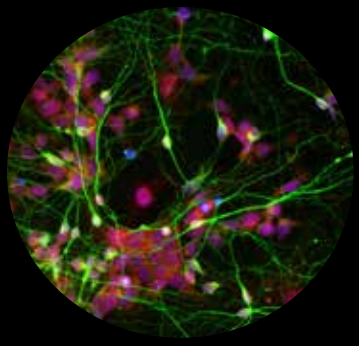

MARYLAND

Stem Cell Research Fund

Annual Report

2019

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Chulan Kwon, Ph.D.
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Marta Lipinski, Ph.D.
University of Maryland, Baltimore
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Xiaobo Mao, Ph.D.
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Nicholas Maragakis, MD
Johns Hopkins University
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Kimberly Stroka, Ph.D.
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Senquan Liu, Ph.D.
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Kathryn Moss, Ph.D.
Johns Hopkins University
Mentor: Ahmet Hoke, MD, Ph.D.
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Rahel Schnellmann, Ph.D.
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Arens Taga, Ph.D.
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Completed MSCRF Awarded Research

Completed Commercial Awards:

Luis Alvarez, Ph.D.
Theradaptive, Inc.
2018 Commercialization Award
Development of a Biphasic MSC Delivery System for the Repair of Osteochondral Defects

Emily English, Ph.D.
Gemstone Biotherapeutics, LLC.
2019 Commercialization Award
Stem Cell Loaded Extracellular Matrix Replacement Scaffolds for Skin Regeneration in Burns

Linhong Li, Ph.D.
MaxCyte, Inc.
2017 Commercialization Award
Translational Development of Gene-Corrected Hematopoietic Stem Cells as Treatment for Sickle Cell Disease (SCD)

Hai-Quan Mao, Ph.D.
LifeSprout, Inc.
2018 Commercialization Award
Delivery of MSCs to Enhance the Replacement and Regeneration of Soft Tissue

Ha Nam Nguyen, Ph.D.
3Dnamics, Inc.
2017 Commercialization Award
Engineering Human Pluripotent Stem Cell-Derived Brain Organoids for Drug Screening and Toxicity Testing

Jonathan Rowley, Ph.D.
RoosterBio, Inc.
2018 Commercialization Award
Closed Systems Enabling Commercially-Viable Stem Cell Manufacturing

William Rust, Ph.D.
Seraxis, Inc.
2017 Commercialization Award
Long-term Function of Stem Cell Grafts for Insulin-Dependent Diabetes

Chengkang Zhang, Ph.D.
Propagenix, Inc.
2017 Commercialization Award
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Completed Validation Awards:

Sharon Gerecht, Ph.D.
Johns Hopkins University
2017 Validation Award
Patient-Specific Small-Diameter Tissue Engineered Vascular Grafts

Sunjay Kaushal, MD
University of Maryland, Baltimore
2017 Validation Award
Neonatal Cardiac Stem Cells for Heart Regeneration

Chulan Kwon, Ph.D.
Johns Hopkins University
2018 Validation Award
Developing Adult Cells from iPSCs

Yunqing Li, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
2017 Validation Award
Lineage Reprogramming of human Fibroblasts into Oligodendrocyte Progenitor Cells

Elias Zambidis, MD, Ph.D.
Johns Hopkins University
2018 Validation Award
MoroPLUR: A Defined Feeder-Free Medium for Enhancing Functionality of Human Pluripotent Stem Cells

Completed Discovery Awards:

Jeff Bulte, Ph.D.
Johns Hopkins University
2017 Discovery Award
Non-Invasive Imaging of Hydrogel Scaffold Biodegradation and Cell Survival

Ivy Dick, Ph.D.
University of Maryland, Baltimore
2017 Discovery Award
Developing a Novel Treatment Strategy for Timothy Syndrome

Aaron James, MD, Ph.D.
Johns Hopkins University
2017 Discovery Award
Regional Specification of Bone-Associated Perivascular MSC

David Kass, MD
Johns Hopkins University
2017 Discovery Award
Conditional Power Switch for Stem Cell-Derived Cardiomyocytes

Gabsang Lee, Ph.D.
Johns Hopkins University
2017 Discovery Award
Pharmacological Cues to Expand Functional Human PAX7::GFP+ Skeletal Muscle Stem/Progenitor Cells

Brady Maher, Ph.D.
Lieber Institute for Brain Development
2017 Discovery Award
Use Human Cellular Models of Pitt-Hopkins Syndrome to Study Neuronal Development and Validate Therapeutic Targets

Michael Nestor, Ph.D.
The Hussman Institute for Autism
2017 Discovery Award
Establishing a 3D Based High-Content Screening Platform for Cellular/Phenotypes in Autism

Linda Resar, MD
Johns Hopkins University
2017 Discovery Award
Developing Stem Cell Technology for Tissue Repair and Goblet Cells for Ulcerative Colitis Patients

Piotr Walczak, MD, Ph.D.
Johns Hopkins University
2017 Discovery Award
Inducing Immunotolerance of Myelinating Progenitor Cells Transplanted into the Brain of Immunocompetent Mice

Mingyao Ying, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
2017 Discovery Award
Highly Efficient Conversion of iPS Cells to Motor Neurons and Oligodendrocytes by Synthetic Modified mRNAs

Completed MSCRF Awarded Research - *Cont'd*

Completed Post Doctoral Fellowship Awards:

Qin Bian, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award
Project Mentor: Patrick Cahan, Ph.D.
Direct Specification of Articular Chondrocytes from iPSC-Derived Lateral Plate Mesoderm

Dongwon Kim, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award
Project Mentor: Luis Garza, Ph.D.
Postdoctoral Training Towards Independence: Testing of Skin Stem Cells to Modify Skin Identity

Josephine Lembong, Ph.D.
RoosterBio, Inc.
2017 Post-Doctoral Fellowship Award
Project Mentor: Jonathan Rowley, Ph.D.
Stem Cell Expansion and Differentiation in Bioreactors via Coupling of Substrate Curvature and Shear Stress

Joseph Mertz, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award
Project Mentor: Donald Zack, Ph.D.
Proteomic Approaches to Study Cell Death Mechanisms in Human Stem Cell-derived Retinal Ganglion Cell

Nikhil Panicker, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award
Project Mentor: Ted Dawson, Ph.D.
Activation of the NLRP3 Inflammasome in Human Dopamine Neurons as a Consequence of Parkin Dysfunction

Marco Santoro, Ph.D.
University of Maryland, College Park
2017 Post-Doctoral Fellowship Award
Project Mentor: John Fisher, Ph.D.
Development of Tissue-Engineered Vascularized Scaffolds via 3D Printing of Endothelial/Stem Cells

Congshan Sun, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award
Project Mentor: Gabsang Lee, Ph.D.
hiPSC Based Compound Screening for Treatment of Duchenne Muscular Dystrophy (DMD)

Aline Thomas, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award
Project Mentor: Jeff Bulte, Ph.D.
Development of Stem Cell Therapies for Multiple Sclerosis using Non-Invasive Biomarkers

Zhao Wei, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award
Project Mentor: Sharon Gerecht, Ph.D.
3D Printing Vascularized Cardiac Constructs

Maryland Stem Cell Research Commisssion

Debra Mathews, Ph.D., M.A. - Chair
(Appointed by Johns Hopkins University)
Assistant Director for Science Programs,
Johns Hopkins Berman Institute of Bioethics;
Associate Professor, Dept. of Pediatrics,
Johns Hopkins School of Medicine

Curt Van Tassell, Ph.D. - Vice Chair
(Appointed by the Speaker of the House of Delegates)
Research Geneticist, USDA-ARS, Beltsville, MD

Scott Bailey, Ph.D.
(Appointed by Johns Hopkins University)
Associate Professor; Biochemistry and Molecular Biology,
Johns Hopkins Bloomberg School of Public Health;
Johns Hopkins School of Medicine

Rachel Brewster, Ph.D.
(Appointed by the University System of Maryland)
Associate Professor; Biological Sciences University of Maryland,
Baltimore County

Margaret Conn Himelfarb
(Appointed by the Governor)
Health Advisory Board and Institutional
Review Board, Johns Hopkins Bloomberg School of
Public Health; Embryonic Stem Cell Research Oversight
Committee, Johns Hopkins School of Medicine

Diane Hoffmann, M.S., J.D.
(Appointed by the University System of Maryland)
Professor of Law, Director Law & Health Care Program,
University of Maryland School of Law

Haig Kazazian, Jr., M.D.
(Appointed by Johns Hopkins University)
Professor of Pediatrics McKusick-Nathans Institute of
Genetic Medicine

David Mosser, Ph.D.
(Appointed by the University System of Maryland)
Department of Cell Biology and Molecular
Genetics, University of Maryland, College Park.

Linda Powers, J.D.
(Appointed by the President of the Senate)
Managing Director of Toucan Capital,
Early & Active Supporter of Biotech Companies

Rabbi Avram Reisner, Ph.D.
(Appointed by the Governor)
Rabbi of Congregation Chevrei Tzedek, Baltimore, Maryland.

Ira Schwartz, Esq.
General Counsel, MD Technology Development Corporation

Bowen Weisheit, Esq.
(Appointed by the Governor)
Attorney, President of the Ensign C. Maryland Kelly, Jr.
Memorial Foundation

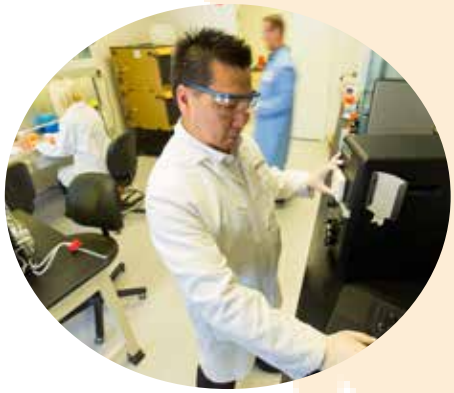
For more information about the commission members, meetings, bylaws and more please visit ***www.MSCRF.org***

Regenerative medicine has been a rapidly evolving field that is transforming healthcare and is delivering on its promise of bringing cures to patients in need.

In 2019, the progress and innovation in this sector has continued. There are currently over 959 regenerative medicine companies worldwide.[1] There are an estimated 1071 regenerative medicine clinical trials ongoing worldwide, impacting over 59,575 patients.[1] On the heels of product approvals and Regenerative Medicine Advance Therapy (RMAT) designations, conversations and innovations around pricing and reimbursement have progressed this year. Regulatory pathways and manufacturing challenges continue to be addressed.

Financings have also been abundant with a reported $7.4 Billion raised as of Q3. [1] In addition, mergers and acquisitions in the field have continued to propel this industry, for example Vertex acquiring Semma therapeutics for $950M (to develop stem cell-based cures for Type 1 Diabetes). This is a really exciting time to be in this field and we, at MSCRF, continue to be inspired by the researchers driving this innovation and we remain dedicated to serving this community and helping Maryland be at the forefront of this momentum.

1. Informa/Alliance for Regenerative Medicine



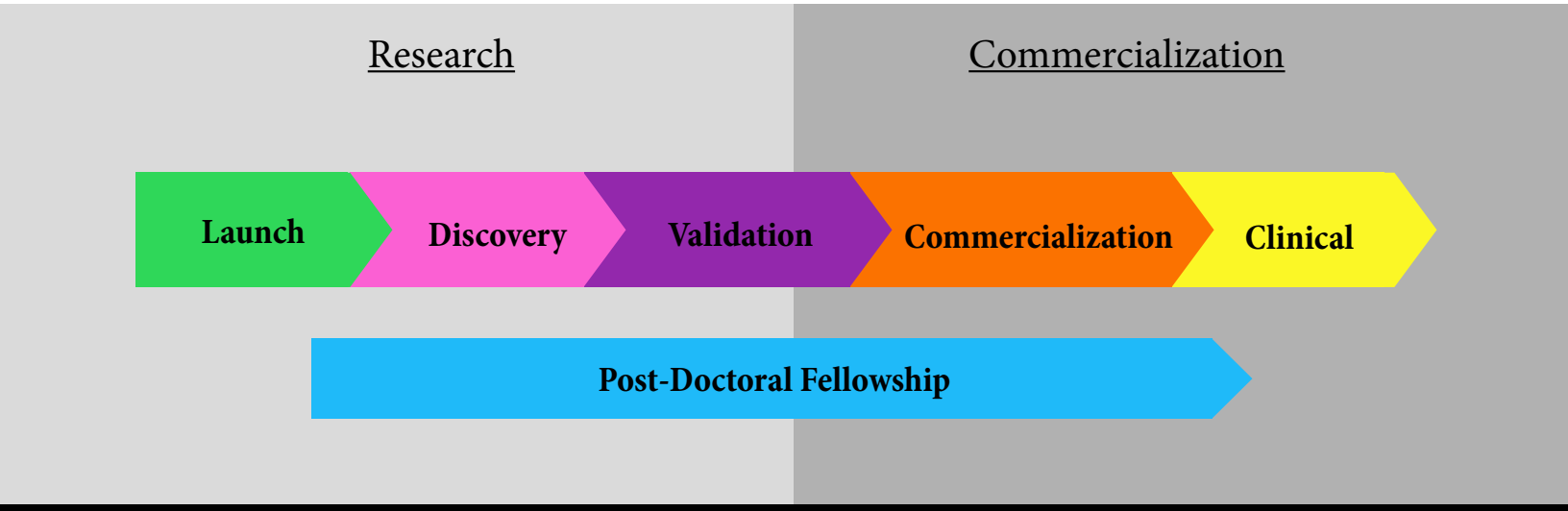
Funding Opportunities

We are committed to identifying and fostering cutting-edge research and innovation in the field of regenerative medicine in MD.

Our Accelerating Cures initiative comprises programs that help transition human stem cell-based technologies from the bench to the bedside as well as mechanisms to build and grow stem cell companies in Maryland. The Maryland Stem Cell Research Commission (Commission) offered five programs in calendar year 2019 to address the needs of our community: Discovery, Validation, Commercialization, Clinical, and Post-doctoral fellowship. The Commission is constantly working to adapt and improve our programs to address the emerging challenges in the field and to meet the evolving needs of our community of researchers, companies and clinicians. After careful evaluation of the different programs, we have introduced a new program called Launch, which is open for our upcoming grant cycle. This program is aimed at new and new-to-the-field faculty to bring novel/orthogonal thought, technologies and expertise to the regenerative medicine field. This program is an addition to the five exciting programs that we continue to offer.

ACCELERATING CURES

Our six programs are designed to catalyze innovation and sequentially transition the most promising discoveries from the labs where the invention occurred, to the clinic where they will be offered to patients. There is an urgent need to accelerate these cures across diverse therapeutic areas in this field.



Launch Program

is for New faculty or researchers who are new to the field of human stem cell research at non-federal Maryland-based Universities/Research Institutes who have not received any prior MSCRF funding. Applicants to these grants may request up to $345,000 over up to 24 months.

Discovery Program

is for faculty at non-federal Maryland-based Universities/ Research Institutes with innovative ideas to develop novel human stem cell-based technologies and cures. Applicants to these grants may request up to $345,000 over up to 24 months.

Validation Program

is for faculty at non-federal Maryland-based Universities/ Research institutes with IP for human stem cell-based technologies that require additional validation before creation of start-up companies. Applicants to these grants may request up to $230,000 over up to 18 months.

Commercialization Program

is for Maryland-based start-up companies or established companies developing new human stem cell-based products. Applicants to these grants may request up to $300,000 over up to 12 months.

Clinical Program

is for Universities /Research Institutes or Companies that wish to conduct human stem cell-based clinical trials in the State of Maryland. Applicants to these grants may request up to $750,000 over up to 24 months. A 1:1 match of non-state money is required. FDA IND clearance is required.

Post-Doctoral Fellowship Program

is for exceptional post-doctoral fellows who wish to conduct human stem cell research in academia or in industry in the State of Maryland. Applicants to these grants may request up to $130,000 over up to 24 months. Applicant must have completed the doctoral degree within the past 3 years.

Introducing the New MSCRF Mobile App!

At the beginning of 2019, we launched our new website. This provides easy access to our funding opportunities, our current awardees and features our portfolio companies. Our website also serves as the go-to source for regenerative medicine news and stories from around the world. We provide our readers with news that we collect and update daily. In addition, we also list events in the field that are of interest to our audience.

Since we noticed that many of our users accessed our website on mobile devices, we decided also to develop an interactive app to allow our community to better engage with the information we provide and have easier access to our stories as well as our events. We released our newly developed app in early December and will continue to enhance its capabilities to address the needs of our community.

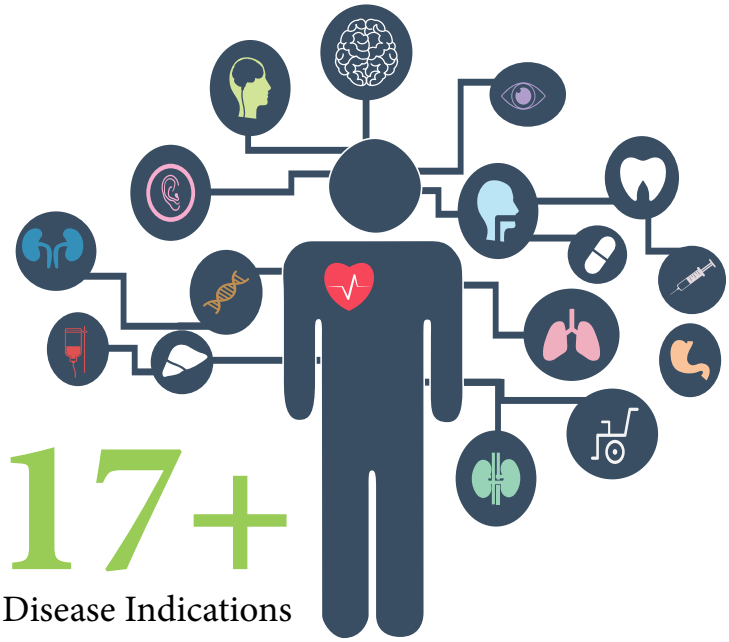
2019 MSCRF by the numbers

$7,974,074

in Funding

10

Organizations

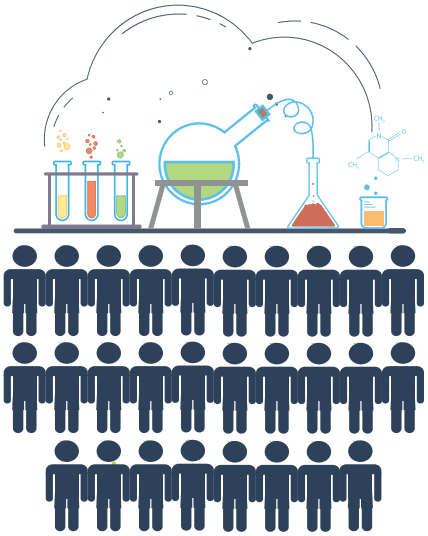


17+

Disease Indications

28

Grants Awarded






MSCRF is Paving the Way for Cures

We serve as much more than a funding source to the numerous researchers in our community. We support each project, every scientist and provide guidance and expertise tailored to each unique need. Our leadership and support serves to connect researchers across our state as well as on a national and global scale to build a strong community to advance regenerative medicine therapies.

We engage with our researchers and companies throughout the year at various events that we organize, collaborate on, promote or attend in the region and around the nation. We will continue to bring the best events to our awardees and provide value at every stage of their research to ensure that each project, scientist and company has the best chance to succeed.

We engaged with the local community at over 12 events in the past year. We held funding briefings at JHU and UMB to continue our interactions with researchers at different universities to help them understand and apply to our various programs. We also discussed regenerative medicine funding opportunities with companies at a recent innovation forum organized by MD Tech Council. We routinely support various stem cell scientific seminars/symposiums held at universities, organized by faculty. We discussed the opportunities and challenges in the field on a panel with NIH and FDA, organized by Biobuzz/BioTrac. We also spoke at and advised companies at local incubators (FITCI and GIC). We participated in and supported our portfolio at events such as the Bioinnovation Conference, the Entrepreneur Expo, the BioHealth Capital Region (BHCR) Forum as well as the BHCR investment conference. We continue to partner with BioTrac to offer hands-on training and courses in the field of stem cell biology.

In addition to these local events we were invited to provide thought leadership and insight at various internationally recognized key stem cell meetings. We joined the 12th annual Business of Regenerative Medicine at the Harvard Business School to help define and create value, while engaging with CEOs, venture capitalists and experts in biomedical research. We helped organize key sessions at the Biotech week Boston Cell and Gene therapy Bioprocessing and Commercialization conference, overseeing the tracks discussing Pre-clinical, Clinical Development and Commercialization for Cell, Gene and CAR-T therapies. We co-chair and are invited keynote speakers at the World Stem Cell Summit. Several members of our portfolio participated in the International Society for Stem Cell Research and the Cell and Gene meeting on the Mesa to promote the scientific advances we have made in Maryland. Finally, MSCRF joined the Maryland delegation at International Bio promoting our region to international companies seeking to expand or relocate to Maryland. We haveput MSCRF on the world stage and this increased visibility and global recognition of our expertise and our portfolio has accelerated growth and investment into our regional regenerative medicine ecosystem.With our increased program offerings and our tireless efforts to further our mission, we will continue to build this community and accelerate cures to patients in Maryland and around the world.

Maryland Stem Cell Research Commission

In July 2019 the Commission elected a new chair and vice chair to serve for the next two years.
We are honored to have Dr. Debra Mathews and Dr. Curtis Van Tassell lead our initiative and further our mission.

