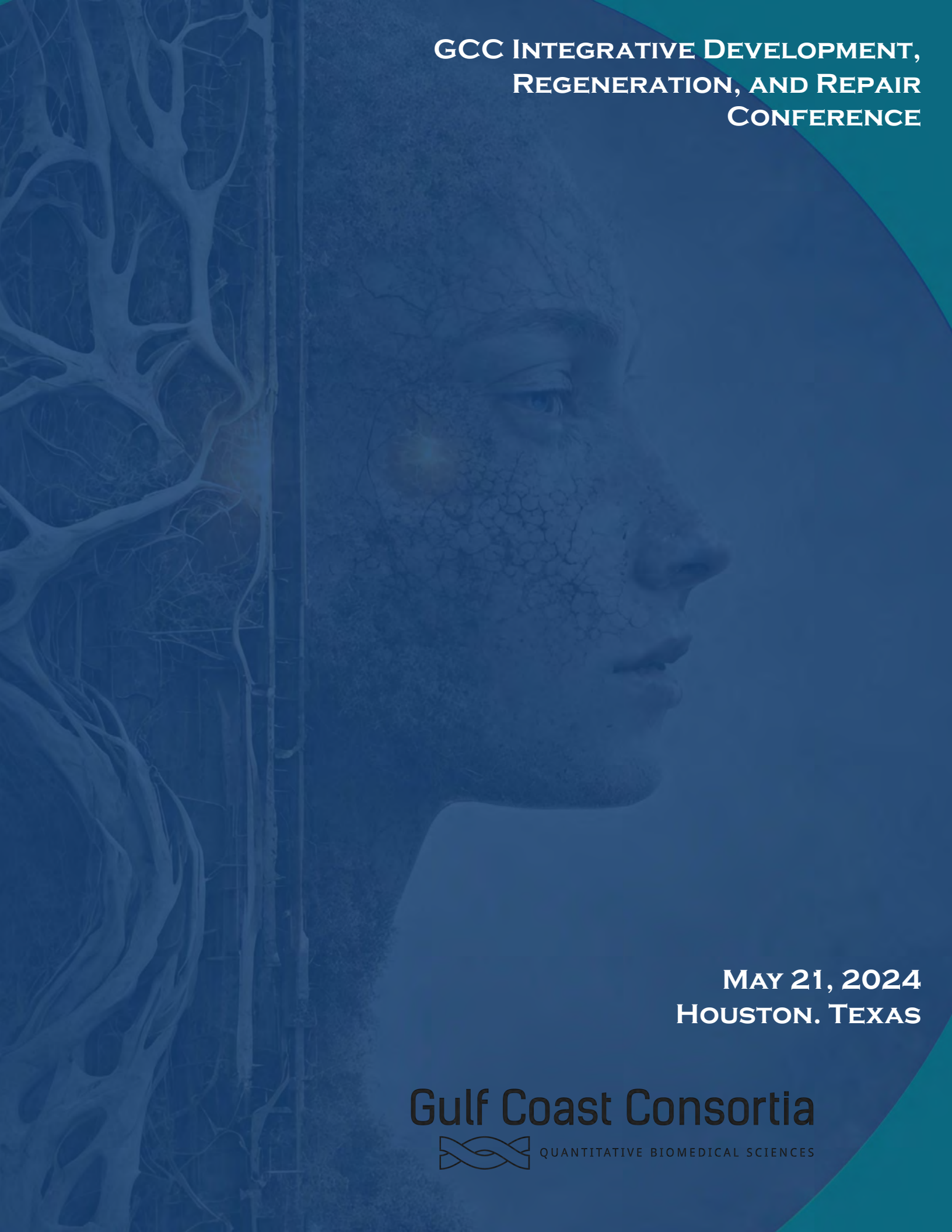

GCC Integrative Development, Regeneration, and Repair Conference

May 21, 2024
Houston. Texas

Gulf Coast Consortia
QUANTITATIVE BIOMEDICAL SCIENCES

Gulf Coast Consortia
QUANTITATIVE BIOMEDICAL SCIENCES

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Agenda

8:30 Breakfast and poster set-up

8:50 Welcoming remarks
George Eisenhoffer, MD Anderson Cancer Center
Phil Horner, Houston Methodist Research Institute

Session 1: Inflammation, Fibrosis, and Immunobiology
Conveners: **George Eisenhoffer**, MD Anderson Cancer Center
Phil Horner, Houston Methodist Research Institute

9:00-9:25 *Using the Salamander to Guide Latent Tissue Regeneration in Mammals: Understanding Nerve-Immune Signaling Controlling Fibrosis and Regeneration Potential*
James Godwin, Mount Desert Island Biological Laboratory (MDIBL) and Jackson Laboratories

9:25-9:50 *Immune-Epithelial Crosstalk in Tissue Adaptation and Maladaptation*
Piotr Konieczny, New York University Grossman School of Medicine

9:50-10:15 *How to Tune the Immune System Towards Tissue Restoration*
Francesca Taraballi, Houston Methodist Research Institute

10:15 Break

Session 2: Aging and Rejuvenation
Conveners: Rachel Arey, Baylor College of Medicine
Robert Krencik, Houston Methodist Research Institute

10:30-10:55 *Exercise Hormone Irisin Protects Against Alzheimer's Disease in 3D Culture*
Se Hoon Choi, Harvard Medical School

10:55-11:20 *Neuroprotection on the Treadmill: Exercise, Brain Aging and Alzheimer's Disease*
Constanza Cortes, University of Southern California

11:20-11:45 *Discovering a Model for Organismal Rejuvenation in an 'Immortal' Flatworm*
Blair Benham-Pyle, Baylor College of Medicine

11:45-1:15 Lunch and poster session (Event Hall)
11:45-12:15 Lunch
12:15-1:15 Poster session

Session 3: Tissue Engineering, Cell, and Organ Transplantation
Conveners: Cindy Farach-Carson, University of Texas Health Science Center Houston
Doris Taylor

1:15-1:40 *Regenerative Therapeutics: From Discovery to Product to Market*
Caralynn Nowinski Collens, CEO of Dimension Inx

1:40-2:05 *Regulated Progress: The University of Maryland Cardiac Xenotransplantation Experience*
Andrew Tully, University of Maryland School of Medicine

2:05-2:30 *Integrating Imaging and Scaffold Design to Enhance Musculoskeletal Regeneration*

Warren Grayson, Johns Hopkins University

Session 4: Developmental Mechanisms in Regeneration
Conveners: Ross Poche, Baylor College of Medicine
Amy Sater, University of Houston

2:30-2:55 *Decoding the Metabolic Requirements for Appendage Regeneration*
Andrea Willis, University of Washington

2:55-3:20 *Hippo-Signaling in Cardiac Regeneration*
Jim Martin, Baylor College of Medicine

3:20-3:45 *Regeneration of Rod Photoreceptors in a Zebrafish Model of Chronic Retinal Degeneration*
John O'Brien, Univ of Houston

3:45 Break

Session 5: Engineering Tools and Methods
Conveners: Dan Harrington, University of Texas Health Science Center Houston
Feng Zhao, Texas A&M University

4:00-4:25 *Engineering Biomaterials and Millifluidic Systems for Investigating Enteric Infections*
K. Jane Grande-Allen, Rice University

4:25-4:50 *Pumps, Pipes and AI: Engineering Vascular & Cancer Medicine with Organ-Chips*
Abhishek Jain, Texas A&M University

4:50-5:15 *Engineering Systems-Level Sensorimotor Plasticity Through Co-Adaptive Neural Interfaces*
Amy Orsborn, University of Washington

5:15-6:15 Final remarks followed by reception (reception is in the event hall)

Presenters in alphabetical order

Blair Benham-Pyle, MD, PhD

Assistant Professor
Molecular and Cellular Biology
Baylor College of Medicine

Discovering a Model for Organismal Rejuvenation in an 'Immortal' Flatworm

Blair Benham-Pyle is an Assistant Professor of Molecular and Cellular Biology at Baylor College of Medicine, with appointments in the Stem Cell and Regenerative Medicine Center and Dan L. Duncan Comprehensive Cancer Research Center. Prior to joining the faculty at Baylor College of Medicine, Blair received a joint B.S./M.S. in Biochemistry and Biophysics from Yale University and a PhD in Cancer Biology from Stanford University. While at Stanford, she worked with W. James Nelson and Beth Pruitt to study how cell-cell adhesions translate mechanical force into cell behavior. She then moved to the Stowers Institute for Medical Research, where she complete her postdoctoral work with Alejandro Sánchez Alvarado studying mechanisms by which post-mitotic cells regulate regeneration, scaling, and behavior. Highlights from her postdoctoral work include the first molecular dissection of asexual reproduction behavior in planaria, a single cell atlas of planarian regeneration that identified transient regeneration-activated cell states (TRACS), and a spatial atlas of planarian tissue that identified candidate stem cell microenvironments. Dr. Benham-Pyle joined Baylor College of Medicine as an Assistant Professor in 2022. Her research group (benhampylelab.org) leverages the remarkable biology of planarian flatworms to study stem cells, regeneration, aging, and cancer resistance.

