Topical: The Importance of Complex Human *in vitro* Models to Future Human Spaceflight

Dr. Rihana S. Bokhari
NASA Research and Education Support Services
202-967-0907; Rihana.s.bokhari@nasa.gov

Co-authors:

Dr. Dorit Donoviel
Executive Director of the Translational Research Institute for Space Health
Associate Professor, Center for Space Medicine Baylor College of Medicine Houston

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In the next several years NASA intends to move towards longer, deep space missions beyond Low Earth Orbit (LEO). To insure successful missions, it is critical to understand spaceflight stressors on human physiology and evaluate possible countermeasures to safeguard the health of crew members. Current plans within the next several years have small manned missions, two to four crew members, transiting beyond LEO. This will aid in collecting data on spaceflight effects on the human beyond LEO; however, in order to achieve statistical significance, the number of crew members needed surpasses mission capability. One method to collect sufficient data to understand the mechanisms of relevant spaceflight phenomena is the use of complex human *in vitro* models. These new biological analogs are comprised of 3D tissues and can be either self-organizing, called organoids, or engineered to create biological analogs of organs and organ systems, called microphysiological systems (MPS) or tissue chips (Kim et al., 2020; Vunjak-Novakovic et al., 2021). These approaches present a distinct opportunity to study spaceflight phenomena and mechanisms and thus should be considered a topic of importance in NASA's future research endeavors. Conducting research in space has clear constraints, some of which these platforms may be able to circumvent.

Complex human *in vitro* models represent a chance to utilize the spaceflight venue without requiring burdensome physical space and crew time. These platforms also present an opportunity to study biological phenomenon without animal sacrifice. Although early 3D tissues were less representative of human physiology, the new biological analogs are becoming more sophisticated and therefore have greater realistic utility. Finally, NASA, the Translational Institute for Space Health (TRISH), and the International Space Station National Labs (ISSNL) have recently invested significantly in the improvement of these biological analog platforms leading to quality research and advancements in the technology. For these many reasons, complex human *in vitro* models hold promise for swift, targeted, high "n" science to prepare NASA for longer human spaceflight missions beyond LEO.

Current spaceflight research has significant constraints in terms of venue availability, crew time, tissue collection capabilities, etc. Samples from astronauts are extremely difficult for researchers to obtain and typically limited to blood, serum, saliva and urine samples or small diagnostic imaging devices. Due to the minimal availability of astronaut samples and crew time for experiment participation, NASA has long employed animal and human *in vitro* models for spaceflight in order to develop mechanistic understanding of observed phenomena. Mice have played a key role in this area due to their small size, short lifespan and cost effectiveness. Live animals flown to space are also, unfortunately, in low supply and thus insufficient to answer all of the necessary mechanistic questions. While cell culture is possible on the space station it is by nature self-limiting in understanding complex spaceflight phenomenon observed in intact physiological systems. Though the spaceflight venue itself is often minimally accessible to researchers, there are many widely accepted spaceflight analogs that can mimic microgravity and radiation conditions in ways that are relevant for some physiological systems. However, it can be difficult to definitively determine mechanistic pathways through these models as they are often not perfect analogs for spaceflight. In addition to the challenge of imperfect spaceflight analogs,

animal models, due to interspecies variation, have limited success in translating to human physiology. For instance, nearly 90% of clinical trials fail despite successful results in the mouse model (Ingber, 2020). Complex human *in vitro* models present a unique opportunity to study biological mechanistic changes at a more complex tissue level than is possible in cell culture alone without the use of animals or human subjects/samples. This has advantages for spaceflight research in that the mechanisms involved in and phenomenon as a result of microgravity, radiation and other spaceflight environment effects have the potential to be tested and better understood in a realistic setting while not using precious astronaut or rodent samples. Further, additional advantage to complex human *in vitro* models over animal models is that a greater sample size could likely be achieved in a smaller physical space.

