Topical white paper:

Human organs-on-a-chip platforms for developing countermeasures to space radiation

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This topical white paper is in response to the call of the National Academies and NASA to help inform U.S. priorities for research in space for the coming decade, and define our vision and strategy for a decade of transformative scientific research that is critical for supporting human space exploration, with additional major benefits to human wellbeing on Earth. We highlight the capability of recent advances at the cross-sections of biomedical engineering, nanotechnology and radiation biology/physics to address the grand challenge of *determining and counter-acting the effects of space radiation on human organs, within one decade*.

*We suggest that currently-available Organs on a Chip (OOC) (**Figure 1**) represent a technologically practical and mechanistically validated methodology for a) assessing space-radiation risk models, and b) testing space-radiation countermeasures. Currently available OOC technology will facilitate these two central goals not only for "average" individuals, but also for specific individual travelers in space. We emphasize that appropriate OOC technology is available now, allowing for dramatic short cuts in the time scale required both for space-radiation risk estimation and for countermeasure development and validation.*

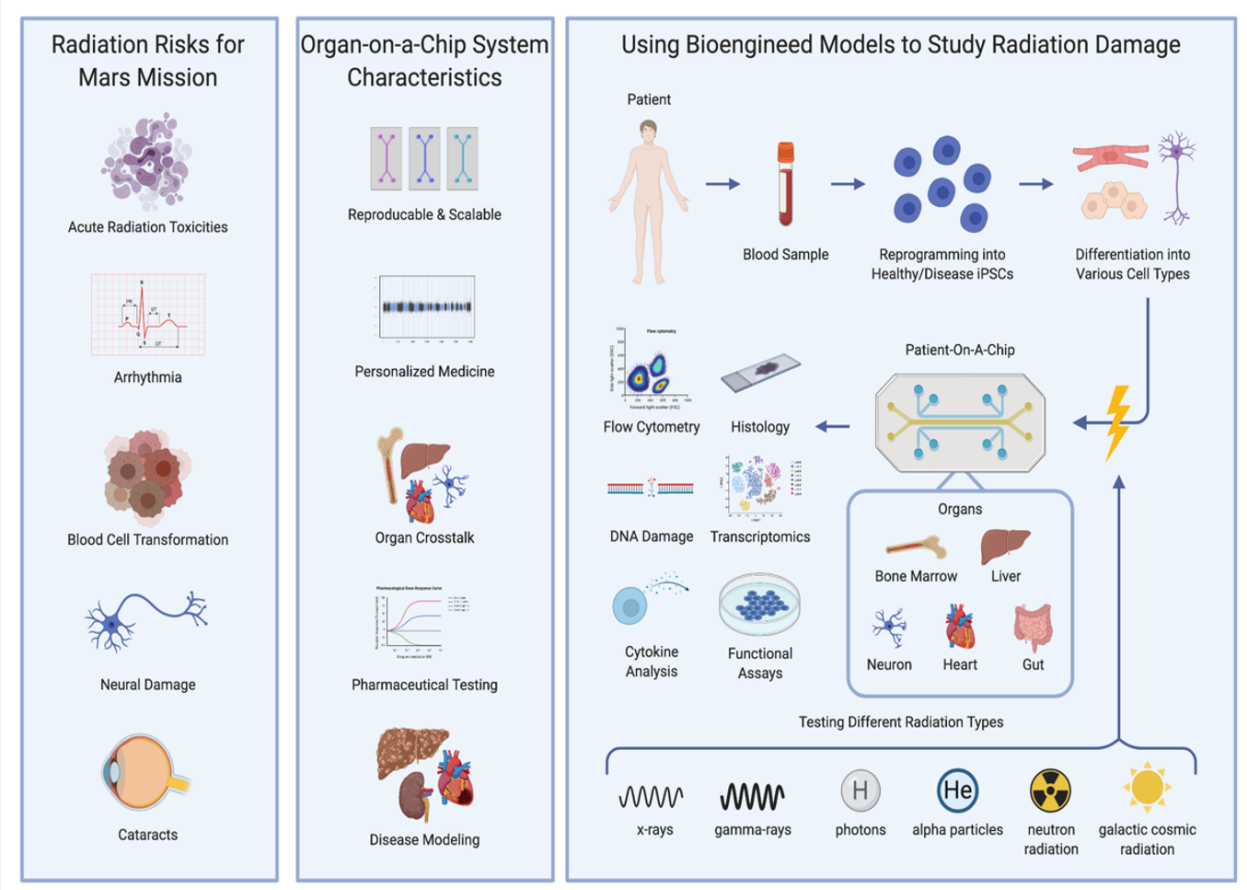

Figure 1. Overview of the Organs on a Chip technology

The health effects associated with the exposure to radiation during long-term deep-space missions are critically important for the NASA mission and are still poorly understood, preventing the development of countermeasures to the damage caused by space radiation. There is a compelling need to accurately determine the safety thresholds and mechanisms of various

types of cosmic radiation damage at the molecular (DNA), cellular, tissue, and organ levels, to inform the development and validation of safe and effective radiation countermeasures during extended space travel. For long space flights (mission to Mars), *determining the exact regime for mitigating the radiation damage for each individual on mission will be critical*, given the individual differences in responses to stress and countermeasures and the need to maintain the wellbeing of astronauts over long time and under extreme conditions.

A critical requirement for addressing this major challenge is the availability of experimental models utilizing *functional human tissues capable of modeling organ level functions under normal physiological conditions and in response to stress*. Organs on a Chip (OOC) evolved over the last 10 years, to meet the need for human tissue models that can accurately recapitulate cell/tissue/organ functions in health and disease. These models are a powerful tool to enable quantitative assessments of tissue responses to environmental factors and therapeutic measures in human tissue settings. Current OOC, with multiple miniature tissues grown in vitro and integrated by vascular perfusion, are being increasingly used to model human patho/physiology [1-3].