Debra Mathews, Ph.D., M.A. - Chair

Curt Van Tassell, Ph.D. - Vice Chair

For more information about the commission members, meetings, bylaws and more please visit ***www.MSCRF.org***

MSCRF

Annual Report
2019

Commercialization Grant Awards

Taby Ahsan, Ph.D.
RoosterBio, Inc.
Award Amount: $300,000
Disease Target: Tool for CV, Ortho, Metabolic, & Immunomodulator

Luis Alvarez, Ph.D.
Theradaptive, Inc.
Award Amount: $299,990
Disease Target: Osteochondral Defect

Simplified Kit for EV Production in Scalable Systems

There have been over 900 registered clinical trials using mesenchymal stem/stromal cells (MSCs) for various therapeutic applications, showing MSCs as a well-tolerated, safe therapy. Additionally, MSC-derived extracellular vesicles (MSC-EVs) are being increasingly investigated as a clinical therapy for a broad range of indications due to their similar therapeutic effects to MSCs and its potential as a key bioactive agent in regenerative medicine applications. With these growing numbers of MSC-EV clinical applications, there is a critical need for an economical biomanufacturing process capable of generating the EV numbers necessary to meet the demand for clinical doses.

Building on RoosterBios expertise in commercialization of scalable hMSC products, this proposal will develop a new product paradigm for scalable MSC-EV production in bioreactors. This will be achieved through the development of RoosterAdhere, a cryopreserved product with hMSCs pre-seeded onto microcarriers in a thaw-and-culture format. The funding from this proposal will support the development of this novel technology and its incorporation with existing RBI products (i.e. standardized hMSCs, high-performance media), as well as RBIs scalable bioreactor process that are designed for clinical manufacturing use, to generate a specialized product kit that addresses a new and growing facet of the stem cell industry: extracellular vesicles (EVs) and exosomes. By incorporating well characterized, consistent raw materials into this product, the MSC-EV Rapid Bioreactor Development Kit is designed to simplify EV production, reduce cost, and therefore accelearte timelines to development of stem cell-based clinical therapies.

Development of a Biphasic MSC Delivery System for The Repair of Osteochondral Defects

Theradaptive has developed a novel therapeutic delivery platform to repair cartilage called ConForma. This platform can control the dose and timing of release of protein therapeutics at implant sites. Key features of ConForma are: (1) its 3D-printed scaffold is mechanically tunable with physical properties mimicking native cartilage, (2) it supports the delivery of a wide variety of small molecules, peptides and proteins, and (3) it has demonstrated an excellent targeted release performance in a challenging large animal model. Our ongoing 2018 MSCRF commercialization grant accelerated the selection of the therapeutic proteins, enabled our 3D-printing capability to manufacture the scaffold component of the implant, and to validate an antimicrobial agent that can vastly reduce incidence site infection. In this proposal, we plan to: (1) complete the optimization and production of ConForma, and (2) to evaluate cartilage repair of ConForma in large animals. The incorporation of progenitor cells (MSCOC) with high osteochondrogenic potential into ConForma offers a breakthrough technology in reconstructive medicine. With Theradaptives new MSCOC- ConForma, orthopedic surgeons can for the first time offer patients a potent, viable and regenerative restoration for osteochondral defects without the need for marrow isolation.

Jamie Niland
NeoProgen, Inc.
FY2020 Commercialization Award (1st Funding Cycle)
Award Amount: $300,000
Disease Target: Heart Failure

Neonatal Cardiac Stem Cells for Heart Tissue Regeneration

After a myocardial infarction, the mammalian heart undergoes only minimal levels of regeneration. While medical and surgical interventions are effective in limiting damage, these ischemic patients suffer from heart failure (HF) symptoms that grow worse over time and can lead to disability and death. The most promising approaches to regenerate muscle and vasculature are cell-based therapies. However, even successful autologous cell therapies face obstacles to widespread adoption, including high expense, delays, and variable effectiveness. Allogenic cardiac stem/progenitor cells (CPCs) generated from neonatal tissue offer an attractive alternative. The Kaushal lab has identified a novel type of CPC in the healthy myocardium of neonates that express the cell-surface signaling protein c-kit and discovered that these c-kit+ CPCs are able to stimulate the regeneration of cardiac muscle and restore heart function significantly more than various cell types tested in a rodent model of myocardial ischemia (MI) and now in a large acute MI porcine animal model. This stem cell population can be developed into a therapy that incorporates streamlined manufacturing techniques to keep costs down and offers reproducible, well-defined benefits. These are key elements of a commercially-viable regenerative cell therapy. In this application, we propose to validate the safety and functional abilities of allogenic, neonatal, c-kit+ CPCs in human HF patients. The Kaushal lab complies with bioethical requirements and expectations of the University of Maryland, School of Medicine. The neonatal cardiac progenitor/stem cells (nCPCs) were obtained from discarded cardiac tissue biopsies from neonates, with the family's consent obtained in accordance with the University of Maryland's IRB procedures. All animal work was approved by IACUC at the University of Maryland. As suggested by one of the reviewers, significant swine testing has been completed and additional swine testing will be completed prior to initiating the company's human clinical study. NeoProgen Phase I/II clinical investigation will be conducted at an independent investigational site by an interventional cardiologist with extensive experience of investigating stem cell therapies in patients with heart failure. The investigation will be performed in accordance with a written protocol approved by the hospitals local ethics committee and authorized by the local competent authority. The company is not aware of an ISO standard specifically related to clinical investigations of stem cells so the plan is to design the study in accordance with ISO-14155;2011 Clinical investigation of medical devices for human subjects - Good Clinical Practice which provides guidance to ensure the ethical protection of human subjects. Data will be analyzed by investigators blinded to group assignment. All data will be reviewed by CMO, CSO and CTO. Data will be disaggregated to enable assessment of possible sex differences.

Brian Pollok, Ph.D.
Propagenix, Inc.
FY2020 Commercialization Award (1st Funding Cycle).
Award Amount: $299,500
Disease Target: Pulmonary Disease, Cancer Infectious Disease

Apical Surface-Outward (ASO) Airway Organoids: A Novel Cell System for Drug Discovery & Personalized Medicine

Basic biomedical research and drug discovery work has relied on in vitro cellular models to represent normal and disease biology since the 1950s. Historically, such cell models have been configured as 2-dimensional cultures where cells subsist in a planal layer on the bottom of a plastic tissue culture vessel. It is now becoming quite evident at both a molecular and functional level that 2D cell culture models fail to accurately embody the normal in vivo microenvironment where variation in tissue ultrastructure, cell-cell communication, and metabolic conditions exist. Recent advances to create more biologically-relevant cell models have focused on use of primary cells (rather than transformed cell lines) together with methods to form a 3D architecture; most notable of these advances has been organoid technology using expanded adult stem cells to form differentiated, structured cell aggregates of defined tissue types. Organoid technology has experienced robust commercial interest from life science companies selling DIY reagents, biorepositories distributing patient-specific organoid models, and (limited) biopharma groups using organoid cell models for drug discovery. However, a major issue with the current organoid technology is that the cells face their apical surface inwards to the lumen of the organoid rather than the normal outward orientation. Propagenix has recently discovered and filed provisional patents on a scalable method to generate epithelial cell organoids positioned in their biologically normal orientation, termed apical surface-outward (ASO) organoids. Having epithelial organoids with a normal cellular orientation such that their apical surface points outwards enables an entire new set of research interrogations including cell-virus and cell-bacteria interactions, drug transport, and protein secretion. ASO organoids also have features that have potential importance for diagnostic and therapeutic use. This Commercialization Grant proposal is focused on generating application data that will drive commercial success of our ASO organoid technology in multiple markets.

Amir Saberi, Ph.D.
Domicell, Inc.
Award Amount: $300,000
Disease Target: Heart Failure

Application of Mesenchymal Stem Cell Spheroids in an Implantable Bioreactor

The Stem Cell Implantable Bioreactor (SCIB) is a novel platform for the in vivo production and delivery of stem cell paracrine factors (PFs). Stem cell therapy administered after myocardial infarction (MI) is intended to reduce heart failure by promoting anti-inflammatory and regenerative responses in the injured myocardium. Growing evidence suggest that these beneficial effects are largely mediated by se-creted PFs. To harness the beneficial effects of stem cell PFs we designed and built the SCIB and validated its design as a customizable and implantable platform for sus-tained PF production in vivo. The SCIB consists of a catheter-based, selectively per-meable cell chamber that protects contained cells from washout and immune attack while allowing free release of PFs and exosomes in response to host signals. We showed in prior studies that deploying allogeneic mesenchymal stem cells (MSCs) in a prototype SCIB after MI reduced adverse cardiac remodeling in pigs. We have now built a clinical-grade SCIB and the next critical step in preparing this technology for clinical studies is optimization of the SCIB cellular component in order to optimize the production and release of PFs. Evidence suggests that MSCs better retain their stemness and therapeutic factor production in tri-dimensional culture. In this pro-posal, we will optimize deployment of MSCs as multi-cellular spheroid clusters in the clinical-grade SCIB and compare angiogenic and anti-inflammatory PF output relative to standard single cell suspensions. MSC spheroids increase the SCIB MSC capacity, limit replicative senescence, and are safer than alternative MSC 3D constructs that employ foreign materials. Validation of deployment of MSC sphe-roids in the SCIB will inform the optimal MSC format and dosage for the pivotal pre-clinical animal efficacy study, further de-risk this technology, and accelerate the path to clinical trials and commercialization.

Ines Silva, Ph.D.
Reprocell, USA
FY2020 Commercialization Award (1st Funding Cycle)
Award Amount: $292,580
Disease Target: Parkinson, Alzheimer's, ALS

Building a Commercial Neural Cell Bank from Patient-derived Induced Pluripotent Stem Cells

REPROCELL is a global regenerative medicine company that specializes in the providing biologically relevant human tissue models for drug discovery and development. REPROCELL offers a range of cell products and services encompassing biobanking, human induced pluripotent stem cell (iPSC) line development and services to differentiate cells into hematopoietic progenitor cells (HPC). By adapting this technology to develop additional lineages, REPROCELL aims to develop a diverse portfolio of differentiated human iPSCs to meet the demand for preclinical tests and drug discovery screening in several disease areas with an emphasis on the central nervous system. Affecting millions of people worldwide, with no cure and current treatments often insufficient in controlling symptoms, neurodegenerative diseases, such as Parkinsons Disease, are a primary focus. A staggering 90% of new drugs that reach the clinical trial stage fail to proceed to the clinic and patient application. Consequently, interest in the development of new effective therapies is high. To increase the success rate and implementation of new therapies, it is imperative that researchers develop biologically relevant in vitro models that are capable of mimicking human neurodegenerative diseases and evaluating pre-therapeutic outcomes. We believe that iPSC derived human cells from normal and affected individuals will provide a cost effective, biologically relevant, model for screening new therapies. We propose to expand REPROCELLs current portfolio to include a well-characterized tissue bank of human samples derived from patients with neurodegenerative diseases and of iPSCs reprogrammed from patients derived cells. We will optimize protocols to differentiate cells into various neural cell types. Finally, we will generate a biobank of differentiated neural cells that will be commercially available and a portfolio of services to support new drug development for neurodegenerative disease. Such commercially available research tools will prove invaluable in the development of future treatments for neurodegenerative diseases.

MSCRF

ANNUAL REPORT
2019

Discovery Grant Awards

Seth Ament, Ph.D.
University of Maryland, Baltimore
Award Amount: $344,587
Disease Target: Schizophrenia

Molecular, Neurodevelopmental, Connectomic, and Behavioral Consequences of a Psychiatric Risk Gene in a Population Isolate

We propose to validate a psychiatric risk gene, SETD1A, via multi-level convergent neuroscience experiments using stem cells, human brain imaging, and integrative systems biology. Loss-of-function and missense variants in SETD1A -- a chromatin remodeling gene highly expressed in the fetal brain -- are strongly associated with risk for psychiatric and neurodevelopmental disorders. We identified two protein-coding variants in SETD1A that are rare in the general population yet are >10-fold enriched in the Old Order Amish (OOA), a unique population isolate for which we have collected deep genetic and phenotypic information from >7,000 individuals. With existing funding, we will assess structural and functional brain connectivity and cognitive and psychiatric behavioral traits in OOA individuals with these SETD1A variants. Using funds from MSCRF, we will characterize the molecular, cellular, and neurodevelopmental consequences of the SETD1A variants using patient-derived, induced pluripotent stem cells and genome-editing in human embryonic stem cells (Aim 1). SETD1As effects on psychiatric phenotypes are likely to involve perturbations in downstream gene regulatory networks. We will characterize these networks by RNA-seq and ChIP-seq and test the hypothesis that the genes in the SETD1A regulatory network are associated with psychiatric and connectomic phenotypes (Aim 2a). Finally, we will integrate these data with transcriptomic and epigenomic data from human brain development to trace the connections from gene to molecular network to neurodevelopmental cell types to structural and functional brain connectivity to behavior (Aim 2b). Our approach could easily be extended to 45 additional coding variants in known psychiatric risk genes that we have discovered are up to 600-fold enriched in the Old Order Amish cohort.

Specific Aim 1. Test the hypothesis that Amish-enriched coding variants in SETD1A influences neural stem cell differentiation via its effects on chromatin states and gene expression during key cell fate decisions. We have generated iPSC lines from Old Order Amish individuals homozygous for naturally-occurring SETD1A coding variants and their relatives who do not have these variants. In addition, we generated genome-edited cell lines in which we have produced SETD1A loss-of-function variants on an isogenic background. We will differentiate cells of each genotype to neural precursors and characterize the effects of SETD1A variants on cellular lineages, transcriptomic states, and chromatin states.

Specific Aim 2. Test the hypothesis that SETD1A variants and the SETD1A gene regulatory network influence severe mental illness via effects on brain gene expression, brain connectome, and cognition. There are two sub-Aims. In Aim 2a, we will test for effects of SETD1A variants and the SETD1A gene regulatory network on risk for psychiatric disorders and on cognitive and brain connectome phenotypes in the Old Order Amish population isolate, as well as in publicly available GWAS. In Aim 2b, we will integrate transcriptomic and epigenomic data from Aim 1 and genetic associations from Aim 2a with transcriptomic and epigenomic data from the developing human brain. By interpreting the SETD1A gene regulatory network in this context, we will establish links from gene to molecular network to brain cell development to structural and functional brain connectivity to behavior.

Ola Awad, Ph.D.
University of Maryland, Baltimore
Award Amount: $345,000
Disease Target: Parkinson's Disease

Enhancing TFEB Activity to Ameliorate Neurodegenerative Changes in iPSC Models of Parkinson's Disease

Parkinson's disease (PD) is a common, progressive neurodegenerative disorder that is currently incurable. Dysfunction of the autophagy-lysosomal pathway (ALP) plays a key role in PD pathogenesis. Understanding the molecular basis of ALP dysfunction in PD has been hindered by the lack of relevant experimental models to study the diseases. Induced-pluripotent stem cell (iPSC) models provided a unique opportunity to investigate ALP dysfunction in PD and identify novel therapeutic targets. Using Gaucher's disease (GD) iPSC lines, we found that ALP alterations in GBA1 mutant neurons are mediated by deregulation of the transcription factor EB (TFEB), the master regulator of lysosomal biogenesis and autophagy. GBA1 mutations are also the most frequent genetic risk factor for PD, thus this novel mechanism maybe involved in PD pathogenesis. Our recent studies showed that TFEB dysfunction in GBA1 neurons is mediated by hyperactivity of the mammalian target of rapamycin (mTOR), the key negative regulator of TFEB. Pharmacological mTOR inhibition restored TFEB regulation of the ALP, indicating that this approach can be of therapeutic value. Enhancement of TFEB activity is shown to promote protein clearance and neuroprotection in animal models of PD, which makes it a promising therapeutic target. However, in order to develop TFEB-based therapies, the molecular regulation of TFEB in PD has to be fully understood. The overall goal of this proposal is to investigate TFEB regulation of the ALP in human iPSC models of genetic PD and determine the effects of enhancing TFEB activity on PD phenotypes. This is the first step in our target validation plan to develop TFEB-based treatment for PD. We will generate dopaminergic (DA) neurons from PD patients iPSC lines harboring the two most common genetic PD mutations (GBA1 and LRRK2) and use it to achieve the following aims: 1) To determine TFEB activity and regulation of the ALP in PD iPSC-DA neurons. We found that perpetuation of mTOR signaling by GBA1 mutations deregulates TFEB, which results in ALP dysfunctions. To investigate TFEB regulation and functions in PD iPSC-DA neurons we will determine: 1) TFEB protein levels, stability, nuclear localization, phosphorylation status and transcriptional activity. 2) TFEB-mediated lysosomal biogenesis, autophagic flux and clearance of autophagic substrates. We will also determine mTOR kinase activity and perform the above-mentioned assays in the presence of pharmacological mTOR inhibitors/activators. 2) To determine the effects of enhancing TFEB activity on PD phenotypes in iPSC-DA neurons. To enhance TFEB activity, we will bypass the negative regulatory effects of mTOR on TFEB using pharmacological or genetic approaches. We will then determine the effects of treatment on: Alpha-synuclein accumulation, DA neurons survival, dendritic length, and calcium homeostasis. To validate our results we will use isogenic gene-edited PD iPSC lines and test the effects of correcting the inherited genetic mutations on TFEB functions and regulations. Our study will uncover the relationship between mTOR deregulation, TFEB activity and PD pathogenesis. It will also identify pharmacological reagents capable of ameliorating neurodegenerative changes in DA neurons, which will pave the way for future development of effective treatment for PD.

Mohamed Farah, Ph.D.
Johns Hopkins University
Award Amount: $345,000
Disease Target: Motor Neuron Diseases /ALS

Distal Axon Regeneration of iPSC-Derived Motor Neurons

Amyotrophic Lateral Sclerosis (ALS) is a fatal and devastating motor neuron disease with very few treatments available that do little to alter the course of the disease. An early event in the pathogenesis of ALS is die-back of motor axons and denervation of skeletal muscle, before death of motor neurons. This proposal details a research strategy to examine distal axon degeneration and regeneration in human induced Pluripotent Stem Cell (iPSC)-derived motor neurons. We plan to examine human iPSC-derived motor neurons in microfluidic chambers to determine: 1) potential for ALS-linked mutant iPSC-derived motor neurons to regenerate and innervate muscle; and 2) the efficacy of BACE1 inhibitors in enhancing axonal outgrowth and study the basic pathobiology of the long, distal motor axon in ALS patient-derived motor neurons. Additionally, we plan to investigate the requirement of known BACE1 substrates involved in axonal outgrowth for enhanced distal axon regeneration of human iPSC-derived motor neurons.

Motor neurons derived from human iPSCs (hMNs) hold promise for advancing the field of ALS research. Animal models, while extremely valuable and completely indispensable at this stage, have so far failed to yield treatments for ALS that make it significantly less horrific. The most commonly used, and most well-characterized model of ALS, the SOD1G93A transgenic mouse, has been used in research for over 20 years. However, the current treatments for ALS patients, Riluzole and Edavarone, only do little to ameliorate symptoms and may extend life for a few months. Any treatments that improve life for ALS patients are much-needed, and it is likely that the future of ALS research involves research with hMNs and other patient-derived cells to lead the way in discovering new therapies. Targeting axonal outgrowth and sprouting is a novel approach for improving the quality-of-life for ALS patients, as most therapies up to this point have focused on neuroprotection. Biologic characteristics of patient hMNs must be thoroughly explored; the length of axons, their ability to regenerate, and mutant-specific innervation of NMJs by these human neurons is not characterized. Therefore, it is important to perform experiments that specifically examine the distal motor axons in patient-derived cells.

We are addressing this knowledge gap by utilizing microfluidic chambers that separate axons from neuronal cell bodies in order to study the basic pathobiology of the long, distal motor axon of ALS patient neurons. We also propose to test whether small molecule BACE1 inhibitors can enhance axonal outgrowth of hMNs. Using the microfluidic devices, we will be able to trace individual axons that are millimeters in length, and introduce different cell types into separate compartments, such as muscle fibers. This is a collaborative project that utilizes expertise from three different labs. The Maragakis lab is skilled in differentiation of patient iPSCs and we have access to iPSCs from over 50 different ALS patients. The Venkatesan lab has extensive experience with microfluidic devices and modeling axonal injury. We (Farah lab) have extensive expertise in axonal regeneration and drug treatment in models of neurodegenerative disease.

David Hackam, M.D., Ph.D.
Johns Hopkins University
Award Amount: $345,000
Disease Target: Short Bowel Syndrome

Single Cell RNA-Seq of Intestinal Stem Cells in the Generation of a Humanized Artificial Intestine

This project addresses the stated objective of this RFA by offering new and innovative hypotheses, approaches and models that differ from current thinking in the stem cell field by developing an artificial intestine for the treatment of short bowel syndrome. Further, we will use single cell RNA-seq of human intestinal stem cells to identify new pathways that regulate their proliferation and differentiation in this disease. Short bowel syndrome (SBS) is the condition in which the body cannot sufficiently absorb nutrients to sustain growth. Current treatment is often ineffective, and may require intestinal transplantation, a procedure limited by a lack of donor availability. Importantly, the remaining, shortened intestine in patients and experimental animals with short bowel syndrome undergoes hyper-proliferation of the residual stem cells and lengthening of the remaining villi. These findings illustrate that an understanding of the factors that lead to adaptation may be harnessed in order to make an artificial intestine for children with this devastating disease. Hypothesis: Previous studies towards the generation of an artificial intestine have been limited by an inability to recreate the complex three dimensional architecture of the required bioscaffold, and a lack of understanding of the cellular processes that regulate intestinal stem cell proliferation in human cells. Recently, our laboratory and collaborators have developed a novel three dimensional bioscaffold design, which we have integrated with intestinal stem cells obtained from human intestinal samples obtained at the time of surgery. We now seek to test the hypothesis that intestinal stem cells can be harvested from the human intestine, propagated outside the body, and implanted on a novel bioscaffold that bears remarkable similarities to the native human intestine to support nutrient absorption. We further hypothesize that the use of single cell RNA-seq will allow us to identify novel genes that drive normal with human intestinal cell differentiation and development, which can be harnessed in order to direct intestinal stem cell fate. To test these hypotheses we now propose the following specific aims:

Aim 1: to evaluate the ability of an artificial intestine derived from human intestinal stem cells to treat short bowel syndrome in mice. We will harvest human intestinal stem cells, culture them on a novel bioscaffold with a 3D structure that mimics the native intestine, and evaluate the ability of this artificial intestine to treat experimental short bowel syndrome in mice.

