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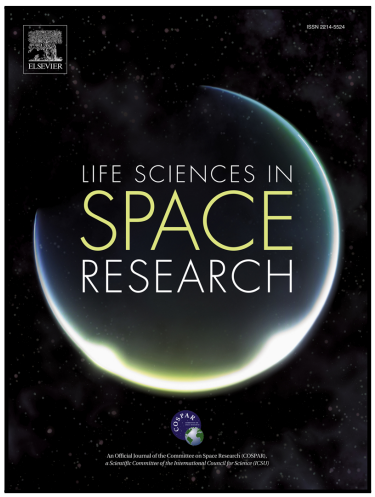

Looking on the Horizon; Potential and Unique Approaches to Developing Radiation Countermeasures for Deep Space Travel

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Title: Looking on the Horizon; Potential and Unique Approaches to Developing Radiation Countermeasures for Deep Space Travel

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Abstract

Future lunar missions and beyond will require new and innovative approaches to radiation countermeasures. The Translational Research Institute for Space Health (TRISH) is focused on identifying and supporting unique approaches to reduce risks to human health and performance on future missions beyond low Earth orbit. This paper will describe three funded and complementary avenues for reducing the risk to humans from radiation exposure experienced in deep space. The first focus is on identifying new therapeutic targets to reduce the damaging effects of radiation by focusing on high throughput genetic screens in accessible, sometimes called lower, organism models. The second focus is to design innovative approaches for countermeasure development with special attention to nucleotide-based methodologies that may constitute a more agile way to design therapeutics. The final focus is to develop new and innovative ways to test radiation countermeasures in a human model system. While animal studies continue to be beneficial in the study of space radiation, they can have imperfect translation to humans. The use of three-dimensional (3D) complex in vitro models is a promising approach to aid the development of new countermeasures and personalized assessments of radiation risks. These three distinct and unique approaches complement

traditional space radiation efforts and should provide future space explorers with more options to safeguard their short and long-term health.

Key-Words
Space Radiation, 3D tissue, Organoid, Extremophile, Nucleotide-based Approaches, Radiation Countermeasure

1.1 Background

Space radiation differs from commonly encountered forms of radiation emitteed by diagnostic and therapeutic procedures (X-rays and gamma-rays) because it comprises a mixed field of high-energy protons and nuclei components originating from either solar particle events (SPEs) or galactic cosmic rays (GCR), which are made of high atomic number and energy (HZE) radiation particles. HZE radiation particles are particularly concerning because their track structure, the particle's energy deposition as it passes through a tissue, leaves significant complex damage both along the track structure and from energetic electrons that are formed due to the particle's interactions with the tissue (1). The result of this damage in living tissues is mutations and sometimes cell death. Space radiation exposure (unless from SPEs) occurs at a low dose rate, of approximately 0.5 mGy/day. The complex nature of GCR is attributable to its composition of numerous particle species, making them potentially harmful even at low dose rates. The major GCR particle types include H (protons, approximately 87%), He (approximately 12%), about 2% positrons and electrons, and about 1% of HZE particles with broad energy spectra of interest (from Li to Zn) primarily from ~10 MeV/n to 10,000 MeV/n (2). On the other hand, SPEs are composed chiefly of protons and have a broad energy spectrum extending out to a few hundred MeV. On Earth, life is shielded from space radiation by the Earth's magnetic field. Astronauts in low Earth orbit (LEO) have partially benefited from this protection, although their exposure is greater than those on Earth (3).

On future missions to the moon and Mars, astronauts will experience prolonged exposure to radiation at a low dose and low fluence (4). Given this dose rate at the expected energies is not replicable on Earth, the true health effects of space radiation remain largely unknown. Although HZE particles represent only 1% of the GCR spectrum, they will likely contribute most of the absorbed dose. Exposure to HZE particles may result in significant effects on astronauts; thus studying the effects of such particles at total doses similar to that of an entire mission has been prioritized (5-9). However, because it is not feasible to deliver a realistic replication of GCR on Earth at the dose rate and fluence expected in space, and it is unethical to purposefully expose humans to such radiation, the human health and performance effects of long-term exposure to GCR beyond LEO remains unknown. Because of the unique nature of space radiation and the enormous engineering challenges associated with adequately shielding in spaceflight, the current plan is to depend on exposure limits and a reliance on the very limited set of known countermeasures that have shown promise in preventing damage from space radiation in animal studies (9). There is a need for innovative countermeasure development approaches to ensure safe space travel for astronauts on exploration class missions.

