ISS National Laboratory
CENTER FOR THE ADVANCEMENT OF SCIENCE IN SPACE

TISSUE ENGINEERING AND REGENERATIVE MEDICINE ONBOARD THE ISS

Virtual Seminar Series Report

The seminar series was hosted on July 29, August 4, and August 6, 2020, by the International Space Station (ISS) U.S. National Laboratory, managed by the Center for the Advancement of Science in Space (CASIS).

TABLE OF CONTENTS

About the Organizer:

In 2005, Congress designated the U.S. portion of the International Space Station (ISS) as the nation's newest national laboratory to optimize its use for improving quality of life on Earth, promoting collaboration among diverse users, and advancing science, technology, engineering, and mathematics (STEM) education. This unique laboratory environment is available for use by non-NASA U.S. government agencies, academic institutions, and the private sector. The Center for the Advancement of Science in Space (CASIS) manages the ISS National Lab, under Cooperative Agreement with NASA, facilitating access to the permanent microgravity research environment, a powerful vantage point in low Earth orbit, and the extreme and varied conditions of space. To learn more about the ISS National Lab, visit www.ISSNationalLab.org.

The CASIS mission is to manage the ISS National Lab as a public service in order to benefit the U.S. taxpayer and to foster a scalable and sustainable low Earth orbit economy. We leverage our core competencies, facilitate public-private partnerships, and utilize the platform capabilities and unique operating environment of the space station. We create demand, incubate in-space business ventures, provide access for and awareness of discovery science and technological innovation, and promote science literacy of the future workforce.

The CASIS Vision is to be the Center of Excellence advancing U.S. leadership in commercial space, fostering science and innovation in microgravity and inspiring the next generation.

I. INTRODUCTION

The Center for the Advancement of Science in Space (CASIS), manager of the International Space Station (ISS) U.S. National Laboratory, hosted a three-day online seminar series focused on tissue engineering and regenerative medicine. The National Institutes of Health (NIH), the National Science Foundation (NSF), NASA, and commercial partners have all invested resources in life sciences research leveraging the ISS National Lab to improve life on Earth—specifically in the areas of tissue engineering and regenerative medicine. This research seeks to understand the effects of microgravity conditions on stem cell behavior, 3D cell culture, the construction of complex tissues, and disease modeling (see this white paper for an overview of these efforts).

Building on previous efforts by CASIS to bring together thought leaders in the field of bioengineering, the seminar series reviewed key tissue engineering and regenerative medicine research conducted on the ISS over the past 15 years and examined how the future of this important research could advance biomedical discovery. The series also discussed specific areas of research and new capabilities that enable life sciences research and in-space production on the ISS.

The 2020 virtual seminar series took place over three days, with each day focusing on a specific topic area: Biofabrication (July 29), Organoids and Microphysiological Systems (August 4), and Stem Cells (August 6). CASIS invited terrestrial and microgravity research experts from industry, academia, and government to present their work and views on current trends, research, and challenges specific to their interests. The seminars were moderated by a CASIS staff member, and each two-hour session was open to the public with free registration. This report summarizes of each of the three seminars and provides links to recordings of the virtual events.

At the conclusion of each seminar, an invite-only breakout group met virtually for one hour. The invited members of each breakout group were notified that the primary goal of the breakout session was to convene experts for an open discussion focused on identifying barriers to enabling research and technology development (R&D) in low Earth orbit (LEO) that benefits the biomedical community. Specifically, participants came prepared to share thoughts on the value of a reference mission and/or improvements to a hardware platform or research facility that might advance tissue engineering and regenerative medicine R&D in space. To be seriously considered, a proposed reference mission or facility must be feasible, be ready to implement in three to five years, and offer clearly defined solutions for barriers to using the LEO research environment. Breakout participants included stakeholders from government agencies, academia, industry, and companies developing products and services for the emerging space economy. This report catalogues the lists of all breakout participants and provides a summary of key discussion points.

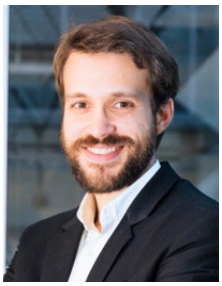
Finally, a collection of open-source resources germane to the topics of this seminar series can be found in the Section VI of this document. CASIS is committed to helping the nation effectively utilize the ISS National Lab for the betterment of humankind. We are grateful to the participants of this seminar series for their contributions and hope this seminar series will serve as a catalyst to further space-based R&D efforts in tissue engineering and regenerative medicine.

II. DAY 1: BIOFABRICATION SESSION

JULY 20, 2020

Seminar Summary: In the past year, the first American bioprinter was sent to the ISS. Additionally, CASIS and NASA have worked in partnership with industry to increase biological additive manufacturing capabilities in LEO. Looking to the future of bioprinting in space and its application for treating diseases on Earth, it is important to reconsider the value of additive manufacturing in microgravity and identify the fundamental questions that have yet to be answered, as well as the technical hurdles that can be addressed by investments in space-based research. This session discussed the future of biofabrication in space.

Session Presenters



Ricky Solorzano, CEO, Allev

Ricky Solorzano is the co-founder and CEO of Allevi, Inc. Allevi creates tools and solutions to design, engineer, and build with life. The company's 3D bioprinters and bioinks are used around the world to find solutions to humanity's most difficult problems—to cure disease, test novel drugs, and eliminate the organ transplant waiting list. Founded in 2014, Allevi's mission is to make it easy to design and engineer 3D tissues. The company created its desktop 3D bioprinters to be the most versatile, powerful, and easy-to-use bioprinters on the market. Allevi is trusted by leading researchers and industry giants in hundreds of labs globally in the fields of tissue engineering, organ-on-a-chip research, pharmaceutical validation, biomaterial development, and regenerative medicine.

Nicole Wagner, Ph.D., CEO, LambdaVision

Nicole Wagner earned her Ph.D. in molecular and cell biology from the University of Connecticut. During the course of her Ph.D. research, she played a critical role in the proof-of-concept experiments that helped to found LambdaVision in May 2009. Through the use of site-directed mutagenesis, site-specific saturation mutagenesis, and directed evolution, Wagner was able to genetically engineer the protein bacteriorhodopsin for a variety of device applications, including protein-based holographic and three-dimensional memories, a chemical detection sensor, and most recently, a protein-based artificial retina. Wagner is an accomplished scientist and entrepreneur with numerous peer-reviewed publications, and she has presented her research at both national and international meetings. In 2012, Wagner received the Connecticut Technology Council's Women of Innovation "Collegian Innovation and Leadership Award" for her work with LambdaVision and was listed as one of CT Magazine's 40 under 40 for the class of 2015. Wagner serves on the board of directors of the New England Women in Science Executive's Club as well as the CT Technology Council. Wagner has been with LambdaVision since its inception and serves as the company's president and CEO. Since taking on the role of CEO, Wagner has been successful in securing more than $8.6M in local, state, and government funding to accelerate the research, development, and commercialization of LambdaVision's artificial retina.

Eugene Boland, Ph.D., Chief Scientist Techshot, Inc.

Eugene Boland has more than 25 years of experience in laboratory research, with a specific focus on developing engineering solutions for cardiovascular diseases as well as chronic wounds. His materials expertise extends from bioinert metals and ceramics to bioactive and bioresorbable electrospun polymers and proteins. After receiving his Bachelor of Science in biomedical engineering from Marquette University in 1994, Boland went on to receive his Ph.D. in biomedical engineering from Virginia Commonwealth University in 2004 after six years in the cardiovascular medical device field. Boland held senior engineering positions with companies such as St. Jude Medical Inc., Cordis, and Cryolife before completing his doctorate. More recently, he held the positions of principal scientist with Tissue Genesis, Inc. and chief of regenerative medicine at the University of Louisville's Cardiovascular Innovation Institute before joining Techshot in the role of chief scientist in 2013. Boland is currently leading a collaborative team managing the Techshot BioFabrication Facility (BFF) installed onboard the International Space Station both for Techshot's own tissue and neo-organ commercial efforts and as a materials R&D platform for the microgravity community. In addition, he is leading efforts at Techshot to develop biologically derived inks (mimetic bioinks) to take advantage of the unique capability that microgravity offers in tissue development, as well as a method to induce, expand, and differentiate human induced pluripotent stem cells in a microgravity environment.

Orchid Garcia, Ph.D., Research Fellow and Lead for 3D Bioprinting and Tissue Regen Technologies, Johnson & Johnson

As a Johnson & Johnson (J&J) research fellow and lead for 3D Bioprinting and Tissue Regen Technologies, Orquidea (Orchid) Garcia is the technical lead for 3D bioprinting and related tissue regen technology development. She is responsible for evaluation and execution of technical strategies and new technologies integration to develop a new class of next-generation healthcare solutions. Garcia works closely with internal business partners as well as technology, academia, and government partners to develop the Centers of Excellence for bioprinting capabilities. Garcia has extensive experience identifying novel technologies through scientific discovery and translating them into patentable, marketable technologies both in industry and academia. Having served as the scientific subject matter expert on numerous initiatives, she brings a keen understanding of worldwide technical, scientific, regulatory, and policy issues that face the business. Previously, Garcia held various positions at J&J in Clinical Affairs and Regulatory Affairs. She received a Bachelor of Science degree in biochemistry and cellular biology from the University of California San Diego; a Master of Science degree in microbiology from California State University, Los Angeles; and a Ph.D. in medical biology from the University of Southern California Keck School of Medicine. She is a fellow of the California Institute of Regenerative Medicine (CIRM) and is based in Irvine, CA.

Session Moderator

Rachel Clemens, Ph.D., Commercial Innovation Manager, CASIS

Rachel Clemens is focused on advancing science and product development through experiments in space. She earned a Ph.D. in molecular biology at Oregon Health & Science University (OHSU) before moving on to NASA's Ames Research Center as a research scientist. In her current role at CASIS, Clemens helps send life science research to LEO to benefit life on Earth. She is passionate about finding new solutions to earthbound problems.

Summary of Session

Recording of full session available online

Ricky Solorzano, Allevi: Plug-and-Play Bioprinting Extruder in Space

Recording available online

Allevi's vision is to provide scientists from academia and industry a more accessible way to test biomaterials in space. Allevi strongly believes that the addition of a plug-and-play biomaterial extruder to the suite of hardware available on the ISS will help lower the barrier of entry to performing bioprinting in space. This speaker presented thoughts about how Allevi, as a terrestrial-based company, can enhance services for its users as well as provide further bioprinting capabilities in space to benefit science on the ground (Figure 1). The design considerations included ISS factors such as being lightweight, having small footprint, and being easily transportable; the design also considers bioprinting capabilities such 4-160oC control micro-volume extrusion and photocuring (visible and UV). The first generation of the Allevi ZeroG will be an extruder focused on biomaterials (i.e., collagen, GelMA, PEGDA, silk, graphene, and hyaluronic acid) without cells, thus enabling studies to achieve complex structures with low-viscosity biomaterials that are not possible on Earth.