Aim 2: to identify and functionally characterize novel genes that regulate human intestinal stem cell proliferation and differentiation on bio-scaffolds in experimental short bowel syndrome using single cell RNAseq. We will perform single cell RNAseq to identify and characterize novel genes that regulate human intestinal stem cell proliferation in experimental short bowel syndrome, and test whether target genes can improve artificial intestine function in mice. Through these studies, we seek to develop a discovery platform for the development of an artificial intestine based on human intestinal stem cells, and to also harness the powers of single cell RNAseq to discover novel cellular processes that regulate human intestinal stem cell function.

Charles Hong, Ph.D.
University of Maryland, Baltimore
Award Amount: $333,957
Disease Target: Heart Disease

Reduced Graphene Oxide (rGO)-Mattress System for High-Throughput iPSC-Derived Cardiomyocyte-Based Screening Platform

Heart failure (HF) is a public health epidemic that affects over 6 million patients in the United States and costs an estimated $30 billion annually for medical care and hospitalization. Notwithstanding the recent introduction of sacubitril/valsartan (a combination neprilysin inhibitor/angiotensin receptor blocker) and ivabradine (a sinoatrial node modulator), 5-year HF mortality remains high at 50%. It is imperative to develop new HF drugs to improve heart function. Despite this urgent need, little progress has been made in HF drug development due to the lack of an in vitro model of human cardiomyocytes that recapitulates the contractile and relaxation functions of the heart at a cellular level and the lack of a method for rapid and precise screening of potential HF drugs. Conventional single-cell methods, like patch-clamp recording and calcium handling, are very slow (even experts can only examine 2 to 3 drugs per day) and technically challenging to execute in a high-throughput system. Current high-throughput systems, based on impedance and field potential, are costly and, although valuable, cannot be used to directly study contractile function because the electrical impedance signal generated from cells in a monolayer does not correspond to the bona fide contractility of cardiomyocytes. Moreover, these systems use electrode-based pacing, which is potentially detrimental to the intracellular electrical network and, by averaging readout signals, misses important data on cell-to-cell variations. These shortcomings limit the utility of current high-throughput systems in HF drug screens/tests. Recently developed optogenetic methods may circumvent these limitations but require manipulation of cell behavior by genetic modification of exogenous transmembrane ion-conducting proteins. The research proposed here aims to use innovative technologies to improve and facilitate HF drug screening. These technologies include reduced graphene oxide (rGO), an outstanding optoelectric material, and Matrigel Mattress, a culture technique that promotes cardiac maturity. rGO and Matrigel Mattress will be combined to create a new hybrid platform, the rGO-Mattress, with which to measure the contractile and relaxation functions of individual cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs) under noninvasive electrical stimulation by light. The proposed rGO-Mattress is innovative because it is expected to overcome the limitations of current high-throughput systems due to its ability to noninvasively stimulate an iPSC-CM while simultaneously measuring the iPSC-CMs systolic and diastolic functions, action potentials, and Ca2+ activity. Thus, the proposed research should have the strong positive impact of reducing the time required and increasing the cost-effectiveness of preclinical drug screening of candidate HF medications.

Aaron James, Ph.D.
Johns Hopkins University
Award Amount: $345,000
Disease Target: Bone and Soft Tissue Defects

CD107a Divides Fat From Bone-Forming Progenitors Within Adipose Tissue

Autologous stem cell therapies hold great promise for the treatment of defects of bone and soft tissue. Recent clinical trials using MSC (mesenchymal stem cell) based tissue engineering approaches have demonstrated suboptimal or inconsistent results. This has led our research group to the overarching tenant that development of a translatable cell-based therapy for bone or soft tissue regeneration hinges on the identification of a more well-defined progenitor cell subpopulation. Our team has a long-standing interest in adipose tissue-derived progenitor cells for tissue engineering, as fat represents a dispensable and easily accessible autologous tissue source. In fact, the perivascular identity of adipose tissue MSC was first described by our group. We have isolated, typified and functionally characterized two related cell populations of perivascular MSC, most commonly referred to under the umbrella term perivascular stem cells. Despite their purification by fluorescence activated cell sorting, when examined in a clonal fashion PSC represent a mixed population, with single cells of unipotent, bipotent, or multipotent properties. Until recently, additional cell surface markers to better resolve this mixed perivascular stromal population had remained elusive. Recent single cell transcriptional analysis of 178 perivascular cells has identified CD107a as a novel cell surface marker to further segregate adipose tissue PSC. CD107a is predominantly expressed in lysosomes and other intracellular vesicles. Cell surface CD107a is either the result of either nascent protein trafficking or the fusion of late endosomes and lysosomes to the cell membrane. Our findings suggest that cell surface CD107a marks a population of progenitor cells uniquely primed for adipogenesis. In contrast, CD107a negative progenitor cells have a strong and robust predilection for osteogenic differentiation. The molecule CD107a has never been studied in any context related to the current application. This award seeks to overturn conventional thinking in the field, with the central hypothesis that cell surface CD107a can be used to separate functionally distinct progenitor cells capable of more robust bone or soft tissue formation.

Aim 1: Define the bone forming advantage of CD107a negative perivascular progenitor cells. First, cell surface CD107a will be used to isolate two subpopulations among human adipose total stromal cells or perivascular stem/stromal cells. Bone regeneration incited by either CD107a positive or negative populations will assayed, using our previously validated calvarial defect model. Healing of bone defects in Scid mice occurs over an 8 week period as assessed in a temporospatial fashion by microcomputed tomography, histology, and immunohistochemical techniques. Coordinate studies will examine the consequences of CD107a knockdown with lentiviral encoded shRNA, using previously validated techniques.

Aim 2: Harness the fat forming advantage of CD107a positive perivascular progenitor cells. The same human CD107a+ and CD107a- progenitor cell preparations will be used in a clinically relevant, cell-augmented fat grafting model of adipose tissue engineering. Cell augmented lipotransfer in Scid mice will be characterized by magnetic resonance imaging, histology, histomorphometry, and immunohistochemical means over a standardized 12 week period. Many studies in the translation of adipose-derived MSC therapies have met with mixed results. These shortcomings necessitate use of a more defined progenitor cell product. The identification of adipose-derived stromal cells that are primed for either bone or fat formation has large translational applications in the field of tissue engineering and regenerative medicine.

Miroslaw Janowski, Ph.D.
University of Maryland, Baltimore
Award Amount: $345,000
Disease Target: Stroke

Image-Guided, Intra-Arterial Delivery of Human MSC-Derived Extracellular Vesicles for Treatment of Ischemic Stroke

Stroke is the leading cause of long-term, severe disability in the United States, with over 800,000 of survivors affected annually. Therefore, it is not only the source of suffer for patients, but also causes a tremendous financial burden to the society. Very recently, several clinical trials have shown an effectiveness of mechanical clot removal (thrombectomy) through intra-arterial catheter for emergent large vessel occlusion (ELVO). Since thrombectomy is accomplished through intra-arterial catheter, it gives an excellent opportunity for subsequent infusion of adjuvant therapeutic agents to further improve stroke outcome. Mesenchymal stem cells (MSCs) have been shown effective in animal models of stroke but the high cost, complex logistics and the potential risks of microinfarcts impeded fast clinical translation. It has been recently shown that intravenously delivered MSC-derived extracellular vesicles (EVs) are equally effective as MSCs themselves in animal model of stroke. The favorable character of EVs such as the small size (excludes microinfarct formation), the lack of immune active antigens (excludes need for immunosuppression or the matching of donor to host) and relative resistance to freeze-thaw cycle makes them a very attractive off-the-shelf therapeutic agent. However, the cost of production of EVs as any biologic is not negligible, thus any effort to minimize the therapeutic dose is of high importance. Since, the intra-arterial route is capable to provide the EVs directly to the infarcted area of the brain, there is a great chance that it will allow to decrease the dose at least 100 times [the intra-arterial administration of contrast agent (ferumoxytol) diluted 1:100 still gives stronger signal in the brain than intravenous administration of undiluted contrast agent]. Thus, the lower dose of EVs due to lower price is expected to contribute for much wider future clinical application. Importantly, this low dose could be administered during the same intra-arterial procedure of thrombectomy, immediately after the clot removal, using the catheter being still in place, what makes the entire therapy highly applicable clinically. There was also expressed a high enthusiasm to this approach by Dr. Pearl, a co-investigator and clinician, who routinely perform thrombectomies in patients with stroke at Johns Hopkins Hospital, and who found a compelling need for such adjuvant therapy, and would be willing to translate it to clinic, once proven effective in animal studies. We propose to reproduce positive results of intravenous EV infusion and compare them to the intra-arterial route, as well as to combination of intravenous and intra-arterial administration. Our preliminary data has shown positive effects of EVs on decreasing a stroke-induced neuroinflammation. The preparation of EVs for the administration will be produced under supervision of an expert for production, isolation and characterization of EVs for diagnostic purposes, and serves as a secretary general for the International Society for Extracellular Vesicles (ISEV). We will use typical animal model of stroke induced through occlusion of middle cerebral artery (MCAO) and intra-arterial EV administration using our unique real-time MRI-guidance, which facilitates high precision of delivery of therapeutic agents to the brain. The therapeutic effects will be evaluated by MRI, behavioral tests and post mortem analysis (histochemistry and immunofluorescence). The distribution of EVs will be assessed in the separate groups of animals sacrificed a day after EV delivery, also using an advanced label-free method of in situ hybridization of anti-human miR-941, and then correlated with the achieved therapeutic outcome. Summarizing, the proposed study will allow for comprehensive assessment of therapeutic potential of human MSC-derived EVs in treatment of stroke in acute phase, in particular as adjuvant therapy after thrombectomy. However, in future this approach could also be extended to patients with stroke not qualified for thrombectomy, which currently lack therapeutic options.

Chulan Kwon, Ph.D.
Johns Hopkins University
Award Amount: $345,000
Disease Target: Heart Disease

A Gene Regulatory Network Controlling Human iPSC-Myocyte Maturation

Pluripotent stem cell-derived cardiomyocytes (PSC-CMs) have great potential for a wide range of preclinical and clinical applications, including cardiac disease modeling, drug discovery, and regenerative medicine. However, the inability to produce mature CMs from PSCs has emerged as a fundamental and practical challenge for its broad applicability. We and others recently demonstrated that nascent PSC-CMs do not mature beyond the stage equivalent to late embryonic CMs in culture. Unlike PSC-CMs, endogenous CMs undergo significant levels of morphological, structural, and functional changes until adolescence. However, factors and mechanisms governing CM maturation are largely unknown, making it difficult to develop a strategy to produce mature PSC-CMs in vitro. By conducting a large-scale metatranscriptomic analysis and taking multiple bioinformatics approaches, we identified a group of nuclear receptors as a stage-independent gene regulatory network regulating CM maturation. The nuclear receptors were found inactive in cultured PSC-CMs, and our readout system indicated that their activation may enable PSC-CMs to mature to a juvenile state. Based on these findings, this proposal will focus on investigating the biological role and mechanisms of the nuclear receptors in human induced PSC-CMs (hiPSC-CMs). By completing this study, we expect to interrogate the roles and mechanisms by which nuclear receptors affect the maturation of hiPSC-CMs. This will provide fundamental insights into understanding hiPSC-CM maturation and help us develop a novel and robust strategy to produce mature hiPSC-CMs in a dish. Relationship between the proposed research and the etiology of human diseases or conditions: Heart disease remains the number one killer in the States. With methodological advances in generating patient-specific induced pluripotent stem cells (iPSCs) into cardiomyocytes, current cardiac PSC research is centering on modeling heart muscle diseases (cardiomyopathy), a leading cause of heart failure. However, generation of adult-like, mature cardiomyocytes from PSCs remains intractable, making it difficult to recapitulate the disease phenotype and to validate the efficacy of drugs for cardiomyopathy, which occurs predominantly in adult stages. The proposed research aims to develop a novel and robust method to generate mature cardiomyocytes from human iPSCs. (ii) How clinical practice and treatment of human diseases or conditions will be advanced by the proposed research: The proposed research is expected to significantly advance the current cardiac regenerative medicine by enabling us to generate mature and homogeneous cardiomyocytes from iPSCs with a defined condition. The resulting iPSC-derived myocytes can be utilized for (1) modeling cardiomyopathies, (2) drug discovery for late-onset heart diseases, and (3) iPSC-based repair of adolescent/adult heart disease. How the proposed research may contribute to new medical therapies or test new therapies in human patients: More than 80% of drugs or treatments shown to be effective and safe in animal studies fail in human trials. The present study offers an opportunity to use mature human cells for preclinical trials, which can be translated to effective drug development for the treatment of human diseases. (iv) How the proposed research will translate prior research results into new medical therapies or test new therapies in human patients, and the projected time line for accomplishing such clinical application(s): To date, no method is available to generate mature cardiomyocytes from PSCs, and current PSC-based drug screening assays rely on immature cells. Thus, the newly obtained knowledge is expected to be immediately applicable for ongoing PSC-based applications upon successful completion.

Erin Lavik, Ph.D.
University of Maryland, Baltimore County
Award Amount: $339,140
Disease Target: Neurodegenerative Diseases / Autism Spectrum

Vascularized Hydrogel System Modeling Neural Networks in Autism Suitable for High Throughput Screening

With the development of human, induced pluripotent stem cells (hiPSCs) in 2006, creating representative models of healthy and diseased tissues has never been closer. High throughput screening methods amenable to three dimensional cultures opens the possibility of efficiently investigating disease models and therapeutic interventions. However, the ability to build three dimensional models for these high throughput systems has been primarily based on spheroid culture, photolithography, and bioprinting. These methods allow one to develop patterns and architectures that are seen in vivo, but they are time consuming, require specialized materials, and the processes are not always compatible with the cell types of interest due to exposure to UV light or shear forces. We have developed an approach that is highly scalable and avoids extrusion issues associated with bioprinting. Taking a cue from the electronics industry, our approach uses screen printing to make highly scalable hydrogel-cell tissue models. Screen printing offers several advantages. (1) It can model tissue comprising many types of materials and cells including those that are sensitive extrusion. (2) These materials and cells can be patterned within a wide range of three-dimensional, multilamellar structures. (3) By varying the screen structure, layers can be created across a wide range of thicknesses. (4) These models can be built quickly and at low cost. Neural cells, in particular, are challenging to print with traditional bioprinting systems and exhibit reduced survival and can exhibit genotypic and phenotypic changes following printing. In this proposal, we focus on applying the screen printing approach to the development of models for autism based on neural cells. In aim 1, we will focus on printing cocultures of endothelial cells and neural stem cells (NSCs) to model the NSC niche. The NSC niche provides an important platform for models of autism spectrum disorder (ASD) and neural diseases more broadly. A number of reports have suggested that there are either differences in NSCs in ASD or that the niche environment may be different. By building and validating the NSC niche, we will be able to investigate the potential differences and their implications using high throughput screening approaches. In aim 2, we will extend the work to building models of inhibitory and excitatory neurons to investigate the hypothesis that the etiology of ASD is the lack of balance between excitation and inhibition in the cortex. Although there is evidence from both mouse models and humans that neurobiological phenotypes may arise from the mismatch of excitatory glutamatergic and GABAergic inhibitory inputs (specifically PV-expressing interneurons), to date, there is no hiPSC model that acutely tests these observations. This proposal will lead to a rapidly translatable and commercializable technology for high throughput screening to understand disease and screen for therapeutic interventions.

Marta Lipinski, Ph.D.
University of Maryland, Baltimore
Award Amount: $345,000
Disease Target: Parkinson's Disease

Inhibition of the PARK10 gene USP24 As A Neuroprotective Treatment in Parkinson's Disease

Defects in autophagy, a lysosome-dependent cellular catabolic pathway, are causatively linked to human neurodegenerative diseases, including Parkinsons disease (PD). Furthermore, up-regulation of autophagy has been proposed as a potential prevention and treatment strategy against PD. Since PD patient derived induced pluripotent stem cells (iPSCs) differentiated into dopaminergic (DA) neurons closely recapitulate many of the PD associated cellular phenotypes, including autophagy defects and time-dependent neurite degeneration, they represent an excellent model for studying PD mechanisms and evaluating potential neuroprotective strategies. Recent genomic studies led to the discovery of many Parkinsons disease (PD) risk genes. However, lack of understanding of their cellular and molecular functions and the mechanisms of their involvement in PD prevents exploitation of these genes for development of PD treatments. One of these genes is the ubiquitin-specific peptidase 24 (USP24) located on chromosome 1p in the PARK10 locus associated with late-onset PD. Using previous MSCRF funding (2014-MSCRFE-0587) we identified USP24 as a negative regulator of autophagy in cell lines and human iPSCs differentiated into DA neurons and defined the molecular mechanism of its function. Our preliminary data also indicated that USP24 may specifically affect function of Parkin, a product of the familial PD gene PARK2 and a known mediator of mitochondria-specific autophagy (mitophagy). We also found that USP24 levels are increased in the substantial nigra of subpopulation of non-familial PD patients at the mRNA and protein levels, suggesting that USP24 may also contribute to idiopathic PD. Importantly, we discovered that while inactivation of USP24 had no short-term effect on either viability of differentiation of iPSC derived DA neurons, long-term USP24 knock-down led to improved maintenance of neuronal processes in aged iPSC neurons. Based on these data we hypothesize that inhibition of USP24 may be a novel approach to protect DA neurons in PD. In accordance with the MSCRF goal of developing new strategies for prevention and treatment of human diseases through human stem cell research, we propose to determine if inhibition of USP24 can protect PD patient iPSC derived DA neurons from degeneration in long-term cultures. Furthermore, our data indicate that USP24 may interact with other genes involved in PD. These interactions may affect whether specific PD patient populations may respond favorably to USP24 inhibition. To determine these interactions will use isogenic iPSC lined with CRISPR generated mutations in PD associated genes to determine if their function is necessary for neuroprotection after USP24 inhibition. We expect that our data will demonstrate inhibition of USP24 as a potential neuroprotective treatment against PD and define which other genes involved in PD may be necessary for the mediation of this effect. Following in vivo testing of USP24 inhibition as a PD therapy we plan to perform a high-throughput screen (HTS) to identify small molecule USP24 inhibitors with high translational potential. We would than establish a partnership with local Maryland biotechnology and pharmaceutical companies in order to expedite translation of our findings.

Xiaobo Mao, Ph.D.
Johns Hopkins University
Award Amount: $345,000
Disease Target: Parkinson's Disease

Resistance of Pathologic alpha-Synuclein in LAG3 Deficient Human Dopaminergic Neurons

Cell replacement therapy for Parkinson's disease (PD) has shown therapeutic efficacy in animal models and clinical trials. HiPSC-derived human dopaminergic (hDA) neurons provide a unique resource for autologous transplantation. Currently, a major challenge is the host cytotoxicity caused by pathologic alpha-synuclein (a-syn) cell-to-cell transmission, which significantly impedes long-term survival and functions of hDA grafts.

We have discovered that lymphocyte-activation gene 3 (LAG3) is an essential receptor of pathologic a-syn. Depletion of LAG3 can significantly block a-syn internalization and transmission in mouse DA neurons. LAG3 neutralizing antibody also blocks a-syn internalization in hiPSCs-derived hDA neurons. Furthermore, depletion of LAG3 (LAG3-/-) did not affect DA neurons viability and functions in vitro and in vivo. These results support that depletion of LAG3 may render DA neurons more resistance to a-syn transmission without changing neuron functions. Thus, we hypothesize that, LAG3-/- hDA neurons derived from hiPSCs are resistant to a-syn transmission and toxicity, exhibiting enhanced therapeutic efficacy in PD models, and providing a better cell resource to autologous transplantation for PD therapy.

Our goal is to improve the long-term efficacy of iPSC-derived hDA neurons against host cytotoxicity for cell replacement therapy. The specific aims of this project are (1) determine if LAG3-/- hDA neurons that are resistant to pathologic a-syn transmission in vitro; and (2) examine if LAG3-/- hDA grafts that are resistant to host cytotoxicity by pathologic a-syn in vivo and show better therapeutic efficacy in the 6-OHDA-induced PD mouse model. If successful, this project will establish a novel strategy to make hDA neurons with enhanced therapeutic efficacy in the toxic host environment. The project will also establish role of LAG3 as a therapeutic target against pathologic a-syn spreading in hDA neurons. The corresponding therapeutic development on LAG3 inhibitors (antibody and small molecules) could be followed up. The methodology established here will facilitate the development of various transplantable neurons or neural stem cells for more effective cell replacement therapy in neurodegenerative diseases with prion-like spreading (e.g., Alzheimer's disease). Overall, our proposed research will strengthen stem cell research and biotechnology development for regenerative medicine in Maryland.