While earlier 3D tissue models were rudimentary, complex human *in vitro* models are becoming more sophisticated representations of human physiology. These platforms began in the 1990s as significantly smaller and simpler than their native tissue counterparts; however, they have advanced significantly since then to be capable of approximating distinct molecular, structural and functional tissue phenotypes (Vunjak-Novakovic et al., 2021). Although grouped together for discussion here, these platforms can have different and relevant applications. Organoids can be derived from stem or progenitor cells taken from an organ of interest (Ingber, 2020). They will self-organize into 3D tissues/organs due to self-renewal and differentiation capacities of the stem cells themselves (Ingber, 2020). These models have been used for disease modeling, drug testing and personalized medicine (Kim et al., 2020; Low & Giulianotti, 2019). At present, there are patient derived organoids/organoids on a chip being used in clinical trials for cancer therapy (Ingber, 2020; Kim et al., 2020). MPS are microscale cell culture platforms for *in vitro* modeling that employ engineered microfluidic systems with controlled dynamic environments capable of mimicking the physiological aspects of the tissue/organ that is vital to its function or pathophysiological condition (Ingber, 2020). MPS platforms provide similar capabilities as organoids listed above and may be composed of mono-cultures, or co-cultures of multiple cell types to more accurately recapitulate the tissue/organ.

To provide context to the rapid advancement of this research area, many tissue/organ platforms are now commercially available and the global organ on chips market was valued at $50.8 million in 2020 and is expected to grow to $350.8 million by 2030 (*Organ-On-Chip Market Trends Lead To The Technology Entering Space Research*, 2021). While animal model replacement may not happen for years, these platforms are currently being used in clinical trials with the goal of replacing animal models. The Director of NIH, Francis Collins, stated during the 2017 Senate appropriations hearing that, “Animal safety testing for environmental chemicals and drugs will largely be replaced by tissue chips and iPS cells in 10 years (*Hearing on FY2017 National Institutes of Health Budget Request*, 2016).” Many federal agencies have invested in advancing these in vitro models including NIH, EPA, CDC, DOD, DOE, DARPA, FDA, and BARDA (*'Chipping' Away at Personalized Medicine*, 2021). The EPA stated its intent to eliminate animal model testing by 2035 at the National Academy of Sciences Microphysiological Systems (MPS) Bridging Human and Animal Research Workshop, in January of 2021. The FDA has established an Alternative Methods Working Group to examine systems such as 3D tissues/MPS as substitutes for animal testing for regulatory testing (*'Chipping' Away at*

Personalized Medicine, 2021). FDA is now encouraging the submission of tissue chip data for regulatory submission not just as a supplement to traditional datasets. NCATS has been addressing many technical issues that limit the use of these platforms including establishing Tissue Chip Validation Centers and issuing Clinical Trials on Chip (*'Chipping' Away at Personalized Medicine*, 2021; Low & Giulianotti, 2019; Yeung et al., 2020). The rapid advancement and success of these *in vitro* models has attracted the attention of pharmaceutical companies to incorporate into their drug development pipelines (Ingber, 2020; Kim et al., 2020). Many of the goals other agencies have invested in have relevance to spaceflight related health outcomes. As NASA continues to explore the moon and other planetary bodies in the future, the agency will benefit from these advancements and investments by agency partners and commercial organizations to gain a better scientific understanding of the impact of spaceflight stressors on human health. These biological analogs can be used to understand human physiology in new and unique environments such as those expected to be encountered on planetary bodies outside of LEO. An advantage of these models is that they can be designed to focus on one physiological phenomenon and can result in direct assessment of the effects of genetic and environmental factors on the chosen cellular and tissue function (Vunjak-Novakovic et al., 2021). Mechanistic understanding of space environment effects on many human tissues has been established especially for LEO. However, with advancement of human spaceflight beyond LEO it will be important to quickly understand whether the findings collected over many years are similar in lunar environments and beyond. Future utility of these systems in space goes beyond mechanistic understanding of space environments and can aid in personalized medicine for future crew members. With medicine in space and on earth moving in this direction already, complex human *in vitro* models have a natural role in understanding of drug metabolism and even therapeutic screening which could be necessary to understand the effects of a new drug in space (Vunjak-Novakovic et al., 2021). The utility of these *in vitro* platforms goes beyond single human tissues/organs-on-chip, it is now possible to link together organoids, MPS or combinations together to allow the creation of human-on-chip or body-on-chip systems providing a more human-like model (Ingber, 2020). Currently, more than ten tissue/organ models can be linked to create human-like models and can be probed with standard analytic techniques for evaluating in vitro models or for animal studies, including high resolution microscopy, flow cytometry, - omics, histology and confocal imaging. MPS can also be instrumented with in-line electrical, chemical, mechanical and optical sensors able to incorporate real-time readouts which would be valuable when sending these types of platforms on missions where crew tending is not feasible (Clarke et al., 2021; Zhang et al., 2017). 3D tissues and MPS can play a significant role in accelerating NASA's understanding of spaceflight stressors at the mechanistic level for individual organs and for a system of organ models connected.