The current body of knowledge on radiation countermeasures is limited (10). The majority of published studies on ionizing radiation health effects in humans, are not GCR relevant, thereby creating a challenge for the applicability of traditional pharmacological countermeasures developed for terrestrial radiation to the space context. Rodent models have long served as an acceptable option for studying space radiation effects, with the assumption being that much of these results will translate to humans. Samples derived from astronauts exposed to space radiation and rodents exposed to simulated space radiation do show similar responses in triggering DNA damage response, oxidative stress, inflammation, and lipid peroxidation (11-13). However, inbred rodent models are known to be more radioresistant than humans, though this can vary with strain, and generally, animal models have been found to imperfectly translate to human physiology for various reasons (14). For instance, it is estimated that there is a nearly 90% failure rate of drug testing using preclinical animal models, which is related to both efficacy and safety problems when brought to clinical trials (15-17). Continuing to rely on animal models alone to understand the effects of space radiation on humans does not provide a complete picture. Hence, it is vital to develop innovative experimental approaches to bridge the translational relevance of animal studies to human response.

Both adherent and non-adherent human cell cultures have been used to understand the cell biology and disease mechanisms induced by radiation. However, monolayer cultures do not mimic the cell-cell and cell-extracellular matrix (ECM) interactions. *In vivo,* these interactions are essential for cellular functions such as differentiation, proliferation, and fate-associated gene expression. Because of the lack of tissue-like architecture, monolayer cultures are not set up to respond to nutrients or metabolic gradients (18). One alternative is human complex microphysiological systems that have the potential for improved translatability as they are developed with normal human cells. Recent advances in their complexity, longevity, reproducibility, and recapitulation of *in vivo* anatomical features and differentiation markers, as well as physiological activities, promise whole organism translatability.

This paper discusses funded projects by the Translational Research Institute for Space Health (TRISH) that address novel radiation countermeasure development approaches. TRISH is a NASA-funded institute with a focus on disruptive, high-risk, high-reward research aimed at pushing the field of human spaceflight forward in great leaps as opposed to incremental steps. This three-pronged approach by TRISH focuses on advancement in radiation countermeasure target identification, development, and testing. One way in which TRISH is supporting innovative work on this front is focusing on identifying new targets for radiation resistance by studying accessible organism models, sometimes called lower organisms, and leveraging adaptations by other organisms, extremophile, to increased radiation exposure. Another strategy is the use of innovative nucleotide-based therapeutic methods to mitigate the impacts of radiation on humans. Finally, TRISH has supported work using three-dimensional (3D) complex microphysiological systems, which may prove to be not only more translatable to humans but also advantageous for research in space. With NASA planning missions beyond LEO where there is the unprecedented possibility of testing countermeasures in an actual GCR, and possibly SPE, environment, it becomes more crucial to identify innovative strategies for radiation countermeasures that can be tested in these unique opportunities.

2.1 High-Risk, High-Reward Approaches to the Identification of New Radiation Countermeasure Targets

One arm of TRISH's approach to new radiation countermeasure development is to fund research that focuses on identifying novel targets. Extremophile organisms, like tardigrades, have adapted to survive in extreme environments, including under conditions of increased radiation exposure. Identifying the genes that make them resilient and validating that they can confer resistance is a reasonable approach. However, extremophile organisms do not yet lend themselves to genetic manipulation that are the strengths of accessible organisms such as bacteria, yeast, and fruit flies. To circumvent this challenge, Dr. Donald Fox's group successfully developed a novel platform for identifying radiation-resistance genes from an extremophile, the tardigrade *R. varieornatus* in a host organism (19). The platform involves expressing tardigrade genes in a genetically accessible model organism, the fruit fly *Drosophila melanogaster.* By using this approach, 74 genes have been screened that are either unique to tardigrades or are part of a stress response gene family thought to confer resistance to environmental stressors in tardigrades. Candidate tardigrade transgenes that improve *Drosophila* survival following either low (X-ray) or high (^{56}Fe ions) LET radiation have been identified using this approach. These studies were the first to expose fruit flies to space radiation. Promising screen hits are being identified and tested in human cells for their ability to confer radiation resistance.