Se Hoon Choi, PhD
Assistant Professor
Harvard Medical School
Exercise Hormone Irisin Protects Against Alzheimer's Disease in 3D Culture

Se Hoon Choi, PhD, is an Assistant Professor of Neurology at Massachusetts General Hospital (MGH) and Harvard Medical School. Dr. Choi received his PhD in Neurobiology under Dr. Sangram Sisodia at the University of Chicago. He joined Dr. Rudolph Tanzi's lab at MGH and Harvard Medical School for his post-doc training. In the pursuit to understand Alzheimer's disease (AD), his current overall research objectives are to explore the roles of adult-generated neurons (adult hippocampal neurogenesis) in AD, to develop three-dimensional (3D) human neural cell culture models with application to AD in order to reveal its pathological mechanisms and find novel therapeutic targets, and to uncover the molecular mechanisms linking exercise hormone irisin to neuroprotection in AD. As his ultimate goal, he aims to identify novel, alternative druggable targets to treat cognitive decline in aging and neurodegenerative diseases.

Constanza Cortes, PhD
Assistant Professor
Gerontology
University of Southern California

Neuroprotection on the Treadmill: Exercise, Brain Aging and Alzheimer's Disease

Dr. Cortes obtained her PhD at the University of Chicago and has been working in the field of neurodegenerative diseases during her entire career. Her lab focuses on exercise as a novel intervention against neurodegenerative diseases and brain aging, focusing on the muscle-to-brain axis and circulating factors that can modify disease progression. Her research is funded by the National Institute of Aging, the Alzheimer's Association and the American Association for Parkinson's Disease.

James Godwin, PhD
Assistant Professor
Mount Desert Island Biological Laboratory and the Jackson Laboratory

Using the Salamander to Guide Latent Tissue Regeneration in Mammals: Understanding Nerve-Immune Signaling Controlling Fibrosis and Regeneration Potential

Dr James Godwin is an assistant professor with a dual appointment at both the Mount Desert Island Biological laboratory (MDIBL) and the Jackson Laboratory (Jax) in Maine USA where he studies the gulf in regenerative potential between salamanders and mice. His focus is on the role of the immune system and its interactions with nerve signaling required for functional regeneration and regulation of scarring. He performed his postdoctoral training in the laboratory of Professor Nadia Rosenthal at the Australian Regenerative Medicine Institute (ARMI) using mouse and salamander regeneration models after postdoctoral training with Professor Jeremy Brockes at University College London (UCL) studying salamander limb regeneration. His PhD was earned in clinical immunology at the University of Melbourne, and St Vincent's Hospital Australia with Professor Peter Cowan and his undergraduate studies at University of Melbourne.

Jane Grande-Allen, PhD
Cameron Professor
Bioengineering
Rice University

Engineering Biomaterials and Millifluidic Systems for Investigating Enteric Infections

Jane Grande-Allen is the Isabel Cameron Professor of Bioengineering at Rice University. She also serves as Associate Dean for Faculty Development in the School of Engineering. Her research group investigates the structure-function-environment relationship of soft connective tissues through bioengineering analyses of the extracellular matrix and cell mechanobiology, with a focus on cardiovascular and intestinal diseases, as described in >180 peer-reviewed publications. Dr. Grande-Allen received a BA in Mathematics and Biology from Transylvania University in 1991 and a PhD in Bioengineering from the University of Washington in 1998. After postdoctoral research in Biomedical Engineering at the Cleveland Clinic, she joined Rice University in 2003 and was promoted to full professor in 2013. Dr. Grande-Allen is a Fellow of AIMBE, IAMBE, BMES, AAAS, AHA, and the Society for Experimental Mechanics. She served on the BMES Board of Directors and Executive Board from 2009-2022 and was BMES Secretary from 2020-2022. Dr. Grande-Allen also serves on the research committee for the American Heart Association.

Warren Grayson, PhD
Professor, Vice-Chair of Faculty Affairs
Biomedical Engineering
Johns Hopkins Univ.
Integrating Imaging and Scaffold Design to Enhance Musculoskeletal Regeneration

Dr. Warren Grayson is a Professor, Vice-Chair of Faculty Affairs in the Department of Biomedical Engineering at Johns Hopkins University. Dr. Grayson is also a founding member and the current Director of the Translational Tissue Engineering Center – a multidisciplinary center that houses nine labs with close to 150 researchers. His Laboratory for Craniofacial and Orthopaedic Tissue Engineering focuses on developing advanced therapeutics for the regeneration of bone and skeletal muscle.

Dr. Grayson obtained his B.Sc. in Chemical & Process Engineering at The University of the West Indies (Trinidad), his Ph.D. in Biomedical Engineering from Florida State University, and completed his postdoctoral training at Columbia University in New York. Dr. Grayson's work on bioreactor design and engineering anatomically shaped bone grafts received national and international coverage in various news agencies including the New York Times, BBC, and Corriere della Serra and led to the formation of the company, EpiBone.

Dr. Grayson's scientific contributions and impact have been recognized by various entities. He received the Maryland Science Center Outstanding Young Engineer award (2010) and awards from the Orthopaedic Research Society (2007), the American Society for Bone and Mineral Research (2013), Young Investigator Award from TERMIS (2014), and the prestigious Early Faculty CAREER Award from the National Science Foundation (2014). In 2019, he was elected as a fellow of the American Institute of Medical and Biological Engineering. He has also been recognized by the National Academy of Medicine as an Emerging Leader in Health and Medicine.

Abhishek Jain, PhD
Associate Professor
Biomedical Engineering
Texas A&M University
Pumps, Pipes and AI: Engineering Vascular & Cancer Medicine with Organ-Chips

Dr. Abhishek Jain is an Associate Professor of Biomedical Engineering and holds the Barbara and Ralph Cox'53 faculty fellow position at Texas A&M University. He directs the Bioinspired Translational Microsystems (BioinSyst) lab that specializes in making microengineered models of vascular and hematologic diseases. The overarching theme of his lab is to harness tools from engineering, biology, and mathematics, in order to reconstruct the in vivo functionality of human tissues and organs in microfluidic devices (organs-on-a-chip). With this platform, his lab has put efforts to advance physiology, drug development and humane science. Dr. Jain has won numerous awards, including the NSF CAREER Award, NIBIB Trailblazer Award, Dean of Engineering Excellence Award and a TEES Young Faculty Fellow Award. Dr. Jain did B.Tech in Mechanical Engineering from Indian Institute of Technology-Delhi. After graduation and a short stint in industry, he went to Boston University and Harvard Medical School to get his PhD in Biomedical Engineering. Following that, he accomplished his postdoctoral fellowship with Dr. Don Ingber at Harvard's Wyss Institute of Biologically Inspired Engineering.

Abstract: The perpetual rising cost of healthcare is one of the biggest socioeconomic problems of our globe. Part of the challenge is that productivity of drug companies is declining, and relatively fewer drugs are reaching market. This is partly so because drug discovery largely rests on the results from animal studies, which can turn into negative outcomes in human clinical trials. The Jain lab creates microphysiological systems and associated technologies that predict human physiology and complement in vivo studies with an emphasis on hematological and cardiovascular disorders. They have made contributions in advancing preclinical research in sickle cell disease, diabetes, vein thrombosis, ovarian cancer, and lymphedema. Here, few examples of their approach will be presented.

Piotr Konieczny, PhD
Postdoctoral Fellow
New York University Grossman School of Medicine
Immune-Epithelial Crosstalk in Tissue Adaptation and Maladaptation

Piotr is a senior postdoctoral fellow at the New York Grossman School of Medicine in Dr. Shruti Naik's laboratory. He received his Ph.D. from Jagiellonian University in Poland, where he investigated the role of Regnase-1 in skin physiology and pathophysiology. In 2019, he joined the Naik lab. His postdoctoral training focused on decoding the crosstalk between immune and epithelial cells during tissue repair and regeneration. In doing so, he discovered a new repair mechanism that directs the metabolic rewiring of damaged epithelium towards a program of glycolysis to fuel migration and tissue repair. Additionally, using an interdisciplinary approach, he has demonstrated that understanding homeostatic repair holds translational potential, offering new therapeutic opportunities for epithelial inflammatory diseases.

Piotr has been recognized for his work and received several awards, including the NIH Pathway to Independence Award, the Research Award for Young Researchers from Lilly Company, the European Society for Dermatological Research Etiuda Fellowship from the Polish National Science Center, and the National Psoriasis Foundation Early Career Research Grant.