Figure 1: Image of Allevi ZeroG bio-extruder. Image courtesy of Allevi

Nicole Wagner, LambdaVision: Enhancement of the Performance and Stability of a Protein-Based Artificial Retina by Manufacturing in Microgravity

Recording available online

LambdaVision has developed a protein-based artificial retina to restore vision to the millions of people who are blinded by retinal degenerative diseases, including retinitis pigmentosa (RP) and age-related macular degeneration (AMD) (Figure 2). Preclinical evaluation of the technology, including ex vivo extracellular recording experiments and in vivo surgical development, demonstrated the ability to reproducibly stimulate degenerated retinal tissue and insert the artificial retina into the subretinal space of both rats and pigs. These milestones provide a foundation for further work to test the safety, biocompatibility, and in vivo efficacy of the technology; however, the outcome of future efforts is dependent on the quality and efficiency of the company's manufacturing methodology. The implants are manufactured using a layer-by-layer (LBL) assembly technique, in which alternating layers of the light-activated protein, bacteriorhodopsin (BR), and a polycation binder are sequentially deposited onto an ion-

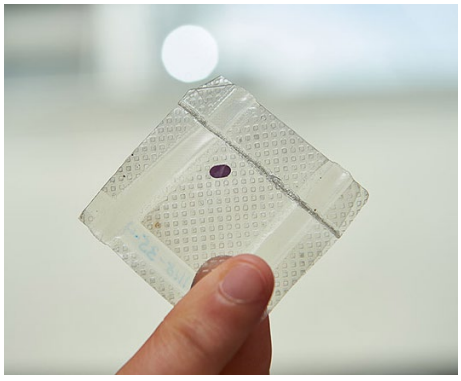


Figure 2: LambdaVision's tiny protein-based artificial retina (the small purple dot), which is about the size of a paper hole punch. Image courtesy of Peter Morenus/UConn Photo

permeable film. The current terrestrial LBL approach is influenced by gravity, in which sedimentation and gradients of solutions interfere with homogeneity and uniformity of the multilayered implants. The company hypothesizes that manufacturing in a microgravity environment will improve the quality of the films and, as a result, will enhance stability and performance of the implant for future preclinical and clinical trials. A pilot manufacturing trial was completed on the ISS via SpaceX's 16th Commercial Resupply Services (CRS) mission and through initial funding from CASIS and Boeing, which led to the miniaturization of a LBL device and the proof of concept for creating multilayered thin films using a LEO platform.

LambdaVision, along with Implementation Partner Space Tango, was recently awarded a $5 million NASA Commercialization award to continue work on the ISS to establish pilot-scale production systems for the artificial retina and future biomedical applications across a number of diverse industries.

Eugene Boland, Techshot, Inc.: Preliminary Experience and Future Direction of the Techshot BioFabrication Facility

Recording available online

Techshot presented initial findings from the maiden flight of its BioFabrication Facility (BFF), which operated onboard the ISS after its launch on the SpaceX CRS-18 and SpaceX CRS-19 missions, as well as lessons learned.

Techshot believes 3D printing in microgravity provides a unique opportunity to utilize biological materials as the foundation for tissue and organ construction. Techshot began developing novel bioinks based partly on cell selection and partly on the fluid physics with the bioprinter to print a neonatal-sized ventricle with human stem cells. The original hypothesis held that structures can be printed with low-viscosity inks to allow cell motility within the structures and rapid fusion of layers. Knowing the viscosity range is only the first hurdle, as the microenvironment of the cell must be able to maintain its viability until the vasculature develops. This is harder in the diffusion-limited environment of microgravity. Nutrients and factors must be both sequestered and actively transported throughout the construct.

The experimental tissues are printed using the Techshot purpose-built, three-locker-sized bioprinter capable of multicomponent printing using its four-independent-head design (Figure 3). The human extracellular matrix-derived components are blended with both partially and terminally differentiated cells to form the custom bioinks. These bioinks are printed into a proprietary bioreactor developed by Techshot that is housed within an Advanced Space Experiment Processor (ADSEP) tissue cassette. The tissue cassette will be placed directly below multiple print heads located on a gantry system with three axes (X, Y, Z) of control. Once a print is complete, the tissue will be stimulated inside of the bioreactor. Fluidic controls, mechanical and electrical stimulation, within the ADSEP tissue cassette are handled in a manner like Techshot's many past space ADSEP projects, with a series of logic-controlled valves, micro-pumps, bags, and solenoids (Figure 3).

Anticipated for this first technology demonstration flight onboard the ISS, the Techshot BFF will print multiple test structures, as well as structural cardiac tissue. Detailed physical and histopathological examinations will compare space-printed and ground-printed samples.

Ultimately, this muscularized tissue could be placed within a patient's damaged cardiac tissue and be inosculated into the coronary blood flow and regain muscular tone. These early demonstrations are the precursor for future microgravity whole organ printing investigations. This 3D bioprinting capability on the ISS will lead to significant commercial advancements in the healthcare industry while also helping humankind explore beyond LEO.

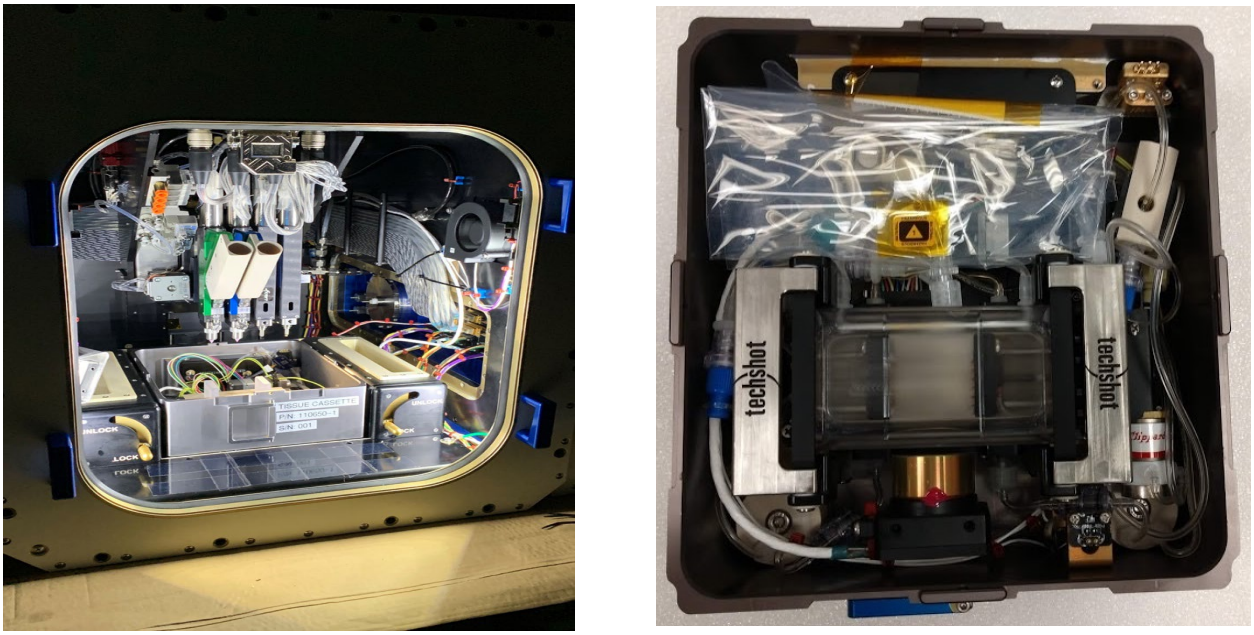


Figure 3: Left figure showing the inside of the BFF, specifically the bioprinter component that is capable of multicomponent printing using its four-independent-head design. Right figure is the tissue culture chamber that the printed material is moved to. Figures courtesy of Techshot, Inc.

Orchid Garcia, Johnson & Johnson: The Promise of Bioprinting: Evolving How We Create and Deliver Personalized Products and Solutions

Recording available online

While still early in development, bioprinting is rapidly evolving as a promising new option to produce, repair, and regenerate human tissue. In this presentation, Orchid Garcia explained Johnson & Johnson's approach to developing a deeper expertise in tissue regeneration and bioprinting technologies to create new ways of diagnosing and treating patients.

Garcia discussed near-term applications, opportunities, and challenges, including next-generation medical implants that enable cellular growth and tissue regeneration and biological tissue models that could potentially make medical products and drug development more efficient and effective (Figure 4). She also discussed Johnson & Johnson's strategy of partnering with top minds around the world in the area of bioprinting and investing in research that brings value to the patient, while also working on more complex tissues and structures in the long term.

Figure 4: Slide presented by Garcia outlining her perspective on the current challenges and opportunities for Johnson & Johnson in the area of bioprinting. Image courtesy of Orchid Garcia (Johnson & Johnson)

Panel Session

After all the speakers presented, a panel session was conducted. A few of the main points discussed included:

- The panel discussed some future experiments they would like to see performed on the ISS in the area of biofabrication. The ideas proposed ranged from materials testing (i.e., examining how differences in viscosity and photo cross-linkers are affected in the microgravity environment) to understanding the interaction of stem cells in their microenvironment and evaluating quality control systems in orbit for monitoring biofabrication in space.
- The panel discussed how conducting experiments in space changes some of the experimental design questions, introducing considerations for surface and fluid interactions as well as miniaturization and automation.

Invite-Only Breakout Session

At the conclusion of the public seminar, a small breakout group met virtually for one hour. The invited members of the breakout group were notified that the primary goal of the breakout session was to convene experts for an open discussion focused on identifying barriers to enabling research in LEO that benefits the biomedical community. Specifically, the participants were encouraged to come prepared to share thoughts on the value of a reference mission and/or improvements to a hardware platform or research facility that might advance the field. The participants were also encouraged to come prepared to talk about how their ideas could be supported by collaborative research efforts, enable real-time or almost real-time iterative science, lead to future projects in the field, and, where possible, identify a dual terrestrial and exploration benefit. A list of the participants and a summary of some of the recommendations from the group is provided below.

BREAKOUT GROUP

Eugene Boland, Ph.D., *Chief Scientist, Techshot, Inc.*

Orchid Garcia, Ph.D., *Research Fellow – Lead, 3D Bioprinting and Tissue Regen Technologies, Johnson & Johnson*

Laurel Kuxhaus, Ph.D., *Program Director, National Science Foundation*

Yu Shrike Zhang, Ph.D., *Assistant Professor, Harvard Medical School*

Nicole Wagner, Ph.D., *CEO, LambdaVision*

MODERATOR

Rachel Clemens Ph.D., *Commercial Innovation Manager, CASIS*

Summary of Discussion Points

- The participants discussed whether a reference mission could be used to address regulator questions. The goal would be to buy down risk for others that might want to manufacture products in space for clinical use on Earth. From an industrial perspective, regulation of additively manufactured bioproducts is ill-defined and, therefore, represents a major hurdle for any of these products to be commercialized. Although regulation of future products is a complicated thing to incorporate into a reference mission, there are clear benefits to leveraging a collaborative science approach to build historical data that can be used in future FDA applications.
- The participants suggested designing an open science experiment around biofabrication in space. The program would involve a collaborative group of investigators, bringing together experts in fluid dynamics, stem cells, bio inks, materials, cardiac tissues, etc.
- The participants addressed the question: How do we view the total product lifecycle of biofabricated products? If any part of that cycle can be accelerated in the LEO environment, it would generate value that could be demonstrated to the community. The U.S. Food and Drug Administration (FDA) is trying to come up with new ways to minimize the time for trials.

- Standardization is a critical issue in the field of bioprinting on Earth. It is challenging to find even two papers with same printing parameters for bioinks. Everything is different, which makes it hard to compare results and advance knowledge. There is an opportunity for space bioprinting to standardize from the beginning.
- Computational tools could be utilized to identify the specific areas of biofabrication that would benefit the most from a sustained LEO environment.

III. DAY 2: ORGANOIDS AND MICROPHYSIOLOGICAL SYSTEMS (MPS) SESSION

AUGUST 4, 2020

Seminar Summary: Microphysiological systems (MPS, also known as tissue chips or organs-on-a-chip) are multicellular engineered platforms that allow researchers to develop in vitro models of human organs. These systems have been used to model rare diseases, test for drug toxicity, screen for cancer, and understand metabolism, among other applications. In 2016, CASIS partnered with the National Institutes of Health (NIH) to develop the Tissue Chips in Space Initiative. The program uses MPS to demonstrate how microgravity could be used to accelerate the induction of diseases such as sarcopenia and cardiomyopathy and then examines how MPS in microgravity could be used to expedite the development of therapeutics for use back on Earth. In recent years, the use of organoids (self-organized three-dimensional tissue cultures) for disease modeling and fundamental biology research has expanded; this includes their integration into MPS devices. This session reviewed highlights from the past year, discussed the future of MPS and organoid research in LEO, and identified potential focus areas for utilization of the ISS National Lab to translate discovery into new products.