The power of using accessible organisms such as the common bacterium *Escherichia coli* has been demonstrated in a screen for genes that promote and prevent endogenous cellular damage when slightly overexpressed. By using this approach, Drs. Susan Rosenberg and Kyle Miller have previously identified the bacterial genes' human homologs that may play a role in cancer (20). To use this same method to identify novel targets for space radiation countermeasures, *E. coli* cells were exposed to ionizing radiation to look for overexpressed genes that confer resistance. The screen was successful and human homologs were identified and are currently being tested. Many of these targets are brand new and have never before been identified as potential radiation countermeasures.

Both these approaches leverage accessible organisms that lend themselves to high-throughput genetic screening and manipulation to expand the existing pool of radiation countermeasure targets.

3.1 High-Risk, High-Reward Approaches to Developing Radiation Countermeasures

Today, most drugs on the market have been developed using traditional drug discovery approaches, which require decades of preclinical and clinical research and billions of dollars in funding. The failure rate is so high that the market size has overshadowed the need in the decision to move forward in drug development. Because the space radiation market is negligible, there are no financial incentives to develop novel drugs to protect humans in space. TRISH has invested in several **nucleotide-based approaches** because they are likely to be

developed faster and at a lower cost. The recent success of Luxturna, an adeno-associated viral (AAV) vector-based gene therapy that cures an inherited form of blindness, invigorated the field of nucleotide-based therapeutics. Another recent success, RNA-based vaccines, has proven invaluable in fighting the recent COVID-19 pandemic due to, amongst other benefits, the speed by which they could be developed. TRISH has funded several programs aimed at improving faster, lower cost nucleotide-based therapeutic approaches for radiation countermeasures.

Dr. Dawn Bowles explores the use of gene therapy as a method for delivering a prophylactic radiation protectant. The group is optimizing gene-carrying AAV vectors for nucleotide-based radiation countermeasure delivery and safety. There are many advantages of using the non-pathogenic AAV in gene therapy applications; however, from the NASA perspective perhaps the most important advantage is that vectors based on AAV confer sustained expression (up to years) of therapeutic nucleic acids in target tissues (21). Packaging a genetic cargo in this type of delivery vehicle could be used as a prophylactic and initiated well in advance of the mission and thus radiation exposure. This approach would also obviate the need to bring, store, and continuously administer pharmaceutical agent during habitable volume-limited deep space missions. The Bowles group focused on developing a therapeutic for GCR induced cardiovascular dysfunction packaged in a cardiac enhanced AAV capsid (22, 23).

Dr. Niles Pierce focuses on dynamic RNA nanotechnology to enable cell-selective and programmable gene therapies. Guide RNAs (gRNAs) play a central role in CRISPR technologies by directing the function of Cas protein effectors to a target gene of choice, providing a versatile programmable platform for engineering diverse modes of synthetic regulation (edit, silence, induce) across species (from bacteria to humans) (24). However, the fact that gRNAs are constitutively active is a significant limitation, making it challenging to confine gRNA activity to a desired location and time within an organism. To achieve programmable control over the scope of gRNA activity, the Pierce Lab is utilizing principles from dynamic RNA nanotechnology to engineer conditional guide RNAs (cgRNAs) that change conformation in response to an RNA trigger X, which make it possible to conditionally direct the function of Cas to an independent target gene Y (24, 25). Candidate genes for radiation countermeasures could be targeted simultaneously in a tissue-specific manner. For each gene, an endogenous RNA trigger X would be selected to confine the regulation of target gene Y to the desired tissue type(s).

Dr. Afshin Beheshti's lab has adpoted a systems biology approach to develop novel nucleotide-based radiation countermeasures. The putative targets are miRNAs (i- for interfering; small non-coding RNA with a negative post-transcriptional effect on gene expression) which may act as systemic regulators of responses to stressors, including microgravity, oxidative stress, and potentially radiation-induced DNA damage (26). It is known that each miRNA can target hundreds of mRNAs and regulate immunity, cardiovascular disease, muscle degeneration, central nervous system (CNS) related diseases, and cancer (26). In addition, specific miRNAs have been shown to influence and increase DNA repair efficiency (27). Radioresistant lung cancer cell lines express increased levels of miRNAs after ionizing radiation, which suggests that this may be a mechanism of radioresistance (28). The group has recently shown that a distinct circulating spaceflight miRNA signature found in the serum is correlated with cardiovascular

disease and muscle degeneration (29-31).