James F. Martin, MD, PhD
Vice Chairman/Professor
Molecular Physiology and Biophysics
Baylor College of Medicine
Hippo-Signaling in Cardiac Regeneration

Dr. Martin is an internationally recognized developmental and regenerative biologist who has contributed fundamentally to our understanding of development, disease, and regeneration. His work aims to obtain an in-depth understanding of how signaling pathways are connected to adult tissue regeneration to develop ways to treat congenital diseases and regenerate heart muscle and other adult tissues. He has authored over 195 peer-reviewed papers in top journals such as Nature, Science, Cell, Developmental Cell, Plos Genetics, Development, and PNAS. His groundbreaking work on the Hippo pathway in heart size regulation is a landmark study that led to the insight that the Hippo pathway is an inhibitor of adult heart muscle regeneration. Dr. Martin's insights revealed new avenues for treating human heart failure. His group recently completed a study to determine whether Hippo deficiency can improve functional outcomes in a swine model of heart failure. Dr. Martin has made fundamental insights into the role of the transcription factor Pitx2 in atrial fibrillation, the most common sustained arrhythmia in the human population. He uses the mouse model to investigate Pitx2 in atrial homeostasis and left-right asymmetric morphogenesis, which is essential for human development.

Dr. Martin's studies investigating Pitx2 function in craniofacial development provided insight into the molecular basis of Rieger syndrome. His group discovered that Hippo signaling inhibits the cardiac injury response by maintaining the resting state of cardiac fibroblasts and, most recently, transitioned into single-cell genomics and computational biology to interrogate complex biologic systems. Using these methods, they have published several high-impact papers that address an array of critical biological questions important for human development and diseases. His group's most recent study investigated human congenital heart disease using single-cell multi-omics approaches. Dr. Martin's studies are highly cited and are reported on by the lay media.

Caralynn Nowinski Collens, MD, MBA
Co-founder and CEO
Dimension Inx
Regenerative Therapeutics: From Discovery to Product to Market

Dr. Caralynn Nowinski Collens is the co-founder and CEO of Dimension Inc, a biotech company developing regenerative therapeutics that direct cells to rebuild healthy tissues. She is passionate about building teams and organizations that harness technology to drive transformational change and improve the quality of people's lives. Caralynn is also an Independent Director of Fathom Digital Manufacturing [NYSE:FATH] and serves on the Executive Council of Granite Creek Capital Partners. Previously, she was co-founder and CEO of UI LABS, a first-of-its-kind innovation collaborative that launched MxD, the national digital manufacturing institute.

Caralynn has advised leaders from startups to Fortune 100 companies, universities, and all levels of government. She loves to problem-solve and engage on the challenges of technology transfer and commercial innovation. Notable speaking invitations include the U.S. Congress, The Atlantic Council, McKinsey & Company, The Economist, The New York Times, Forbes, TEDx, and The Financial Times. After starting her first company while a joint medical/business student, Caralynn spent her early career in venture capital and corporate finance, primarily focused on technology-based university spin-outs.

John O'Brien, PhD
Professor, Vision Sciences
University of Houston

Regeneration of Rod Photoreceptors in a Zebrafish Model of Chronic Retinal Degeneration

Dr. John O'Brien is a Professor of Vision Science at the University of Houston College of Optometry. He earned a B.A. in Biochemistry at Bowdoin College in Brunswick, Maine and a Ph.D. in Biochemistry at the University of California, San Diego. Dr. O'Brien has been engaged in vision research since the mid-1990's. His research program is focused on elucidating the molecular mechanisms of plasticity in retinal electrical synapses. Dr. O'Brien has recently begun to study regeneration of rod photoreceptors in a transgenic zebrafish model of Retinitis Pigmentosa developed in his lab. His group has applied single-cell transcriptomics to map out transcriptional pathways involved in the proliferation and differentiation of progenitor cells to form new rods and to study the role of microglia in retinal regeneration.

Amy L. Orsborn, PhD
Clare Boothe Luce Assistant Professor
Electrical & Computer Engineering
Bioengineering
University of Washington

Engineering Systems-Level Sensorimotor Plasticity Through Co-Adaptive Neural Interfaces

Dr. Orsborn is a Clare Boothe Luce Assistant Professor in the departments of Electrical & Computer Engineering and Bioengineering at the University of Washington. Her research explores sensorimotor plasticity in brain-computer interfaces and how plasticity is influenced by the algorithms used. Her long-term goal is to develop "smart" algorithms that can shape plasticity to improve devices to restore and rehabilitate motor function after injury. She completed her Ph.D. at the UC Berkeley/UCSF Joint Graduate Program in Bioengineering and her postdoctoral training at NYU's Center for Neural Science. Her work has been supported by a range of governmental (NSF, the American Heart Association, NIH) and philanthropic (L'Oreal USA, Simons Foundation) organizations, along with industry (Google, Meta).

Francesca Taraballi, PhD
Assistant Professor of Orthopedic Surgery, Academic Institute
Assistant Member, Research Institute
Director, Center for Musculoskeletal Regeneration
Houston Methodist
How to Tune the Immune System Towards Tissue Restoration

Dr. Taraballi earned her B.S. in Biological Sciences and the first M.S. in Biochemistry at University of Milan – Bicocca, Italy. After another M.S. in Structural Biochemistry, she earned her Ph.D. in Nanostructures and Nanotechnologies from a joint program of the Materials Science Department of University of Milan – Bicocca with the Lawrence National Berkeley Laboratory (LBNL) in 2009 and the MIT.

Dr. Taraballi is currently the Director of the Center for Musculoskeletal Regeneration at Houston Methodist Research Institute affiliated to the Department of Orthopedics and Sports Medicine at Houston Methodist Hospital.

Dr. Taraballi's research is focused on the development of translational biomaterials, both injectable and implantable, to target the immune system toward tissue restoration specifically for musculoskeletal tissues. She is author of more than 100 papers as well as multiple patents (9), she is a member of different NIH study sections as well as reviewer panelist for grants issued by European committee.

Andrew Tully, MD
Cardiac Xenotransplant Fellow
Univ. of Maryland School of Medicine
Regulated Progress: The University of Maryland Cardiac Xenotransplantation Experience

Andy Tully earned his BA at University of Chicago and his MD at Loyola University Chicago. He completed general surgery residency training at University of Illinois at Chicago while working on questions of trauma and burns at Cook County Hospital as well as limb transplant with the Wertheim Lab at Northwestern University. He completed a fellowship in surgical Structural Heart and Valve Therapy at Emory University before joining Dr. Muhammad Mohiuddin's and Dr. Bartley Griffith's Cardiac Xenotransplantation Team at the University of Maryland as an NIH T32 Fellow. Upon completion, he is pursuing a career in cardiac and pulmonary transplant.

Andrea Wills, PhD
Associate Professor
Biochemistry
Univ. of Washington
Decoding the Metabolic Requirements for Appendage Regeneration

Andrea Wills started her scientific career as an undergraduate at Pomona College, where she developed an early love of regeneration and tissue patterning working with the small aquatic animal Hydra. During her graduate studies at Berkeley, she studied axial patterning in early Xenopus embryogenesis with Richard Harland, receiving her PhD in 2009. From there she moved to Stanford for her postdoc, continuing to study early embryonic patterning using frogs, mice, and embryonic stem cells in the lab of Julie Baker. While doing her postdoc research she became interested in the similarities and differences between the embryonic development of a structure and that structure's regeneration. This question brought her back to her early interest in regeneration, and has continued to motivate studies in her own lab, which she started as an Assistant Professor at the University of Washington's Department of Biochemistry in 2015, followed by promotion to Associate Professor in 2022.

Poster presenters in alphabetical order

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Alexander	Ferrer	University of Houston	The Transcriptional Analysis of Response to Focal Injury in the Xenopus Tadpole Midbrain	18
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Xiaolei	Zhao	UTHealth Science Center Houston	Yap/Taz Function as New Mediators of FGF Signaling Regulating Neural Crest-Derived Cranial Suture Mesenchymal Cells	11

Poster 19

The Role of Retinoic Acid in the Initiation of Gliogenesis in *Xenopus laevis*.

Mahmoud M. Alhomouz, Christina H. Ulrich, and Amy K. Sater[1]

1. University of Houston

Corresponding author: Amy K. Sater, Department of Biology and Biochemistry, University of Houston, Houston, TX 77204, USA, Email: asater@uh.edu.