Session Presenters

Jeanne Loring, Ph.D., Chief Scientific Officer, Aspen Neuroscience

Jeanne Loring is a world-renowned stem cell scientist and co-founder of Aspen Neuroscience. Loring's work provided the methods for differentiation of autologous induced pluripotent stem cells (iPSCs) into dopaminergic neurons, which formed the basis for Aspen Neuroscience. Loring has a wealth of biotech industry experience including founding Arcos Bioscience (now Viacyte) and leading the development of Hana Biologics, GenPharm International, Molecular Dynamics, and Incyte Genomics. She was the founding director of the stem cell center in the Center for Regenerative Medicine at The Scripps Research Institute and co-founder of the stem cell center at the Sanford Burnham Prebys Discovery Research Institute. Loring is professor emeritus at The Scripps Research Institute, scientific advisor for Summit for Stem Cell Foundation, research fellow of the San Diego Zoo, adjunct professor in Human Genetics at Sanford Burnham Prebys Discovery Research Institute, and adjunct professor in the School of Public Health at San Diego State University. Loring has provided advanced training in human stem cell biology for more than 400 scientists over the last 15 years. She is author of the "Human Stem Cell Manual" and has an issued patent for PluriTest®, a novel bioinformatic test for pluripotency that is publicly available and has been used more than 30,000 times globally.

Deok-Ho Kim, Ph.D., Associate Professor, Johns Hopkins School of Biomedical Engineering

Deok-Ho Kim's group research spans the disciplinary boundaries between nanotechnology, biomaterials, and mechanobiology, with an emphasis on applications to tissue engineering and regenerative medicine. Kim is also a founder of NanoSurface Biomedical (now Curi Bio), which is focused on integrating human iPSC-derived cells, tissue-specific biosystems, and AI-enabled data analytics to accelerate the discovery of new therapeutics. Recently, Kim and his team sent an engineered heat tissue platform to the ISS.

Stefanie Countryman, Director, BioServe Space Technologies

Stefanie Countryman is the director of BioServe Space Technologies and a research associate within the Ann and HJ Smead Aerospace Engineering and Science Department at the University of Colorado Boulder. Countryman has worked for BioServe for more than 20 years. As director, she leads a wide variety of activities including developing and overseeing the strategic direction of BioServe, grant writing, and technical and programmatic oversight of space life science investigations and engineering projects. Countryman has been involved in or directly responsible for the development and/or management of more than 50 different space life science experiments, ranging from the simple to the very complex. As such, she is intimately familiar with the process for developing, launching, and operating life science experiments in space as well as the development of the supporting space flight hardware. Countryman has expertise in translating ground-based life science research into space-based life science research. While she has experience with the translation of many different types of life science experiments, her area of expertise and focus is mammalian cell culture. Recently, Countryman has been involved with experiments examining microgravity's effects on proximal tubule epithelial cells of the kidneys, iPSCs, mesenchymal stem cells, cardiomyocytes, and endothelial cells. Each experiment seeks to utilize the microgravity environment as a model for different types of human disease, including aging, in order to seek more effective treatments for people on Earth.

Lucie Low, Ph.D., Scientific Program Manager, National Center for Advancing Translational Sciences (NCATS)

Lucie Low is a scientific program manager for the NIH Tissue Chips for Drug Development program at the National Center for Advancing Translational Sciences (NCATS) at NIH, where she manages up to 50 transdisciplinary teams funded by NIH to develop tissue chips, or organs on chips—potentially transformational tools for drug development. She is also the NIH and NASA-U.S. Department of Health and Human Services liaison point of contact, working to discuss and facilitate collaborations between NIH and other agencies (e.g., the FDA and the Centers for Disease Control and Prevention [CDC]) and NASA on areas of overlapping agency interest. Prior to joining NCATS in 2016, Low was a research fellow at the National Center for Complementary and Integrative Health at NIH, researching nonpharmacological interventions for the prevention and reversal of chronic pain and the interactions between pain, emotion, and cognition. She obtained her master's and Ph.D. in neuroscience from University College London in the United Kingdom after completing her undergraduate degree at Oxford University.

Session Moderator

Marc Giulianotti, Ph.D., Senior Program Director, CASIS

At CASIS, part of Marc Giulianotti's activities include managing the Tissue Chips in Space initiative, cosponsored by CASIS and NCATS, as well as the Tissue Engineering and Mechanobiology in Space initiative, cosponsored by CASIS and NSF. Prior to joining CASIS, Giulianotti spent more than 20 years working in early drug discovery efforts at the Torrey Pines Institute for Molecular Studies. He received his Bachelor of Science in chemistry/biochemistry from the University of California San Diego, his MBA from San Diego State University, and his Ph.D. in chemistry from the University of South Florida.

Summary of Session

Recording of full session available online

Jeanne Loring, Aspen Neuroscience: Neural Organoids for Neurodegenerative Disease Research in Microgravity

Recording available online

Parkinson's disease (PD) and multiple sclerosis (MS) are neurodegenerative diseases characterized by neuroinflammation. Jeanne Loring and her research team, which includes researchers from the New York Stem Cell Foundation Research Institute and the National Stem Cell Foundation, are using iPSCs derived from donors with PD and MS to investigate the effects of microgravity on neuroinflammation. To create cellular models of these diseases, the team generated three-dimensional spherical organoids composed of iPSC-derived dopamine neurons for PD and cortical neurons for MS. Microglia, the brain's migratory immune cells, were derived from the same iPSC lines and added to both types of organoids to determine whether microgravity affected their gene expression and migration. The team developed a unique culture system to maintain healthy organoids over the month-long experiment on the ISS. The experiment was delivered to the ISS on SpaceX CRS-19, which launched on December 5, 2019. After the organoids were returned to Earth in January 2020, they were analyzed for their composition and gene expression and are being compared to identical cultures maintained on Earth. Preliminary analysis shows that the neurons survived well and matured in the organoids. Detailed analyses of the structure of the organoids and their gene expression were delayed by the pandemic outbreak but are currently in progress (Figure 5).

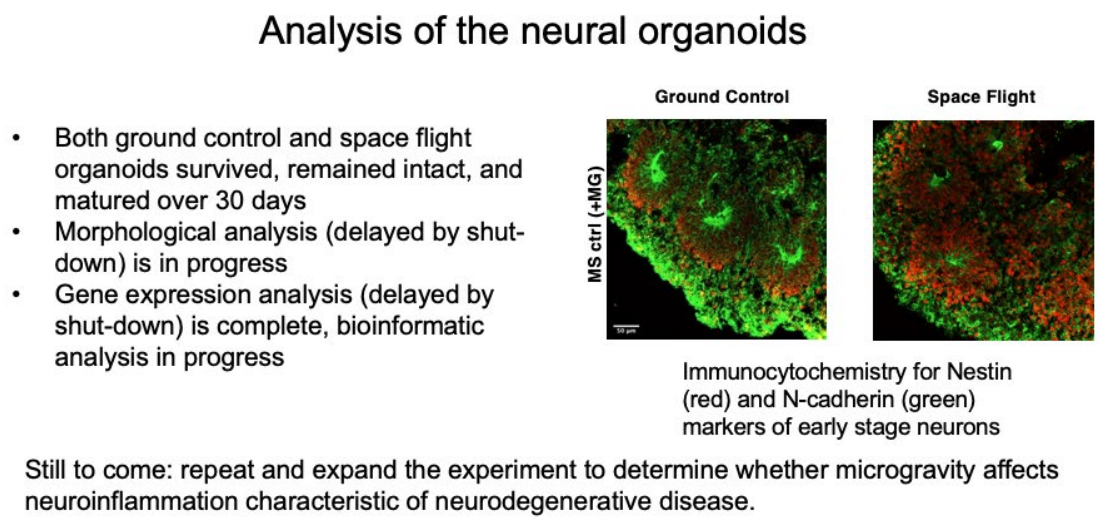


Figure 5: Current status of the neural organoid analysis. Images courtesy of David Marotta (New York Stem Cell Foundation Research Institute) and Jeanne Loring (Aspen Neuroscience)

Deok-Ho Kim, Johns Hopkins School of Biomedical Engineering: A Human iPSC-based 3D Microphysiological System for Modeling Cardiac Dysfunction in Microgravity

Recording available online

Spaceflight has been shown to have a negative impact on the heart and cardiovascular system. Additionally, the effects of spaceflight on the human body appear to mimic an accelerated aging process. Given that heart disease is one of the leading causes of death in adults in the U.S., an understanding of the cardiogenic effects of microgravity may have implications for helping to treat millions of heart disease patients on Earth. Unfortunately, much is still unknown regarding the effect of spaceflight on the cardiovascular system and, in particular, the heart. To address this issue, Kim and his research team have developed a high-throughput MPS model of human cardiac muscle, derived from human iPSCs, to study the effects of microgravity on cardiac tissue structure and physiological function. To generate engineered heart tissues (EHTs) that are physiologically representative of human myocardium, a biocompatible and cardiac-specific decellularized extracellular matrix (dECM)-based electroconductive composite scaffold was used to promote the maturation of cultured cells. These dECM hydrogels contain tissue-specific ECM proteins post-decellularization, and when combined with reduced graphene oxide (rGO), feature native tissue-like stiffness and electroconductivity. EHTs generated with dECM-rGO hydrogel scaffolds exhibited significantly increased force production and action potential conduction velocity compared with tissues generated from dECM-only and collagen scaffolds. A magnetic force sensor system was developed to capture real-time data on tissue contraction and force generation. The team's magnetic approach records the deflection of the posts caused by the contractions of EHTs using giant magnetoresistive (GMR) sensors. They have finalized the integration of their EHTs and GMRs with the spaceflight hardware developed by BioServe.

Specifically, they have stress-tested the culture chambers that will house the tissues to ensure that the tissues will remain viable in the face of unforeseen circumstances during flight and have validated the compatibility of the chambers with maintenance and analytical procedures. Experiment Validation Test (EVT) results indicated that the proposed protocols and procedures for tissue fabrication, histology, and RNA preservation should lead to mission success, and that the custom hardware and software was able to sustain tissue viability and record tissue function over a period exceeding 28 days (Figure 6).

The team successfully launched the investigation on SpaceX CRS-20 on March 6, 2020. EHTs remained on station for approximately 28 days before their return to Earth. A subset of the tissues were returned viable and analyzed for postflight changes in structure and transcriptome (Figure 7). The technologies developed during this study will facilitate the generation of mature 3D engineered cardiac tissues that recapitulate the microarchitecture and function of human myocardium, and the data collected using this platform onboard the ISS will provide a better understanding of how prolonged microgravity affects the structure and function of the human heart.