Dr. Beheshti's group is attempting to target miRNAs as a novel therapeutic approach to radiation resistance (29, 31). Antagonists to certain miRNAs termed antagomirs delivered before radiation exposure effectively prevented angiogenesis damage (29). Delivery was mediated using a unique technology developed by a company called AUM Biotech termed "AUMantagomirs" (32, 33). Antagomirs delivered to a 3D microvessel tissue model 24 h before exposure to 0.5 Gy simulated GCR prevented the complete collapse of the vessels. Molecular analyses suggest that the effect may be partly mediated by reducing p53 binding protein 1 repair foci and increasing DNA repair activity (34).

An analogous but distinct nucleotide-based therapeutic approach involves the Nanoligomer™ (Sachi Bioworks) platform developed by Drs. Anushree Chatterjee and Prashant Nagpal's team (35). A Nanoligomer is composed of six design elements, including a peptide nucleic acid (PNA), a synthetic DNA-analog where the phosphodiester bond is replaced with 2-N-aminoethylglycine units, as the nucleic acid binding domain, and an engineered nanoparticle for facile cellular and tissue delivery of the naked molecule (36, 37). Nanoligomers offer improved stability, facile delivery and internalization, high target specificity (on-target) and minimal off-target activity. They can be designed to rapidly create a library of many therapeutic molecules that can be screened (36, 37). This approach is a high-throughput method to customize peptide-nucleic acid-based gene-specific reversible therapeutics with high precision. The platform has accelerated design, building, testing, and learn-based iteration cycles, enabling the timeframe from drug discovery to testing to reduce from weeks/months to days. The procedure uses bioinformatics to predict the optimal sequence of Nanoligomers to exert inhibition (targeting mRNA or DNA) or activation (targeting DNA) of gene expression of specific targets. The information is used to synthesize a library consisting of hundreds of molecules per round within days. A novel nanoparticle-based transport system allows for efficient delivery of Nanoligomers to host cells at the sub-micromolar range which is a therapeutic dose. Screening of the Nanoligomer library can be performed in a high-throughput manner. Using machine learning algorithms, the platform analyzes the gene expression readouts from the treated cells to optimize the particular combination of Nanoligomers that serve as effective countermeasures. Thus far, Nanoligomers have not caused cytotoxicity *in vivo* across different routes of administration, including intranasal, intraperitoneal, and intravenous delivery. The team has used this approach to develop Nanoligomers that are potential radiation protectants.

The COVID-19 pandemic afforded an opportunity to test the development of Nanoligomers as antivirals against SARS-CoV-2. Efficacy was demonstrated in both *in vitro* and *in vivo* models (37, 38). This proof of concept exemplifies the versatility of the platform and its broad applicability. Having this capability on a deep space spacecraft would enable the crew to develop countermeasures to any novel threats during exploration space missions. The accelerated design, build, test, and learn cycles and safety and efficacy of Nanoligomers allow for customized rapid just-in-time therapies.

The limited market and the costs of new drug development have hindered traditional drug discovery in radiation countermeasures. Even repurposing approved medications for a novel indication such as exposure to space radiation may require costly and lengthy studies (10). This is precisely why TRISH has funded nucleotide-based approaches. With new molecular tools that have improved delivery, programmability, tissue-specificity, and speed of synthesis, nucleotide-based countermeasures now present realistic platform capabilities for deep space missions. Without access to resupply from Earth, an exploration mission crew could synthesize just-in-time therapeutics to any health threat with agility, speed, and specificity.

4.1 High-Risk, High-Reward Approaches to Modeling Human Organs

There is room to innovate in developing research model systems that more closely recapitulate the healthy human who will travel in deep space and experience the unique space radiation environment. Rodent models are typically used in radiation research and have yielded much knowledge. The translatability to the human is less certain. 3D complex microphysiological systems that utilize normal cells from human donors present a unique opportunity to study mechanistic biological changes in response to space radiation and test potential countermeasures in a more physiologically relevant manner (39). This approach has advantages for spaceflight research. Human microphysiological systems serve as organ-specific 'sentinels' due to their limited repair capacity and tolerance to stressors. Hence, they reveal cascades that fail early in disease development. As such they have the potential to identify not only the effects of acute radiation but also long-term or delayed radiation effects in a shorter timeframe, where such outcomes may take months or years to arise in a model organism. Another innovative approach is the use of humanized" mouse models, immunodeficient mice engrafted with functional human cells and tissues, which can provide an improvement in the fidelity with human physiology (40). These advantages have motivated TRISH to fund several research projects using complex human or humanized rodent models to develop unique radiation countermeasures spanning multiple different organ systems.