Nicholas Maragakis, M.D.
Johns Hopkins University
Award Amount: $344,999
Disease Target: Amyotrophic Lateral Sclerosis

iPSC-spinal Cord Astrocyte/Motor Neuron Co-Culture Platform Investigating Hemichannel-Mediated Toxicity and Neuroprotection in Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is the most common form of adult motor neuron disease. The prognosis of patients with ALS remains poor, with the mean duration of disease from onset to death approximately 2 to 5 years. One of the most enduring themes in ALS pathobiology is centered around the concept that astrocytes and other non-neuronal cells are responsible for the propagation of ALS anatomically and temporally after disease onset. This is particularly relevant because when ALS patients seek medical attention, it is during this critical time period when astrocyte-mediated toxicity to motor neurons is most relevant. This offers the potential for novel therapeutic strategies for intervention. Astrocytes form a highly coupled intercellular network in the central nervous system (CNS) through gap junctions. Connexin 43 is the major protein that makes up gap junction (GJ) and hemichannels (HC) in astrocytes. We have observed in human ALS spinal cord that connexin 43 expression correlates with disease progression. We have also verified, using in vitro rodent ALS astrocytes and in an in vivo rodent ALS model (SOD1G93A mouse), that Cx43 HC contribute to motor neuron pathology and reducing Cx43 is neuroprotective and slows disease progression anatomically and temporally. However, rodent models of ALS have limitations in that they represent only a small subset of ALS patients that carry specific hereditary forms of ALS (less than 5% of all patients). This represents a gap in our ability to translate findings to patients with sporadic ALS. The current proposal will focus on using human iPSC-derived astrocytes from these ALS patients, to investigate whether astrocyte-mediated neurotoxicity occurs through Cx43 HCs. Therefore, we will seek to identify whether our previous observations in ALS tissues and SOD1G93A mice is also true in human ALS iPSC-derived astrocytes from sporadic ALS patients. This is powerful because we will be able to tease out whether Cx43 HC-mediated neuronal toxicity is relevant to all forms of ALS, or just a subset. We will utilize several methods to examine HC-mediated release of factors that are toxic to iPSC-motor neurons using several novel astrocyte/neuron co-culture techniques. This will be combined with multielectrode array analyses of the role Cx43 HC play in iPSC motor neuron physiology, and finally, begin to investigate whether the HC-specific blocker, tonabersat, can modulate ALS iPSC-Astrocyte-mediated neuron toxicity. This is relevant as tonabersat has previously been in early phase studies in patients with migraine. We believe therefore, that if our observations in this proposal are meaningful, this compound could be used in patients with ALS. This proposal will bring investigators from the ALS Center for Cell Therapy and Regeneration Research at Johns Hopkins as well as a collaborator with expertise in gap junction physiology together to provide the best resources for establishing the potential significance of Cx43 HC on motor neuron pathology. With these combined resources and scientific expertise, we believe that this proposal provides an environment for translating the current project to a clinical trial targeting astrocyte hemichannels in ALS patients.

Jamie Spangler, Ph.D.
Johns Hopkins University
Award Amount: $345,000
Disease Target: Critical-Sized Bone Defects

An Engineered Orthogonal Growth Factor for Targeted Stimulation of Bone Repair

Bone is the second most transplanted tissue after blood, and are over 2 million bone grafting procedures annually with estimated costs exceeding $3 billion. The demand for bone transplants significantly outpaces the supply of donor tissue, and the gap continues to widen due to factors such as obesity and increased life expectancy. In response to this growing public health problem, tissue engineering approaches have emerged as promising alternatives to bone transplant. Such approaches incorporate stem cells into biomaterial scaffolds, and also require use of signaling proteins to provide pro-regenerative cues. However, signaling proteins are rarely used in clinical tissue engineering scaffolds due to systemic toxicity concerns, which has drastically limited translation of bone tissue engineering strategies. Marrow-derived mesenchymal stem cells (MSCs) are most commonly studied for bone tissue engineering, but are scarce. Adipose-derived stem cells (ASCs) are much more abundant and can also differentiate into bone. Our team recently showed that the platelet-derived growth factor (PDGF) ligand PDGF-BB robustly induces osteogenesis of ASCs, motivating incorporation of this growth factor into ASC-based bone tissue engineering regimens. PDGF ligands signal through cognate receptors (PDGFRs) to mediate various physiological processes including bone regeneration. The powerful pleiotropic effects of PDGF-BB complicate its use in bone repair scaffolds. Additional concerns are raised for PDGF-BB due to its implications in diseases such as cancer, vascular disorders, and fibrotic disorders, limit its clinical use. Thus, the design of a selective version of PDGF-BB that exclusively stimulates osteogenic responses in transplanted ASCs would tremendously advance the field of bone tissue engineering. Here, we propose to overcome the limitations of PDGF therapy in bone regeneration through development of a new molecular engineered ligand/receptor pair. Advances in protein engineering allow for the generation of orthogonal protein technologies: ligand/receptor pairs that are genetically mutated to interact exclusively with one another, and not with any endogenous proteins. We will engineer an orthogonal PDGF ligand that exclusively interacts with transplanted ASCs engineered to express an orthogonal PDGF receptor to allow, for the first time, targeted activation of regenerative pathways, realizing the vast potential of growth factor therapy for regenerative medicine. Our work will proceed as follows:

Aim 1: Engineer an orthogonal PDGF ligand/receptor pair. Synthesizing computational and experimental techniques, we will remodel the interface between PDGF-BB and PDGFR to create an orthogonal signaling pair, and biophysically validate exclusivity of the ligand/receptor interaction.

Aim 2: Functionally characterize orthogonal PDGF pair in human stem cells. We will transduce human ASCs with our engineered orthogonal PDGFR, demonstrate the signaling capacity of orthogonal PDGF on these cells, and assess osteogenic responses. Our multidisciplinary team includes PI Prof. Jamie Spangler, a new investigator excited to apply her biomolecular engineering expertise to regenerative medicine applications, co-I Prof. Jeffrey Gray, an expert in computational protein design, and co-I Prof. Warren Grayson, an established leader in bone tissue engineering. Our safe and effective growth factor delivery strategy will revolutionize stem cell engineering, serving as a blueprint for developing targeted proteins for an array of regenerative medicine objectives.

Kimberly Stroka, Ph.D.
University of Maryland, College Park
Award Amount: $345,000
Disease Target: Metastatic Cancer

Role of Mechanobiological Forces in Engineering Induced Pluripotent Stem Cell-Derived Brain Endothelial Cells

The blood-brain barrier (BBB) is a highly selective endothelial cell barrier that regulates the flow of ions, nutrients, and cells between blood and interstitial fluid of the brain. BBB dysfunction is believed to occur in many diseases, including infectious disease, stroke, multiple sclerosis, HIV, tumor metastasis, and Alzheimers disease. However, it is difficult to accurately model the BBB in vitro using primary endothelial cell sources on non-physiological substrates. Recently, human induced pluripotent stem cell-derived brain microvascular endothelial cells (hiPSC-derived BMECs) have been shown to possess markedly improved barrier function over primary cells in vitro. Our goal is to incorporate hiPSC-derived BMECs into a BBB-on-a-chip and evaluate the effects of physiological mechanical cues (e.g., matrix stiffness and shear stress) on vascular function; this integrated microsystem will be used for disease modeling, drug screening, and tumor metastasis risk assessment applications. Notably, we and others have demonstrated that external mechanical cues play a critical role in regulating endothelial cell migration, morphology, barrier function, interactions with immune cells, and mechanobiology. This proposal seeks to evaluate how mechanical stimuli affect hiPSC-derived BMEC differentiation, mechanobiology, and barrier function. Given the significant effect of mechanical cues on mechanobiology of peripheral endothelial cells and also in the differentiation of other stem cells (e.g., mesenchymal stem cells), we hypothesize that mechanical cues are also implicated in the differentiation and barrier function of hiPSC-derived BMECs. We therefore propose two aims: Aim 1. Evaluate how mechanical cues (i.e., matrix stiffness and shear stress) affect the differentiation of hiPSCs into BMECs. As matrix stiffness and shear stress are varied individually or in combination, we will assess differentiation markers at various steps of the differentiation process. Results of these experiments will indicate whether there exists an optimal combination of mechanical cues to drive differentiation of hiPSCs into BMECs. Aim 2. Quantify the effects of mechanical cues (i.e., matrix stiffness and shear stress) on hiPSC-derived BMEC barrier function and mechanobiology. First, we will assess whether these mechanical cues influence (a) the ability of hiPSC-derived BMECs to maintain expression of requisite BBB markers, and (b) hiPSC-derived BMEC morphology, actin cytoskeleton arrangement, contractility, or barrier function, and if so, what molecular mechanisms are involved. Results of these experiments will indicate whether there exists an optimal combination of mechanical cues to maintain pure, functional populations of hiPSC-derived BMECs. In future work, we will use this integrated microsystem to investigate the mechanisms of tumor cell metastasis across the BBB and related biomarkers, to develop patient-specific therapeutics for tumor metastasis, and to assess whether specific patients are at risk for brain metastasis. This microsystem could also be extended to explore BBB dysfunction in other diseases. Furthermore, endothelial cells are used as therapeutic agents in a variety of applications, such as vascular grafts, and hence knowledge of what microenvironmental cues are necessary to maintain iPSC-derived BMECs in their differentiated state is absolutely critical. The proposed work will determine the role that mechanical cues play in regulating hiPSC-derived BMEC differentiation and barrier function and will establish an updated microsystem to model the BBB.

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Validation Grant Award

Sheikh Amer Riazuddin, Ph.D.
Johns Hopkins University
FY2020 Validation Award (1st Funding Cycle)
Award Amount: $229,320
Disease Target: Critical-Sized Bone Defects

An Engineered Orthogonal Growth Factor for Targeted Stimulation of Bone Repair

The cornea is the outermost, transparent tissue of the eye composed of five layers. The corneal endothelium (CE) is the innermost layer composed of hexagonal cells that are critical for maintaining corneal clarity by mediating hydration through barrier and pump functions. The corneal endothelial cell (CEC) density is approximately 2500 cells/mm2 in adult cornea and the physiological functioning is substantially compromised below a CEC density of 500 cells/mm2 resulting in corneal edema. Corneal endothelial dystrophies are the leading cause of corneal transplantation performed in the US each year, and although keratoplasty has been successful in treating corneal edema, the worldwide shortage of transplantable-grade donor CE remains an insurmountable obstacle in reducing corneal blindness. Pluripotent stem cells have the potential to differentiate into any cell type of the human body including CECs. We previously generated CECs from human embryonic stem cells (hESCs) and demonstrated that cryopreservation does not affect the cardinal features of CECs.

We now have established the efficacy of hESC-derived CECs as an alternative to donor CE in transplant surgeries using rabbits and monkeys as preclinical models. To this end, our data confirm that cryopreserved hESC-derived CECs can form a functional CE when injected into the anterior chamber of the eyes of these models. In here, we propose to validate four elements critical for the commercial utility of hESC-derived CECs. These include (a) validate the shelf life of cryopreserved hESC-derived CECs; b) validate the stability of hESC-derived CECs during shipment on dry ice; c) validate the amount of hESC-derived CECs per injection necessary to form a functional layer; and d) validate the protocol for the preparation of hESC-derived CECs injections. Validation of the above-mentioned will bring us one step closer to the commercial utility of hESC-derived CECs reducing dependency on keratoplasty-grade donor corneas.

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Post-Doctoral Fellowship Grant Awards

Chengyan Chu, Ph.D.
Johns Hopkins University
Mentor: Piotr Walczak, Ph.D.
Award Amount: $130,000
Disease Target: Brain Tumor and Neural Repair

Transplantation of Stem Cells to Prevent Brain Toxicity Following High-Dose Intraarterial Chemotherapy of Glioblastoma

Lack of treatment options for highly aggressive, unresponsive CNS tumors motivates the search for novel therapeutic approaches. One reason behind the failures of chemotherapy of brain tumors is insufficient drug dose at the tumor site and excessive systemic side effects that limit elevation of thatdose. This project proposes to deliver high-dose chemotherapy selectively to the tumor using imageguided, targeted intra-arterial route while minimizing systemic exposure and thus assuring low systemic toxicity. To achieve this goal, interventional MRI will be used to guide targeted and local intra-arterial (IA) injection. Temozolomide (TMZ), the oral agent used clinically for the treatment of glioblastoma, is selected as the chemotherapeutic agent. The capacity of high-dose chemotherapy to fully eradicate glioblastoma will be assessed by MRI and bioluminescence imaging. However, this strategy is expected to inevitably result in the injury of healthy tissues adjacent to the tumor, in particular for those highlysensitive proliferative cells such as astrocytes or oligodendrocytes. Therefore, we will assess extent of the damage induced by high-dose chemotherapy, using MRI, behavioral tests and histology. We will then evaluate reparative approach and attempt to restore chemotherapy-induced brain through intraarterial transplantation of glial restricted progenitors (GRPs). We previously demonstrated potential of GRPs to engraft, differentiate towards mature glial phenotypes and exert significant therapeutic benefit. MRI, behavioral tests, histology and animal survival will be used to evaluate the therapeutic effects. Notably, the progenitors are manufactured by our collaborating biotechnology company Q Therapeutics, who received FDA approval for their use. Overall, the purpose of this study is to eradicate tumors with local high-dose chemotherapy, to determine the extent of chemotherapy-induced damage and to evaluate whether GRPs are able to repair that damage. If we are successful, this approach will improve the quality of life and functional independence of cancer survivors. Dr. Pearl, co-mentor for Dr. Chu is an Associate Professor in the Interventional Neuroradiology Division and a PI for an active Phase I clinical trial, "Intra-arterial Chemotherapy for the Treatment of Progressive Diffuse Intrinsic Pontine Gliomas", NCT01688401. She will provide guidance on clinical applicability of the proposed regenerative approaches. Once feasibility of our approach is established, she will be a driving force for translating this strategy into clinical practice.

Dong Won (Thomas) Kim, Ph.D.
Johns Hopkins University
Mentor: Seth Blackshaw, Ph.D.
Award Amount: $130,000
Disease Target: Sleep Disorder

Directed Differentiation of Human Hypothalamic Neurons that Regulate Sleep

Although sleep is essential for all aspects of mental and physical health, the neural mechanisms controlling sleep onset and maintenance are still poorly understood. Our group has recently identified a new type of sleep-promoting neurons in the dorsolateral hypothalamus, which express the LIM homeodomain transcription factor Lhx6 (referred to here as Lhx6+ neurons)1. Hypothalamic Lhx6+ neurons are activated in response to sleep pressure and this activation is necessary and sufficient to promote sleep. Hypothalamic Lhx6+ neurons promote sleep by sending local inhibitory signals to wake-promoting hypocretin (Hcrt)-expressing neurons in the lateral hypothalamus. While hypothalamic Lhx6+ neurons are observed in other vertebrate species including humans2, their role in sleep regulation has not been characterised.

We seek to directly study the cellular, molecular, and functional properties of human Lhx6+ hypothalamic neurons. Using the results of a large-scale gene expression screen that we have recently conducted in developing mouse hypothalamus as a starting point, we first aim to develop a robust protocol for differentiating these neurons from human ES cells using guided growth factor-mediated differentiation or direct differentiation mediated by transcription factor overexpression (Aim1). Once this has been achieved, we will study the role of the ES-derived neurons in sleep regulation using cell transplantation (Aim 2).

Ultimately, by developing similar strategies for differentiating other subtypes of hypothalamic sleep/wake-regulatory neurons (i.e. Hcrt+ neurons), we aim to functionally reconstruct human hypothalamic sleep regulatory circuitry in vitro, and to use this as a tool for rapid identification of drugs and genetic variants that regulate sleep onset and duration.

Senquan Liu, Ph.D.
Johns Hopkins University
Mentor: Linzhao Cheng, Ph.D.
Award Amount: $130,000
Disease Target: Irradiation Related Injury; Degeneration

Investigating the Therapeutic Potential of Human iPSC-Derived Extracellular Vesicles In-Vitro and In-Vivo

Extracellular vesicles (EVs) are small membrane-enclosed anucleate biological particles (30-1,000 nanometers in diameter). Mounting evidence demonstrates that EVs-carried cargos such as proteins and mRNAs can be delivered into various recipient cells and exert distinct biological activities. While many scientists are actively studying EVs roles in disease pathology and early diagnostics, I am interested in investigation of unique properties and functions of EVs produced by human stem cells. During my PhD training, I acquired considerable experience in manipulating human induced pluripotent stem cells (iPSCs), including reprogramming, cell proliferation, differentiation and genome editing. For my postdoctoral training starting in July 2017 in the Johns Hopkins, I have been focusing on investigating the properties and functions of iPSC-derived EVs. In the past 1.5 years, I made great progress on the collection, purification and characterization of human iPSC-EVs. We found that human iPSCs can produce much more EVs than mesenchymal stem cells (MSCs) under a completely defined medium without exogenous EVs present in serum, and that highly purified iPSC-EVs are capable to reduce cellular reactive oxygen species levels and alleviated aging phenotypes. In this proposal, I plan to further advance my studies to investigate the therapeutic effects of human iPSC-EVs on alleviating radiation damage to bone marrow cells. I will evaluate the effect of human iPSC-EVs on MSC support of human hematopoietic stem/progenitor cells after irradiation in vitro (Aim 1). In Aim 2, I will examine the therapeutic potential of human iPSC-EVs in alleviating irradiation induced damage to the hematopoietic system in vivo. The success of this project will provide key evidence of using EVs from human iPSCs as a novel therapeutic modality for repairing tissue damage and treating degenerative diseases. It will also impact the development of innovative approaches of using human iPSC-EVs for therapeutic applications in regenerative medicine.

Kathryn Moss, Ph.D.
Johns Hopkins University
Mentor: Ahmet Hoke, Ph.D.
Award Amount: $130,000
Disease Target: HNPP and CMT1A

Studying PMP22 Function in HNPP & CMT1A iPSC-Derived Schwann Cells

Hereditary Neuropathy with Liability to Pressure Palsies (HNPP) and Charcot-Marie-Tooth disease, Type 1A (CMT1A) are common inherited peripheral neuropathies that are caused by copy number variations in the PMP22 gene. HNPP patients have one copy and CMT1A patients have three copies. The PMP22 gene encodes a transmembrane glycoprotein that is enriched in compact myelin but little is known about the endogenous function of PMP22 and why its expression needs to be precisely regulated in order to support proper myelination. Our studies will utilize HNPP and CMT1A patient iPSC-derived Schwann cells in order to study PMP22 gene dosage in disease relevant cells from actual patients. Our preliminary findings suggest that excess PMP22 is trafficked to the plasma membrane in CMT1A patient fibroblasts, which corresponds with increased cell substrate adhesion. Additionally, generation of iPSC-derived Schwann cells and preliminary RNAseq data revealed dysregulated expression of several adhesion genes including PCDHGA3, CNTN4, CHL1, PTPRT and COL6A6. Taken together, these preliminary findings suggest that dysfunctional adhesion may contribute to HNPP and CMT1A pathogenesis. The proposed research aims to characterize adhesion phenotypes in HNPP and CMT1A patient iPSC-derived Schwann cells and verify dysregulated gene expression in these cells by RNA sequencing (Aim 1). Candidates for novel treatment strategies will then be identified by evaluating substrate adhesion and myelination rescue of HNPP and CMT1A patient iPSC-derived Schwann cells with attenuation of significantly dysregulated adhesion genes (Aim 2). We hypothesize that HNPP and CMT1A patient iPSC-derived Schwann cells have dysregulated PMP22 plasma membrane trafficking, adhesion and myelination, which can be rescued by attenuating the dysgreulated expression of key genes. Results from these studies will significantly advance our understanding of PMP22 function, help uncover HNPP and CMT1A pathomechanisms and reveal targets for novel therapeutics

Rahel Schnellmann, Ph.D.
Johns Hopkins University
Mentor: Sharon Gerecht, Ph.D.
Award Amount: $130,000
Disease Target: Cardiovascular Diseases

The Role of Mechanosensing Towards the Treatment of Age Mediated Vascular Diseases

Vascular diseases such as peripheral arterial disease (PAD), aortic aneurisms and venous thrombosis, all subtype of cardiovascular diseases (CVDs), are one of the leading causes of death worldwide with age as one of the major risk factors. The extracellular matrix (ECM) is hereby of big importance, because of its capability to regulate vascular morphology and barrier function. Age mediated cross-linking and increased deposition of several ECM components leads to changes in matrix stiffness and architecture with fatal effects on endothelial cells and thus, blood vessel function. Mechano-sensitive signaling pathways, which are activated in endothelial cells upon increase in matrix stiffness, are hereby of big interest. However, these pathways have not been fully uncovered and characterized yet. Nevertheless, a better understanding of ECM mediated signaling pathways is needed to fully understand age related vascular malfunctions and to restore vascular health in affected individuals. The suggested project aims to develop a 3D hydrogel with the capability to gradually increase matrix stiffness over time as a platform for the in vitro modeling of the observed increase in matrix stiffness during vascular aging. Such a model will allow the investigation of the effect of tissue mechanics on endothelial cell signaling in a physiological in vitro environment and will further serve as a platform for effective drug screening to prevent and reverse the effect of tissue stiffness on vascular morphology. Using stem cell derived endothelial cells (ECs) will allow us to generate a more robust and modifiable system to study network and barrier formation. Additionally, stem cells with particular genetic characteristics, enable a deeper mechanistic understanding of disease mediated vascular phenotypes. For this purpose we will embed stem cell derived early vascular cells (EVCs) or endothelial cells (ECs) into methacrylated collagen gels to form networks under soft matrix conditions mimicking healthy tissue. When the network is established the stiffness of the hydrogel will be gradually increased over a given time period mimicking the process of tissue aging. This system will allow the investigation of changes within the ECM of the vessel wall and the screening for the activation of mechano-sensitive cell signaling pathways. The so acquired results will then be used to specifically target the identified pathways with the goal to stop or reverse the negative effects of ECM stiffness on vascular health. Doing so, we will be able to show that changes within the physical properties of the 3D environment affect EC cell signaling and significantly increases cell motility and loss of cell-cell and cell-matrix contacts. The activation of the WNT/β-catenin pathway is hereby of special interest, because it plays a critical role in adult tissue homeostasis and could be implicated in abnormal wound healing and fibrosis. The proposed research strategy involves the combination of tissue engineering techniques and stem cell biology to gain a better understanding of the process of vascular aging. The impact of the study is to develop a 3D matrix mimicking the progression of vascular aging and fibrosis. This system will enable to study progression in vascular stiffening, its underlying mechanism and potential therapeutics. This engineered vascular model will increase our knowledge about blood vessel morphology and regeneration during aging, help us translate the knowledge into clinical applications and benefit healthcare in Maryland and our society.