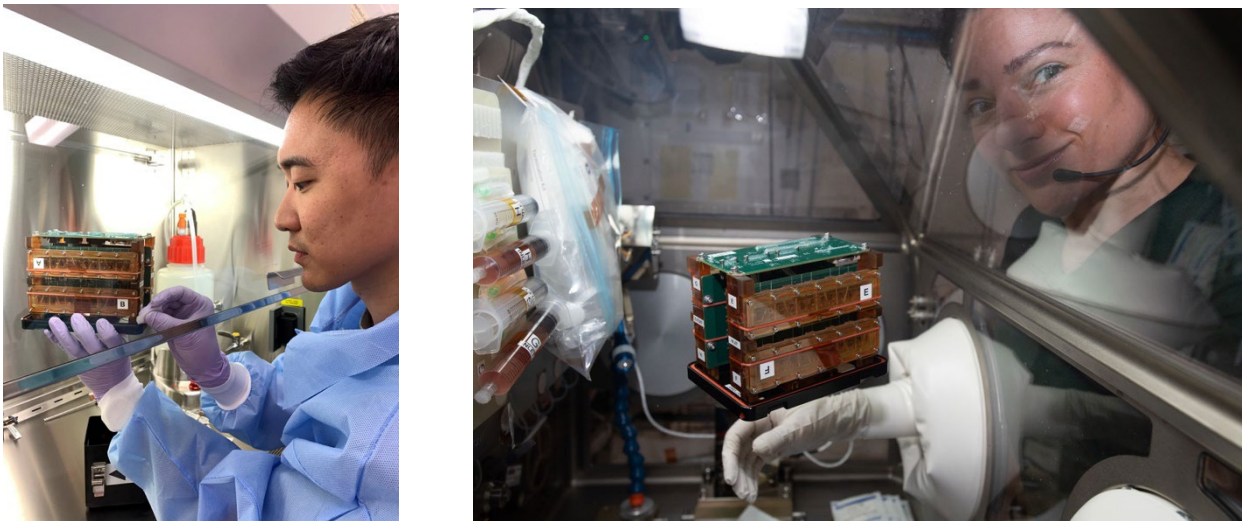


Figure 6: Left: Jonathan Tsui inspecting fully integrated PHAB prior to launch. Right: Astronaut Jessica Meir performing a media exchange in the life sciences glovebox onboard the ISS. Images courtesy of D.-H. Kim (left) and NASA (right)

Stefanie Countryman, BioServe Space Technologies: From benchtop to the International Space Station: supporting MPS and organoid research in a microgravity environment

Recording available online

Translating ground-based experiments utilizing MPS and organoids into a microgravity-based research platform is challenging. Procedures and processes that are relatively simple in a ground-based laboratory are more complex and often difficult to complete successfully in space. Maintaining sterility, liquid containment, appropriate gas exchange, and a stable cell culture atmosphere (37°C, 5% CO_2), as well as managing fluid exchanges while keeping bubbles to a minimum are just a few of the challenges and issues that must be addressed. Countryman discussed the obstacles scientists and spaceflight hardware providers must consider and overcome to successfully conduct these studies in space. Recently, BioServe published an article describing how the company worked with the University of Washington Kidney Chips in Space team to translate ground-based experiments into a viable ISS research platform (Figure 8).

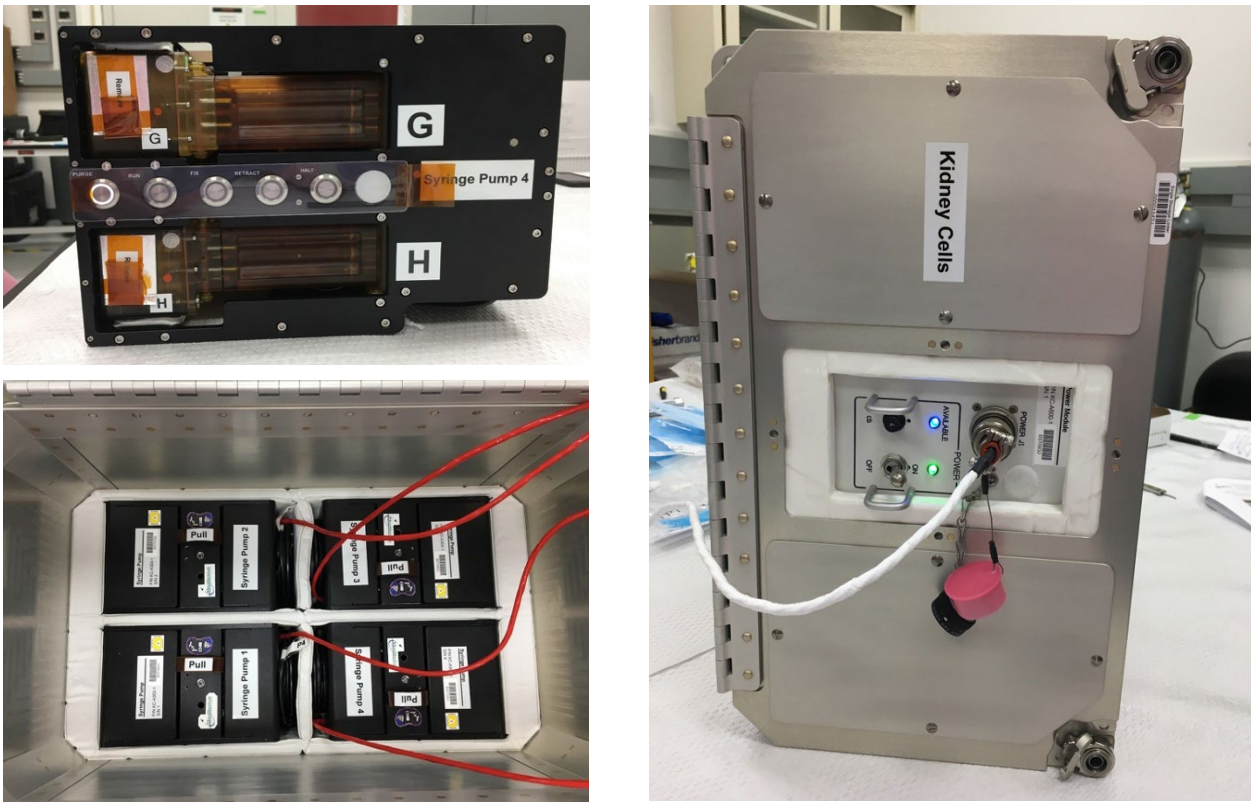


Figure 8: Showing the final hardware configuration for the Kidney Chips in Space ISS payload. The configuration reduced a conventional laboratory setup requiring four syringe pumps, two incubators, and >30.5 m of tubing and a total volume of ~ 1,350 L down to a final installed volume on the ISS of just 45 L. Image courtesy of BioServe Space Technologies

Lucie Low, NCATS: How NCATS Leverages Partnerships to Accelerate Scientific Progress

Recording available online

The National Center for Advancing Translational Sciences (NCATS) at *NIH* aims to advance the process of translational sciences in a disease-agnostic manner, working on common issues that slow down the aim of getting more treatments to patients more quickly than current methods. Currently, drug development can take many years and many billions of dollars to move from promising compounds to approved drugs, and the NIH MPS Program aims to address this. MPS can model human tissues and diseases in vivo to provide useful tools during drug development. The NIH MPS Program has now expanded to nearly 50 multidisciplinary teams under many different trans-NIH initiatives, with a strong emphasis on partnership and collaboration between teams and multiple stakeholders. This includes the Tissue Chips in Space initiative, in collaboration with CASIS. External stakeholders with insight and input into the NIH MPS Program include pharmaceutical industry representation and regulatory bodies—both key end-users of tissue chip technology. The program also focuses on robust validation of the technology at multiple levels, which helps build confidence in the technology and positions it as a transformative technology for multiple applications in the translational sciences. Partnerships and collaborations between academic and industrial parties and engagement across the field of potential MPS end-users helps accelerate and advance the development and uptake of the technology (Figure 9).

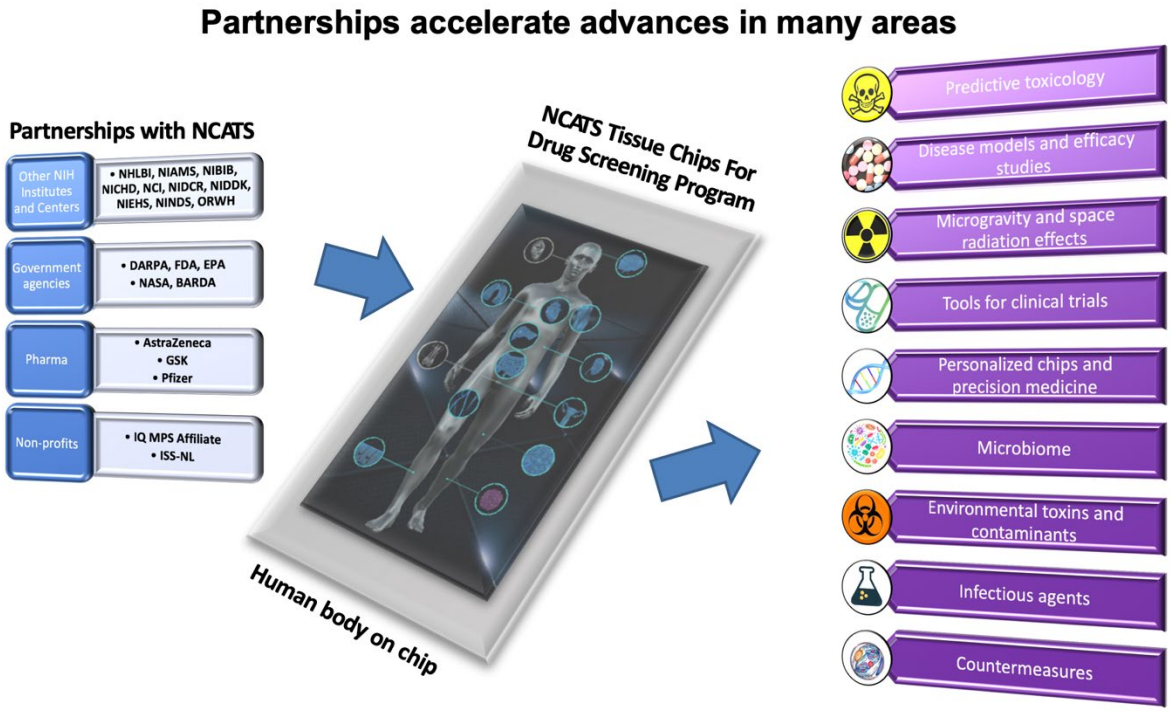


Figure 9: Slide presented by Lucie Low showing how NCATS partners with a wide variety of stakeholders (left side of the figure) to identify the needs of the community for the tissue chip technology. They work together to validate (middle) and apply the technology to goals (right side of the figure) of the stakeholders. The partnerships encourage learning across all the applied goals. Image courtesy of Lucie Low (NCATS)

Panel Session

After all the speakers presented a panel session was conducted. A few of the main points discussed included:

- The panel discussed the trade-offs in complexity of MPS and organoids. While these models are considered to be more in vivo-like than models that utilize systems with 2D single-cell populations, care has to be applied to make sure that the MPS and organoid systems truly recapitulate the desired model and do not introduce nonrelevant artifacts.
- The panel talked about the need to validate all experimental procedures on Earth prior to spaceflight experiments and how these validations help to optimize and advance terrestrial applications such as use of a CO2-independent medium, miniaturization and automation of experimental hardware, and increased reproducibility and viability of models.
- The panel addressed how the research community can work together to share knowledge that advances technology and research while still enabling opportunities for intellectual property and commercialization. Partnerships and consortiums such as the NIH Tissue Chip Consortium are examples of how these efforts can be coordinated.

Invite-Only Breakout Session

At the conclusion of the public seminar, a couple small breakout groups met virtually for one hour. The invited members of each breakout group were notified that the primary goal of the breakout session is to convene experts for an open discussion focused on identifying barriers to enabling research in LEO that benefits the biomedical community. Specifically, the participants were encouraged to come prepared to share thoughts on the value of a reference mission and/or improvements to a hardware platform or research facility that may advance the field. The participants were also encouraged to come prepared to talk about how their ideas could be supported by collaborative research efforts, enable real-time or almost real-time iterative science, lead to future projects in the field, and, where possible, identify a dual terrestrial and exploration benefit. For the organoid and MPS session, two separate breakout groups were formed, as noted below. A summary of some of the recommendations from the groups is also provided below.