4.2 Gastrointestinal System and the Microbiome

Because of high numbers of stem cells and rapid turnover rate, the human small intestinal epithelium is particularly sensitive to ionizing radiation. Prolonged space travel will likely cause small intestine disorders, including intestinal mucosal barrier dysfunction and nutrient malabsorption. Organoid models may prove a platform for studying human gastrointestinal effects (41, 42). To develop a human model for the small intestine, Dr. Sarah Blutt uses tissue samples obtained from surgical specimens, endoscopies, or colonoscopies to generate 3D human tissue stem cell-derived gastrointestinal organoid cultures. They have not altered the pH conditions as the small intestine is thought to be at relatively neutral pH. The group has also recapitulated the steep oxygen gradient seen *in vivo* (43). The crypt like organoids, predominately made of stem cells survive indefinitely and the villus-like organoids only live for about 3-5 days, which is the lifespan *in vivo* in humans.

The intestinal microbiome consists of a community of microorganisms, predominantly bacteria, that reside within the lumen of the intestinal tract (44). Because ofits proximity to the epithelium, the intestinal microbiome is a constant source of signals to the epithelium, including factors that protect, heal, and support intestinal epithelial health. Microbial factors could influence the proliferation and survival of the intestinal stem cell (ISC), the nucleating cell type necessary for regenerating damaged intestinal epithelium, specifically the villus epithelium. Studies in animal models indicate that the presence of the microbiome is associated with a higher mitotic index, intestinal epithelial turnover, cell migration, and ISC proliferation (45-49). Recent work has suggested that the microbiome has the potential to protect from the intestinal side effects observed with anti-cancer radiation treatments and was linked to a reduction in radiation-induced epithelial damage and symptoms (50-53). Microbial products can also activate signaling pathways that protect the ISC from radiation-induced apoptosis (54).

Dr. Blutt's group is using the gastrointestinal microbiome to potentiate the ISC environment by stimulating epithelial repair and regeneration to correct damage induced by simulated space radiation exposure. To date, neither specific microbial communities nor individual candidate organisms or factors responsible for the healing effects have been elucidated. The group has identified secreted factors naturally produced by complex commensal communities derived from healthy human donor stool that exhibit stem cell stimulatory properties. Their approach of culturing gastrointestinal microbiome *ex-vivo* has revealed that microbial factors can stimulate human stem cell activation and proliferation. This ongoing work aims to develop novel, safe, and cost-effective microbiome-based countermeasures with minimal side effects that can be used to protect and stimulate the ISC and manage intestinal dysregulation during long-duration space travel.

4.3 Nervous System

Decades of work using mostly rodents has indicated that the central nervous system is susceptible to very low levels of space radiation exposure (5, 6). The applicability to humans remains to be demonstrated because no human has yet to be exposed to the levels and duration of deep space radiation that will be experienced on an exploration mission planned for the return to the Moon and eventually Mars. The longest Apollo mission was 12 days. Apollo 17 astronauts appeared to have withstood the space radiation exposure without major effects, but it is unclear how they might have fared after 30 days or longer. Dr. Maletic-Savatic's group is examining neurogenesis in human brain organoid models exposed to a low linear energy transfer (LET) proton beam to mimic components of the particle fluence found in SPEs and within GCR (55). These models recapitulate the tissue environment *in vivo,* which has multiple neurogenic sites (rosettes) and diverse cell types including neural stem cells, neurons, astrocytes, oligodendrocytes, and microglia. The group has used two complementary cerebral organoid models and exposed them at different time points and different frequencies of exposure. Each organoid is grown at atmospheric O^2 conditions and physiologic pH of 7.25 and can last up to 18 months in culture. A detailed analysis is underway to examine molecular, metabolic, cellular, and physiological properties of the different cell types that are part of the neurogenic niche (56, 57). The 3D human brain organoid model can be used in a high-

throughput manner for assessing novel small molecules that promote neural stem cell self-renewal and neurogenesis or decreased microglial inflammation, thus targeting multiple elements of the neurogenic niche.