The initiative and a consortium of projects led by the National Center for Advancing Translational Sciences (NCATS), in collaboration with the other NIH institutes and centers, the FDA, and NASA, has accelerated the growth of this new field and resulted in the development, validation, and utilization of various human tissue platforms. The initial focus on evaluation of drug safety has extended to modeling of tissue injury and disease in human tissue models, studies of pain, immune system, infections, and even to clinical trials on a chip. A major part of this initiative is the "Chips in space" project, a collaborative effort between NCATS and the International Space Station U.S. National Laboratory (ISS National Lab). This collaborative program is supporting ground and flight studies of tissue- and organ-on-chip platforms for on-flight experiments, and the translation of the scientific results into benefits for human health. After less than 10 years, the program has already resulted in commercial entities that are already advancing the development and testing of new medicines. These models are now being utilized at the cross-sections of biomedical engineering, nanotechnology, stem cell biology and radiation biology/physics, to support the development of countermeasures tailored for use during long space missions.

Modeling integrated human physiology in vitro is a formidable goal with the potential to transform biological research and eventually healthcare. Current studies largely rely on human cell cultures and small animal models, neither of which provides an adequate representation of cellular and tissue responses in the human body. While such reductionist approach to determining organ level behavior is well established and allows scaling, the insufficient resemblance of human biology provided by these models limits their predictive power. OOC seek to combine the best of both models by culturing human cells in tissue-specific settings designed to recapitulate the multifaceted cellular and extracellular cues – molecular, structural and physical, that are found in vivo [2]. While the term OOC suggests that mini-organs are grown on a chip, it is important to note that this elusive goal has not been achieved. Instead, these systems contain small tissue constructs designed to reproduce just one or a few specific functional properties of the entire organ, as for example: barrier function of the skin, lung vasculature, muscle contractility or liver metabolism. The simplicity of these models is a major advantage, as it allows direct assessments of the effects of genetic and environmental factors on cellular and tissue function [1].