BREAKOUT GROUP 1

Stefanie Countryman, *Director, BioServe Space Technologies*

Deok-Ho Kim, Ph.D., *Associate Professor Johns Hopkins School of Biomedical Engineering.*

Catherine Yeung, Ph.D., *Assistant Professor, Department of Pharmacy Investigator, Kidney Research Institute, Division of Nephrology*

Alysson Muotri, Ph.D., *Professor University of California San Diego*

Dilip Thomas, Ph.D., *Postdoctoral Research Fellow, Stanford Cardiovascular Institute*

Jennifer Fogarty, Ph.D., *NASA Human Research Program Chief Scientist*

GROUP 1 MODERATOR

Marc Giulianotti, Ph.D., *Senior Program Director, CASIS*

BREAKOUT GROUP 2

Valentina Fossati, Ph.D., *Senior Research Investigator, New York Stem Cell Foundation*

Jana Stoudemire, *Commercial Innovation Officer, Space Tango*

Al Grodzinsky, Sc.D., *Professor of Biological, Electrical, and Mechanical Engineering, Massachusetts Institute of Technology*

Kevin Costa, Ph.D., *Associate Professor Icahn School of Medicine at Mount Sinai*

Senior Innovation Scientist, Translational Research Institute for Space Health

Joseph Wu, M.D., Ph.D., *Director, Stanford Cardiovascular Institute*

Siobhan Malany, Ph.D., *Associate Professor, University of Florida College of Pharmacy*

GROUP 2 MODERATOR

Liz Warren, Ph.D., *Senior Program Director, CASIS*

Summary of Discussion Points

- One way to focus a reference mission could be to choose a few tissue types that benefit both life on Earth and astronaut health. Brain, gut, and heart tissues have high relevance in patients on Earth but also overlap heavily with research on countermeasures to protect astronauts. Choosing tissues with this type of crossover would provide a dual benefit.
- It would be good to consider areas where there is a point of convergence, such as vascularization. Every tissue has some type of vasculature, and although not all vasculature is created the same, it is a common thread that touches multiple tissues.
- With a focus on vasculature or a few primary cells, a large dataset of the transcriptomic and proteomic analyses could be collected and used to develop a reference chart that could become the gold standard for any other cell type.
- For standardization, it is important to have the ability to say we have an automated system that we understand the physiology with on the ground before going to space. When going from the ground to space, we have to be careful that there is a translation not just from the bench to space science, but from the bench to automation to space science. Validating an automated system and having standards would go a long way in providing robust data sets that could help in directionality.
- It would be helpful if there were a way to cross-compare data from tissue chips, animal studies, and astronauts and provide some standardization to all the transcriptomic data coming from these types of experiments.
- Utilizing well established cell lines for iPSC derived tissues and organoids is critical for generating data comparable to terrestrial research.

IV. DAY 3: STEM CELL SESSION

AUGUST 6, 2020

Seminar Summary: Some of the earliest CASIS-supported investigations were aimed at understanding the effects of microgravity on stem cell behavior. Through these studies, the research community has made intriguing observations and gained greater understanding of the mechanisms underlying stem cell biology. This session reviewed highlights from the past year, discussed what the future of stem cell research in LEO might look like, and identified potential focus areas for the ISS National Lab and the wider space community.

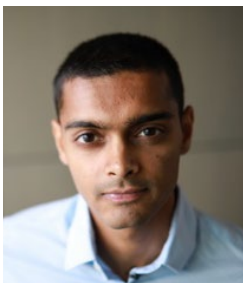
Session Presenters

Abba Zubair, M.D., Ph.D., Medical Director of Transfusion Medicine and Stem Cell Therapy, Mayo Clinic

The Mayo Clinic division that Abba Zubair oversees includes a blood bank that supports one of the largest liver transplant programs in the world, a state-of-the-art clinical apheresis unit, and an FDA-registered cGMP (Current Good Manufacturing Practices) stem cell therapy laboratory. Zubair is a certified international inspector of bone marrow transplant centers and blood banks for the Foundation for the Accreditation of Cellular Therapy (FACT) and the American Association of Blood Banks (AABB). He is currently a consultant in transfusion medicine and a professor at Mayo Clinic College of Medicine. Zubair is also the associate dean of the Mayo Clinic School of Health Sciences. He carried out his clinical residency at the Hospital of the University of Pennsylvania in clinical pathology and undertook a fellowship in transfusion medicine at the Harvard Medical Center in Boston. Zubair obtained a master's degree in clinical trials and principles of drug development at Harvard University and MIT. After his training, he joined the transfusion medicine staff at Brigham and Women's Hospital and the Dana Farber Cancer Institute as a clinical instructor. Zubair received his education at Ahmadu Bello University Medical School in Zaria, Nigeria. He also studied homing and trafficking of human lymphoma cells at the Sheffield University Cancer Institute in England and obtained his Ph.D. in cancer immunobiology from the University of Sheffield in 1995.

Jana Stoudemire, Commercial Innovation Officer, Space Tango

As the commercial innovation officer at Space Tango, Jana Stoudemire leads the commercial market creation for biomedical and technology manufacturing applications in LEO. She transitioned from working in pharma to leading life science research in microgravity, initially as part of CASIS, the organization that manages the ISS National Lab, and then at Space Tango, to focus on building an emerging market in LEO. Stoudemire is a member of the National Academies of Sciences, Engineering, and Medicine Committee on Biological and Physical Science in Space, a member of the Regenerative Medicine Manufacturing Society, and a Women In Advanced Therapies (WIAT) leadership mentor. Previously, she was a member of the New Organ Alliance Oversight Committee and co-chair of the Microgravity Enabling Technology Committee.

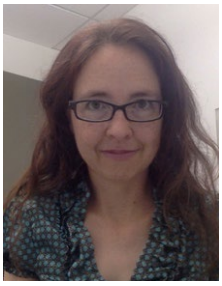


Arun Sharma, Ph.D., Senior Research Fellow, Cedars-Sinai Medical Center

Prior to his current position, Arun Sharma was a postdoctoral research fellow at the Harvard Medical School Department of Genetics. His research focuses on the applications of iPSCs for studying cardiovascular biology, modeling diseases "in a dish," and high-throughput screening of drug toxicity. As a graduate student at Stanford University, Sharma led an effort to send a sample of human iPSC-derived cardiomyocytes to the ISS to investigate the effects of microgravity on human heart cell function.

Stephen Lin, Ph.D., Senior Science Officer, California Institute for Regenerative Medicine (CIRM)

Stephen Lin is a senior science officer at California's stem cell agency, CIRM. He oversaw creation of its iPSC Repository, from tissue collection to line generation and distribution infrastructure. Lin is also the program lead on CIRM's genomics initiative, a consortium involving more than 20 laboratories that applies genomics approaches to stem cell research, driving the creation of a centralized bioinformatics hub for the initiative. In therapy development, he is program lead on a preclinical research organization termed the Translating Center focused on preparing stem cell therapy candidates for clinical trials and has organized conferences on cell therapy manufacturing. Lin manages several discovery- and translational-stage stem cell awards plus CIRM's SPARK internship training program. Prior to CIRM, he was a scientist at Thermo Fisher Scientific and StemCells, Inc of California, developing new methods for genetic analysis and cell therapies for the liver. Lin received his Ph.D. from Washington University in St. Louis and did his postdoctoral research at Harvard University.



Kelly Shepard, Ph.D., Associate Director of Discovery and Translation, California Institute for Regenerative Medicine (CIRM)

Kelly Shepard serves as associate director of discovery and translation at CIRM, where her responsibilities include scientifically administering a large portfolio of early- and translational-stage research awards, overseeing CIRM's state-wide undergraduate and master's-level training programs, and curating CIRM's progress and outcomes for public dissemination. Most recently, Shepard's efforts have focused on helping teams anticipate and address the unique scientific and technical hurdles involved in translating a stem cell-based therapy, from fundamental knowledge gaps to the challenges of process development, manufacturing, and regulatory strategy. Prior to joining CIRM in 2009, Shepard used a variety of multidisciplinary approaches to investigate biological mechanisms underlying cell behavior and function, ranging from the regulation of mitochondrial inheritance and morphology to the study of RNA localization as means of gene regulation. After leaving academia, Shepard led an effort at Parallel Synthesis Technologies, Inc. to adapt a new optical encoding platform for use by biologists in high-throughput screening applications. She has also acted as an independent contractor and biotechnology consultant. Shepard received her Ph.D. from the University of California San Diego and conducted postdoctoral studies at the University of California, San Francisco as a fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

Session Moderator

Liz Warren, Ph.D., Senior Program Director, CASIS

Liz Warren has been involved in spaceflight research for nearly three decades. She was born and raised in the San Francisco Bay Area and attended the University of California at Davis, where she earned both her undergraduate and doctoral degrees in physiology. For her doctoral work, Warren investigated the effects of gravity as a continuum on energy balance in rats. She completed postdoctoral work in cancer biology at the San Francisco Veterans Affairs Laboratory of Cell Growth and in the Neuroscience Laboratory at NASA's Johnson Space Center. Warren has performed a variety of roles at NASA, including serving as deputy project scientist for the NASA Bed Rest and Artificial Gravity Projects. Warren also spent several years as an operations lead in Mission Control for the ISS Medical Project. She is a passionate science communicator and an advocate for human spaceflight and science, technology, engineering, and mathematics (STEM).

Summary of Session

Full session recording available online

Abba Zubair, Mayo Clinic: Expansion of Human MSC for the Benefit of Mankind on Earth and Beyond

Recording available online

Growing stem cells on Earth is challenging and limited to a few population doublings. The standard two-dimensional culture environment is an unnatural condition for cell growth. Therefore, culturing stem cells onboard the ISS under a microgravity environment may provide a more natural three-dimensional environment for stem cell expansion and organ development. In Zubair's study, human-derived mesenchymal stem cells (MSCs) grown in space were evaluated to determine their potential use for future clinical applications on Earth and during long-term spaceflight. MSCs were flown in Plate Habitats for transportation to the ISS. The MSCs were imaged every 24 to 48 hours and harvested at 7 and 14 days. Conditioned media samples were frozen at –80°C and cells were either cryopreserved in 5% dimethyl sulfoxide, RNAprotect, or paraformaldehyde. After return to Earth, MSCs were characterized to establish their identity and cell cycle status. In addition, cell proliferation, differentiation, cytokines, and growth factors' secretion were assessed. To evaluate the risk of malignant transformation, the space-grown MSCs were subjected to chromosomal, DNA damage, and tumorigenicity assays. The research team found that microgravity had a significant impact on the capacity of the MSCs to secrete cytokines and growth factors. The MSCs appeared to be more potent in terms of immunosuppressive capacity compared with their identical ground control (Figure 10). Chromosomal, DNA damage, and tumorigenicity assays showed no evidence of malignant transformation. Therefore, it is feasible and potentially safe to grow MSCs onboard the ISS for potential future clinical applications. The first set of results from this experiment were published in June 2020 in *Nature Microgravity*.