The group has recently discovered a mechanism to rescue neural stem cells and increase neurogenesis from damage using an endogenously produced monounsaturated fatty acid, oleic acid, which binds to an "orphan" nuclear receptor TLX (NR2E1), a transcription factor essential for neural stem cell self-renewal and proliferation (58). To understand further in developing this naturally occurring fatty acid as a therapeutic, the team applied novel synthetic chemistry approaches based on Fragment-Based Drug Discovery (FBDD) to identify traditional small molecules that can modulate the function of the Oleic Acid Receptor (59). In addition to molecular countermeasures, transient bursts of electrical stimulation are being tested for increasing neurogenesis. This is a promising new therapeutic approach to test and validate space radiation countermeasures in an untransformed *in vitro* model for the human nervous system.

4.4 Cardiovascular System

Changes in the cardiovascular system can impact many organs since vascular integrity is fundamental to for their function. This is of major concern since simulated space radiation has been shown to affect cardiovascular health and both rodents in larger animals (7, 60). Terrestrial radiation exposure has been shown to affect the cardiovascular system in cancer survivors, nuclear workers, and the Hiroshima and Nagasaki atomic bomb survivors (61-65). Hence, it is important to assess space radiation effects on human experimental models.

Dr. Sharon Gerecht and her team utilize a 3D human vascular multicellular model to study how space radiation modulates tissue integrity and function. Specifically, they are using their novel tissue-engineered small vascular grafts (sVGs) based on a process that involves electrospinning a natural polymer that is then seeded with vascular cells derived from human induced pluripotent stem cells (iPSCs) (66-68). This model system recapitulates cellular, structural, and physiochemical features of the human vasculature and is easily modulated to generate a range of blood vessel sizes and types, from microvasculature to arteries. Cultures are maintained in atmospheric conditions in physiological pH and have been tested for up to 20 days. The group is currently studying endothelial cell barrier function, vascular injury, inflammatory readouts, smooth muscle cells contractility, and necrosis in response to simulated space radiation.

By using the sVGs model system, the group is uniquely positioned to also test potential countermeasures to vascular damage from space radiation. The high mobility group box 1 (HMGB1) protein is normally localized to the nucleus but is secreted from the cell in response to damage or stress, and likely also radiation exposure (69). Secreted HMGB1 serves as an endogenous danger-associated molecular pattern (DAMP) protein, binding to pattern recognition receptors (PRRs) to activate inflammatory pathways of the innate immune system (70, 71). Since unresolved inflammation associated with HMGB1 can initiate pathogenesis of the vasculature and cause vascular disorders, autoimmune disease, arthritis, heart disease, and

neurodegenerative disease, HMGB1 is a promising drug target (71, 72). Dr. Gerecht's team is developing novel high-affinity antagonists of HMGB1. To achieve this, they are employing molecular evolution approaches to produce putative antagonist proteins that block the activities of HMGB1. By using natural HMGB1 as an engineering template, they apply structure-based molecular design (73) to generate a library of variants for selection using the yeast display platform (74, 75). Iterative rounds of magnetic-activated cell sorting (MACS) and fluorescence-activated cell sorting (FACS) on yeast cells are used to isolate high-affinity antagonists that potently block inflammatory HMGB1 signaling. MACS and FACS are also used to optimize antagonists. Although the present work focuses on HMGB1 inhibition, the Gerecht group's versatile engineering approach can be applied to other protein targets in pathways activated by radiation.

Another TRISH-funded team of investigators from Colorado State University, Stanford University, and the University of Colorado is also developing a cardiovascular model, specifically engineering human heart tissues (EHT) (76). 3D-engineered cardiac models have been shown to have less variability and hypersensitivity to drugs compared to 2D monolayers with the same cellular composition (77). EHTs resemble native anisotropic myocardial tissue and are derived from iPSCs differentiated into different lineages: cardiomyocytes, endothelial cells (EC), and cardiac fibroblasts. These cultures mimic the extracellular tissue-like environment of the heart and its multicellular composition. Arterial oxygen concentration is ~14%, whereas in the myocardium, it is less than 10% (78, 79). *In vitro*, standard culture conditions are typically maintained at 20% oxygen, which represents hyperoxia compared to the oxygen tension within myocardial niches. Higher oxygen levels *in vitro* could blunt physiological processes such as proliferation and metabolism (80). However, higher oxygen tension helps in the maintenance of a homeostatic environment for the human engineered cardiac tissues, driving oxidative phosphorylation pathways critical for maturation in culture. The EHTs are cultured at buffered physiological pH (7-7.6) with regular medium replenishment to prevent acidification. Characterization of these cardiac models using the current protocols demonstrate comparable structural and molecular features to its stage-matched *in vivo* counterpart. The EHT platform enables physiological analyses such as contractility, electrophysiology, and calcium transport. In a semi high-throughput manner, one can test countermeasures against radiation-induced damage of various cardiac cell types. The EHT have been studied in culture for up to 90 days with long-term culture beyond 3 months under current investigation.