OOC are harnessing the established approaches from tissue engineering that utilize tissue-specific scaffolds (serving as structural and informational templates for tissue formation) and the provision of molecular and physical signals that regulate cell function and tissue development. Through these features, OOCs provide consistency of tissue structure and phenotypes for studies of organ-level functions, while often incorporating only a few cell types found in the native organ. OOC currently in use include heart muscle, liver, alveolar unit of the lung, blood-brain-barrier, kidney glomerulus and proximal tubule, neuromuscular junction, vascular networks, skin, retina, pancreas, gut, bone marrow, and placenta, with a continually growing list of tissue models emerging. These OOC are being used to study tissue maturation, regeneration and disease. Coupling multiple tissues together by vascular perfusion, one can study organ-organ interactions and systemic diseases such as cancer, inflammation or infection (please see details in reference [1]).

Human tissue platforms for *in vitro* studies of integrated human physiology in health and disease are becoming increasingly predictive of clinical data. Not surprisingly, numerous OOC start-ups launched from academic laboratories are starting to fill the commercial space and to be used for drug testing in pharmaceutical industry [4] including GSK [5], Roche [6] and Pfizer [7]. This technology is ready for application in space-related research. However, there are no widely adopted ground-based human tissue models that would enable predictive studies of the risks associated with long space flights and the mitigation of these risks. OOC have capability to address this critical gap, through the use of an already established highly innovative human "organs on a chip" model of radiation exposure for assessing cell and tissue damage and investigating radiation countermeasures.

Over the last decade, our team has bioengineered a range of human tissues starting from induced pluripotent stem cells (iPSCs) (heart, liver, bone, bone marrow, skin, sensory neurons, motor neurons, skeletal muscle, midbrain). These tissues can be matured to display many aspects of the adult human phenotypes, and can be studied either individually (to recapitulate specific tissue or organ functions), or physiologically connected to each other (to investigate systemic conditions and responses). Most multi-tissue OOCs include liver, as the primary site of nutrient and drug metabolism. It is also possible to include immune cells, or to provide sources of immune cells by including immune organs (e.g., bone marrow module). More advanced systems are now being developed to provide a tissue specific niche for each tissue module, mimic the systemic vascular network, and include routing of culture media and biosensors for on-line readouts. Such systems allow scaling of organ volumes and blood flow to match the in vivo situation, while enabling high throughput screening and extended culture times. Linking multiple tissues by vascular perfusion has important biological impacts on each tissue (via cellular cross-talk inherent to the in vivo environment), and also requires specialized designs that allow the preservation of the individual tissue phenotypes [1].

This area of research resides at the cross-sections of many disciplines and also requires expertise in stem cell biology, systems biology, computational modeling, nanotechnologies design and testing of radiation countermeasures. Our team has greatly benefited from our highly diverse and complementary expertise, combined resources and the history of collaboration that resulted in a strong and cohesive team. We believe that these requirements will be universally important for the envisioned decade of transformative scientific research that is needed to support human space exploration. Some additional features are important for most OOCs:

(i) *Modular design* allows configurability, such that the same platform can be used to accommodate different types and numbers of tissues, depending on the question being studied. The selection of tissue types will vary from one study to another, and the use of the same platform that is broadly available, and can be standardized and easily manufactured is a significant advantage. Modularity also allows changing the order of tissues and the direction of flow of blood substitute connecting the tissue chambers. In the context of radiation studies for space missions, an already established modular platform can be extended to studies of other or additional tissue/organ systems of interest.

(ii) *Derivation of all tissues from the same source of human cells*. Induced pluripotent stem cells (iPSCs) is a practical cell source, given their broad availability and robust protocols for their derivation from small blood samples and differentiation into multiple linages of tissue-specific and supporting cells. This approach provides defined genetic background and allows insights into the diversity of responses, by studying sex, age, race and state of health or disease as experimental variables. In the context of long space missions, OOC enable an individualized approach for studying an "astronaut on a chip".

(iii) *On-line functional readouts* Noninvasive data acquisition in longitudinal studies where the same biological sample is repeatedly evaluated over time, are invaluable for increasing consistency of experimentation and capturing the biological dynamics. This way, each sample becomes an experiment by itself ("n=1 study") allowing monitoring of the progression of events, and the dynamics of responses to a perturbation or treatments. Examples include measuring in real time the contraction frequency and force generation of heart and skeletal muscle, and calcium flux measurements established for a number of tissue systems.