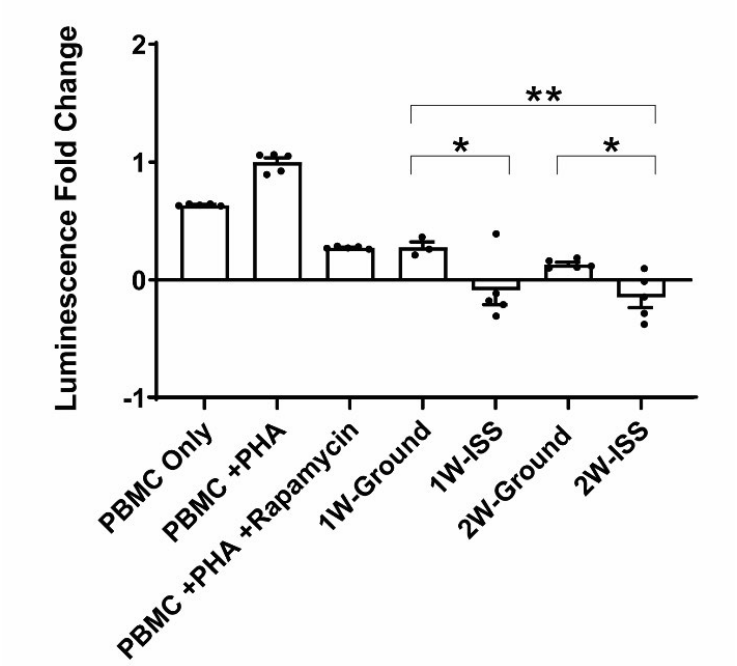


Figure 10: Provided by Abba Zubair (and included in Zubair's recent paper). The figure shows that the measured luminescence fold change was significantly reduced in the ISS samples (1W-ISS and 2W-ISS) compared to the ground samples (1W-Ground and 2W-Ground) and controls (first three bars). The reduction in luminescence corresponds to reduction in the ATP content which is proportional to the number of metabolically active cells, this in turn indicates that the cells cultured in microgravity were significantly more immunosuppressive. Each condition was seeded and measured in quintuplicate. Statistics determined by one-way ANOVA ($n = 5$, $^{*}P < 0.05$, $^{**}P < 0.005$). Error bars represent standard deviation (SD). 1W = 1 week, 2W = 2 weeks, PBMC = peripheral blood mononuclear cells, and PHA = phytohemagglutinin. Source: Huang, P., Russell, A.L., Lefavor, R. et al. Feasibility, potency, and safety of growing human mesenchymal stem cells in space for clinical application. npj Microgravity 6, 16 (2020). https://doi.org/10.1038/s41526-020-0106-z; Creative Commons License: CC BY 4.0

Jana Stoudemire, Space Tango: Expanding Capabilities and Partnerships for Stem Cell Discovery and Manufacturing Applications in Microgravity

Recording available online

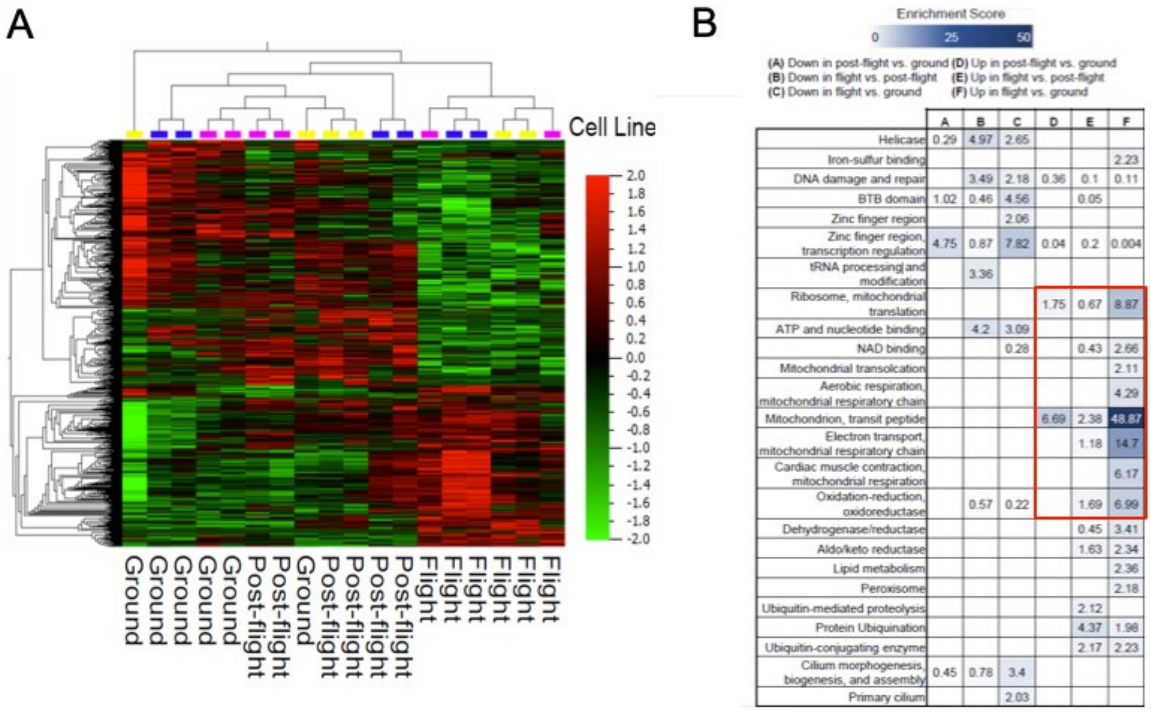
Space Tango is recognized for its expertise in automated R&D and in-orbit manufacturing systems. The company's vision is to inspire, innovate, and create a better future for humanity utilizing the unique environment of space (Figure 11). Current stem cell projects include NIH NCATS Tissue Chips in Space blood-brain-barrier, gut, muscle, and lung/bone marrow projects; NSF Tissue Engineering cardiovascular and bone projects, and brain organoid projects with the National Stem Cell Foundation and University of California San Diego/Arthur C. Clarke Center for Human Imagination evaluating neurodevelopmental and neurodegenerative disease. Additionally, recent partnerships with Cedars-Sinai and the University of California San Diego Health/Sanford Consortium of Regenerative Medicine focus on defining parameters for stem cell manufacturing in orbit and creating capabilities for an integrated space stem cell orbital research laboratory for stem cell translational regenerative medicine applications. Space Tango's mission is to manufacture health and technology products in space that create value and transformational solutions.

Figure 11: Left: Preparing automated cell culture Space Tango CubeLab prior to a SpaceX launch (Image courtesy of Space Tango). Upper right: Space Tango facility onboard the ISS (Image courtesy of NASA). Lower right: Space Tango's Ground Control Center (Image courtesy of Space Tango).

Arun Sharma: Using Human Pluripotent Stem Cells to Model the Effects of Spaceflight on the Heart

Recording available online

With extended stays onboard the ISS becoming commonplace as humanity prepares for exploration-class space missions, the need to better understand the effects of microgravity on cardiac function during spaceflight is critical. However, primary human heart tissues, which would be useful for in vitro studies on heart function, are difficult to obtain and maintain. As a model system, Sharma's research team utilized cardiomyocytes derived from human iPSCs to study the effects of microgravity on human cardiac function and gene expression at the cellular level (Figure 12). This study represented the first time that human iPSC technology has been used to study the effects of spaceflight on human cardiomyocyte function, and results demonstrated that long-term stem cell culture onboard the ISS is feasible. Some results from the study were published in December 2019. Future studies will utilize three-dimensional and tissue-engineered cardiac culture as an improved model system.



	A	B	C	D	E	F
Helicase	0.29	4.97	2.65			
Iron-sulfur binding						2.23
DNA damage and repair		3.49	2.18	0.36	0.1	0.11
BTB domain	1.02	0.46	4.56		0.05	
Zinc finger region			2.06			
Zinc finger region, transcription regulation	4.75	0.87	7.82	0.04	0.2	0.004
tRNA processing and modification		3.36				
Ribosome, mitochondrial translation				1.75	0.67	8.87
ATP and nucleotide binding		4.2	3.09			
NAD binding			0.28		0.43	2.66
Mitochondrial transolcation						2.11
Aerobic respiration, mitochondrial respiratory chain						4.29
Mitochondrion, transit peptide				6.69	2.38	48.87
Electron transport, mitochondrial respiratory chain					1.18	14.7
Cardiac muscle contraction, mitochondrial respiration						6.17
Oxidation-reduction, oxidoreductase		0.57	0.22		1.69	6.99
Dehydrogenase/reductase					0.45	3.41
Aldo/keto reductase					1.63	2.34
Lipid metabolism						2.36
Peroxisome						2.18
Ubiquitin-mediated proteolysis					2.12	
Protein Ubiquination					4.37	1.98
Ubiquitin-conjugating enzyme					2.17	2.23
Cilium morphogenesis, biogenesis, and assembly	0.45	0.78	3.4			
Primary cilium			2.03			

Figure 12: RNA-sequencing data comparing flight (4.5 weeks) and postflight/groundside samples of human iPSC-cardiomyocytes (10 days after return from the ISS). Panel A is a heat map showing which genes were upregulated (red) vs. downregulated (green), note the difference between flight and ground samples. Panel B identifies some of the key genes altered by spaceflight. Source: Wnorowski A, Sharma A, Chen H, et al. Effects of Spaceflight on Human Induced Pluripotent Stem Cell-Derived Cardiomyocyte Structure and Function. Stem Cell Rep. 2019;13(6):960-969. https://doi.org/10.1016/j.stemcr.2019.10.006 ; Creative Commons License: CC BY 4.0

Stephen Lin and Kelly Shepard, CIRM: Stem Cells for Predictive Modeling and Therapies

Recording available online

The mission of the California Institute for Regenerative Medicine (CIRM) is to accelerate stem cell treatments to patients with unmet medical needs. Starting with $3 billion, CIRM has awarded more than 1,000 grants and supported 64 clinical trials in cell and gene medicine, covering a broad range of diseases from cancers to COVID-19. In support of its mission, CIRM has supported five main pillars of investment: infrastructure, discovery, translation, clinical, and education. Discovery, translation, and clinical awards constitute CIRM's core programs, where the expected outcomes establish a pipeline to advance promising discoveries from bench to bedside. Several cell therapies supported by CIRM that are currently in first-in-human trials originated from its early discovery-stage programs and encompass a wide range of cell modalities, including gene therapy, blood-derived stem cell progenitors, and a variety of pluripotent stem cell derivatives. In addition to therapeutics, CIRM's programs have supported the advancement of knowledge in stem cell biology and the development of stem-cell related tools to promote further discovery and translation. Several CIRM investigators have set up projects to study their models in space onboard the ISS, raising exciting possibilities for research and discoveries that can be uniquely enabled in a microgravity environment. Possibilities include using stem cells to model physiological systems for predicting how individuals would react to microgravity and utilizing the microgravity environment to overcome bottlenecks in stem cell technology (Figure 13). Many of these applications utilize iPSCs, which can be created from individual donors. Recent advancements allow cell villages of more than 100 genetically distinct iPSCs to be compared within a single culture in vitro, which could be a platform to conduct population studies onboard the ISS. The studies take advantage of an infrastructure program set up by CIRM to establish an iPSC repository that contains cell lines representing thousands of individuals with various diseases. CIRM anticipates one funding opportunity in 2020 that could support California-based projects for microgravity and space studies.