Astrocytes play vital roles in the central nervous system, such as maintaining neuronal homeostasis, blood-brain-barrier modulation, neurotransmission, and synapse regulation. Because of the multitude of functions carried out by astrocytes, dysregulation of their genes contributes to numerous neurological disorders, such as amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and Huntington's disease. Astrocytes also play a major role in the response cascade to central nervous system (CNS) injuries. During neural development, glial lineages typically arise from neuroprogenitor cells later than neuronal lineages, and the capacity to initiate glial development, which often referred to as the gliogenic switch, is marked by the onset of astroglial glutamate transporter, *glast* (aka *slc1a3*) expression. The gliogenic switch regulators in *Xenopus laevis* are yet to be described. Explants of neural plate ectoderm isolated from mid-gastrula embryos (NP)will later express both neuronal and astroglial genes, while animal caps overexpressing noggin (NOG AC) will only initiate expression of neuronal genes. Previous data from our lab used these explant systems to show that overexpression of the transcription factors associated with the initiation of mammalian gliogenesis, *sox9 and nfia*, is insufficient to drive expression of the astroglial glutamate transporter *glast* in animal ectoderm overexpressing noggin. Previous data from our lab demonstrated that expression of *glast* is first detected in the anterior spinal cord, suggesting a relationship between anteroposterior regionalization and the initiation of gliogenesis. We tested the hypothesis that the anteroposterior (AP) patterning factor retinoic acid (RA) could regulate expression of *glast*. Treatment of late gastrula embryos with all-trans-RA led to upregulation of *glast* and *sox9*; in whole embryos and NOG AC, all-trans-RA elicited expression of *glast* and *sox9* as well as a reduction in the expression of *neurod1*. RA inhibition using RA antagonist or by overexpressing a dominant negative for RARα (DN-RARα) decreased expression of both neuronal and astroglial-associated genes in NPs. Finally, RA dysregulation affected the spatial distribution of *glast* transcript along the AP axis. Our findings suggest that RA signaling is a major regulator of astroglial development in *X. laevis*. Understanding factors regulating the development of astrocytes could provide insights into how astrocytes are activated or deactivated following an injury or a disease, given the overlapping factors regulating both processes.

Acknowledgements: College of Natural Sciences and Mathematics, University of Houston Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation, The Texas Research Initiative Program, and Sigma Xi in Aid of Research (GIAR).

Poster 1

Enhancing Osteoarthritis Cell Therapy with Biomedical Engineering: Evaluating Fat Pad-derived Mesenchymal Stem in 3D Spheroid Culture and on Collagen-based Scaffold.

Federica Banche-Niclot[1,2], Michael E. Williams[1,2], Humza Rehman[1,2], Jaesang Lim[1,2], Patrick McCulloch[2], Francesca Taraballi[1,2]

1. Center for Musculoskeletal Regeneration, Houston Methodist Research Institute
2. Orthopedics and Sports Medicine, Houston Methodist Hospital

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The current cell therapy clinical standard for osteoarthritis (OA) uses bone marrow-derived mesenchymal stem cells (BM-MSC) to address clinical needs and promote healing. However, a valid alternate source is infrapatellar fat-pad-derived MSCs (FP-MSC) offering less invasive harvesting method. In particular, since OA is an inflammatory-based disease, the immunosuppressive potential of MSCs is key for regenerative medicine, fostering a microenvironment conducive to optimal cartilage repair by suppressing excessive inflammation. Moreover, MSCs grown in three-dimensional (3D) culture systems, such as spheroids, have been shown to increase their stemness and can result in stronger immune-modulatory capabilities when compared to traditional 2D cell culture. In addition, 3D biomimetic scaffolds can maximize the efficacy of regenerative therapies by allowing the spatiotemporal control over signaling molecules at the nanoscale.

Based on these considerations, we first assessed the feasibility of human FP-MSCs for cell therapy by comparing their stemness and immunomodulatory capability to human BM-MSCs. Then, the cell culture conditions were optimized to create stable 3D spheroids whose viability was evaluated through CellTiter-Glo® and Live/Dead assays. Specific stem marker characterization (CD105+, CD73+, CD90+, and CD45- phenotype) was carried out via flow cytometry.

The simulation of inflammatory condition was also tuned setting 40 ng/mL as the optimal concentration of combination interferon-gamma (IFN-γ) and tumor necrosis factor (TNF-α). Then, we demonstrated that 3D FP-MSC spheroids have greater immunosuppressive potential characterized by superior PTGES, PTGS2, and IDO1 expression when compared to traditional 2D culture.

Finally, we enhanced the biomimicry using a freeze-dried collagen scaffold as a substrate for 3D spheroid culture. A seeding titration approach was employed to determine the optimal spheroid density. Viability was evaluated using CellTiter-Glo® and Live/Dead assays.

Poster 15

Engineered Lymphatic Flap: A Promising Approach for Treating Lymphedema

Alvis Chiu[1], Wenkai Jia[1], Yumeng Sun[1], Jeremy Goldman[2], Feng Zhao[1]

1. Department of Biomedical Engineering, Texas A&M University
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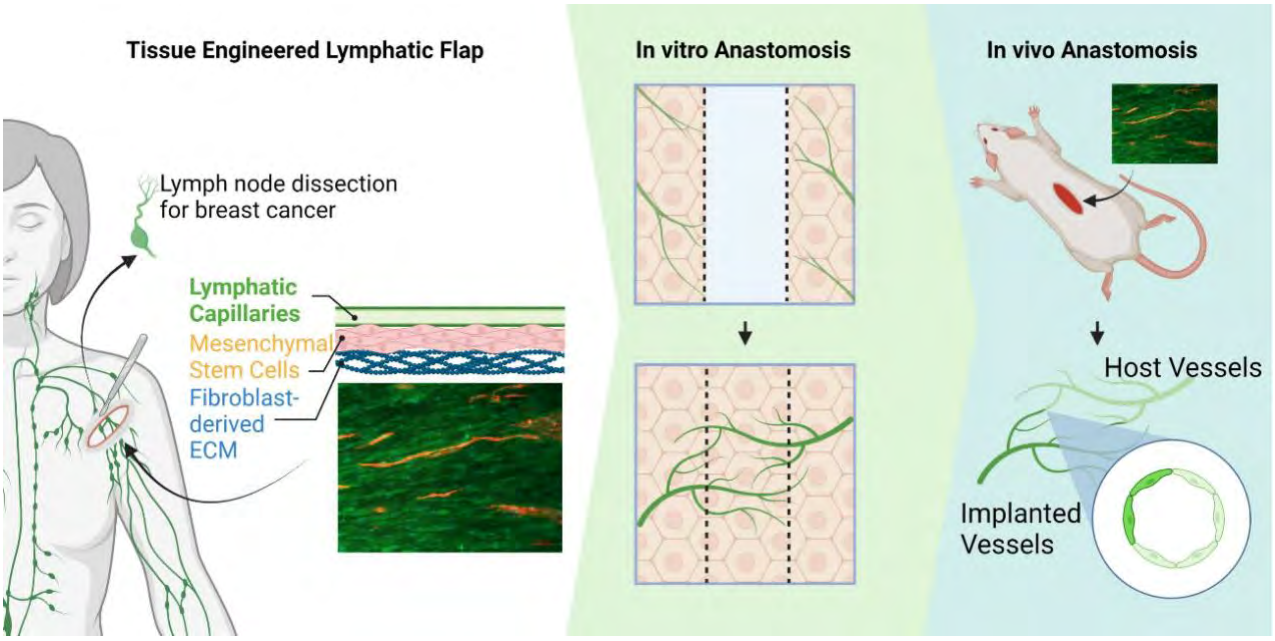
A healthy lymphatic system is required to return excess interstitial fluid back to the venous circulation. However, up to 49% of breast cancer survivors eventually develop breast cancer-related lymphedema years after the cancer goes into remission due to the progressive lymphatic degeneration caused by the damage from the initial lymph node dissections or biopsies performed to treat cancer. While early-stage lymphedema can be ameliorated by manual lymph drainage, no cure exists for late-stage lymphedema when lymph vessels become completely dysfunctional. A viable late-stage treatment is the autotransplantation of functional lymphatic vessels. Here we report on a novel engineered lymphatic flap that may eventually replace the skin flaps used in vascularized lymph vessel transfers. The engineered flap mimics the dermal lymphatics of the skin by guiding multi-layered tissue organization of mesenchymal stem cells and lymphatic endothelial cells with an aligned decellularized fibroblast matrix. The construct was tested in a novel in vitro bilayered wound healing model and implanted into athymic nude rats. The in vitro model demonstrated capillary invasion into the wound gaps following the deposition of extracellular matrix fibers by fibroblasts into the wound, which may guide anastomosis and vascular integration of the graft during wound healing. The construct successfully anastomosed in vivo, forming chimeric vessels composed of human and rat lymphatic endothelial cells. Overall, our flap replacement has high potential for treating lymphedema.