Arens Taga, Ph.D.
Johns Hopkins University
Mentor: Nicholas Maragakis , Ph.D.
Award Amount: $130,000
Disease Target: Amyotrophic Lateral Sclerosis (ALS)

Regional Diversity of hIPSC-Derived Astrocytes and its Contribution to Non-Cell Autonomous Toxicity in ALS

Despite growing evidence suggesting that glia are regionally heterogeneous in vivo, little attention has been directed to human induced pluripotent stem cells (hIPSC) for modeling such diversity in vitro. As a result, hIPSC-derived astrocytes (hIPSC-A) are still largely used as a relatively homogenous and interchangeable cell type.
In normal states, astrocytes contribute to neuronal function by promoting synaptogenesis. Additionally, in vivo studies have suggested that astrocyte-neuron interactions may be influenced by their respective positional identities. However, it is not known whether regionally-specific hIPSC-A can model such interactions.

In neurological disease models, the relevance of positional identity is prototypically displayed by amyotrophic lateral sclerosis (ALS), where neurodegeneration is primarily segregated into distinct anatomical regions (i.e. motor cortex/anterior horns of the spinal cord) and neuronal cell subtypes (i.e. upper/lower motor neurons). Astrocytes are thought to contribute to neuronal death through non-cell autonomous toxicity, as demonstrated by different experimental models, including, by hIPSC-based paradigms. However, little is known whether regional astrocyte identity, i.e. cortical vs spinal cord, is relevant to their toxicity towards motor neurons. This is of particular relevance to understanding the pathobiology of a region-specific disease such as ALS, and translates to therapeutic strategies targeting symptoms restricted to specific anatomic regions, such as spasticity for corticospinal dysfunction and cramps for lower motor neuron impairment.

In the current proposal, we hypothesize that hIPSC-A regional specification between cortical (hIPSC-cA) and spinal cord (hIPSC-scA) subtypes influences the function and survival of region-specific hIPSC spinal cord motor neurons (hIPSC-MN) in control and ALS samples. To test this hypothesis, we first aim to generate and characterize regionally-specific hIPSC-cA and -scA. We then seek to co-culture these hIPSC-A subtypes, from control and ALS patients, with control hIPSC-MN and to determine whether hIPSC-A regional specification influences key neuronal functions, including, synaptogenesis and neuronal electrical activity, and neuronal survival.

Renjun Zhu, Ph.D.
Johns Hopkins University
Mentor: Chulan Kwon, Ph.D.
Award Amount: $130,000
Disease Target: Multiple

Multiplexed Gene Knock-in with CRISPR/Cas9 in Human Pluripotent Stem Cells

Human pluripotent stem cells (hPSCs), including embryonic and induced pluripotent stem cells, are an increasingly valuable resource in both basic and translational research. Their ability to give rise to all cell types in the human body makes hPSCs a promising platform for drug screening, regenerative medicine and disease modeling1. The utility of hPSCs has been made even more powerful by combining gene editing technologies. For example, high-throughput gene knockout methods allow large-scale functional genomic screens, and transgenic reporters such as fluorescent protein fusions enable isolation of specific hPSC-derived cell types and monitoring of their biological and pathological processes2. The CRISPR/Cas9 system is a simple and powerful tool for gene editing that provides significantly higher accessibility to researchers than previous methods based on zinc finger or TAL effector nucleases3. Despite technological advances in adapting CRISPR/Cas9 for high-throughput genome-scale knockouts4,5, generating reporter knock-in lines in hPSCs remains a time-consuming and expensive process6, accounting for its slow adaptation by both academia and industry. Targeted gene knock-in using CRISPR/Cas9 involves the simultaneous delivery of the Cas9 enzyme, the synthetic single guide RNAs (sgRNAs), and a "repair template" DNA molecule containing the knockin gene and homology arms. While the Cas9 enzyme and sgRNAs can be encoded and delivered by a single vector that is sufficient for inducing gene knockouts, repair templates are often delivered separately to the target cells, virtually eliminating the chance that a repair template with site-specific homology arms would transfect the target cell simultaneously with the correct site-specific sgRNA if multiple pairs of repair templates and sgRNAs were used. Further, electroporation or lentiviral-based delivery systems can lead to uncontrolled genomic integration of exogenous DNA, increased off-target mutagenesis, and ultimately limiting the potential of the resulting cell lines for use in research or clinic. The goal of this proposal is to develop a novel technology for multiplex and scalable generation of knock-in hPSC lines using the CRISPR/Cas9 system. To do this, I have developed a novel methodology that a) uses single-vector modified baculoviruses, which are non-integrating insect viruses with large packaging capacity, to transiently deliver all CRISPR/Cas9 knock-in components, and b) uses a library of these all-in-one vectors to perform knock-ins for fluorescently tagging multiple genes in hPSCs. By performing transfection, isolation and identification in parallel for multiple targets, this strategy enables the production of knock-in hPSC lines in a high-throughput fashion.

To optimize this system for multiplex gene knock-in in hPSCs, I propose the following specific aims:

Aim 1: **Develop a single-vector delivery system for CRISPR/Cas9-mediated gene knock-in in hPSCs.** This aim will evaluate the utility of modified baculovirus in CRISPR/Cas9-mediated gene knock-in, which is an advantageous system over electroporation or lentivirus-based systems with an all-in-one vector design and low uncontrolled integration.

Aim 2: **Test the multiplex gene knock-in strategy by targeting a library of cardiac genes in hPSCs.** In this aim, a fluorescent protein will be inserted downstream of a library of cardiac genes, resulting in fusion protein production. This will allow tracking of their expression and localization. Our lab has expertise in stem cell-based cardiac development. This aim will use cardiac differentiation and maturation-related genes as a platform to develop and optimize the multiplex gene knock-in technology.

The immediate outcome of the proposed project will be fluorescently tagged hPSC cell lines that can be used for studies on cardiomyocyte maturation, heart diseases and drug development. The multiplex knock-in technology developed in the proposed project will have a wider impact in general hPSC-related research by enabling faster and cost-efficient gene editing in hPSCs. These expected outcomes are directly in line with the mission of the MSCRF to promote the promise hPSCs have for the academia and the biotechnology community.

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Completed MSCRF Grant Projects (FY 2019)

Luis Alvarez, Ph.D.
Theradaptive, Inc.
2018 Commercialization Award - *Completed*
Award Amount: $299,005
Disease Target: Cartilage

Development of a Biphasic MSC Delivery System for the Repair of Osteochondral Defects

Adult articular cartilage exhibits extremely limited capacity for self-renewal. Relatively minor injuries that result in small cartilage lesions often lead to progressive cartilage degeneration and eventually to osteoarthritis. Post-traumatic osteoarthritis results in significant pain and disability early in life, and is significant socio-economic burden worldwide. Current treatments focus on symptom relief rather than modifying disease progression. Significant challenges remain in the development and clinical application of osteochondral repair platforms. Theradaptive is a Frederick-based regenerative medicine company that has developed a platform for highly targeted delivery of protein therapeutics that bring about tissue repair in a precisely controlled manner. Under the support of 2018 MSCRF commercialization grant, we proposed to develop ConForma®, a novel biphasic osteochondral scaffold, in which the chondral phase supports chondrogenesis for cartilage regeneration, and the underlying osseous phase serves as a template for osteogenesis in the repair of subchondral bone. ConForma conforms to the defect sites and replaces lost and damaged tissue by encouraging specific growth of the two affected tissue types by loading two different kinds of growth factors simultaneously in different layers, and tightly controlling the dose and timing of release of the protein therapeutics. Our objectives were: 1) Complete the development of the ConForma system, i.e. conforming biphasic scaffolds, engineered growth factors that can be tightly tethered onto the scaffolds, and MSCs, and 2) Characterize ConForma® performance in terms of its i) biocompatibility, ii) chondro- and osteo-inductivity, and iii) cell loading capacity for MSCs with high osteochondral inductivity. Results: During this work, we successfully engineered six proteins (BMP2, TGF-β3, IGF1, FGF2, FGF18, and EGF) known for their potent osteochondral repair potential. After processing, all proteins remain bioactive as confirmed by luciferase assays. BMP2 and TGF-β3 were selected due to their outstanding performance for bone and cartilage regeneration, respectively, using an in vitro alkaline phosphatase assay and GAG content assay. A novel, flexible, 3D-printed polymer/ceramic composite scaffold was developed which contains β-tricalcium phosphate ceramic particles bound together with a biocompatible polymer. This composite material contains ceramic powder that serves as a high surface area substrate for attachment of the tetherable growth factors, while the polymer imparts high flexibility and handleability. The scaffold is highly porous allowing high loading of growth factors, long retention of antimicrobial agent, as well as cell penetration. To find out the maximum cell loading capacity of the scaffolds, we performed a static cell seeding experiment using hMSCs (RoosterBio, Frederick, MD). It was determined that 20 million cells was the most efficient and economical dose for a 2 mm tall cylindrical scaffold with 6 mm in diameter (dimensions taken from Agili-CTM, a commercial osteochondal allograft). Robust chondrogenesis was observed when hMSCs were cultured on ConForma for 21 days, confirmed by histological analysis. These promising results set the groundwork for moving ConForma to large animal studies in a follow-on 2019 MSCRF Commercialization Grant. Impact: ConForma® will be the first and only viable, off-the-shelf 3D-printed osteochondral allograft that contains growth factors and cells specific for cartilage and bone repair. This is a paradigm shift in treating focal cartilage defects and mild to moderate osteoarthritis. The most important value that ConForma® can bring to patients is that it requires a single procedure, and can essentially eliminate the need for microfracture or autograft harvesting. Other advantages include: 1) improved outcome due to i) targeted delivery of therapeutic factors and cells, ii) controlled release of growth factors, iii) MSCs for chondral repair; and 2) conformable 3D-printed geometries.

Emily English, Ph.D.
Gemstone Biotherapeutics, LLC.
2019 Commercialization Award (1st Funded Cycle) - *Completed*
Award Amount: $229,998
Disease Target: Severe burns

Stem Cell Loaded Extracellular Matrix Replacement Scaffolds for Skin Regeneration in Burns

In previous work, we developed an acellular extracellular matrix replacement (EMR) scaffold for skin regeneration. Preclinical studies in excisions and third-degree-burns demonstrated that EMR treatment induced regeneration of normal, functional skin, and restored hair follicles, sebaceous glands and reticulated epithelium. The EMR provides a moist environment, promotes autolysis, debrides the wound, and adsorbs wound exudate while functioning as a barrier to further trauma. Others have shown that mesenchymal stem cells (MSCs) amplify the innate wound healing response by recruiting neutrophils and macrophages to the wound, increasing vascular network formation, and restoring sebaceous glands and hair follicles1,2. Therefore, we proposed a Stem Cell-EMR (SC-EMR) that combined human MSCs (hMSCs; RoosterBio, Frederick, MD) with the EMR, to create a cell therapy device to enhance wound healing outcomes in third-degree burns.

Objectives: The three goals of our project were to: 1) evaluate SC-EMR prototypes and MSC donor lots for effects on skin regeneration in a murine third-degree-burn model; 2) optimize the delivery formulation for the SC-EMR scaffold, incorporating the chosen hMSC donor as determined from wound healing results in the murine model; and 3) evaluate optimized SC-EMR in a porcine third-degree-burn model. Analysis of the porcine third-degree-burn model is ongoing. Importantly, we did not observe any toxicity or rejection in any of the wounds. Preliminary results suggest that the SC-EMR promotes faster wound healing than nontreated control wounds. Analyses of tissue structure, protein localization, and gene expression in wounded and healthy tissue from this study are underway.

Translational Potential: The current standards-of-care for severe burns are limited by donor skin availability, dated technologies, and poor aesthetic outcomes. Hospital-based burn centers and their patients are the target customers for our SC-EMR product. This technology will require FDA approval, likely followed by approval for reimbursement through CMS. We have established cGMP manufacturing for our initial acellular product offering, which will be completely commercialized in 2019 for use in dermatology. The RoosterBio hMSCs used in our SC-EMR are also produced in a cGMP process. Identifying an ideal donor hMSC population in this work allows us to focus further development efforts on expanding a bank of these cells, and provides us a foundational protein expression profile to explore for further optimization and development work. Having this knowledge and our well-developed manufacturing capabilities and the forthcoming FDA clearance and sales channels on our acellular product put us in a strong position to translate the SC-EMR from preclinical models to the clinic.

Linhong Li, Ph.D.
MaxCyte, Inc.
2017 Commercialization Award - *Completed*
Award Amount: $300,000
Disease Target: Sickle Cell Disease

Translational Development of Gene-Corrected Hematopoietic Stem Cells as Treatment for Sickle Cell Disease (SCD)

Sickle cell disease (SCD) is a genetic disease of c.20A>T mutation in β-globin gene. There is no cure for most patients. It is our goal to find cure for this disease by using gene correction approach. In this one year grant, we, in collaboration with Dthe leading authorities in sickle cell disease (SCD) and gene therapy, proposed to develop a process to correct the A-T mutation in the first exon of the β-globin gene in plerixafor-mobilized peripheral hematopoietic stem cells (HSC) from SCD patient. The successful correction procedure was first translated to converse health HSC into SCD mutation, by using a newly designed gRNA with one nucleotide change targeting to the same site in health HSC as that of the mutation in SCD. This new gRNA could be used to make a A to T converse, just an opposite case of fixing the mutation by making T to A correction. The developed correction procedure could converse the health HSC into SCD HSC at 20-40% rate assayed by targeted deep sequencing (N=3). Then, the correction procedure was translated to correct SCD HSC in vitro and validated by the recovery of the adult wild type β-hemoglobin expression after the in vitro 17d differentiation of HSC into erythroid. It was found that the correction procedure is consistency in gene correction (N=3), could reach as high as 30-40% in the assumed biallelic correction, and does not decrease with the 17d differentiation. Considering the corrected cells could have both single allelic and biallelic correction for individual cell, the cellular correction efficiency could be about 1.5 folder higher, reaching to 50-60%. The efficient correction results in wild type adult β-hemoglobin expression to a level as high as 92% among all hemoglobin, when the fetal hemoglobin induced by in vitro differentiation procedure is ignored. After the gene correction could reach to the therapeutic level in vitro, the IND-enabling study of animal engraftment and scale up becomes a task in reality. The efficient SCD gene correction and the high wild type adult β-hemoglobin expression after gene correction have motivated MaxCyte and NHLBI, NIH to enter into a Collaborative Research and Development Agreement (CRADA) to further translate this finding into clinical. We are currently ready to perform IND-enabling study of animal engraftment and scale up. We have successfully achieved the scientific milestones outlined in our research proposal. Based on clinical data from allogeneic transplantation outcomes, 20% level of engraftment of single allelic correction in human subjects has been established as being clinically 'curative' for SCD patients. Our long-term engraftment outcomes in animals with the similar gene correction approach in CGD could reach ≥70% of gene correction rate after long-term engraftment relative to that without going through animal engraftment, the current ~50-60% cellular correction efficiency of SCD in vitro and the high percentage of ~90% of wild type adult β-hemoglobin expression after differentiation from the corrected SCD HSC to erythroid give us the hope to be optimistic that the gene correction procedure developed by the support of this grant may reach to the curative window for curing SCD patients. These results have been presented and published in peer-reviewed scientific community. We will continue to translate this finding to clinical with our collaborator Drs. John Tisdale in NHLBI and Harry Malech in NIAID, NIH under a CRADA between MaxCyte and NHLBI, NIH established through the work supported by this grant.

Hai-Quan Mao, Ph.D.
LifeSprout, Inc.
2018 Commercialization Award - *Completed*
Award Amount: $300,000
Disease Target: Multiple

Delivery of MSCs to Enhance the Replacement & Regeneration of Soft Tissue

LifeSprout is commercializing a novel injectable and bioresorbable nanofiber-hydrogel composite (NHC) for the replacement and reconstruction of soft tissue defects. The composite holds promise to improve clinical outcomes for reconstruction of soft tissue loss following cancer surgery, trauma, and birth defects which affect more than half a million patients each year. As demonstrated in multiple in vivo preclinical models, the injectable composite provides immediate volumization and soft tissue feel by simulating the viscoelastic property of natural soft tissue, and more importantly promotes extensive revascularization at the tissue repair site. The overall objective of LifeSprout's Maryland Stem Cell Research Fund (MSCRF) Commercialization Grant is to extend the utility of the acellular NHC microbeads by (1) optimizing the matrix for the delivery of mesenchymal stem cells (MSCs) and (2) validating the key product features and competitive advantages of LifeSprout's novel MSC delivery matrix compared to the market benchmark for large volume soft tissue reconstruction. The LifeSprout team produced twelve injectable NHC microbeads of varying stiffness and porosity by adjusting fiber loading levels, crosslinking density, and interfacial bonding in comparison to HA controls. All twelve formulations were tested in vivo via subcutaneous injection, first without cells seeded, in a rat model and compared to Allergan's class-leading commercial injectable filler. The LifeSprout formulation variants were rank-ordered based on degree of cellular remodeling, neovascularization, and volume retention over a 244-day duration. MRI imaging also enabled assessment of swelling and volume retention. The LifeSprout formulations exhibit limited swelling compared to Juvederm Voluma XC (15% vs. 150%) and were equivalent in comparison of percentage reduction from the peak volume compared to Juvederm Voluma XC through Day 244. The preferred NHC microbead formulation variant was then cultured with human mesenchymal stem cells (hMSCs, obtained from RoosterBio) over a 7-day period. Immunostaining of the in vitro cell culture indicates successful hMSC adhesion to NHC microbeads. Over time, cells proliferate and compact the NHC microbeads, populating the microbead both on the surface and into the core. The Juvederm injection showed little or no tissue ingrowth compared to all LifeSprout groups. Compared to Juvederm Voluma XC and LifeSprout's acellular scaffold only group, all hMSC NHC groups exhibited a protein rich core due to ECM deposition. Incorporation of adhesion peptide RGD has no noticeable impact on cell proliferation in vivo, and both groups with 7-day pre-culture demonstrated similar host tissue ingrowth, volume retention, and a protein rich core that was substantially stiffer at day 72. In comparison, 1-day pre-cultured hMSC-NHC showed improved host tissue infiltration with a non-protein deposited core. A 2-day hMSC-NHC microbead culture was determined optimal for balancing the rate of cellular infiltrate and stiffness of the implant. The optimized 2-day cell cultured composite was then compared per graft retention, fibrosis, necrosis, and angiogenesis to fat grafting and Voluma XC in a rabbit model, demonstrating similar trends to the rat modeling. The incorporation of RoosterBio MSCs into LifeSprout's injectable NHC microbeads offers a potential breakthrough technology in reconstructive medicine. With the new MSC-seeded composite, reconstructive surgeons could for the first time offer patients a minimally invasive, cost-efficient, and regenerative restoration for medium and large three-dimensional soft tissue losses.

Ha Nam Nguyen, Ph.D.
3Dnamics, Inc.
2017 Commercialization Award - *Completed*
Award Amount: $300,000
Disease Target: Alzheimer's

Engineering Human Pluripotent Stem Cell-Derived Brain Organoids for Drug Screening and Toxicity Testing

The quest to find novel therapeutics for neurological disorders has been hindered by the lack of access to live human brain samples and relevant experimental models. Currently, roughly 0.1% of drugs that show promise in preclinical testing make it to Phase 1 clinical trials, and 90% of those drugs go on to fail FDA approval. One of the reasons for such a high failure rate is that standard animal and two-dimensional cell culture models cannot fully recapitulate genuine disease phenotypes and are not good enough predictors of how drugs will work in humans. Progress in three-dimensional (3D) tissue engineering offers a promising new platform that may be the key in accelerating and improving current drug developmental trend. These 3D tissues or organoids are differentiated from human pluripotent stem cells (hPSCs) to resemble specific parts of the human brain, which include architecture composition and physiology. Thus, the aims of this project were 1) to scale-up 3D brain region-specific organoids for drug screening and toxicity testing and 2) to commercialize 3D brain organoids and 3D cell culture bioreactors. We launched 3Dnamics Inc. in September of 2017 and, currently, the company has leased lab and office spaces in Fastforward Bioincubator, Baltimore City. We have successfully developed a method to scale up midbrain organoids (10,000+). We have tested and shown that brain organoids can model Parkinson's disease and can serve as ideal platform for compound and toxicity testing. Furthermore, we have obtained clients and partnerships in Pharma, National Institute of Health, and academia. Human brain organoids derived from hPSCs can model human diseases (e.g., Alzheimer's disease) that are not amenable in other systems, such as animals, monolayer cell culture, and other cell-free systems. For this reason, brain organoids can be used to further understand disease mechanisms and develop specific assays for targeted drug screening and toxicity testing. A more accurate human brain organoid disease model and drug screening platform can lead to discoveries of novel medicines for intractable neurological diseases and can reduce human suffering and potentially save lives.

Jonathan Rowley, Ph.D.
RoosterBio, Inc.
2018 Commercialization Award - *Completed*
Award Amount: $300,000
Disease Target: Multiple

Closed Systems Enabling Commercially-Viable Stem Cell Manufacturing

Currently there are over 800 active clinical trials involving mesenchymal stem/stromal cells (MSCs), indicating an upcoming wave of demand for clinical-grade cells for future regenerative medicine therapies. As such, there is a critical need for economical biomanufacturing processes capable of generating billions to trillions of cells per manufacturing lot to meet the demand of these future commercial applications. The need for affordable production of cells in closed scalable systems has also been identified as a "Current Challenge" in the Technology Roadmap established by the National Institute of Standards and Technology -funded Cell Manufacturing Consortium. One major component required for realizing scalable platform systems capable of large scale human MSC production is the availability of a closed-system working cell bank , since CS processes compared to open systems lower the inherent risks of contamination. Furthermore, while traditional cryovial WCB configurations for seeding 2D T-flasks or multi-Layer vessels are manageable for production lot sizes of <30B cells, cryovials become impractical for seeding large-scale 3D bioreactor platforms where the number of cells required during seeding increases markedly. Therefore, the goal of this project was to develop a scalable CS-WCB in a cryobag configuration containing xeno-free hMSCs that are of comparable health, quality, and function to hMSCs vialed in traditional cryovial formats in order to support future large scale hMSC production for regenerative medicine therapies.This project was divided into two aims: Aim 1 was to develop the process for generating a CS-WCB and Aim 2 was to verify the suitability of the CS-WCB product. Future work will involve tech transfer of the process to a cGMP manufacturing suite to produce FFM CS-WCBs for production of clinical therapies. As a result of this MSCRF Commercialization Grant, a complete manufacturing process and quality control assays were transferred to the RoosterBio Operations department to create a new product: RoosterBank™-hM-100M-XF (Part # MSC-035A). In addition, a total of three production runs were executed as part of this project yielding a total of 86 units of product. QC safety (e.g. sterility, mycoplasma) and functional testing will be performed prior to the expected product launch and release in Q1 of 2019. In addition, stability testing will be performed at scheduled intervals up to 30 months to establish an expiry/ shelf-life for the product. A poster showcasing the product/process development process and comparability data generated was submitted and presented at the TEDCO/MSCRF 2018 Entrepreneur Expo and Stem Cell Symposium.

