRNA treatment of formed hS/PC clusters demonstrated an 84% mean reduction in ω compared to its control. Treatment of preformed hS/PC clusters with CD49α resulted in a 65% reduction in ω when compared to its control.

Conclusions: Secretion of laminin and collagen IV from hS/PCs as single cells in HA based hydrogels ensures early activation of β₁ integrin with its bioactive ligand. β₁ integrin activation is essential for cell proliferation and multicellular morphogenesis. Coordinated cellular motility, rather than disorganized cellular dynamics, is associated with organization of de novo basement membranes surrounding multicellular clusters. hS/PCs retain cellular machinery necessary to respond to altering mechanical cues during growth and maturation in a new microenvironment.

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Human Pancreatic Niche Stimulates Differentiation of hESC-derived Progenitors Into β Cells and Reveals New Signals For Endocrine Cell Maturation

Yang D1*, Chmielowiec J2*, Scavuzzo MA3, Wamble K2, Cimborowska J4, Sabek O5, & Borowiak M1,2,3,6

1Molecular and Cellular Biology Department, Baylor College of Medicine, Houston, TX USA
2Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children’s Hospital, and Houston Methodist Hospital, Houston, TX 77030, USA; Stem Cell and Regenerative Medicine Center, Baylor College of Medicine, Houston, TX USA
3Program in Developmental Biology, Baylor College of Medicine, Houston, TX USA
4Department of Bioinformatics, Institute of Molecular Biology and Evolution, Faculty of Biology, Adam Mickiewicz University in Poznan, Umultowska Poland
5Department of Surgery, Houston Methodist Hospital, Houston, TX USA
6McNair Medical Institute, Baylor College of Medicine, Houston, TX USA

*equal contribution

Corresponding author: Malgorzata Borowiak, Center for Cell and Gene Therapy, Baylor College of Medicine, One Baylor Plaza, Houston, TX, E-mail: borowiak@bcm.edu

Insulin-secreting pancreatic β cells generated from human embryonic stem cells (hESC) might not only benefit type 1 diabetic patients by replacing lost β cells, but also serve as a model to study human β cell development. Current in vitro differentiation protocols have been developed based on knowledge gained from pancreatic development of mice and other model organisms. However, differentiation and maturation of either murine or human endocrine progenitors (EP) to β cells is not well understood, resulting in poor differentiation of hESCs. To better understand β cell formation it is important to study the interactions between the surrounding pancreatic niche and EPs. We hypothesized that stage specific signals from the human pancreatic niche are important for β cell specification and maturation.

Here, we established various human fetal pancreatic mesenchymal-endothelial cell (pME) lines at different stages of development to delineate the contribution of the pancreatic niche on differentiating β cells. We demonstrated that co-culture of stage specific human pancreatic niche at Wk17.5h and 20.1, but not Wk9.1, significantly improves β cell formation and, importantly, in vitro glucose stimulated insulin secretion, which overcomes a long-lasting obstacle in the field. Microarray analysis was performed to identify secreting factors from Wk17.5 and Wk20.1 pME cell lines. We selected FGF7, HGF, PDNP, SERPINF1, WNT5A, THBS2, IGF1, and Endocan, which are upregulated at least 20 fold in both Wk17.5 and Wk20.1 cells and tested whether their treatment can effectively increase EPs or β cells. We have found that FGF7, HGF, and IGFI expand EPs by increasing ISLET1+ cells, while SERPINF1, WNT5A, THBS2, PDNP and Endocan promote β cell differentiation by increasing Chromogranin A and insulin+ cells. We further show WNT5A is an endothelial-derived β cell-differentiating factor, which acts through the noncanonical WNT pathway and interacts with the BMP pathway. Together, we identified novel signals from the human pancreatic niche that promote β cell differentiation, and this knowledge benefits regenerative cell therapy for diabetes by improving the in vitro derivation of human β cells from hESCs.

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Combined bFGF and Synoviocyte Derived-Extracellular Matrix Scaffold Effects on Human Articular Chondrocyte (HAC) Proliferation and Re-Differentiation Capacity

Yazdi A1, Kean T1, Dennis J1
1. Department of Orthopedic Surgery, Baylor College of Medicine

OBJECTIVES: Our previous work on chondrocyte expansion showed positive results from both the addition of bFGF1 and by culture on synoviocyte matrix2 to mitigate the limited capacity to self-repair. Even with these positive results, additional improvements are needed to make joint resurfacing procedures from a single biopsy possible. To address this, bFGF combined with porcine synoviocyte-derived extracellular matrix scaffold was assessed for its ability to increase HAC expansion and retain re-differentiation potential over multiple passages.