EHTs are being exposed to chronic low dose rate ^{137}Cs γ-rays and 14 MeV neutrons mimicking the space radiation field. This experimental strategy was enabled by TRISH-funded modification of an existing radiation facility at Colorado State University by the addition of a D-T neutron generator, enabling for the first-time, protracted exposures of cultured tissues. Collaborations are enabling the testing of potential radiation countermeasure targets identified through genomics, metabolomics, and proteomic analyses will be tested via adeno-associated virus delivery and Nanoligomer delivery (described above) using the EHT model.

4.5 Hematopoietic System

Studies in animals and humans have demonstrated that the hematopoietic system is highly sensitive to radiation damage (81). To create a model for human hematopoiesis that could be used to test space radiation countermeasures, Dr. Christopher Porada's group developed mouse "avatars" whose endogenous murine hematopoietic systems are replaced with human hematopoietic stem/progenitor cells (HSC) from healthy donors of approximate astronaut age. By using this mouse model, they demonstrated that exposure to Mars mission-relevant doses of SPE protons or GCR ions profoundly affects human hematopoiesis and introduces mutations in genes associated with hematopoiesis distinct from those induced by gamma radiation. Perhaps most concerning is that exposure to some of the higher energy HZE ions, *e.g.*, ^{56}Fe ions, resulted in the generation of human T-cell acute lymphoblastic leukemia *in vivo* (82). This ability to reconstitute human hematopoiesis and perform *in vivo* exposures to Mars mission equivalent doses and ion species of radiation provides a powerful experimental model system that can be used to explore possible LET effects. The humanized mice have now been exposed to single ion beams of ^{16}O ions and ^{28}Si ions, as well as the NASA Space Radiation Laboratory's simplified 5-ion GCR Simulator at Brookhaven National Lab. This model may be useful in identifying space radiation damage biomarkers, developing mission-compatible multi-omics platforms to enable screening of astronauts for just-in-time radiation-induced stress/damage. The humanized mice can be used to test potential, radiation countermeasures. As an example, the group has preliminary data to indicate that curcumin is an effective radiation countermeasure when the solubility and bioavailability were enhanced by packaging it in nanolipoprotein particles (NLPs) in the hematopoiesis-humanized mice.

4.6 Multi-Organ Systems

Although looking at individual organ system effects via organoids can be insightful, radiation damage would affect the entire body of a crew member, and damage to specific organs would be likely to impact other organs through multiple mechanisms of cellular signaling. To replicate some aspects of the whole-body system, Dr. Gordana Vunjak-Novakovic's group enables the humoral communication between distinct bioengineered tissues representing different organ systems. This integration of multiple 3D complex microphysiological systems was developed over many years (83, 84). Each tissue type is provided with its own specialized environment and is connected to other tissues by vascular perfusion, as a selectively permeable endothelial barrier can distinctly separate the vascular and tissue compartments. The tissues are fabricated using human cells (both primary and iPSCs) and tissue-specific biomaterial scaffolds, and are matured individually over 4-6 weeks before being placed into the platform. The bioengineered tissues are linked by perfusion and cultured for an additional 4 weeks, thereby allowing for cross-talk between each individual organ (85).

For studies of the effects of GCR radiation on human body, this body-on-a-chip platform contains engineered human tissue models of bone marrow (site of hematopoiesis and acute radiation toxicity), cardiac muscle (site of chronic radiation damage), liver (site of metabolism), and vasculature (barrier for transport of signals throughout the system). The adult-like human cardiac muscle is engineered from human iPSCs and matured in culture by electromechanical conditioning (86). After only 4 weeks of culture, cardiac tissues displayed adult-like gene

expression profiles, remarkably organized ultrastructure, oxidative metabolism, and contractile properties. The human bone marrow model can produce downstream blood and immune cells, as well as incorporates the complexity of multiple cell populations, with human mesenchymal stem and stromal cells, osteoblasts, endothelial cells, and CD34+ hematopoietic stem/progenitor cells introduced within a decellularized bone scaffold (87). The group can control oxygen level in these platforms, from 0.1% to 21% oxygen. Studies on the effects of GCR have been conducted in normoxia (21% oxygen). The body-on-a-chip platform can be regularly maintained for 2 months; however, the bone marrow system can last for 3 months and the cardiac muscle system can last up to 8 months specifically (85).