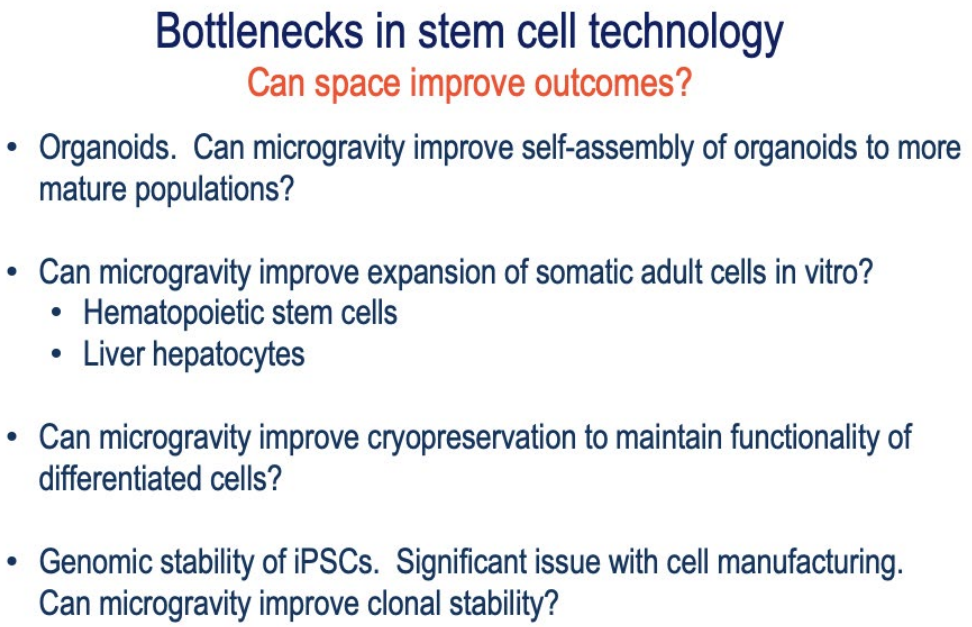


Figure 13: Slide presented by Stephen Lin listing potential areas that microgravity stem cell research might help overcome some of the terrestrial bottlenecks in stem cell technology. Image courtesy of Kelly Shepard and Stephen Lin (CIRM)

Panel Session

After all the speakers presented, a panel session was conducted. A few of the main points discussed included:

- The panel discussed how real-time in space read-outs of immunosuppression and T-cell activation could further advance the knowledge of spaceflight's effects on the expansion of MSCs.
- The panel talked about the potential of using spheroid models to increase understanding of multicellular interactions.
- The panel discussed how spaceflight's accelerated aging effect translates to modeling human diseases here on Earth.

Invite-Only Breakout Session

At the conclusion of the public seminar a small breakout group met virtually for one hour. The invited members of the breakout group were notified that the primary goal of the breakout session is to convene experts for an open discussion focused on identifying barriers to enabling research in LEO that benefits the biomedical community. Specifically, the participants were encouraged to come prepared to share thoughts on the value of a reference mission and/or improvements to a hardware platform or research facility that may advance the field. The participants were also encouraged to come prepared to talk about how their ideas could be supported by collaborative research efforts, enable real-time or almost real-time iterative science, lead to future projects in the field, and where possible identify a dual terrestrial and exploration benefit. A list of the participants and a summary of some of the recommendations from the group is provided below.

BREAKOUT GROUP

Abba Zubair, M.D., Ph.D., *Medical Director of Transfusion Medicine and Stem Cell Therapy, Mayo Clinic Florida.*

Jana Stoudemire, *Commercial Innovation Officer, Space Tango*

Arun Sharma, Ph.D., *Senior Research Fellow, Cedars-Sinai Medical Center*

Stephen Lin, Ph.D., *Senior Science Officer, CIRM*

Kelly Shepard, Ph.D., *Associate Director of Discovery and Translation, CIRM*

Mary Kearns-Jonker, Ph.D., *Associate Professor Co-Director, Cancer, Development and Regenerative Biology Program, Loma Linda University School of Medicine*

Stefanie Countryman, *Director, BioServe Space Technologies*

Chunhui Xu, Ph.D., *Associate Professor, Emory University School of Medicine*

Elizabeth Blaber, Ph.D., *Assistant Professor, Rensselaer Polytechnical Institute*

Araceli Espinosa-Jeffrey, Ph.D., *Research Neurobiologist, UCLA*

Steven Zehnder, Ph.D., *Science Analyst, NSF*

MODERATOR

Marc Giulianotti, Ph.D., *Senior Program Director, CASIS*

Summary of Discussion Points

- Recently, the technology for single-cell profiling has been made more readily accessible, resulting in a dramatic increase in the number of publicly available datasets that can be used to characterize cells. A reference mission—particularly one that focuses on cell lineage and cell identity—that prioritizes projects that may be able to leverage these existing datasets would be powerful because the data is free, easily accessible, and widely available for comparative studies.
- A reference mission that allowed for observation of cell types as they form in space would help provide a better fundamental understanding of the differences in stem cell development in microgravity versus on Earth. The ability to set up complex stem cell studies with differentiation conditions on the ISS and actually observe the dynamics in space—rather than observing the effects after the cell type has been produced—would be very informative.
- Experiments to address fundamental issues such as maintaining pluripotency and maintaining the progenitor state are important because if cells cannot be maintained at the pluripotent state, it is difficult to do anything beyond that.
- The ability to conduct longer-duration experiments is needed, as some processes take months (e.g., maturation or the culture of organoids), and 30-day experiments do not provide sufficient time. Modular systems that allow astronauts to change media cartridges and maintain cell cultures for longer periods could enable longer-duration experiments that provide valuable insight.
- It is important to examine the function of cells in vivo after return from spaceflight. A lot of genomic data from spaceflight studies points to enhanced stemness and the potential for better functional efficacy in in vivo models for repair; however, these need to be tested. There is a lot of genomics testing but very little functional testing after return, and more functional testing is needed.
- In developing good manufacturing practices (GMP), it is important to monitor the production process through various assays that demonstrate that certain criteria are met—closed systems have been developed for this purpose and are capable of performing analyses with minimal input from the user. Such a system could be adapted for use on the ISS to monitor samples and analyze functional parameters over time.

V. THE PATH FORWARD

The Future of Regenerative Medicine and Tissue Engineering in Space

As we prepare to celebrate the 20th anniversary of humans living continuously in space, we can reflect on the marvel that the ISS is a look forward to the future of space-based research. One of the key advantages of the ISS is its sustained microgravity environment. The ability to conduct long-term research in microgravity has enabled opportunities for fundamental studies in topics including organic and inorganic crystal formation, fluid dynamics and transport phenomena, and biological sciences ranging from single-cell to whole-organism studies. Recently, a number of other government agencies, such as NIH, NSF, and the Department of Defense (DoD), have initiated funding programs that utilize the ISS National Lab.

Removing the effects of gravity has contributed significantly to the collective fundamental knowledge of cellular behavior, cell-cell interactions, tissue development and regeneration, as well as the aggregate interactions in the context of a whole organism. Pioneering bioengineering experiments on the ISS coupled with ground-based studies have demonstrated that microgravity enables the study of novel features not attainable under unit gravity conditions, including changes to stem cell proliferation rates

and differentiation. Printing biological materials in microgravity also promises advantages in the use of lower-viscosity biomaterials or bioinks and the ability to fabricate diaphanous, intricate structures as well as perform uniform thin-layer protein deposition. These processes are heavily reliant on biomechanical and mechanobiological cues that are affected by the gravitational field. In this way, the ISS provides an unprecedented opportunity to advance fundamental research in biomechanics and mechanobiology.

We are now at a critical phase in ISS operations, reaching the stage of full utilization of the ISS National Laboratory and now planning for potential next-generation space stations and National Labs that will likely require the establishment of public-private collaborations. Planning must now include resources for fundamental research as well as provide opportunities to prove out commercial opportunities in the areas of tissue engineering and regenerative medicine. Moving into this next phase, CASIS looks forward to working with the community to plan these activities that will shape the future of space-based R&D facilities.

VI. ADDITIONAL REFERENCE MATERIALS

Biofabrication Resources

Publications: Biofabrication (most recent publications)

Aleshcheva G, Bauer J, Hemmersbach R, et al. Scaffold-free Tissue Formation Under Real and Simulated Microgravity Conditions. Basic Clin Pharmacol Toxicol. 2016;119 Suppl 3:26-33.

Anil-Inevi M, Yaman S, Yildiz AA, et al. Biofabrication of in situ Self Assembled 3D Cell Cultures in a Weightlessness Environment Generated using Magnetic Levitation. Sci Rep. 2018;8(1):7239. (Simulated microgravity)

Avitabile E, Fusco L, Minardi S, Orecchioni M, et al. Bioinspired Scaffold Action Under the Extreme Physiological Conditions of Simulated Space Flights: Osteogenesis Enhancing Under Microgravity. Front Bioeng Biotechnol. 2020;8:722. (Simulated microgravity)

DiStefano T, Chen HY, Panebianco C, et al. Accelerated and Improved Differentiation of Retinal Organoids from Pluripotent Stem Cells in Rotating-Wall Vessel Bioreactors. Stem Cell Rep. 2018;10(1):300-313. Epub 2017 Dec 7. (Simulated microgravity)

Grimm D, Egli M, Krüger M, et al. Tissue Engineering Under Microgravity Conditions-Use of Stem Cells and Specialized Cells. Stem Cells Dev. 2018;27(12):787-804. Epub 2018 Mar 29. (Review; not open access)

Grimm D, Wehland M, Pietsch J, et al. Growing Tissues in Real and Simulated Microgravity: New Methods for Tissue Engineering. Tissue Eng Part B Rev. 2014;20(6):555-66. Epub 2014 Apr 4.

Mann V, Grimm D, Corydon TJ, et al. Changes in Human Foetal Osteoblasts Exposed to the Random Positioning Machine and Bone Construct Tissue Engineering. Int J Mol Sci. 2019;20(6):1357. (Simulated microgravity)

Parfenov VA, Khesuani YD, Petrov SV, et al. Magnetic levitational bioassembly of 3D tissue construct in space. Sci Adv. 2020;6(29):eaba4174.

Stamenković V, Keller G, Nesic D, et al. Neocartilage Formation in 1 g, Simulated, and Microgravity Environments: Implications for Tissue Engineering. Tissue Eng Part A. 2010;16(5):1729-36. (Not open access)

Biofabrication Investigations on the International Space Station: NASA Mission Pages

Assessing Osteoblast Response to Tetranite™ in Microgravity Conditions to Induce Osteoporosis (Affiliation: LaunchPad Medical LLC, Principal Investigator (PI): Brian Hess, Sponsoring Organization: ISS National Lab) Assessing Osteoblas

BFF Assembled Next-gen Development of Collagenous Allograft Meniscal Prosthetics aboard the International Space Station (Affiliation: The Geneva Foundation, PI: Joel Gaston, Sponsoring Organization: ISS National Lab)

Maturation Study of Biofabricated Myocyte Construct (Affiliation: Techshot, Inc., PI: Eugene Boland, Sponsoring Organization: ISS National Lab)

The Effect of Microgravity on Stem Cell Mediated Recellularization (Affiliation: Galveston National Laboratory, PI: Joan Nichols, Sponsoring Organization: ISS National Lab)

Additional Resources for Biofabrication

- Techshot Inc. press release: Success: 3D Bioprinter in Space Prints with Human Heart Cells
- ISS National Lab ISS360 article: 3D Printer for Human Tissue Now Available for Research Onboard the ISS National Laboratory
- ISS National Lab ISS360 article: Our BFF Gets to Know the Knee
- NASA article: Three-Dimensional Bioprinting in Space
- ISS National Lab workshop report: Organ Bioengineering Research in Microgravity

Organoids and Microphysiological Systems (MPS) Resources

Publications: Organoids and MPS (most recent publications)

Giulianotti MA, Low LA, McLamb WT, et al. Tissue Chips in Space. IAC-19 A2.7.5x53999 2019.

Low LA, Giulianotti MA. Tissue Chips in Space: Modeling Human Diseases in Microgravity. Pharm Res. 2019;37(1):8. PMID: 31848830. (Not open access)

Mattei C, Alshawaf A, D'Abaco G, et al. Generation of Neural Organoids from Human Embryonic Stem Cells Using the Rotary Cell Culture System: Effects of Microgravity on Neural Progenitor Cell Fate. Stem Cells Dev. 2018;27(12):848-857. Epub 2018 Apr 16.