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Poster 2

Amniotic Stem Cell-derived Extracellular Vesicles Promote *In-utero* Regeneration in a Spina Bifida Murine Model.

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Spina bifida (SB) is a devastating birth defect caused by abnormal closure of the posterior neural tube that results in significant disability. Although public health efforts have reduced the prevalence of SB in the U.S., there are still approximately 35 cases per 100,000 live births annually. The etiology of SB is complex and multifactorial, and several hypotheses have attempted to explain its pathophysiology. The two-hit hypothesis postulates that the initial defect, caused by failure of the neural tube folds to fuse during the third week of development, is worsened as gestation progresses and the primary lesion is exposed to the intrauterine environment. This hypothesis provides a strong rationale for prenatal SB repair to prevent further damage. However, intrauterine surgical repair is sometimes unable to prevent disability, as some patients require shunts and other procedures postnatally. Proper healing of the SB lesion is vital to prevent further neurological damage, and thus, promoting a pro-regenerative environment could optimize surgical outcomes and reduce the phenotypic severity postnatally.

Extracellular vesicles (EVs) are membrane-bound cargo-rich vesicles secreted by a variety of cells as a means of cell-cell communication. EVs isolated from stem cells maintain parental cell properties, often carrying signaling moieties that exert therapeutic effects. We hypothesize that EVs isolated from amniotic fluid mesenchymal stem cells (AF-MCSs) can promote an *in-utero* pro-regenerative environment to enhance wound healing and reduce the severity of SB lesions, ultimately ameliorating the phenotype. $Fkbp8^{Gt(neo)}$ homozygous null mice develop SB and are phenotypically similar to human patients, developing similar lesions and lower body paralysis postnatally. This model is an excellent opportunity to test the efficacy of AF-MSC EVs to prevent further damage to a genetically induced SB lesion.

EVs secreted from human AF-MCSs were isolated from culture media and characterized. EVs, or a PBS control, were injected intraperitoneally into pregnant Fkbp8 heterozygous dams (n=5/group) daily from E5.5 to E9.5, to cover the neurulation period. Pregnant dams were euthanized and embryos were harvested at E15.5 and designated as affected (SB) or normal. Embryo weight and length were noted, and fixed embryos were further analyzed under the microscope to quantify lesion size. While affected embryos are generally smaller than their normal littermates, our data shows that EV-treated embryos with SB have a partial rescue in terms of weight when compared to PBS-treated SB controls (421 ± 55 and 338 ± 36 mg, respectively). Moreover, EV-treated Fkbp8 homozygous null embryos show a reduced posterior SB lesion size compared to PBS-treated controls (13.9 ± 0.67 and 8.47 ± 0.74 mm^2, respectively) with reduced posterior herniation and a less translucent exterior lesion surface. Although preliminary, our findings support the potential of AF-MSC EVs to promote a pro-regenerative environment in utero, paving the way for the development of minimally invasive strategies to reduce SB severity.

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Poster 3

Exploring the Role of *Yap/Taz* in Cardiac Neural Crest Cells

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Congenital heart defects (CHDs), the most common type of birth defect, affect one in every 100 newborns. Cardiac neural crest cells (cNCCs), a migratory and multipotent cell population, are vital for proper heart formation and have been clinically linked to CHDs. Patient data indicates that CHDs can be associated with alterations of the fundamental Hippo signaling pathway, yet its role in cNCC-derived heart development remains largely unknown. Conditional knockout of *Yap/Taz* (*$Yap^{+/-};Taz^{-/-}$*), the downstream effectors of the canonical Hippo pathway, resulted in mouse embryos with various CHDs, including cardiac outflow tract (OFT) and ventricular septum defects. Notably, *$Yap^{+/-};Taz^{-/-}$* hearts also had ectopic cartilage tissue in, an atypical cell population to be found in the embryonic cardiac system, suggesting a cNCC fate alternation. In addition, *ex vivo* cardiac OFT culture and *in vitro* NCC migration assays indicated reduced migration capabilities due to Yap/Taz reduction. Furthermore, ultra-low bulk RNA-sequencing of cardiac OFTs from control and *$Yap^{-/-};Taz^{-/-}$* E10.5 mouse embryos indicated that compared to controls, *$Yap^{-/-};Taz^{-/-}$* OFTs had altered expression of genes regulating cell movement, extracellular matrix organization, stress response, and differentiation, all critical components of mechanical signaling regulation. Ongoing studies aim to validate novel candidate sequencing results using in vivo and human embryonic stem cell-derived NCCs to investigate cNCC fate mechanisms throughout development. Together, our data indicate that Yap and Taz are required for proper cNCC-derived heart formation.

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Poster 18

Title: The Transcriptional Analysis of Response to Focal Impact Injury in the *Xenopus* tadpole midbrain

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We seek to develop the Xenopus tadpole as a scalable model for investigation and therapeutic discovery for Traumatic Brain Injury (TBI). As a proof-of-concept, we have shown that tamoxifen (TMX), a Selective Estrogen Receptor Modulator that is neuroprotective in mammalian spinal cord injury, promotes recovery from focal impact injury in our TBI model, based on cellular and behavioral assays. We carried out transcriptome profiling to evaluate the time course of transcriptional responses to Focal Impact Injury and the effects of tamoxifen in the tadpole midbrain. Metamorphic tadpoles at st. 56 were subjected to either focal impact injury to the dorsolateral midbrain or sham treatment, and injured tadpoles were injected intraventricularly with either TMX or ethanol (vehicle). Tadpoles were allowed to recover over a 7-day period, and euthanized for retrieval of midbrains at 3 hours, 24 hours, 48 hours, and 7 days following injury. Midbrain RNA was isolated for library preparation and examined via bulk transcriptome sequencing, followed by analysis with a pipeline incorporating Kallisto and DESeq2. The most extensive differences were observed at 3 hours and at 7 days. Targets of injury-dependent transcriptional changes included genes involved in inflammation, phagocytosis, and DNA repair. Notably, TMX treatment led to rapid upregulation of c-fos and jun family members and selected cytokines, as well as reductions in the expression of sphingosine-1-phosphate receptor 1 (s1pr1) and key inflammation pathway components. Ongoing studies will investigate the mechanisms by which TMX mediates neural repair in the Xenopus midbrain.

Poster 20

Examining the Effects of Pollution on Cardiac Health

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Air pollution, an ever-increasing challenge in the developing world, poses a threat to individual and societal health due to deposition of particulate matter (PM) from industrial processes, smoke, and general pollution that, when inhaled, can permeate into the bloodstream and travel to the heart. PM has been identified as an independent risk factor for cardiovascular morbidities and mortalities, including an increased risk for cardiac fibrosis, myocardial infarction, and atherosclerosis[1]. Chronic damage to the heart occurs after prolonged exposure; pro-inflammatory signaling pathways and oxidative stress caused by PM lead to damaged cells and altered morphology, gene expression, and extracellular matrix stiffness[2]. From a clinical perspective, studies have been found that highlight the rate of birth defects to ambient levels of particulate matter, but studying the changes at a cellular level remains a challenge[3]. We will identify key changes in cell functionality and observe trends of response to different concentrations of PM exposure. In this study we utilize a heart-on chip platform to investigate the pollution driven mechanisms that contribute to cardiovascular diseases. We will examine gene expression profiles, mechanical modulus and functional responses to exposure to particulate matter. This study will highlight the utility of tunable *in vitro* platforms for disease investigations. Further, this work has the potential to identify novel pathways involved in pollution derived from heart diseases.

Acknowledgements: This work was supported by the John S. Dunn Foundation and the Oak Ridge Associated Universities Ralph Powe Award.

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Poster 4

Assessment of Stemness and Phenotype of Primary Human Salivary Stem/Progenitor Cells for Tissue Engineering Applications

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Objectives: Cultured cells used in tissue engineering applications must meet strict criteria for implant into humans. This study aimed to assess the purity, stability, stemness and phenotype of primary human salivary stem/progenitor cells (hS/PCs). The stem cell marker CD44 was used for primary magnetic sorting and both $CD44^+$ and $CD44^-$ subpopulations were assessed for various lineage and phenotypic markers. Experimental Methods: Primary hS/PCs were isolated and expanded from parotid specimens from patients undergoing surgical resection procedures and expanded to produce millions of cells. Cell suspensions were sorted by CD44+ expression using magnetic cell-sorting with anti-CD44 microbeads. Epithelial purity was assessed by flow cytometry using CD133/PROM1 and anti-fibroblast markers. Flow cytometry, qPCR and immunocytochemistry (ICC) were used to assess phenotype in non-sorted, sorted CD44+ and sorted CD44- cell subpopulations. Transcript levels of CD44, and pluripotency-related biomarker p63 and epithelial marker CK14 were assessed in all three populations. Results: Sorted CD44+ cells exhibited significantly higher expression of CD44 and p63 than CD44- populations. CD44 expression was high (> 95 %) in the unsorted cells, while expression of CD133 and fibroblast marker were negative, indicating cells are uniformly normal epithelial cells. ICC results confirmed the CD44, CD133 and fibroblast expression patterns seen by flow cytometry. Conclusion: Unsorted hS/PCs and sorted CD44+ cells expressed high levels of stemness marker CD44, were uniformly epithelial, and exhibited features suitable for use in tissue engineering applications. Ongoing studies using these cells in animal models (rat and miniswine) will foster development of salivary gland replacement therapies.