Strong expertise in radiation biology and radiation physics is critical for the use of OOC for studying the effects of space radiation. During deep space missions, astronauts will be exposed to a protracted mix of high-LET (densely ionizing) and low-LET (sparsely ionizing) radiations. While the low-LET radiations dominate by dose, it is anticipatedthat the high-LET radiations may dominate radiation risks. The mission-relevant high-LET radiations are a mix of galactic cosmic ray (GCR) heavy ions and fast neutrons, and are best reproduced by the neutron facilities at Colorado State University and Columbia University, while the best GCR simulation facility is the NSRL at Brookhaven National Laboratory.

Because the various different deep-space missions currently under consideration will result in different overall high-LET and low-LET doses, the investigators follow the dose recommendations from the NASA HERO (Human Explorations Research Opportunities) program that identified common dose parameters for comparisons across current and future research studies, as well as to provide comparison with well-established research data sets.

Using this platform, our team is developing novel modalities for radiation countermeasures that can provide sustained long-term effectiveness during long space missions, that will be tested against mission-relevant doses of high-LET and galactic cosmic rays. To demonstrate the utility of OOC for studies of space radiation, we have established a research platform consisting of four **t**issues: bone marrow (target of acute radiation damage and source of immune cells), heart muscle (target of chronic radiation damage), liver (site of metabolism), connected by vascular perfusion containing circulating cells. The tissues in platform are being studied for weeks to months, with exposure to radiation and radiation countermeasures, allowing the assessment of

the acute and chronic effects of radiation and radiation mitigation strategies. Our group, as well as many others in this space, are constantly looking to benchmark our findings to available data from animal models, astronauts from missions to the International Space Station, and accidental radiation exposure incidents. We hope that we will be able to both identify biomarkers of the acute radiation-induced damage (immediate changes to structure, function, gene expression) as well as long-term effects (i.e. mutational incidence, cancer, fibrosis, chronic inflammation).

In summary, OOC offer unique opportunities to investigate effects of radiation on human tissues and organs and to evaluate the effectiveness of candidate countermeasures. These platforms are modular, configurable, highly controllable and equipped for long-term longitudinal studies with on-line assessment of changes in tissue function. The ability to bioengineer all tissues starting from the same iPS cells provides unprecedented opportunities for individualized ("astronaut on a chip") studies. Given the ongoing translation of OOC into commercial evaluation of drug toxicity and efficacy, and recent space studies of OOC, we anticipate that they will demonstrate their utility in research advancing NASA's mission in less than a decade.

References

1. Vunjak-Novakovic G, Ronaldson-Bouchard K and Radisic M. Organs on a chip models for biological research. ***Cell*** 184: 4597-4611, 2021.
2. Ronaldson-Bouchard K. and Vunjak-Novakovic G. Organs on a Chip: a fast-track for engineered tissues in drug development. ***Cell Stem Cell*** 22(3): 310-324, 2018.
3. Tavakol DN, Fleischer S and Vunjak-Novakovic G. Harnessing organs-on-a-chip to model tissue regeneration. ***Cell Stem Cell*** 28(6): 993-1015, 2021.
4. Zhang B and Radisic M. Organ-on-a-chip devices advance to market. ***Lab on a Chip*** 17: 2395-2420, 2017.
5. Ekert JE, Deakyne J, Pribul-Allen P, Terry R, Schofield C, Jeong CG, Storey J, Mohamet L, Francis J, Naidoo A, Amador A, Klein JL, Rowan W. Recommended Guidelines for Developing, Qualifying, and Implementing Complex In Vitro Models (CIVMs) for Drug Discovery. ***SLAS Discov***. 25(10):1174-1190, 2020
6. https://www.roche.com/research_and_development/what_we_are_working_on/research_technologies/institute-for-translational-bioengineering.htm
7. Kopec AK, Yokokawa R, Khan N, Horii I, Finley JE, Bono CP, Donovan C, Roy J, Harney J, Burdick AD, Jessen B, Lu S, Collinge M, Sadeghian RB, Derzi M, Tomlinson L, Burkhardt JE. Microphysiological systems in early stage drug development: Perspectives on current applications and future impact. ***J Toxicol Sci.*** 46(3):99-114, 2021.