William Rust, Ph.D.
Seraxis, Inc.
2017 Commercialization Award - *Completed*
Award Amount: $298,960
Disease Target: Diabetes

Long-term Function of Stem Cell Grafts for Insulin-Dependent Diabetes

Insulin-dependent diabetes can be cured in rodent models by the implant of human insulin-producing cells encapsulated within devices that protect the cells from the host immune system. These promising pre-clinical results have stimulated the launch of SERAXIS, INC, a cell-replacement therapy company targeting type 1 diabetes. To transform the preclinical concept to a clinically validated and practical therapy, the cell/device combination must be demonstrated to be safe, stable and implantable without encumbering the patient. The goal of this project is to evaluate the impact of a macro-device surgically implanted to the omentum of rats on the long-term stability of that organ. The purpose is to show that engraftment to the omentum enables the device to associate with the host vasculature without initiating scar formation, tumorigenesis, or other safety risks to the patient. Results: The safety of the cells used in this project was validated by a third party by verification of purity, genomic stability, and lack of contamination by viruses, bacteria, fungus, and mycoplasma using Good Manufacturing Practice MP) compliant tests. The long-term implant of the Seraxis device to the omentum of diabetic rodents did not stimulate genetic or cellular alteration to the omentum that is known to initiate or contribute to cancer. The implant did cause structural alteration to the omentum that was evident by the proximity of blood vessels to the implant and the deposition of collagen. These alterations were not suggestive of safety risk to the animals. Further studies evaluating the safety of the implants on the remaining organ systems are ongoing. In total, these studies encompass a major step towards the regulatory acceptance of the proposed therapy for testing in human patients. These results comprise completion of originally stated aims of the project.

Chengkang Zhang, Ph.D.
Propagenix, Inc.
2017 Commercialization Award - *Completed*
Award Amount: $300,000
Disease Target: Multiple

Building Commercial Path for EpiX™ Technology - A Breakthrough in Expanding & Utilizing Tissue - Resident Stem Cells

With the support of MSCRF commercialization grant, we successfully built the commercial path for the EpiX™ technology – a breakthrough in culturing tissue-resident epithelial stem and progenitor cells from diverse tissues for studying epithelial cell biology in vitro and unleashing their potential for regenerative cell therapy. In this project, our work aimed at 1) illustrating the unparalleled competitive advantage of EpiX technology over conventional methods using human airway epithelial cells as an example; 2) demonstrating the utility of EpiX technology for personalized medicine applications by expanding nasal epithelial cells out of minimal-invasive nasal brushing samples obtained from cystic fibrosis patients; and 3) scaling up EpiX medium production from bench scale to lot size of 100 liters, to provide support for moving forward with full commercialization. In AIM 1, we verified that the EpiX medium supported the expansion of primary airway epithelial cells from two different donors for over 50 population doublings, while retaining consistent functionality as assessed by various molecular and cellular characterization assays by the end of 1Q 2018. This is a significant improvement over current practice, and allows researchers to generate billion-fold more airway epithelial cells for their research study than using the convention methods. In AIM 2, we tested a personalized medicine approach for cystic fibrosis patients by using patient-derived nasal epithelial cells for functional in vitro drug response evaluation assay. We successfully generated 50–100 millions of nasal epithelial cells using brushing samples from 7 adolescent CF patients (4 males and 3 females, 7/7, 100% success), and tested their responses to Ivacaftor and Lumacaftor using the Ussing chamber assay, a "gold standard" drug efficacy evaluation assay for CFTR-directed therapeutics. The results revealed distinct responses towards the same drug despite that these patients share the same CFTR mutation. In AIM 3, we have successfully established the production SOPs and QC specifications for the EpiX medium, including analytical and functional quality tests and specifications such as standard pH, sterility, osmolality, endotoxin level and support of cell growth and differentiation. So far, we have manufactured 4 lots of EpiX medium at a scale of 100 liters and delivered high-quality, reproducible products for many researchers working with airway epithelial cells, including international customers.

Sharon Gerecht, Ph.D.
Johns Hopkins University
2017 Validation Award - *Completed*
Award Amount: $230,000
Disease Target: Coronary Artery Diseases

Patient-Specific Small-Diameter Tissue Engineered Vascular Grafts

Despite significant research efforts, clinical practice for arterial bypass surgery has been stagnant. Here, we sought to develop and apply a durable small diameter vascular graft with tailored regenerative capacity.

Results: Employing surface topography in a hollow fibrin microfiber tube, we enable immediate, controlled perfusion and formation of a confluent endothelium both in vitro and in vivo within 1 week. Implantation of acellular or endothelized grafts with an external ultrathin poly(ε-caprolactone) fibrous sheath in the mouse abdominal aorta supports normal blood flow and vessel patency for 24 weeks. Mechanical properties of the implanted grafts closely approximate the native abdominal aorta properties after just one week in vivo. Fibrin mediated cellular remodeling, stable tunica intima and media formation, and abundant matrix deposition with organized collagen layers and wavy elastin lamellae. Endothelialized grafts evidenced controlled healthy remodeling with delayed and reduced macrophage infiltration alongside neo vasa vasorum-like structure formation, reduced calcification, and accelerated tunica media formation.

Impact: Our studies establish a small diameter graft that mediates neotissue formation and incorporation into the native vascular tissue and matches the native vessel size and mechanical properties, overcoming main challenges in arterial bypass surgery.

Sunjay Kaushal, M.D.
University of Maryland - Baltimore
2017 Validation Award - *Completed*
Award Amount: $230,000
Disease Target: Cardiac Disease

Neonatal Cardiac Stem Cells for Heart Regeneration

After a myocardial infarction, the mammalian heart undergoes only minimal levels of regeneration. While medical and surgical interventions are effective in limiting damage, these ischemic patients suffer from heart failure symptoms that grow worse over time, and can lead to disability and death. The most promising approaches to regenerate muscle and vasculature are cell-based therapies. Successful autologous cell therapies face obstacles to widespread adoption, including high expense, delays, and variable effectiveness. Allogeneic cardiac stem/progenitor cells (CPCs) generated from neonatal tissue offer an attractive alternative. The Kaushal lab has identified a novel type of CPC that express the cell-surface signaling protein c-kit. We recently discovered that these c-kit+ CPCs are able to stimulate the regeneration of cardiac muscle and restore heart function in a rodent model of myocardial ischemia. This stem cell population can be developed into a therapy that incorporates streamlined manufacturing techniques, keeps costs down, and offers reproducible, well-defined benefits. These are key elements of a commercially-viable regenerative cell therapy. In this application, we propose to validate the safety and functional abilities of allogenic, neonatal, cloned, c-kit+ CPCs in a well-established swine acute myocardial infarction model. The study will be comprised of three groups of four pigs: high-dose CPC, low-dose CPC, and a control group. At study completion, we will have a powerful proof-of-concept validation that will serve as the template for the GLP study that will be a crucial step towards submitting an Investigational New Drug (IND) application for the use of neonatal-derived, c-kit+ CPCs in treating post-MI heart failure. NeoProgen is committed to the commercial development of cardiac stem cell technology for therapeutic treatments in adults suffering from ischemic heart failure. We are developing an allogeneic product based on primary nCPCs generated from neonatal heart biopsy tissue. Our hypothesis is that intracoronary delivered allogeneic nCPCs when injected at the time of catherization will improve the function of the left ventricle in adult ischemic patients, as measured over the short and long term. The next step along this path is a pivotal study to establish effective cell preparation and storage conditions and optimal cell dosing in the pig to demonstrate safety, feasibility, and efficacy in a large animal model of MI. The results from this study has clarified a commercialization path to move forward. We have recently received the MSCRF Commercialization grant to perform those objectives.

Chulan Kwon, Ph.D.
Johns Hopkins University
2018 Validation Award - *Completed*
Award Amount: $230,000
Disease Target: Multiple

Developing Adult Cells from iPSCs

The major goal of this proposal was to determine the scalability of an in vivo iPSC-derived cell maturation technology with the aim of turning it into a drug evaluation platform for the pre-clinical drug industry. By harnessing the power of postnatal organ maturation, we have previously demonstrated that human PSC-derived cardiomyocytes undergo full maturation and become adult cardiomyocytes in 4-6 weeks when transplanted into neonatal rodent hearts. The specific aims of this validation proposal were: (1) To determine the scalability of the technology by evaluating maturation of human iPSC-derived cardiomyocytes in a large animal model and (2) to test a therapeutic on in vivomatured cardiomyocytes generated from patients with Arrhythmogenic Right Ventricular Dysplasia/ Cardiomyopathy (ARVD/C). Milestone 1A: We developed a robust protocol that enables us to generate >80% pure Isl1+CPCs, meeting the anticipated endpoint (89%) for this aim. Milestone 1B: This aim was to determine the capacity of the neonatal pig heart to engraft iPSC-derived CPCs with the plan to introduce a total of 10 million cells via 10 injections at 1mL CPCs/injection. Unexpectedly, an additional amendment was required to our pig protocol for injecting cells into piglets younger than 2 weeks. This process took almost 5 months to be approved by the Johns Hopkins ACUC, thus we had to revise our planed timeline for this milestone from 4–6 months to 11 months. We optimized our injection strategy by testing the effect of increasing numbers of CPCs injected in the left ventricular myocardium. This procedure enabled us to increase the amount of cells to 100 million CPCs/heart from the original estimation of 10 million cells/heart. By analyzing % of engrafted RFP+ cells in the left ventricle, we found that approximately 10% of injected CPCs engraft in the heart. Milestone 1C: 4 weeks following transplantation we evaluated the maturation status of engrafted human cardiomyocytes. The majority of RFP+ cells showed all the characteristics of adult cardiomyocytes, demonstrating that the pig heart serves as an excellent bioreactor for human iPSC-derived cardiomyocyte maturation. To determine maturation status, we developed a novel technique to isolate live adult human cardiomyocytes, which remained a technical hurdle in the field. Milestone 1D: The new isolation technique enabled us to isolate single adult human cardiomyoctyes for RNA-sequencing to confirm the maturation status of human cardiomyocytes. The endpoints in milestone 2 were focused on evaluation of iPSC-derived cardiomyocytes generated from patients diagnosed with ARVD/C. We are currently in the progress of evaluating response to Calpeptin, a potent inhibitor of CAPN1 activation, which is linked to exercise triggered AIFtruncation in ARVD/C patients, which may avert necrosis and disease progression in arrhythmogenic cardiomyopathies. Together, we have concluded that piglets represent an excellent host for the organ-based iPSC-derived cell maturation technology. Impact on public health/translational potential: The combination of continuing rise in development cost and high failure rate of new drugs has prompted the development of more effective and efficient pre-clinical platforms to better assess drug efficacy and toxicity in humans. iPSC-based drug screening platforms have experienced rapid growth due to their ability to generate patient- and tissue-specific cell populations that can model healthy and diseased tissues. With the capability of producing and testing fully mature adult cells from patient-derived stem cells, this technology has great potential to reduce the rate of clinical trial withdrawals.

Yunqing Li, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
2017 Validation Award - *Completed*
Award Amount: $230,000
Disease Target: CNS Myelin Disorders

Lineage Reprogramming of human Fibroblasts into Oligodendrocyte Progenitor Cells

Cell transplantation therapy using oligodendrocyte progenitor cells (OPCs) and or functional oligodendrocytes (OL) for treating dysmyelinating and demyelinating CNS disorder is largely limited by the inability to obtain a pure self-renewal OPCs. Several approaches have been established to derive OPCs from the fetal brain or human embryonic stem cells (hESCs) as well as from human induced pluripotent cells (hiPSCs). However, these approaches are time-consuming, and inefficient, generating heterogeneous cells and tumorigenic potential, and thus have limitations for regenerative medicine. The goal of this project is to establish and validate a protocol which can generate induced (iOPCs) and OLs (iOLs) from hiPSCs and human fibroblasts in a clinically applicable timeframe. We have established and validated an efficient hiPSCs-derived iOPCs protocol using transcription factor mRNA cocktail (i.e. ASCL1, Olig2 and Sox10) combined with the oligodendrocyte specification signaling. Using this protocol, >70% PDGFR+ cells can be generated within 10 days from hiPSCs, > 50% MBP+ cells can be generated within 30 days from hiPSCs. Moreover, with some modifications, human fibroblasts can be first convert to stably expandable induced neural stem-like cells (iNSCs) within 20 days using non-integrating Sendai Reprogramming kit, and iNSCs then convert to induced OPCs using transcription factor mRNA cocktail. This proposal for the first time enables us to generate iOPCs and iOLs using mRNA cocktail without genetic manipulation from hiPSCs and human somatic fibroblasts in a clinically applicable timeframe. This work generates a patentable procedure for the generation of a cell source that can be used for iOPCs-based transplantation, central nervous system (CNS) demyelinating disorder modeling and drug screening for therapeutic purposes.

Elias Zambidis, M.D., Ph.D.
Johns Hopkins University
2018 Validation Award - *Completed*
Award Amount: $230,000
Disease Target: Multiple

MoroPLUR: A Defined Feeder-Free Medium for Enhancing Functionality of Human Pluripotent Stem Cells

Unlike human pluripotent stem cells (hPSC), mouse ESC (mESC) can generate chimeric animals when mixed with equivalent staged embryos; which is the most stringent measure of their functional pluripotency. This project's goal was the commercial development of 'MoroPLUR', a defined, optimized feeder-free (FF) cell culture medium that reverts conventional human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESC) to a naïve-like mESC-like state capable of contributing human tissues into developing mouse embryos. FF MoroPLUR is based on the LIF-3i tankyrase inhibitor small molecule approach originally described by the Zambidis Lab (Zimmerlin et al, 2016) http://dev.biologists.org/content/143/23/4368, and consists of a novel recipe of LIF, four small molecules, and defined media components. Conventional hPSC cultured in MoroPLUR in standard FF conditions are reverted in bulk with to a stable human naïve-like state. Naïve hPSC can be either cryopreserved or used directly for differentiation experiments without further manipulation. In addition to enhanced functionality, MoroPLUR-reverted naïve hPSC possess multiple advantages over conventional hPSC culture that includes ease of single cell passaging in a defined, reproducible FF culture system. FF MoroPLUR is only for pre-clinical, IRB/ISCRO/IACUC-approved research where a defined and reproducible method for versatile naïve reversion of an hESC/hiPSC line is desired. Upon completion of this project, a broad repertoire of seven conventional hESC/hiPSC lines growing in standard FF conditions were validated for reversion with FF MoroPLUR medium, and quantitatively tested for their differentiation capacities. These naïve-reverted hPSC were further validated in vivo in a murine-human fetal chimera pluripotency assay developed in the Zambidis Lab. Directed interspecies development with FF MoroPLUR N-hPSC will ultimately pave the way for humanized animal models of disease, and future development of hPSC-derived transplantable adult-stage human organs. Our media components are currently being tested and prepared for commercialization through an exclusive licensing agreement between Johns Hopkins University Technology Transfer and ThermoFisher, Life Technologies. The goal of this commercialization collaboration is to not only make the formula widely available to investigators for research-only purposes, but following additional minor modifications to our formula, to make our novel system completely cGMP-grade for future FDA/IND-approved patient use in pluripotent stem cell-based regenerative medicine therapies

Jeff W.M. Bulte, Ph.D.
Johns Hopkins University
2017 Discovery Award - *Completed*
Award Amount: $345,000
Disease Target: ALS

Non-Invasive Imaging of Hydrogel Scaffold Biodegradation and Cell Survival

Specific Aim 1: To perform CEST MRI of gelatin decomposition and 2-color NIR optical imaging for the validation of gelatin vs. HA decomposition. Using hydrogels with different compositions and mechanical strengths, we hypothesize that the magnitude of the gel CEST MRI signal can be used as a surrogate marker of gelatin stability. Work summary: We assessed the rheological properties of the composite hydrogel by varying both the dilution of hydrogel components, thiolated Gelatin and polydiacrylate in water and the Gelin-S:HA mixing ratio. The in vitro enzymatic degradation of the composite hydrogels with collagenase was assessed by 2-color NIR imaging after conjugating each component of hydrogels with two distinct LI-COR infrared dyes . CEST MRI of various hydrogel phantoms was assessed using a 11.7T Bruker Avance spectrometer. In vivo degradation was monitored for 42 days by both CEST MRI and 2-color NIR after injecting the dye conjugated hydrogels into the mice brain. Significant results: Hydrogels with various formulations were characterized for their rheological properties. We showed that CEST MRI can be used as a label-free imaging platform for monitoring the degradation of crosslinked hydrogels containing gelatin and hyaluronic acid , of which the stiffness can be fine-tuned by varying the ratio of the Gel:HA. By labeling in-S and HA with two different NIR dyes having distinct emission excitation frequencies, we showed that the HA signal remains stable for 42 days, while the Gelin-S signal gradually decreases to <25% of its initial value at this time point. Both imaging modalities were in excellent agreement for both the time course and relative value of CEST MRI and NIR signals . Our paper based on these results has been accepted and is on line.

Specific Aim 2: To assess the relation between the relative gelatin decomposition and transplanted cell survival for the different hydrogels. Using a transgenic SOD1G39A mouse model of ALS, where the survival of transplanted naked cells is notoriously poor, we will determine the relation between the BLI signal as biomarker for transplanted GRP survival and the MRI/NIR signal from Aim 1 as a surrogate marker for gelatin/HA decomposition. Work summary: GFP+ GRPs transduced with firefly luciferase were scaffolded within composite hydrogels at composite formulations ranging from 5-20 mg/ml. In vitro assessments of cell survival post-injection were made in an optimized hydrogel formulation to assess hydrogel scaffold mediated protection from cell death due to mechanical stresses. The graft survival of scaffolded/naked GRPs transplanted in immunocompetent BALB/c mice brain striatum was assessed with bioluminescent imaging (BLI). The graft morphology and immune reaction post cell transplantation was assessed by T2 weighted MRI and immunohistochemistry (IHC) respectively. The expression of glial/oligodendrocyte phenotypes with/without hydrogel scaffolding of the transplanted GRPs after final BLI time point of 32 days was also assessed by IHC.

Specific Aim 3: (Exploratory): Similar as Aim 2 but then using sGFP as a potential bimodal CEST/optical reporter gene in lieu of luciferase. We will assess if the magnitude of the GFP CEST MRI signal can be used as a possible surrogate marker of cell survival using BLI as gold standard. Work summary: Due to the tight time frame between the notification of the award, posting the job ad, and credentialing/start date of the postdoc at Hopkins, we were unable to pursue this aim. Conclusions: Both in vitro and in vivo time-course studies demonstrate that a decay in CEST MRI signal is caused by gelatin degradation, as validated using two-color NIR imaging studies. Our results suggest that the composite HA hydrogel formulations and mechanical characteristics can be tailored to positively influence the injection and post transplantation survival of GRPs.

Ivy Dick, Ph.D.
University of Maryland - Baltimore
2017 Discovery Award - *Completed*
Award Amount: $345,000
Disease Target: Timothy Syndrome

Developing a Novel Treatment Strategy for Timothy Syndrome

Timothy syndrome (TS) is a particularly severe subset of Long-QT syndrome (LQTS). As with other types of LQTS, TS prolongs the cardiac action potential, leaving patients vulnerable to life-threatening cardiac arrhythmias. Despite the myriad of available pharmacological agents designed to treat LQTS, successful treatment of TS patients has remained challenging. Here, we aim to understand this lack of efficacy and enable new treatment options for these patients. To accomplish this, we first evaluated the potential of currently available LQTS treatments for efficacy in TS using cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs) derived from TS patients. These cells exhibit severe AP prolongation and arrhythmogenic tendencies, allowing us to evaluate the efficacy of current treatment options in a model system which recapitulates the critical elements of TS. Second, we utilized this model system to develop a novel therapeutic treatment for TS. We did this by adapting a technique known as exon skipping, currently used in the treatment of Duchene's Muscular Dystrophy. By applying a similar strategy to TS, we expect to suppress the production of the deleterious form of the calcium channel responsible for TS. Significant progress has been made in evaluating and overcoming the therapeutic challenges to the treatment of Timothy syndrome (TS). Optical mapping of induced pluripotent stem cells derived cardiomyocytes (iPSC-CMs) has been used to evaluate the efficacy of small molecule drugs on TS cells. Importantly, we have found a better-than expected separation of the phenotypes of WT vs. TS iPSC-CMs within our experimental model. This large separation between the control and TS cardiomyocytes has enabled evaluation of multiple reagents, including calcium channel blockers. Interestingly, these blockers exhibited a larger than expected effect on the WT iPSC-CMs, prompting us to improve our model system in order to more closely recapitulate patient responses. We have thus altered our culture protocols for these cells, resulting in a more adult-like phenotype which not only supports the evaluation of therapeutics for TS, but empowers multiple iPSC based projects in the lab. These cells have also been used to design new therapeutic strategies for the treatment of TS based on an exon skipping technique. To this end, we have designed new antisense oligonucleotide (AON) sequences targeting the portion of the calcium channel containing the deleterious TS mutation. Using our iPSC-CMs, we have identified multiple candidate designs with promising results. Importantly, the preliminary date from these experiments has enabled the submission of a new R01 proposal which received a highly competitive score. Timothy Syndrome is a devastating form of long QT syndrome, which is resistant to conventional treatments. Here, we aim to not only understand the underlying basis for the imitated efficacy of current treatment options, but to guide the choice of therapeutics though a careful screen of currently available treatment options, as well as develop a novel strategy for the treatment of this disease. An R01 utilizing preliminary results from this grant is expected to be funded based on the score received. Work from this study was presented at several venues during the course of the grant: This project has not yet generated any publications; however, we expect that the data collected will be included in two planned manuscripts.