Columbia's Radiological Research Accelerator is used by Dr. Vunjak-Novakovic's group to test the effects of neutron radiation on extended-living cultures of the body-on-a-chip system, and to observe differences in the cultures' responses to acute and protracted exposures. The value of this model to study space radiation effects on the human hematopoietic system was further demonstrated through characterization of myeloid cell subtypes, the extracellular matrix remodeling, and leukocyte chemotaxis typically associated with activated myeloid cells. These studies helped establish a platform for studying human hematopoiesis *in vitro* and developing novel radiation protectants.

The discussed individual platforms have the potential to improve our mechanistic understanding of radiation damage to tissues. Importantly, as they are improved and better recapitulate the human organ, they may serve as effective test beds for possible radiation therapeutic countermeasures complementing the animal studies and improving translation to humans. With the ability to connect multiple tissue-on-a-chip systems to make a body-on-a-chip, it is possible to test even more accurately the realistic effects of an intervention on a particular human. A body on a chip can be derived from stem cells obtained from an astronaut who is selected for a deep space exploration mission. NASA has recently partnered with the NIH and other agencies to fund work to extend the longevity of these tissue-on-a-chip and body-on-a-chip platforms. These models hold great promise for space radiation protections as well as for many other applications in drug discovery.

5.1 Future Directions

TRISH has been charged by NASA to help it solve the challenges of human deep space exploration by funding disruptive more high-risk research. A top concern for human health beyond LEO is space radiation. Much work to date has focused on rodent models and traditional countermeasures. TRISH chose to take three different new approaches. First, by focusing on genetically accessible lower-organisms, for which genomic manipulation can be performed with high-throughput and extremophiles that can survive in extreme radiation environments, TRISH is identifying new targets for radiation countermeasures. Second, TRISH believes that nucleotide-based therapeutic approaches are more agile as their synthesis can be potentially accommodated off the planet. This provides an alternative to the strategy undertaken by NASA to repurpose FDA-approved medications that prove effective radioprotectors or radiomitigators for space-radiation induced carcinogenesis (10). Clinical

applications for nucleotide-based therapeutic approaches is still in its infancy, but it is important to make investments to drive these fields forward. Third, TRISH has invested in developing models based on human systems. The humanized mice and the human organoid and complex microphysiological systems (organs on a chip) complement animal studies. Together, we will have a more complete understanding of the effects of radiation during deep space travel and be able to provide each crew member with appropriate and effective personalized countermeasures.

For example, the tissues-on-a-chip systems could be used to understand individual radiation risk and drug efficacy. Since the effects of the space environment, specifically radiation, are highly variable among individuals; the ability to test each person's sensitivity to space radiation would be a game-changer (88). Patient-derived organoids or organs-on-a-chip are already being used in clinical trials for cancer therapy (15, 89). In the space context, individuals bound for a deep space mission would provide samples to derive iPSCs, which would be used to generate organoids or organs-on-a-chip. The cultures would then be exposed to real or simulated space radiation for testing. Given that the systemic physiological responses can differ quite significantly from one person to another, this would enable the personalization of risk profiles and the customization and testing of radiation countermeasures by using an individualized "astronaut-on-a-chip" approach.

Future tissue-on-a-chip research can also lead to a more accurate investigation of real-time GCR exposure testing since they are small enough to be included in autonomous payloads exposed to deep space. More work is needed to increase the longevity of these micro-physiological systems and to develop the technologies that allow the automation of culturing and reporting of physiology and function of these "human avatars" while they are in space. NASA has recently partnered with other government agencies such as the NIH to solicit for research that will address longevity of tissues-on-a-chip model systems. If these cultures could continue for up to 6 months, they could be tested as autonomous payloads beyond LEO to determine the tissue donor's own susceptibility to radiation exposure. Knowledge ahead of a deep space mission about susceptibility of the crew would inform a personalized mitigation strategy.

The investments made in tissue-engineering, new accessible organism model systems, and nucleotide-based therapeutic approaches are at present considered high-risk research. If successful, some of these technologies could be impactful for not only reducing the health impacts to humans exploring deep space but also to the humans who remain on Earth in their pursuit of health and longevity.

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