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Poster 5

Testing Human Astrocyte Influence Upon Synapses within an Alzheimer's Disease Organoid Model

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Disclosures- SSL-None, FT-None, MDP-None, RK-None.

Keywords- Organoids, Astrocytes, synapses, Alzheimer's disease.

Astrocytes contribute to the initiation and progression of neurodegeneration in Alzheimer's disease (AD). However, the specific link between astrocyte reactivity within the amyloid microenvironment and the impact on synapses is obscure due to the lack of state-of-the-art tools. Here, we harnessed the combinatorial potential of two emerging technologies that could dissect the relationship of astrocyte reactivity with synapse density and activity. First, we engineered human pluripotent stem cell (hPSC)-derived neurons to express a synaptic reporter. Specifically, the presynaptic protein, synaptophysin, was linked to a genetically encoded calcium sensor (i.e., SYP-jGCaMP8s) to monitor synaptic calcium transients as a readout of activity. We cocultured this reporter with hPSC-derived astrocytes in the form of neural organoids (i.e., Asteroids) and this revealed that astrocytes accelerate the presence of synchronized synaptic calcium transients throughout the entire population. Second, we optimized expansion microscopy techniques to determine if the astrocyte-induced effect on activity is due to increased synapse density. Expansion microscopy decrowds synaptic areas for binding of antibodies before immunolabeling, and increases image resolution by physically expanded the tissue. By applying this technique to Asteroids, we were able to expand neurons and astrocytes by several fold. We are currently combining these approaches to examine the consequence of amyloid oligomers on astrocyte reactivity and synapse activity as a organoid-based AD model system. We expect that optimization and validation of this experimental platform will open avenues to develop biomarkers and test drugs that could help modulate synapse activity, synaptic trafficking and plasticity.

Poster 6

Midbrain Asteroids: Neural Organoid Approach to Investigate the Impact of Astrocytes in Parkinson's Disease

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Parkinson's Disease (PD) is a neurodegenerative disorder characterized by the depletion of dopaminergic neurons (DA) in the midbrain, leading to the onset of the triad of bradykinesia, rigidity, and tremor. Much focus has been directed toward studying the mechanism of DA neuronal death, but less is known about the influence of astrocytes. Our lab has previously established a human pluripotent stem cell-derived neural organoid model (aka Asteroids) composed of neurons and astrocytes that are directly differentiated via transcription factor induction. Thus, this Asteroid model could also enable the investigation of astrocyte-neuron intercellular signaling in PD. Here, we first tested multiple protocols to produce midbrain organoids containing high numbers of DA neurons, as defined by tyrosine hydroxylase (TH) immunostaining. Our studies concluded that sequential treatment of small molecules (SAG, purmorphamine, CHIR99021), ventral midbrain-restricted transcription factor induction (ASCL1-2A LMX1B-2A-NURR1), and use of media that promotes DA neuron maturation altogether produced the highest number of TH-positive cells within organoids. We are currently using this method to test the effect of astrocytes on DA neuron viability and function, in the context of GBA mutations and astrocyte reactivity. We expect that the utilization of this novel midbrain Asteroid approach offers a promising model system for elucidating the intricate underlying mechanisms related to PD and other neurodegenerative disorders. Further, this model could pave the way for the development and discovery of targeted therapies and interventions.

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Poster 12

Neurostimulation Drives Microglial Neuroprotective Phenotype in a Cervical Spinal Cord Injury Model

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ABSTRACT

Microglia play an important role in maintaining and repairing the central nervous system (CNS) after traumatic injuries. Recent work has demonstrated that the suppression of microglia during spinal cord injury (SCI) led to a decrease in neuroregeneration, less gene diversity in the remaining cell-populations, and overall worsening of functional outcomes. Moreover, modulation of microglia phenotype through genetic and pharmaceutical models shows promising results in understanding the role of microglia during SCI. Neurostimulation/electrical stimulation is one of the few clinically-relevant treatments for functional recovery after SCI but the exact mechanism(s) that takes place during this treatment are under-explored. Moreover, there is little work characterizing microglial phenotype *in vivo* during neurostimulation. This presentation shows preliminary data that demonstrate phenotypic changes in microglia during cervical SCI and electrical stimulation and how that may lead to functional improvements.

In our *Rattus Norvegicus* model, a cervical spinal cord contusion injury at C4 was induced with the Ohio State impactor and epidural electrodes were implanted at C6 one-week post-injury. Electrical stimulation occurred for one week of 20Hz stimulation (1 hour/day) followed by immediate isolation of the stimulated spinal segment. A sham device was used for the control group.

Through bulk RNAseq of whole spinal cord and immune cells specifically, we observe significant upregulation of anti-inflammatory pathways and downregulation of pro-inflammatory pathways as a result of neurostimulation compared to controls. These findings were further confirmed through microglial morphology using stereological techniques. Further experiments need to be done to decipher the exact role electrical stimulation has on microglia to be able to cause this phenotypic shift but we hypothesize this is due to the activation of L-type Voltage-gated calcium channels as we observe a location specific upregulation of gene expression for many of them. This work helps us start to decipher the role of microglia after SCI and how we can possibly use neurostimulation to improve recovery.

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Poster 7

Human Astrocytes Rapidly Generate and Maintain Synchronized Network Activity Across Large-Scale Neural Organoid Ensembles

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Megh Patel: None. Sailee Lavekar: None. Suki Oji: None. Philip J Horner: None. Robert Krencik: None.

THEME AND TOPIC: Theme B: Neural Excitability, Synapses, and Glia. B.09.f. Glia-neuron interactions in physiology

KEYWORDS: Human Pluripotent Stem Cells, Astrocytes, Organoids

Abstract

It is well established that rodent astrocytes promote neural network maturation in the nervous system through a combination of pro-synaptogenic components and neuroprotective mechanisms. Do human astrocytes have similar capabilities in promoting human neural network activity? To address this question, we optimized and tested a human-specific model with bioengineered neural organoids composed of rapidly matured astrocytes and glutamatergic neurons from human pluripotent stem cells (hPSCs) (i.e., Asteroids, Cvetkovic et. al.). We found that Asteroids more rapidly exhibit organoid-wide synchronous burst activity compared to neuron-only organoids as measured by GCaMP-based calcium imaging and multi-electrode array (MEA) analysis. To determine which astrocyte-derived components underlie this effect, we tested candidates identified in RNAsequencing and proteomic screens of Asteroid-conditioned media. A subset of these candidates was sufficient to accelerate neural network activity of neuron-only organoids. Next, we tested whether astrocyte-mediated neuroprotection maintains network activity via improved viability. We found that Asteroids have improved viability in suboptimal media and are protective against glutamate-induced excitotoxicity when compared to neuron-only organoids. Given improved network formation and maintenance, we tested if astrocytes enable generation of functionally interconnected ensembles of organoids. Ensembles of multiple interconnected Asteroids (i.e., Asteroid Belts) revealed synchronous burst activity within 24 hours with physical projections extending into neighboring tissue. Taken together, our work highlights the importance of astrocytic mechanisms in rapidly generating and maintaining human neural networks. The robust and rapid functional interconnectivity of neural networks delivers a new human *in vitro* experimental approach that is expected to extend to models of various distinct regional networks and disease context.