Aaron James, Ph.D.
Johns Hopkins University
2017 DiscoveryAward - *Completed*
Award Amount: $345,000
Disease Target: Bone/Cartilage Disorders

Regional Specification of Bone-Associated Perivascular MSC

With support from the MSCRF, the major goals of the project were to isolate, typify, and functionally characterize skeletal pericytes from human tissues. Pericytes have characteristics of mesenchymal progenitor cells (mesenchymal stem cells or MSC), but also have emerging tissue-specific characteristics. For example, our group previously reported that pericytes associated with the knee joint have a tendency to form cartilage tissue under appropriate conditions. In brief, our aims were to define the regional specification of skeletal human pericytes (Aim 1), and determine how skeletal origin impacts the regenerative phenotype of human pericytes (Aim 2). Our first result was that skeletal (periosteal) derived human pericytes have a marked tendency to form bone and cartilage in vitro. For example, periosteal pericytes have increased bone-forming potential in comparison to unpurified total periosteal cells, as well increased bone-forming potential in comparison to pericytes derived for non- skeletal cell depots. For the purpose of this comparison, we used human pericytes obtained from either human adipose tissue or human dermis. Periosteal pericytes had canonical features of pericytes, including characteristic cell surface marker expression, induction of endothelial cells to form blood vessels in vitro, as well as the ability to form bone, cartilage and fat tissue under appropriate conditions. This result was in agreement with our understanding of skeletal pericytes as an MSC population, but also a cell population with tissue specific properties. A second striking finding was that human periosteal pericytes form bone and recruit marrow elements when placed in an immunocompromised mouse. This was in contrast to soft tissue-associated human pericytes which did not perform either function to a significant degree. Going a step further, microarray analyses were performed to compare gene expression between human pericytes derived from skeletal and soft tissue sources. Pathway analysis demonstrated widespread differences in the transcriptome of skeletal versus soft tissue associated pericytes. This new data has informed our next set of studies, currently underway, in which we have leveraged this information to identify a subset of human pericytes with marked bone-forming tendencies irrespective of tissue of origin. In this way, our funded MSCRF studies have led to additional insight that has directed our next line of investigation. In aggregate, pericytes represent a MSC population with great promise in skeletal repair. MSC/progenitor cell therapies have high potential for the treatment of common conditions such as osteoarthritis, bone fracture, non-healing skeletal defects, and fibrous non-unions. Our results place emphasis on the tissue-specific properties of human pericytes, but also demonstrate that further understanding of this regional specification may help inform and improve our methods for tissue engineering and regenerative medicine.

David Kass, M.D.
Johns Hopkins University
2017 Discovery Award - *Completed*
Award Amount: $313,013
Disease Target: Heart MI

Conditional Power Switch for Stem Cell-Derived Cardiomyocytes

The primary goal of this project was to generate a genetically engineered "power switch" that could be inserted into human progenitor stem cells that would subsequently be differentiated into cardiomyocytes. With exposure to doxycycline, the gene expression vector would produce the small peptide – REM1 – that would inhibit the L-type calcium channel, effectively terminating excitation contraction coupling in the myocyte so it would no longer contract. This would then be established in cells used for cardiac stem cell therapy to test their integration into the myocardium for effective generation of force. This latter issue remains remarkably controversial despite nearly two decades of stem cell therapy research, up to and including multiple clinical trials. The effort proved a bit more difficult than we first anticipated, primarily because while we could get the expression vector for Rem1 to express in PSCs, upon differentiation into cardiomyocytes, the gene was being epigenetically silenced. We tried multiple avenues to circumvent this, placing it in a DNA safe harbor, but this did not work. Ultimately, we were successful, and at present are moving forward with full implementation of this concept for stem cells and into a novel in vivo model. The details of the successful approach follow. The work has been successfully converted into two awarded grants, a innovative investigator award from the American Heart Association, and a K08 (both awarded to Emmanouil Tampakakis, MD, and Assist Professor at Johns Hopkins who took over this project).

Gabsang Lee, Ph.D.
Johns Hopkins University
2017 Discovery Award - *Completed*
Award Amount: $345,000
Disease Target: Duchenne Muscular Dystrophy (DMD)

Pharmacological Cues to Expand Functional Human PAX7::GFP+ Skeletal Muscle Stem/Progenitor Cells

Duchenne muscular dystrophy (DMD) is one of the lethal pediatric muscle disorders. It genetically inherited as an X-linked disease caused by mutations of the dystrophin gene. DMD cause progressive muscle degeneration, weakness, and wasting. DMD finally leads to the premature death in affected individuals because of cardiac, or respiratory failure by age of 30. The purpose of this study was to find compounds maintaining muscular progenitor cells for DMD treatment. We conducted compound screening and xylitol was selected for a potential compound maintaining Pax7 positive muscular progenitor cells. Xylitol treatment for 21 days increased Pax7 positive muscular progenitor cell populations. Also, it produced ATP through pentose phosphate pathway. Action mechanisms of xylitol revealed that treatment with xylitol activated Akt/Erk signaling pathway, leading to regulate Pax7 positive cell proliferation. In comparison with FGF treatment, xylitol treatment was more effective on maintenance of Pax7 positive muscular progenitor cells. There results suggest that xyltiol represents a potential novel hit compound maintaining Pax7 positive muscular progenitor cells.

Brady Maher, Ph.D.
Lieber Institute for Brain Development
2017 Discovery Award - *Completed*
Award Amount: $345,000
Disease Target: Pitt-Hopkins Syndrome (PTHS)

Use Human Cellular Models of Pitt-Hopkins Syndrome to Study Neuronal Development & Validate Therapeutic Targets

Autism spectrum disorder (ASD) is debilitating and costly psychiatric disorder for which we have a limited understanding as to the cause and underlying pathophysiology. Current treatments of ASD primarily focuses on behavioral therapy, as effective pharmacotherapy is lacking, with no drugs that can treat core problems. The primary goal of this grant was to differentiate induced pluripotent stem cells (iPSCs) derived from ASD patients into cortical neurons in an effort to create an experimental model of a complex human disorder to identify the underlying pathophysiology in ASD. We focused on Pitt-Hopkins Syndrome (PTHS), a syndromic form of autism caused by a single mutation in the gene transcription factor 4 (TCF4). The main aims of this grant were to: 1) Expand the number of publicly available PTHS iPSCs. 2) Perform an indepth molecular analysis of PTHS neural progenitor cells (NPCs) to identify disrupted molecular pathways. 3) Perform an electrophysiological assessment of cortical neurons derived from PTHS iPSCs, to identify novel human specific phenotypes and to translate a previously observed hypo-excitability phenotype and sodium channel target from a mouse model into a human context. We have accomplished all of the goals and milestones set out by this grant. Aim one was accomplished by reprogramming 36 additional iPSCs lines from 4 PTHS patients with similar point mutations in TCF4 and 5 patient control lines. All of these lines were robustly characterized for markers of pluripotency, neurogenic potential, and will be made publically available to the scientific community. We performed RNA sequencing analysis of NPCs and demonstrate equal developmental progression of iPSCs towards NPCs over the course of 20 days for both PTHS and control lines. During NPC development, we identified 342 differentially expressed genes (DEGs, FDR <0.05) in PTHS lines with truncation mutations and 89 DEGs (FDR <0.05) in PTHS lines with point mutations. Of these DEGs, 67 genes overlapped between the two PTHS groups and these genes are enriched for biological processes associated with protein trafficking and ribosome function. Based on outcome of these results, we developed a Fluidigm panel of gene expression for NPC development to quickly validate and serve as a quality control assay of cortical differentiation in future experiments. Next we performed RNA sequencing on 8-week old cortical neurons and demonstrated that our differentiation protocol leads to neurons that have a similar transcriptional profile to those obtained from RNA sequencing of adult postmortem brain. We have validated this result with additional immunofluorescence labeling for layer specific markers, further demonstrating our 2D model system can produce a heterogeneous population of neurons similar to the human dorsolateral prefrontal cortex. Finally, we performed electrophysiology assays on PTHS neurons and identified a significant decrease in Na+ currents, but unfortunately, we were unable to measure the expression of our sodium channel target, Nav1.8, as it was not expressed in either PTHS or controls neurons. We are now in the process of scaling our stem cell based platform of cortical development for high throughput screening. The results of this grant was presented at several conferences and will eventually be published in a peer-reviewed scientific journal.

Michael Nestor, Ph.D.
The Hussman Institute for Autism
2017 DiscoveryAward - *Completed*
Award Amount: $343,845
Disease Target: Autism Spectrum Disorder

Establishing a 3D Based High-Content Screening Platform for Cellular/Phenotypes in Autism

The prevalence of Autism Spectrum Disorder (ASD) has increased significantly since the 1980's and as of 2018 has reached a prevalence of 1:59 in the United States. The costs for autism treatment are estimated to be approximately $2 million per person, and ongoing treatment represents a significant challenge for both the autistic individual and their caretakers. To date, there are no appreciable screening or therapeutic approaches that have had a significant impact on improving the lives of individuals with autism. One major reason for this is that ASD is a complex genetic disorder that results from the interaction of genetics with environmental factors. In addition, there are not well-developed animal models that can be used to recapitulate behavioral and cellular phenotypes observed in autism. Screening potential therapeutic compounds for treatments requires a robust human cell-based platform, and human induced pluripotent stem cells (hiPSCs) derived into cortical organoids are a promising technology that can be used to model aspects of autism while capturing the breadth of genetic mutations that may underlie ASD. The approach developed in this proposal has advanced a translational platform for phenotypic screening and the research products from this proposal have set the stage for a deeper understanding of the physiology and genetics of ASD. This funding has helped to develop a pipeline for screening therapeutics for autism from repurposed FDA-approved drugs and has the potential to positively impact the lives of individuals with autism. Imbalance in the excitatory and inhibitory (E/I) inputs in the brain is a hypothesis posited to explain some of the behavioral challenges associated with autism. E/I circuit development requires the interactions of diverse numbers of cells within the 3D environment of the developing brain. Therefore, it is paramount to use an iPSC-based autism model that recapitulates this complex environment. We have developed a 3D cortical organoid-like system based on a serum free embryoid body (SFEB). During the funding period we have developed and used an unbiased high-throughput screening approach to observe the expression of excitatory and inhibitory neurons within developing cortical-like circuits within SFEBs to test the E/I balance hypothesis in autism. In this study we used hiPSCs taken from 4 individuals with autism and 4 unrelated control individuals to create SFEBs. We screened SFEBs across several high-throughput cell-based assays for phenotypes that could provide insight into potential connections between the genetics and physiology of neural circuits within individuals with autism in our small cohort.

Linda Resar, M.D.
Johns Hopkins University
2017 Discovery Award - *Completed*
Award Amount: $345,000
Disease Target: Colitis

Piotr Walczak, Ph.D.
Johns Hopkins University
2017 Discovery Award - *Completed*
Award Amount: $345,000
Disease Target: Myelin

Developing Stem Cell Technology for Tissue Repair and Goblet Cells for Ulcerative Colitis Patients

The primary goal of this project was to develop stem cell therapy to regenerate colon tissue for patients with ulcerative colitis (UC). The lining of the small intestine and colon is among the most regenerative tissues, renewing itself every 3-5 days to protect the gut from bacterial pathogens and toxins. Adult stem cells located deep within the crypts are responsible for this remarkable capacity, although factors that govern their behavior are poorly understood. In recently published work, we discovered that the High Mobility Group A chromatin remodeling proteins are key epigenetic regulators that maintain intestinal stem cell number and function within the small intestine. Using innovative mouse and organoid models with overexpression or deficiency of Hmga1, we found that Hmga1 promotes epithelial regeneration in the small intestines. Our preliminary unpublished studies suggest that Hmga1 also promotes stem cell function, tissue repair, and goblet cell differentiation in the colon. Goblet cells are important because they secrete mucous to protect the intestinal lining from pathogenic invasion and inflammation. Notably, there is a decrease in goblet cells and their protective mucous in the colon of patients with UC, which leads to excessive inflammation, tissue damage, and the acquisition of genomic lesions that increase the risk for colon cancer. Our preliminary results indicate that enhancing Hmga1 expression improves colon-specific stem cell function and tissue regeneration in a dextran sulfate sodium model of UC. With MSCRF funding, we therefore sought to test the following hypotheses: 1) HMGA1 is a key developmental factor important in normal stem cell function and tissue repair, and, 2) Stem cell therapy to increase HMGA1 activity will improve colon-specific stem cell function, restore goblet cell number, and enhance tissue repair in UC. To test these hypotheses, we proposed the following Specific Aims: 1) To define molecular mechanisms through which HMGA1 promotes stem cell function, tissue repair, and goblet cell differentiation in colonic epithelium, and, 2) To determine whether Sendai virus can be used to engineer clinically safe, HMGA1-enriched, colon-specific organoids for personalized tissue regeneration in preclinical models of UC. Over the past 2 years, we generated unique, in vivo models to test the function of Hmga1 in stem cells and tissue regeneration, completed our proposed aims, and discovered a previously unknown role for Hmga1 in bone formation and repair.The impact of our Maryland Stem Cell Fund studies has been significant: 1) First, we discovered that increasing Hmga1 in colon stem cells enhances tissue regeneration and repair in a UC model; we propose to use our results from gene expression profiling to identify safe, therapeutic approaches to increase Hmga1 function in the colon for UC patients, 2) Second, we generated a novel model of human aging by engineering mice with global stem cell defects from Hmga1 deficiency, 3) We also found that these mice have poor bone formation, and, we plan to submit a new Maryland Stem Cell Fund grant to identify underlying mechanisms that could be harnessed to improve bone formation with aging, and, 4) Our gene expression data suggests that loss of Hmga1 cause premature cellular senescence; we therefore plan studies to identify strategies to maintain Hmga1 in aging tissues to mitigate aging phenotypes.

Inducing Immunotolerance of Myelinating Progenitor Cells Transplanted into the Brain of Immunocompetent Mice

The major goal of this project was to comparatively evaluate several strategies for immunoprotection of allotransplanted stem cells to identify technique that is the most effective and safe. Initially, we assessed utility of novel method for inducing immunotolerance with co-stimulation blockade. We found that short (6 days) treatment with agents blocking costimulatory signals (IgG anti-mouse-CD154mAb and IgG anti-CTLA-4) is sufficient for preventing immunological rejection over the entire study duration which was until 200 days after transplantation. This effect is impressive and with high level of certainty we interpret it as permanent immunological tolerance. While these observations are very encouraging it was very important to make sure that inflammatory event such as injury to the brain (traumatic brain injury=TBI) occurring late after transplantation when large amounts of allografted glial cells fully integrated, is not reactivating immune response as rapid rejection of large amounts of allografted cells could be life threatening. To address this question we transplanted glial progenitors with tolerance induction protocol and three months later subjected the mice to TBI using cortical impact model. Viability of transplanted cells was monitored non-invasively using BLI and lesion evolution with MRI. Interestingly after injury BLI signal increased indicating the cells proliferated. We monitored the mice over the period of one month and the signal remained high without evidence of rejection. In MRI TBI lesions were clearly detectable on T2w scans and neither the size nor the inflammatory markers were different between transplant and non-transplant groups. Interesting observation was that lesion size in myelin mutant shiverer was smaller compared to wild type control. This could be due to different mechanical properties of brain tissue in mutant mice or myelinspecific molecular cascades initiated by the injury. Behavioral studies revealed more exacerbated anxiety-like behavior in shiverer mice compared to controls with overallreduced exploratory activity compared to WT animals. There was no difference between transplanted and non-transplanted wild type mice indicating no detrimental effect by cell engraftment or costimulatory blockade. After TBI, anxiety-like behavior was increased for all groups but importantly, there was no statistically significant difference between transplanted and non-transplanted mice again indicating lack of detrimental effect of allografting. Histopathological assessment revealed excellent engraftment of glial progenitors and their integration in white matter structures. Histological data corresponds to imaging as there was increased number of cells in TBI injured comparedto control mice. Inflammatory response (infiltration by CD45+ cells) after injury was observed in all groups but interestingly that response was reduced in myelin mutants. There was no difference between costimulatory blockade and control mice. Overall, these results indicate that costimulatory blockade is excellent strategy for immunoprotection of allografted stem cells. Current clinical studies use pharmacological immunosuppression which is toxic to the patient, interfere with differentiation of transplanted stem cells and has to be continued throughout life leading to severe side effects. In contrast, costimulatory block requires only few injections just after transplantation, leads to long term tolerance and is safe. Tolerance is very robust and is not disrupted even by traumatic brain injury. This has important implications for patient care and with these drugs being clinically available translation potential of this stragety is very good.

Mingyao Ying, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger, Inc.
2017 Discovery Award - *Completed*
Award Amount: $345,000
Disease Target: ALS

Highly Efficient Conversion of iPS Cells to Motor Neurons and Oligodendrocytes by Synthetic Modified mRNAs

Spinal cord motor neurons (MNs) and oligodendrocytes (OLs) derived from human induced pluripotent stem cells provide unique cell resources for disease modeling, drug development and cell replacement therapy for neurological disorders, such as amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), and various demyelinating diseases. Our goals are to develop highly efficient strategies for MN and OL generation from iPSCs. Our goal in Aim 1 is to efficiently generate iPSC-derived MNs by using synthetic mRNAs to express two modified transcription factors (Olig2 and Ngn2), and to perform functional characterization of these mRNA-induced MNs (miMNs). The success of this aim will facilitate mechanistic studies of MN biology, and disease modeling and drug testing for various MN disorders. We compared transcription factor expression induced by various forms of synthetic mRNAs and identified the optimal forms of mRNAs for protein induction. Next, we tested various differentiation strategies and found that Olig2-3SA plus Ngn2-SA mRNAs induces the most efficient MN differentiation as determined by neuron yield and the expression of MN markers. We successfully established a 3-day protocol for generating highly pure MNs. This protocol is more efficient than traditional methods using chemical compounds to induce MNs from iPSCs. We also studied other functions of miMNs, e.g. neuromuscular junction formation and engraftment in vivo. In transcriptome analysis, our miMNs showed similarity to other human MNs. A manuscript reporting this novel mRNA-driven strategy for MN generation is in preparation. We will disclosure this invention to the Johns Hopkins Technology Ventures, and will be prepared to file a provisional patent application. Our goal in Aim 2 is to efficiently generate iPSC-derived oligodendrocytes by using synthetic mRNAs to express two modified transcription factors, and to perform functional characterization of these mRNA-induced OLs. We have identified the optimal forms of mRNAs for high and stable protein expression of these transcription factors. We have identified a defined combination of transcription factors to generate mature OLs. Our on-going experiments are studying the functions of these mRNA-induced OLs to determine the similarity of these stem-cell-derived OLs to human primary OLs. We have achieved half of the milestones proposed in Aim 2, and we will optimize the condition for OL maturation. If our on-going experiments are successful, we anticipate publishing this novel OL differentiation strategy in 6 months and also disclosure this invention. We established novel synthetic-mRNA-driven strategies for generating iPSC-derived MNs and OLs in high purity and with validated functions. These miMNs and miOLs have wide applications in mechanistic studies, drug testing and cell replacement therapy for various neurological disorders. Our innovative strategy using synthetic mRNAs will also provide a solid foundation for generating hard-toachieved lineage-specific progenies from human stem cells. We will translate new inventions from this project into products, such as mRNA-induced MN and OL differentiation kit, and ready-to-use iPSC-derived MNs and OLs. Overall, inventions from this project will facilitate the production of MNs, OLs and likely other functional cells from human stem cells, and benefit biotechnology in Maryland.

Qin Bian, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award - *Completed*
Mentor: Patrick Cahan, Ph. D.
Award Amount: $130,000
Disease Target: Osteoarthritis

Direct Specification of Articular Chondrocytes from iPSC-Derived Lateral Plate Mesoderm

Human pluripotent stem cells (hPSCs) represent a potentially unlimited source of autologous cells to treat and model degenerative diseases such as osteoarthritis (OA). While human induced pluripotent stem cells (iPSC) can give rise to all lineages as determined by teratoma formation assays, the ability to directly differentiate them to selected lineages with fidelity and efficiency has proven challenging. Despite the promise of iPSC-derived articular chondrocytes (AC) for modeling and treating OA, there are relatively few directed differentiation protocols purporting to generate PSC-AC. Moreover, the molecular fidelity of the current PSC-AC has not been comprehensively assessed, and their in vitro and in vivo functional properties are not commensurate with native AC. To address these issues, I proposed the following specific aims.

Aim 1: Improve molecular and functional characteristics of PSC-AC through inducible ectopic expression of superficial specific transcription factors (TFs) in PSC-derived lateral plate mesoderm (LPM).

Aim 2: Augment a network biology based computational platform, CellNet, so that it can assess and suggest improvements for engineering PSC-AC.