While other agencies have been investing in complex human *in vitro* models, NASA and other spaceflight partners have not been on the sidelines. One area in which these advanced model systems can certainly improve the understanding of the spaceflight environment is in the realm of the mechanistic effects of radiation on physiologically relevant tissue. The Translational Research Institute for Space Health (TRISH), which holds a Cooperative Agreement from the Human Research Program focused on transformative space life science research, has already invested in research in this area. In 2020, TRISH released the "TRISH Space Radiation

Solicitation" focused on determining whether complex human models can be used as an effective human analog for radiation studies as well as test and characterize countermeasures for efficacy against high LET ionizing radiation (*TRISH Space Radiation Solicitation*, 2020). The research resulting from this solicitation is currently on-going. In 2017, prior to the release of this TRISH solicitation, the ISSNL, working with the National Center for Advancing Translational Sciences (NCATS), took an interest in funding research using 3D MPS in space to address the high failure rate of drugs in clinical trials (Low & Giulianotti, 2019; Yeung et al., 2020). Space focused programs have invested significantly in the improvement and development of these platforms for use in space relevant research. The NASA Space Biology Program has also released a solicitation centered on improving the longevity of such platforms and testing relevant perturbations (*'Chipping' Away at Personalized Medicine*, 2021). With improved longevity and autonomy these platforms can be even more useful in understanding the impacts of spaceflight on physiologically relevant human tissues under similar conditions to those astronauts will face beyond LEO. NASA can leverage these platforms by sending them on long duration deep space missions including free-flyers both in LEO and beyond to assess gravity alterations and differences in radiation spectrum. Additionally, Gateway provides a realistic analog of Mars transit like radiation and spaceflight exposure inside of habitation and logistics outpost (HALO) and can help NASA assess mechanistic effects of spaceflight stressors on biology to support fundamental science and crew health. Complex human *in vitro* models would be especially useful here since NASA does not currently plan to send enough manned missions beyond LEO to establish statistically significant results via human or animal research before moving beyond Lunar travel. The use of these biologic analogs on future Commercial Lunar Payload Services and Artemis missions to the lunar surface would provide valuable data on deep space radiation, albedo radiation effects, thermal and lunar dust challenges, 1/6 gravity and allow for the comparison across the gravity continuum and altered radiation by comparing data to ground based studies and those conduced in LEO. Data generated from the use of biologic analogs in these venues would help increase the statistical significance, improve predictive models and refine NASA's understanding of adverse events for future exploration missions such as Mars, and provide realistic combined spaceflight stressors that cannot be replicated on Earth or in LEO. While accomplishing this effort is not without challenges, 100% autonomy would need to include evaluation of biological end points and monitoring sensors as well as environmental life support on the vehicle containing the platforms, it presents an opportunity to better understand the impacts of the space environment on human physiology at an accelerated time table without the use of large amounts of crew time or live animals.

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