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Poster 16

Let-7 MicroRNA Restrains Alveolar Type 2 Cells Epigenetically to Prevent Ectopic Formation of Fibrogenic Intermediates in Pulmonary Fibrosis

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Stem cells have a critical role in regenerating a tissue niche by self-renewal and differentiation. Recent studies in lung epithelial stem cells have identified unique transition states during alveolar regeneration. Initiation and commitment of stem cells to their proliferative and differentiation trajectories is controlled by a complex system of cell-cell communication networks, epigenetics, transcriptional and post-transcriptional regulation. Recently, impairing alveolar type 2 (AT2) progenitor stem cell function has been linked to pulmonary fibrosis by allowing aberrant persistence of alveolar differentiation intermediates (ADI). Importantly, microRNAs, especially the *let-7* family, have been an emerging field as regulators in stem cell dynamics and pulmonary fibrosis, but their interplay remains unknown. Here, we generated inducible, AT2-specific *let-7*-cluster knockout murine models that spontaneously develop pulmonary fibrosis. Moreover, depletion of *let-7* in AT2 cells led to hyper-proliferation, persistence of ADIs, and failure to terminally differentiate until stress-induced apoptosis. We created a fibrosis-specific, *let-7* targetome to identify leading edge genes associated with stem cell renewal and differentiation. *Let-7* targets were enriched during ADI formation and regulated transition state markers. Importantly, we identified that *let-7* target EZH2 controls many of the dysregulated pathways by chromatin remodeling. To determine whether these phenotypes are intrinsic to *let-7* loss of function in AT2 cells, we generated 3D organoid alveolosphere cultures that showed loss of *let-7* causes increased proliferation, differentiation, and EZH2-mediated histone methylation. Our study provides a new dimension for *let-7* as a potent regulator in alveolar stem cell fate and its target EZH2 governs progression of pulmonary fibrosis epigenetically.

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Poster 13

Investigating Perfusion and Immunogenic Response of Implantable Pre-Vascularized Tissues Mimicking Natural Capillary Networks

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Accomplishing vascularization in engineered anatomical scale three-dimensional (3D) constructs is a key pre-requisite to ensure adequate nutrient and oxygen supply post-implantation. However, current pre-vascularized 3D tissues are limited to only micro-scale hydrogels, lacking anatomical scale and complexity of natural extracellular matrix (ECM) environments. Anatomical scale perfusable constructs are critically needed for translational applications. Addressing this gap, we present a novel approach using human dermal fibroblast-derived ECM scaffolds prevascularized through co-culturing human mesenchymal stem cells (hMSCs) and endothelial cells under optimized oxygen conditions. These prevascularized scaffolds feature a dense microvascular network, serving as building blocks for engineering complex 3D tissues. Our study aims to evaluate the innate immune response to these engineered constructs *in vivo* through macrophage co-culture and subcutaneous implantation in the RNU nude rat model, respectively. Characteristics of the microvascular network (vessel integrity, length, diameter, area, lumen structure) were evaluated *in vitro* with macrophage co-culture using IF staining. Notably, subcutaneously implanted prevascularized constructs demonstrated the viability and formed a functional anastomosis with host vasculature within 3 days post-implantation (Fig. 1). These completely biological and pre-vascularized engineered constructs remain viable and form functional anastomosis after *in vivo* implantation. Therefore, these engineered constructs have a great potential to serve as a building block to engineer perfusable anatomical scale tissues for tissue engineering applications. The regenerative properties of prevascularized ECM sheets are currently evaluated to treat cardiovascular diseases and chronic wounds.

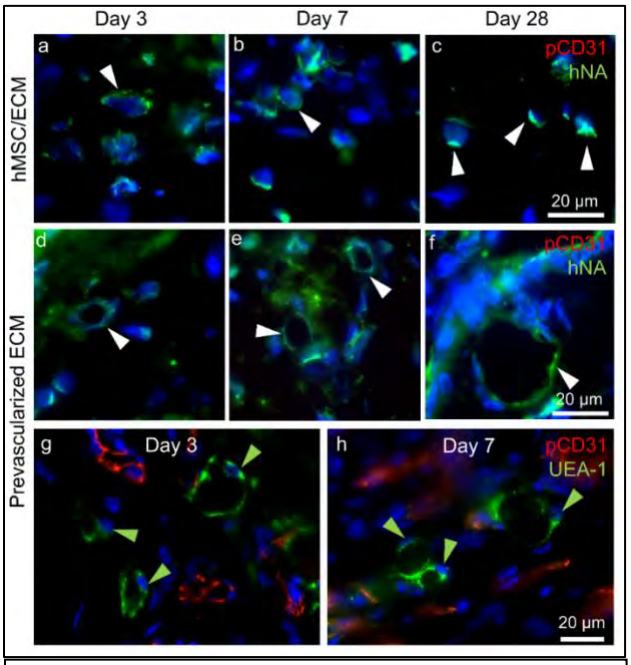

Figure 1. Evaluation of prevascularized constructs post subcutaneous implantation in RNU nude rats. Positive hNA signal (white arrow) indicated presence of human cells from day 3 to day 28 in hMSC-ECM group (a-c) as well as in prevascularized-ECM group (d-f). Prevascularized constructs showed positive signal for human EC specific-UEA-1 lectin (green arrow) and polyclonal CD31 (red) at day 3 (g) and day 7 (f). The lumen structures found in subcutaneous space indicate human capillaries maintained open lumen structure and formed a functional anastomosis with host vessels within one week of implantation.

Poster 13

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Poster 8

Astrocyte Dysfunction in Parkinson's Disease: Unraveling the Impact of Glycosphingolipid Metabolism on Reactivity

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Mutations in the GBA gene represent the highest risk factor for developing Parkinson's Disease (PD), Lewy Body Dementia, and Gaucher Disease. GBA encodes a lysosomal enzyme called Glucosylceramidase (GCase) which in physiological conditions catalyzes glucosylceramides into glucose and ceramide. This reaction sits on the divergence point of pathways that control metabolism, membrane formation, and inflammation. However, the impact of GBA mutations on astrocytes, the main cell type in the brain that regulates inflammation and the trafficking of metabolites, remains poorly understood. To study the consequences of astrocyte GCase dysregulation in the development and progression of PD, we utilized human induced pluripotent stem cells (hPSCs) derived from a PD patient with a GBA mutation (N370S) and a healthy control. We also targeted GBA with CRISPR-Cas9 and generated a complete knockout (KO) line. We engineered these lines to induce direct differentiation into astrocytes (iAstros) and dopaminergic (DA) neurons and incorporated them in 3D midbrain organoids. Patient-derived astrocytes exhibited reduced GCase activity. In contrast, KO astrocytes showed no activity, resulting in the accumulation of Glucosylceramide in the lysosome. Lipidomic analysis revealed reduced levels of triglycerides, and higher levels of sphingolipids in KO astrocytes similar to the inflammatory state of wildtypes astrocytes, however, RNA sequencing of the KO astrocytes did not reveal a correlation with an inflammatory pattern. Palmitic acid treatment, which activates de novo glucoceramide formation, led to a decrease of the electrical activity of neurons as measured by multielectrode arrays. We are currently investigating the mechanism by which dysfunctional sphingolipid metabolism influences astrocyte reactivity and the consequences for the function and survivability of DA neurons in the midbrain organoids. Our findings suggest that astrocyte glycosphingolipid metabolism may play a crucial role in the pathology of PD, making it an important target for the development of new therapeutic approaches.

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Poster 9

Developing a Vascularized Cardiac Patch with Personal iPSCs-Derived Cardiovascular Cells for the Treatment of Myocardial Infarction

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Introduction: The available cardiac patches for the treatment of myocardial infarction in large animal models or in clinical trials were fabricated with xenogeneic or allogeneic iPSCs-derived cardiac cells. Those patches were not embedded with a coronary artery and vein vascular network and most of the cells died at transplantation because no direct nutrients and oxygen were supplied from the host blood stream; their tissues were thin and unable to carry enough fresh cells; patch tissues faced immune response during the process of regenerative remodeling after transplantation. **Objective:** This project is to fabricate a vascularized cardiac patch with personal iPSCs-derived cardiovascular cells for the treatment of myocardial infarction. **Methods:** Cardiomyocytes (CMs), endothelial cells (ECs), smooth muscle cells (SMCs), and fibroblasts (Fbs) are derived from host iPSCs that are generated from host peripheral blood mononuclear cells. The artery-arteriole-capillary-venule-vein coronary vascular network is fabricated with digital light processing of gelatin methacrylate (GelMA) and poly (propylene fumarate) plus iPSCs-SMCs and Fbs, and then coated with iPSCs-ECs. Transplantable cardiac patches are made by casting collagen type I hydrogel plus iPSCs-CMs, ECs, and Fbs with the available coronary vascular system, and then perfused with media. **Results and Discussion:** Non-vascularized cardiac patches were successfully fabricated on day 3 by casting human iPSCs-derived cardiac cells onto collagen type I hydrogel, the patch size was 40 × 40 mm in length and width, and 0.5-0.8 mm in thickness. On day 4, ECG signals were detected, and twitch force measured. The contracting rate of patch tissues was 88 ± 9 bpm (n=7) on day 4, then decreased over time; tissue pacing response frequency was 2.5 ± 0.5 Hz (n=7). The twitch force of the patch tissues increased over time and reached its greatest value on day 16 (1.50 ± 0.10 mN per 300 mm^3 tissue, n=7), and then decreased. Immunofluorescence staining showed rod shape cardiomyocytes with striations in the patch tissues were aligned well towards the direction of tissue contraction. An artery-arteriole-capillary-venule-vein coronary vascular network was fabricated by digital light processing of GelMA. **Conclusions:** A thin and uniformly contracting cardiac patch can be fabricated with human iPSCs-derived cardiac cells, and an artery-arteriole-capillary-venule-vein coronary vascular network is ready to embed into the available cardiac patch to make it as thick and strong as a native heart muscle for the treatment of myocardial infarction.