METHODS: Porcine synoviocyte matrix scaffolds (SCM) were expanded at 21% O2 and ethanol extracted as described.2 5 HAC donors under an IRB approved protocol were thawed and then cultured onto either tissue cultured plastic (TCP) or SCM-coated flasks at physiological (5%) oxygen. The experimental groups were either expanded with regular growth media consisting of DMEM-LG with 10% FBS and 1% Penicillin/Streptomycin or with the same growth media containing 10 ng/mL bFGF. When the cell layer was in a log expansion (70%-90% confluence), the HACs were passaged to a new flask under the same conditions and aggregate cultures were made. The groups were compared for their ability to support human chondrocyte proliferation over 4 passages. Aggregates were compared for their chondrogenic re-differentiation potential by quantifying their glycosaminoglycan (GAG) and collagen content and by histological examination. Statistical analysis was performed using 2-way ANOVA with Newman-Keuls post-hoc test (GraphPad Prism v.6).

RESULTS: SCM, SCM + bFGF, and bFGF showed greater cell expansion than TCP across all passages. TCP flasks took 1.7x – 2x days longer to reach confluence than the other 3 conditions. Hydroxyproline content was significantly greater in passages 3 and 4 for SCM + bFGF compared to all other conditions. There was also increased GAG concentration over passages 1 to 3 in all conditions.

CONCLUSION: Both synoviocyte-derived matrix scaffolds and FGF support the enhanced expansion of human chondrocytes over multiple passages. The aggregates from SCM(+)FGF show that the increased GAG concentrations compared to all other conditions indicate that bFGF and SCM foster an environment that diminishes the detrimental effects of chondrocyte de-differentiation. One surprising result was the enhanced accumulation of GAG with passage in the SCM and FGF-supplemented groups which is contrary to literature3. Total collagen content showed relatively small differences between conditions. Together SCM and bFGF significantly enhances the ability to expand cells with chondrogenic potential for the use in tissue engineering applications like Autologous Chondrocyte Transplantation.
Figure 1: Growth rate and collagen content (hydroxyproline) and GAG content of HAC cultured on tissue culture plastic (TCP), synoviocyte matrix (SCM), SCM(+)FGF and TCP + FGF (FGF). Mean ± S.D. n = 5 human donors. Different letters within the passage indicates statistical significance at $p < 0.05$.

Figure 2: Aggregate histology of passages 1 to 4 with Safranin O & fast green staining to show GAG.

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Mimicking the Osteogenic Niche for Bone Augmentation and Spinal Fusion

Taraballi F¹, Minardi S¹, Bauza G.¹, Cabrera F.¹, Tasciotti E¹,2*

¹ Center for Biomimetic Medicine, Houston Methodist Research Institute, Houston, TX, 77030, USA
² Department of Orthopaedic & Sports Medicine, Houston Methodist Hospital, Houston, TX 77030

* Ennio Tasciotti, Center for Biomimetic Medicine, Houston Methodist Research Institute, Houston, etasciotti@houstonmethodist.org

Several medical applications for bone forming materials have emerged in America’s aging and growing population, including osteoporosis, fracture healing, and spine fusion. Despite the advancements, there is still lack of a unanimous consensus on the most efficient and reliable approach to accomplish bone regeneration. Herein, we propose a novel trabecular bone-mimicking scaffold, consisting of a multi-substituted apatite/ultra-structured type I collagen composite (MHA/Coll). MHA/Coll was synthesized through a biologically inspired process, recapitulating bio-mineralization. MHA/Coll was demonstrated to mimic the chemical, physical and morphological cues of human bone. Spontaneously, the scaffold induced the over-expression of osteogenesis-associated genes of human bone marrow-derived stem cells (hBM-MSC) as evaluated through a broad panel of osteogenic marker genes. We monitored the dynamic expression at early (1wk) and late (3wk) differentiation stages, and observed a significant induction of progenitor genes (RUNX2, SP7, and STAT3) and early osteoblast marker genes (COL1A1 and ALPL) at 1 week, whereas late osteoblast marker genes (SPARC and BGLAP) and osteocyte marker genes (SPP1 and TNFRSF11B) were upregulated at 3 weeks. The osteoinductive potential of MHA/Coll was assessed in a rabbit ectopic site, where we showed the formation of a large mass of new trabecular bone as early as 2 weeks, without the use of heterologous cytokines or cells. The MHA/Coll was tested in a rabbit orthotopic site (spinal fusion), where the scaffold formed new bone, completely integrated in only 6 weeks, and initiated remodeling by forming compact bone. Bone histomorphometry confirmed that the tissue that replaced the scaffold was trabecular bone, and both osteoblasts and osteoclasts were identified. Such bone mass was further analyzed by qPCR to characterize the molecular mechanisms and pathways triggered by the scaffold, and the results were compared to those of mature trabecular bone. In particular, we evaluated the expression of osteogenic (Dlx5, Runx2, Col1a1, Sparc, Spp1), hematopoietic (Kdr, Sele, Cd38, Vcam1) as well as bone marrow stromal cell (Alcam, Itgb1, Vim) markers. We found that all of the osteogenesis associated genes were over-expressed compared to mature trabecular bone, and interestingly that also the expression of both hematopoietic and bone marrow stromal cell markers was comparable to those of native bone. Altogether these data represent a significant step towards the development of acellular off-the-shelf substitutes for bone augmentation not requiring stem cells or growth factors, whose use remains still highly controversial.