Park SE, Georgescu A, Huh D. Organoids on a Chip. Science. 2019;364(6444):960-965. (Terrestrial review)

Yeung CK, Koenig P, Countryman S, et al. Tissue Chips in Space-Challenges and Opportunities. Clin Transl Sci. 2020 Jan;13(1):8- 10. Epub 2019 Sep 16.

Organoid and MPS Investigations on the International Space Station: NASA Mission Pages

Cartilage–Bone–Synovium (CBS) Micro-Physiological System (MPS) Investigation Using the Multi-Purpose Variable-G Platform (MVP) (Affiliation: Center for Biomedical Engineering, PI: Alan Grodzinsky, Sponsoring Organization: ISS National Lab with funding support from NIH)

Culturing of Human Myocytes in Microgravity: An In Vitro Model to Evaluate Therapeutics to Counteract Muscle Wasting (Affiliation: University of Florida, PI: Siobhan Malany, Sponsoring Organization: ISS National Lab)

Development of Advanced 3D Organ Culture System Utilizing the Microgravity Environment (Affiliation: Yokohama City University, PI: Tomomi Tadokoro, Sponsoring Organization: JAXA)

Effects of Microgravity on the Structure and Function of Proximal and Distal Tubule MPS (Affiliation: University of Washington Kidney Research Institute, PI: Jonathan Himmelfarb, Sponsoring Organization: ISS National Lab with funding support from NIH)

Human Muscle Contraction Response in Microgravity (Affiliation: University of Florida, PI: Siobahn Malany, Sponsoring Organization: ISS National Lab with funding support from NIH)

Human iPSC-based 3D Microphysiological System for Modeling Cardiac Dysfunction in Microgravity (Affiliation: Johns Hopkins University, PI: Deok-Ho Kim, Sponsoring Organization: ISS National Lab with funding support from NIH)

Lung Host Defense in Microgravity (Affiliation: Children's Hospital of Philadelphia and the University of Pennsylvania, PI: George Scott Worthen, Sponsoring Organization: ISS National Lab with funding support from NIH)

Microgravity as Model for Immunological Senescence and Its Impact on Tissue Stem Cells and Regeneration (Affiliation: University of California at San Francisco, PI: Sonja Schrepfer, Sponsoring Organization: ISS National Lab with funding support from NIH)

Organ-Chips as a Platform for Studying Effects of Space on Human Enteric Physiology (Affiliation: Emulate Bio, PI: Chris Hinojosa, Sponsoring Organization: ISS National Lab with funding support from NIH)

Organoid Formation from Human Stem Cells (Affiliation: University of Zurich, PI: Oliver Ullrich, Sponsoring Organization: ISS National Lab)

Organs-On-Chips as a Platform for Studying Effects of Microgravity on Human Physiology (Affiliation: Emulate Bio, PI: Chris Hinojosa, Sponsoring Organization: ISS National Lab with funding support from NIH)

The Effect of Microgravity on Human Brain Organoids (Affiliation: University of California San Diego, PI: Alysson Muotri, Sponsoring Organization: ISS National Lab)

The Effects of Microgravity on Microglia 3-Dimensional Models of Parkinson's Disease and Multiple Sclerosis (Affiliation: Aspen Neuroscience, PI: Andres Bratt-Leal, Sponsoring Organization: ISS National Lab)

Additional Resources for Organoids and MPS

- NIH Director's Blog by Dr. Francis Collins: Blast Off! Sending Human Tissue Chips Into Space
- NASA article: Small Tissue Chips in Space a Big Leap Forward for Research
- NIH-NCATS website: Tissue Chips in Space
- NIH-ISS National Lab paper: Tissue Chips in Space (presented at the 2019 International Astronautical Congress)
- New York Times article: Organoids Are Not Brains. How Are They Making Brain Waves?
- NASA article: Growing a Smarter Model for Brain Research in Space

Stem Cell Resources

Related Publications: Stem Cells (most recent publications)

Blaber EA, Finkelstein H, Dvorochkin N, et al. Microgravity Reduces the Differentiation and Regenerative Potential of Embryonic Stem Cells. Stem Cells Dev. 2015;24(22):2605-2621. Epub 2015 Oct 22.

Bradbury P, Wu H, Choi JU, et al. Modeling the Impact of Microgravity at the Cellular Level: Implications for Human Disease. Front Cell Dev Biol. 2020 Feb 21;8:96.

Camberos V, Baio J, Bailey L, et al. Effects of Spaceflight and Simulated Microgravity on YAP1 Expression in Cardiovascular Progenitors: Implications for Cell-Based Repair. Int J Mol Sci. 2019 Jun 4;20(11):2742.

Huang P, Russell AL, Lefavor R, et al. Feasibility, Potency, and Safety of Growing Human Mesenchymal Stem Cells in Space for Clinical Application. NPJ Microgravity. 2020 Jun 1;6:16.

Imura T, Nakagawa K, Kawahara Y, et al. Stem Cell Culture in Microgravity and Its Application in Cell-Based Therapy. Stem Cells Dev. 2018 Sep 15;27(18):1298-1302. Epub 2018 Aug 7. (Review; not open access)

Imura T, Otsuka T, Kawahara Y, et al. "Microgravity" as a Unique and Useful Stem Cell Culture Environment for Cell-based Therapy. Regen Ther. 2019 Apr 22;12:2-5. (Simulated microgravity)

Jha R, Wu Q, Singh M, et al. Simulated Microgravity and 3D Culture Enhance Induction, Viability, Proliferation and Differentiation of Cardiac Progenitors from Human Pluripotent Stem Cells. Sci Rep. 2016 Aug 5;6:30956. (Simulated microgravity)

Lei X, Cao Y, Zhang Y, et al. Effect of Microgravity on Proliferation and Differentiation of Embryonic Stem Cells in an Automated Culturing System During the TZ-1 Space Mission. Cell Prolif. 2018 Oct;51(5):e12466. Epub 2018 Jul 12.

Touchstone H, Bryd R, Loisate S, et al. Recovery of Stem Cell Proliferation by Low Intensity Vibration Under Simulated Microgravity Requires LINC Complex. NPJ Microgravity. 2019 May 15;5:11. (Simulated microgravity)

Wang P, Tian H, Zhang J, et al. Spaceflight/Microgravity Inhibits the Proliferation of Hematopoietic Stem Cells by Decreasing Kit- Ras/cAMP-CREB Pathway Networks as Evidenced by RNA-Seq Assays. FASEB J. 2019 May;33(5):5903-5913. Epub 2019 Feb 5.

Wnorowski A, Sharma A, Chen H, et al. Effects of Spaceflight on Human Induced Pluripotent Stem Cell-Derived Cardiomyocyte Structure and Function. Stem Cell Reports. 2019 Dec 10;13(6):960-969.

Stem Cell Investigations on the International Space Station

BioScience-4 (Affiliation: University of California at Los Angeles, PI: Araceli Espinosa-Jeffrey, Sponsoring Organization: NASA-SLPS)

Conversion of Adipogenic Mesenchymal Stem Cells into Mature Cardiac Myocytes (Affiliation: University of Houston, PI: Robert Schwartz, Sponsoring Organization: ISS National Lab)

Development of Advanced 3D Organ Culture System Utilizing the Microgravity (Affiliation: Yokohama City University, PI: Tomomi Tadokoro, Sponsoring Organization: JAXA)

Differentiation of Bone Marrow Macrophages in Space (Affiliation: University of Florida, PI: Bradley Behnke, Sponsoring Organization: NASA-HEOMD)

Effect of Microgravity on Wound Repair: In Vitro Model of New Blood Vessel Development (Affiliation: The University of Arizona, PI: Stuart Williams, Sponsoring Organization: NASA in collaboration with the U.S. Department of Defense)

Effect of the Space Environment on Fertility of Spermatogonial Stem Cells (Affiliation: Kyoto University, PI: Takashi Shinohara, Sponsoring Organization: JAXA)

Effects of Microgravity on Stem Cell-Derived Heart Cells (Affiliation: Stanford University, PI: Joseph Wu, Sponsoring Organization: ISS National Lab)

Functional Effects of Spaceflight on Cardiovascular Stem Cells (Affiliation: Loma Linda University, PI: Mary Kearns- Jonker, Sponsoring Organization: ISS National Lab)

Generation of Cardiomyocytes from Human Induced Pluripotent Stem Cell-Derived Cardiac Progenitors Expanded in Microgravity (Affiliation: Emory University School of Medicine, PI: Chunhui Xu, Sponsoring Organization: ISS National Lab)

Human iPSC-based 3D Microphysiological System for Modeling Cardiac Dysfunction in Microgravity (Affiliation: Johns Hopkins University, PI: Deok-Ho Kim, Sponsoring Organization: ISS National Lab with funding support from NIH)

Microgravity as Model for Immunological Senescence and Its Impact on Tissue (Affiliation: University of California at San Francisco, PI: Sonja Schrepfer, Sponsoring Organization: ISS National Lab with funding support from NIH)

National Laboratory Pathfinder – Cells (Affiliation: Mayo Clinic, PI: Abba Zubair, Sponsoring Organization: ISS National Lab)

National Laboratory Pathfinder – Cells (Affiliation: United States Department of Agriculture, PI: Neil Talbot, Sponsor: ISS National Lab in collaboration with the U.S. Department of Agriculture)

Organoid Formation from Human Stem Cells (Affiliation: University of Zurich, PI: Oliver Ullrich, Sponsoring Organization: ISS National Lab)

Role of the Edocannabinoid System in Pluripotent Human Stem Cell Reprogramming Under Microgravity Conditions (Affiliation: University of Rome Biomedical Campus, PI: Mauro Maccarrone, Sponsoring Organization: NASA-Italian Space Agency)

Space Tissue Loss - Stem Cell Regeneration (Affiliation: NASA Ames Research Center, PI: Eduardo Almeida, Sponsoring Organization: ISS National Lab in collaboration with the U.S. Department of Defense)

Spaceflight Effects on Vascular Endothelial and Smooth Muscle Cell Processes
(Affiliation: University of Florida, PI: Josephine Allen, Sponsoring Organization: ISS National Lab)

Space Tissue Loss - The Effects Microgravity on Stem Cell-Based Tissue Regeneration: Keratinocyte Differentiation in Wound Healing (Affiliation: NASA Ames Research Center, PI: Eduardo Almeida. Sponsoring Organization: ISS National Lab in collaboration with the U.S. Department of Defense)

Stem Cell Differentiation (Affiliation: University of Milano-Biocca, PI: Silvia Bradamante, Sponsoring Organization: ESA)

Study of Mammalian Pluripotent Stem Cells in Microgravity (Affiliation: University of Minnesota, PI: Bruce Hammer, Sponsoring Organization: NASA-SLPS)

Study on the Effect of Space Environment to Embryonic Stem Cells to Their Development
(Affiliation: Osaka City University, PI: Takashi Morita, Sponsoring Organization: JAXA)

The Effect of Microgravity on Stem Cell Mediated Recellularization (Affiliation: Galveston National Laboratory, PI: Joan Nichols, Sponsoring Organization: ISS National Lab)

WetLab-2 Blaber, part of Cell Science-03 (Affiliation: Rensslaer Polytechnic Institute, PI: Elizabeth Blaber, Sponsoring Organization: NASA- SLPS)

Additional Stem Cell Resources

- ISS National Lab Upward magazine article: Pure of Heart: How Microgravity is Improving Cardiac Cell Quality
- ISS National Lab Upward magazine article: Mending a Broken Heart Using Microgravity: Cardiovascular Progenitor Cells Hold Promise for Regenerative Medicine
- NASA announcement: NASA Selects Proposals for In-Space Development of Projects Including Optical Fibers and Stem Cells and Plan to Enable a Low-Earth Orbit Economy
- NASA article: Stem Cells from Space Could Help Mend Broken Hearts