Poster 14

Wnt/β-catenin Signaling Dynamics in the Aging African Turquoise Killifish Brain

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The dysregulation of the Wnt/β-catenin pathway is associated with human aging and several neurological disorders including Alzheimer's disease, Parkinson's disease, and multiple sclerosis. In the African turquoise killifish (*N.furzeri*) model, I am uncovering how changes in Wnt signaling relate to aging and the spontaneous neurodegeneration process observed in aging killifish. We have established a fluorescent Wnt signaling reporter line in *N.furzeri* in which a 1.1 kb fragment of the zebrafish *sp5a* promoter, a known Wnt responsive element with conserved LEF/TCF binding sites, drives expression of eGFP in areas where Wnt signaling is presumed to be active. This reporter remains active from developmental stages to adulthood, including stages when neurodegeneration is observed. Transgenic founders of an additional fluorescent Wnt reporter line, 7XTCF-Xla.Siam:mCherry, regulated by seven multimerized TCF elements upstream of the *Xenopus* siamois minimal promoter, have a similar pattern of expression in embryonic stages when compared to the *sp5a:eGFP* reporter. These transgenic founders will be outcrossed and screened for germline transmission, followed by a comparative analysis of fluorescence in the adult brain. To interpret changes in fluorescence associated with aging, we adapted the EZ-Clear tissue clearing method to adult fish brains to preserve endogenous fluorescence from the reporter line, while also enabling future immunostaining. Interestingly, eGFP expression in the *sp5a:eGFP* reporter is strongly localized to the habenula, a structure in the brain responsible for many behavioral responses. *In situ* hybridization for Wnt pathway mRNAs (*axin2*, *sp5*, *wnt1*) in the adult brain also shows expression in the habenula with possible age-dependent changes in expression levels. When comparing mRNA expression of 15-week and 25-week-old brain sections, both *sp5* and *axin2* mRNAs were decreased in response to aging across multiple brain regions including areas in the forebrain and in the midbrain optic tectum. Overall, my data suggests a prominent role for Wnt/β-catenin signaling in the adult killifish brain and suggests observable changes in Wnt signaling activity associated with aging.

Poster 10

Defining Network Activity in Human Neural Organoids with Genetically Encoded Voltage Indicators

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Real-time analysis of synchronized neural network formation in experimental models of human tissue will enable the development, testing, and validation of neuromodulatory approaches to promote neuroregeneration in a variety of neurological conditions. With this objective in mind, we previously reported methods for observing and manipulating neural activity in human pluripotent stem cell (hPSC)-derived organoids through use of multielectrode array recordings, genetic encoded calcium indicator imaging, and light-induced stimulation with optogenetics. Here, as an advancement to this approach and to directly observe activity in higher spatial and temporal precision, we are taking advantage of the recent emergence of sensitive genetically encoded voltage indicators (GEVIs). A GEVI approach is expected to enable the direct observance of neuron voltage fluctuations within organoids in real time. In preliminary data, we engineered hPSC lines with recently optimized GEVI variants in a specific safe harbor locus. After purification of transgenic lines, expression of the GEVIs were induced through a TET-on approach during direct differentiation of neurons. Organoids containing these neurons, as well as astrocytes, were successfully generated and we are currently testing functionality with real-time imaging. After defining this model system, in direct subsequent studies we will apply this new model system to investigate human disease and test potential therapeutic modulators of network activity.

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Poster 17

Engineering an Anisotropic Cardiac Patch from a Cardiac-Specific Extracellular Matrix Scaffold

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Extracellular matrix (ECM) fabricated from human induced pluripotent stem cells-derived cardiac fibroblasts (hiPSC-CFs) has potential to act as a biological scaffold for engineered cardiac patches, leveraging both an extensive supply and exceptional reproducibility. hiPSC-CF-derived ECM (hiPSC-CF-ECM) can also be used to enhance the maturation of exogenous cardiomyocytes, such as hiPSC-derived cardiomyocytes (hiPSC-CMs), by providing a cardiac-specific microenvironment rich in biochemical and signaling cues. However, achieving sufficient robustness in hiPSC-CF-ECM presents significant challenges. This study aims to achieve appropriate ECM deposition, scaffold thickness, and aligned hiPSC-CF-ECM mechanical strength by optimizing the culture duration. Ranging from 2 to 10 weeks, hiPSC-CFs were cultured on micro-grated polydimethylsiloxane (PDMS) substrates to direct the alignment of hiPSC-CFs and their secreted ECM. Following analysis, hiPSC-CFs exhibited a production rate of 13.5 µg of ECM per day per 20,000 cells seeded. An anisotropic and nanofibrous hiPSC-CF-ECM scaffold with a thickness of 20.0 ± 2.1 µm was achieved after 6 weeks of culture and decellularization. Moreover, hiPSC-CMs were cultured on the hiPSC-CF-ECM scaffold for 7 days and evaluated for structural and functional markers of maturation. hiPSC-CMs were observed to successfully align with the ECM nanofibers and exhibited mature organization of key structural proteins (cardiac troponin T, connexin 43, F-actin, and sarcomeric alpha actinin) Overall, the culture duration of hiPSC-CFs was successfully refined to yield a robust hiPSC-CF-ECM scaffold containing structural proteins that accurately resemble the native cardiac microenvironment. This entirely biological, anisotropic, and cardiac-specific ECM holds significant promise for cardiac patch engineering.

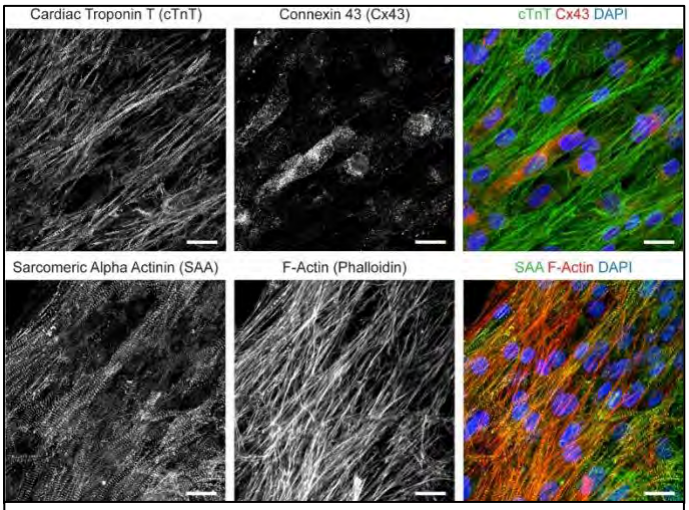

Figure 1. Evaluation of native cardiomyocyte-specific structural and function protein organization in hiPSC-CMs cultured on hiPSC-CF-ECM.

Poster 11

Yap/Taz Function as New Mediators of FGF Signaling Regulating Neural Crest-Derived Cranial Suture Mesenchymal Cells.

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Neural crest cells (NCCs) can give rise to suture mesenchymal cells (SMCs) that are required to keep suture patency and allow for cranial bone growth, repair and regeneration. Studies on syndromic craniosynostosis patients suggested a crucial role of FGF signaling in cranial suture development, yet the detailed pathological mechanisms remain elusive.

By overlaying our CUT&RUN data with published RNA-seq data from NCC-derived frontal SMCs of E18.5 wildtype and FGFR2+/S252W (Apert syndrome mouse model) embryos, we found that over 50% of FGF-regulated genes were potential Yap targets. Additionally, we identified a novel crosstalk between FGF and Hippo-Yap signaling mediated by their downstream effectors p-ERK1/2 and Yap/Taz during cranial suture development. Through in vitro, ex vivo, and in vivo experiments, we observed that FGF signaling activation inhibited NCC-derived SMC osteogenesis and enhanced their stemness, which can be reversed by Yap/Taz deficiency or p-ERK1/2 inhibition. Further comprehensive analysis of our RNA-seq and CUT&RUN datasets indicated that FGF signaling activation enhanced Yap chromatin occupation through p-ERK1/2 during NCC-derived osteogenesis.

Together, our study provides the first evidence that FGF signaling can function through p-ERK1/2 and Yap/Taz to regulate stemness and osteogenesis of NCC-derived SMCs. These findings reveal a novel molecular mechanism underlying NCC-derived SMC development, which opens a new avenue for exploring novel diagnostic and therapeutic methods for cranial suture-related diseases.

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