To identify the superficial specific TFs to be ectopically expressed in PSC-LPM, I performed a study to determine the specific TFs expressed in the articular cartilage development. In detail, I generated single cell RNA sequencing data of knee joints from Gdf5 lineage tracing (GDF5Cre::R26EYFP) male mice at embryonic stages of E12.5, E13.5, E14.5, E15.5 and postnatal early AC maturation stage: Day 5. Gdf5Cre+ cells give rise to most components of the synovial joint, including articular chondrocytes. By removing non-Gdf5 lineage clusters caused by ectopic expression, including muscle cells, immune cells, neural crest cells, and endothelial cells, we identified five super clusters corresponding to successive waves of joint development. Three of these clusters are especially relevant to my project as the detail the specification of permanent chondrocyte progenitors, and their subsequent differentiation. While this stage of the project has taken longer than anticipated, it has identified candidate TFs that contribute to early chondrogenesis, interzone formation, joint cavitation, and early AC maturation. Thus, these discoveries are the foundation for completion of Aim 1. The single cell CellNet is recently published by our lab (Cell systems 2019) which generates most computer software we used for data analysis in our study (Completion of Aim2). Our single-cell transcriptome atlas of developmental synovial joint will boost the fields collective knowledge of joint biology and provide informative clues for OA prevention and treatment in future.

Dongwon Kim, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award - *Completed*
Mentor: Luis Garza, Ph.D.
Award Amount: $130,000
Disease Target: Skin Disease

Postdoctoral Training Towards Independence: Testing of Skin Stem Cells to Modify Skin Identity

The practical goal for this grant is to createectopic volar skin (palm or sole) at the stumpsite of amputees, which leads to theimprovement and enhance of the quality oflive for amputees. To accomplish our goal, wewill transplant autologous fibroblasts stemcells from volar skins into non-volar skin areasand investigate the conversion of skin identity by evaluating KRT9 expression, epidermal thickness, and othervolar specific gene expressions. To accomplish this project, we tested different cellnumbers to increase KRT9 expression using mousemodel before human clinical study.

Specific Aim #1 (Clinical trial); We will perform aclinical trial for gentle curettage of recipient skin to improve autologous human volar fibroblasts induction ofvolar epidermal gene expression.

Specific Aim #2 (Lab efforts); Skin biopsies from Aim#1 will be used for RNA-sequencing to discover uniquemolecules to test ideal degree of epidermal damage for optimum volarization.

Optimized incubation time for imaging ofluciferase positive cells; 10 minutes aftertransplantation. Optimized media for fibroblast stem cell culturingfor transplantation comparing DMEM vs FGM; Cellsgrown in FGM (Fibroblasts growth medium) showbetter engraftment. Tested the numbers of cells from 102 to 106; 106cell injection is optimum, but still with considerable cell signal loss. Multiple injection increase cell survivability compared to single injection. Epidermal thickness is increased after multiple injection of volar FB compared tosingle injection. Collagen with volar fibroblasts increase epidermal thicknes and Krt9 expression. The major experimental goal of this project is to optimize conditions for cell engraftment before human clinicaltrials. Since there is no one studying about this project, we have spent time in examining many parameterssuch as incubation time, cell numbers, injections, and materials. Although there are many things to check andwe are still evaluating critical factors, we believe that we provided essential data to start human clinical trials,which we are currently testing based on the results from this project.

Josephine Lembong, Ph.D.
RoosterBio, Inc.
2017 Post-Doctoral Fellowship Award - *Completed*
Mentor: Jonathan Rowley, Ph.D.
Award Amount: $130,000
Disease Target: Vascular/Bone Disease

Stem Cell Expansion and Differentiation in Bioreactors via Coupling of Substrate Curvature and Shear Stress

The objective of this project is to understand the interactions between human mesenchymal stem / stromal cell (hMSC) expansion, differentiation, and various mechanical cues imposed by their substrate microenvironment. From this understanding, our goal is to create an improved ex vivo culture of stem cells that would enhance the production of large numbers of phenotypically desirable stem cells to facilitate stem cell research and potential regenerative medicine therapies in the future. In order to do this, we developed a dynamic perfusion bioreactor, which have been shown to be beneficial for cell proliferation and differentiation due to the control over environmental parameters, e.g. shear stress imposed on the cell surface, media flow, and oxygen distribution; and studied in details the effect of substrate curvature, surface area, and shear stress on the growth and differentiation of hMSCs in the bioreactors. The major goals/objectives/aims of this project are: 1) Investigate the interaction between hMSC growth, differentiation, substrate curvature, and flow. 2) Produce co-cultures with spatially controlled populations of hMSCs and ECs to optimize vascularization and osteogenesis. Our main accomplishments consist of a successful development of a three-dimensional (3D)-printed fluidic bioreactor chamber for dynamic stem cell culture, with emphasis on control over flow and substrate curvature in a 3D environment, two physiologic features of native tissues. Due to progress in Computer-Aided Design (CAD) and 3D printing, modulation of geometry and fluid dynamic environment in these chambers can be done easily, therefore these cambers can be fabricated with specific geometry in mind for creating tissue model of stem cell environments with particular physiologic features, in addition to providing enhanced proliferation and osteogenic differentiation of hMSC. Our chamber geometry, consisting of an array of vertical cylindrical pillars, facilitates actin-mediated localization of human mesenchymal stem cells (hMSCs) within ~200 mm distance from the pillars, enabling spatial patterning of hMSCs and endothelial cells in cocultures and subsequent modulation of calcium signaling between these two essential cell types in the bone marrow microenvironment. Flow-enhanced osteogenic differentiation of hMSCs in growth media imposes spatial variations of alkaline phosphatase expression, which positively correlates with local shear stress. Proliferation of hMSCs is maintained within the chamber, exceeding the cell expansion in conventional static culture. The capability to manipulate cell spatial patterning, differentiation, and 3D tissue formation through geometry and flow demonstrates the culture chamber's relevant chemo-mechanical cues in stem cell microenvironments, thus providing an easy-to-implement tool to study interactions among substrate curvature, shear stress, and intracellular actin machinery in the tissue-engineered construct. This study highlights the culture chamber's potential as a simple innovative dynamic culture system for creating an in vitro model of a physiologic stem cell microenvironment, which can be easily modulated, scaled up and integrated into large-scale perfusion systems for relevant stem cell applications, or utilized to study various cell-cell and cell-substrate interactions. Such knowledge will be beneficial for future developments of bioreactors or scaffolds for tissue engineering and regenerative medicine applications. For example, our bioreactor can be used to co-culture hMSCs and endothelial cells (ECs) with spatial configurations that will optimize the osteogenic and angiogenic potential of the cells. Such knowledge will be applicable in designing grafts with such spatial control that allows prevascularization of large tissue engineered bone constructs which is crucial prior to patient implantation.

Joseph Mertz, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award - *Completed*
Mentor: Donald Zack, MD, Ph.D.
Award Amount: $130,000
Disease Target: Glaucoma

Proteomic Approaches to Study Cell Death Mechanisms in Human Stem Cell-derived Retinal Ganglion Cell

The two primary scientific aims for which the MSCRF so graciously awarded me a post-doctoral fellowship were to A) characterize the proteome and phosphoproteome of human stem cell derived retinal ganglion cells (hRGCs) during axon injury and pharmacological neuroprotection targeting the MAP triple kinase DLK, and B) to develop targeted mass spectrometry assays for monitoring the MAP kinase pathway at high sensitivity. Regrettably, the second aim was largely unsuccessful. Our first target was a region of the JNK protein with two phosphosites that are commonly used as an indicator for MAPK signaling. I was able to develop reproducible assays targeting this region with all four combinations of phosphorylation state, at limits of detection (LOD) and lower limits of quantification (LLOQ) matching standards in the field. I was, however, unable to detect these species in our hRGC model. After unsuccessfully troubleshooting this by increasing protein input amounts, varying protein extraction strategies, trying different mass spectrometry parameters, and trying an alternate mass spectrometry approach on a different mass spectrometer ('parallel reaction monitoring' as opposed to the 'multiple reaction monitoring' used in the experiments pictured in Fig 1), my mentor and I decided to put this aim on hold. The first aim saw much greater success. Following the design I proposed in our submission to MSCRF, I differentiated approximately 100 million hRGCs and treated them for thirty minutes with 5 conditions: vehicle control, the microtubule disruptor colchicine, colchicine plus a broad spectrum kinase inhibitor VX-680 (Vertex) which has activity against DLK, and colchicine plus a more selectively DLK targeting kinase inhibitor C14 (Genentech). The concentration of colchicine was optimized to kill approximately 90% of unprotected hRGCs at 48hrs and the kinase inhibitor concentrations were optimized to reduce this cell death to approximately 10%. I extracted protein from these samples and labeled them using isobaric tandem mass tags (TMT, Thermo Scientific), enriched phosphopeptides using iron metal affinity chromatography (Fe-IMAC), and analyzed the whole proteome and phosphoproteome separately on the state of the art Orbitrap Fusion Lumos tribrid mass spectrometer (Thermo Scientific). I was able to quantify over 9,000 proteins and 9,000 phosphosites with >75% probability of phosphate assignment, both representing the largest datasets of their kinds in RGCs of any species. Among these, I observed extensive coverage of the MAPK pathway. From our data, I was able to determine meaningfully changed proteins and phosphosites by combined fold change and statistical significance thresholds . With focus on the changes caused by C14 because of its selectivity against DLK, I have been able to extract a strong candidate list for DLK substrates with protective capabilities. This is valuable for determining the mechanism of DLK, which is well established as neuroprotective, and it has guided further experiments and ongoing grant proposals with potential to develop new neuroprotective treatment strategies for glaucoma and other optic neuropathies.

Nikhil Panicker, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award - *Completed*
Mentor: Ted Dawson, MD, Ph.D.
Award Amount: $130,000
Disease Target: Parkinson's / Neurodegenerative Diseases

Activation of the NLRP3 Inflammasome in Human Dopamine Neurons as a Consequence of Parkin Dysfunction

Parkinson's disease (PD) is a neurodegenerative disease characterized by the selective death of dopamine-producing neurons in the ventral midbrain. The molecular mechanisms through which these neurons die is an active area of investigation. We discovered that the Nod like receptor protein-3 (NLRP3) inflammasome, a multiprotein complex known to be present in immune cells was activated in dopamine neurons that lacked Parkin, a PD-associated protein. Parkin is an E-3 ligase, a protein that tags its substrates for degradation in the cell. The major aim of this project was to explore the mechanisms of neuronal NLRP3 inflammasome activation upon Parkin dysfunction. As stated in Aim-1, we conclusively demonstrated mitochondrial-derived reactive oxygen species (mitoROS) generation in Parkin deficient dopamine neurons, which is required for inflammasome assembly. We showed neuronal inflammasome activation in postmortem PD brains to buttress our findings in cultured human dopamine neurons. As described in Aim-2, we proved that We discovered that NLRP3 is a ubiquitination substrate of Parkin, which is why it accumulates in the absence of Parkin. We also discovered that loss of Parkin in neurons also contributes to inflammasome activation by accumulation of another of its substrates, PARIS, a protein that facilitates loss of mitochondrial quality control and mitoROS generation. Hence, the absence of Parkin creates a perfect storm brought on by the loss of its E-3 ligase activity- accumulation of NLRP3 primes the NLRP3 inflammasome, and accumulation of PARIS activates the primed NLRP3 inflammasome. The resulting NLRP3 inflammasome activation contributes to neuron death in PD models. Finally, as stated in Aim 3, we inhibited the NLRP3 inflammasome in vitro and in vivo, preventing dopamine neuron death in PD models. Parkinson's disease is a common neurodegenerative disorder with ~1 million Americans patients as of 2015. all existing drugs provide symptomatic relief but do not halt or slow down the progression of the disease. We discovered that the NLRP3 inflammasome, a complex shown to be assembled in immune cells is activated in dysfunctional dopamine neurons. The mechanisms and consequences of neuronal NLRP3 inflammasome activation have never been described before, and we believe that exploring this pathway may hold significant translational potential in developing novel drugs that might halt or slow the progression of PD.

Marco Santoro, Ph.D.
University of Maryland - College Park
2017 Post-Doctoral Fellowship Award - *Completed*
Mentor: John Fisher, Ph.D.
Award Amount: $130,000
Disease Target: Vascular Disease/Angiogenesis

Development of Tissue-Engineered Vascularized Scaffolds via 3D Printing of Endothelial/Stem Cells

In this proposal we sought to understand the mechanism of vasculature formation within scaffolds fabricated via extrusion-based bioprinting. This technique holds great promise in tissue regeneration as it allows to pattern different cell populations (in this case a vascular network) similarly to what observed in the native organ. In particular, we seek to study the interplay between endothelial cells (ECs, the primary cells forming blood vessels) and either mesenchymal stem cells (MSCs) or mechanical stimuli. Both these factors have been shown to promote vasculature formation in previous studies. Optimal criteria for scaffold design (e.g. EC:MSC ratio, shear levels) would be then used to fabricate a large scaffold to be implanted in a rat animal model for validation and formation of functional vascular network. Results: our studies elucidated how ECs and MSCs crosstalk and reconciled the disagreement present in literature, where MSCs were found to both promote as well as hamper vasculature formation (i.e. angiogenesis). Specifically, we demonstrated that blood vessel formation depends on both the EC:MSC ratio and time. MSCs hinder angiogenesis early on (<2days) but promote it within a week of coculture. Using this information we were able to bioprint large scaffolds using a cell-laden material side-by-side with a hard polymer to provide structural support. Overall, in this project we completed the following aims (as stated in the proposal):

Specific Aim 1: Understanding the collaborative effects of MSC signaling and mechanical stimuli upon angiogenesis and arteriogenesis in a 3D EC:MSC coculture system

Specific Aim 2: Fabrication of a multiphase scaffold that hosts a functional interconnected vascular network. Due to delay in approval of animal protocol as well as unforeseeable technical difficulties in the bioprinting of large scaffolds amenable for animal testing, we did not complete the following aim:

Specific Aim 3: Formation of a fully functional vascularized scaffold in vivo.

Tissue engineers seek to develop implantable scaffolds of clinically relevant size in order to address the shortage of organ donors. Yet, the presence of a vascular supply is essential in such large scaffolds to maintain cell viability. In this proposal we sought to address this critical challenge by combining the use of endothelial/stem cells with the versatility of 3D printing (3DP) technology to fabricate scaffolds hosting a primitive vascular network that can fully develop in vivo. Correspondingly, the proposed research will advance the clinical treatment of virtually all those conditions where a severe tissue defect is present, regardless of how the tissue defect originated. Importantly, the successful realization of the proposed research will accelerate and encourage the use of stem cells into new medical therapies. The proposed system makes use of established materials extensively used in other FDA-approved devices and of autologous cells. Accordingly, we envision that the proposed stem cell-3DP system would be readily applicable to human patients after preclinical safety testing in vitro/in vivo, as per FDA guidelines.

Congshan Sun, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award - *Completed*
Mentor: Gabsang Lee, Ph.D.
Award Amount: $130,000
Disease Target: Duchenne Muscular Dystrophy (DMD)

hiPSC Based Compound Screening for Treatment of Duchenne Muscular Dystrophy (DMD)

Duchenne muscular dystrophy (DMD) is an X-chromosome linked disease that affects 1 in 3500 boys worldwide. The disease is caused by mutation in the DYSTROPHIN gene whose protein anchors muscle cells to extracellular matrix. The main aim of this project is to identify potential small molecule compounds that can ameliorate DMD-related phenotype in the in vitro system. The resulting compounds will include both stop codon 'read-through' compounds as well as other compounds that have beneficial effect for myoblast fusion e.g. DYSTROPHIN protein and/or enhancing myotube formation. Therefore, we are in hope to search for drugs that can treat DMD patients with different mutations. We have recently developed a novel system to differentiate DMD-hiPSC into myoblasts in chemically defined conditions that is free from animal feeder cells, serum or growth factors (Choi et al., 2016). This differentiation protocol involves plating single hiPSCs on defined extracellular matrix material and growing them for 25-30 days in serum-free medium with temporal activation of WNT and inhibition of NOTCH pathways. At day 25-30, myoblasts can be purified by NCAM+/HNK1- cell surface markers. One distinguishable DMD disease phenotype reproducible in vitro with hiPSC-derived myoblasts is the deficiency in myoblast differentiation and fusion.

1) We designed a high content imaging-based screening platform to identify compounds that can correct DMD hPSC-derived myoblasts fusion defects. After performing tiered screening with the Johns Hopkins Clinical Compound Library, two final hit compounds were selected and further studied to elucidate. 2) We studied their mechanism of action with microarray and found out the pathways those two compounds affect in myoblasts. 3) We subsequently tested pre-clinically in mdx mice, demonstrating their effectiveness and therapeutic potential.

Overall, here we performed the first comprehensive study utilizing DMD hiPSC-derived myoblasts, and demonstrate that its feasibility as a platform to identify potential drugs that could be used to treat DMD. Boys who are born with DMD suffer from loss of ambulation in early teenage years. Their life expectancy is 20-40 years. The development of new drugs is very costly, involving the investment of great sums of capital and time. For orphan diseases such as Duchenne muscular dystrophy (DMD), progress in new drug development has been especially limited. Compared with the upcoming gene and cell therapies, pharmacological therapy is less costly, more readily to apply and has shown promises in studies. Therefore, we developed a drug screening platform to seek pharmacological therapy utilizing DMD patients' hiPSC (human induced pluripotent stem cell)-derived myoblasts. Firstly, hiPSCs compared with the primary cell lines or model animals can provide unlimited material for screening. Secondly, the differentiated myoblasts have identified DMD disease phenotype. Therefore, this study advances drug discovery for DMD as well as other rare diseases.

Aline Thomas, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award - *Completed*
Mentor: Jeff W.M. Bulte, Ph.D.
Award Amount: $130,000
Disease Target: Multiple Sclerosis (MS)

Development of Stem Cell Therapies for Multiple Sclerosis using Non-Invasive Biomarkers

In multiple sclerosis (MS) the insulating layer that protects nerves, myelin, is damaged during immune attack resulting in loss of nerve function and disability. The main aim of this project is to stop MS progression by reducing the immune attack and repairing the damaged myelin using transplanted glial progenitors (GPs). We also aim to noninvasively track these cells and to monitor their therapeutic effect using magnetic resonance. We evaluated the dependence of GP survival and therapeutic benefit on transplantation site location using the EAE animal model for MS. We transplanted GPs at the lateral ventricles, wherein the cerebrospinal fluid can aid the circulation of GPs and beneficial factors. We also transplanted GPs in the motor cortex, wherein the signals from regions of the brain and spinal cord are integrated to coordinate movement. We demonstrated that for both transplantation sites, the survival of GPs is transient, yet attenuated the paralysis observed in the model. We used relatively new techniques known as chemical exchange saturation transfer (CEST) MRI and magnetic resonance spectroscopy (MRS) to determine the location of GPs and their therapeutic benefit after transplantation. We evaluated several brain regions proximal and distal to the transplantation site. We observed using MRI that GPs would reduce pathological changes up to 2 mm away from the transplantation site. Histology confirmed the presence of GPs and the reduction of injury at a brain structure near the transplantation site that was indicated using MRI. Multiple sclerosis impacts nearly one million Americans. Evidence has emerged that diffuse injury causes disability to a greater extent than lesions. Lesions are clinically monitored using conventional MRI; however, diffuse injury is "invisible" to these imaging methods. We demonstrated that CEST MRI can identify brain structures damaged during immunological attack, that GPs can protect these structures from that damage, and that CEST MRI can detect the structures protected by GPs. This imaging strategy is compatible with conventional MRI and can readily be implemented in hospitals without the need for additional equipment. Furthermore, CEST MRI can be used to evaluate other candidate therapies, which can aid treatment decisions.

Zhao Wei, Ph.D.
Johns Hopkins University
2017 Post-Doctoral Fellowship Award - *Completed*
Mentor: Sharon Gerecht, Ph.D.
Award Amount: $130,000
Disease Target: Vascular Disease

3D Printing Vascularized Cardiac Constructs

Heart failure including ischemia and subsequently myocardial infarction occurs when the blood supply to heart is impeded by damaged vasculature. The capability to manufacture three-dimensional (3D) vascularized myocardial constructs that replicate the native vascularized cardiac tissue would enable multiple advanced applications in solving the current impact of cardiac dysfunctions, such as in vitro disease modeling, drug screening and tissue repair and regeneration. The blood vessels in the heart are complex and highly organized branched and hierarchical trees with progressively reduced diameter branches of larger roots of main arteries. This multifaceted architecture presents a challenge to fabricate in vitro by available engineered approaches.

Our project targets to develop a 3D myocardial construct with highly organized vessels in vitro. To this end, two pivotal components are required including cell source and the selection of biomaterials as cell scaffolds. ECFCs and Human induced pluripotent stem cells (hiPSCs) which possess the ability to differentiate into both cardiomyocytes, are used as the stem cell source in building the 3D cardiovascular patches. The specific objectives have been completed across the three aims of this Project are: In Aim 1, we developed a novel gelatin-based hydrogel possessed physical and bioactive properties that suitable for both vascular network formation and cardiomyocytes maturation. Aim 2 is to separately encapsulate ECFCs and hiPSC-cardiomyocytes in our hydrogels and track tissue formation of the encapsulated cells. In Aim 3, we co-cultured ECFC and iPSC-derived cardiomyocytes in our hydrogel to obtain vascularized myocardial constructs. The results of the three aims mentioned above have been completed, indicating progress towards cardiovascular engineering. This engineered cardiovascular model will also advance our knowledge about blood vessel assembly and cardiac functions during regeneration, help us translate the knowledge into clinical applications. By using this 3D vascularized cardiac construct, we will be able to build a disease model of myocardial infarction in vitro, testing and screening different drugs or other therapeutics in preclinical studies. Moreover, transplantation of this 3D engineered vascularized cardiac construction provide an opportunity to improve the clinical treatment of the cardiac dysfunctions instead of directing delivering cells in vivo. In addition, for severe heart failure, the establishment of our construction will also offer a potential to fabricate an artificial heart for replacing the severe damaged or diseased heart within patients.


MSCRF
Maryland Stem Cell Research Fund