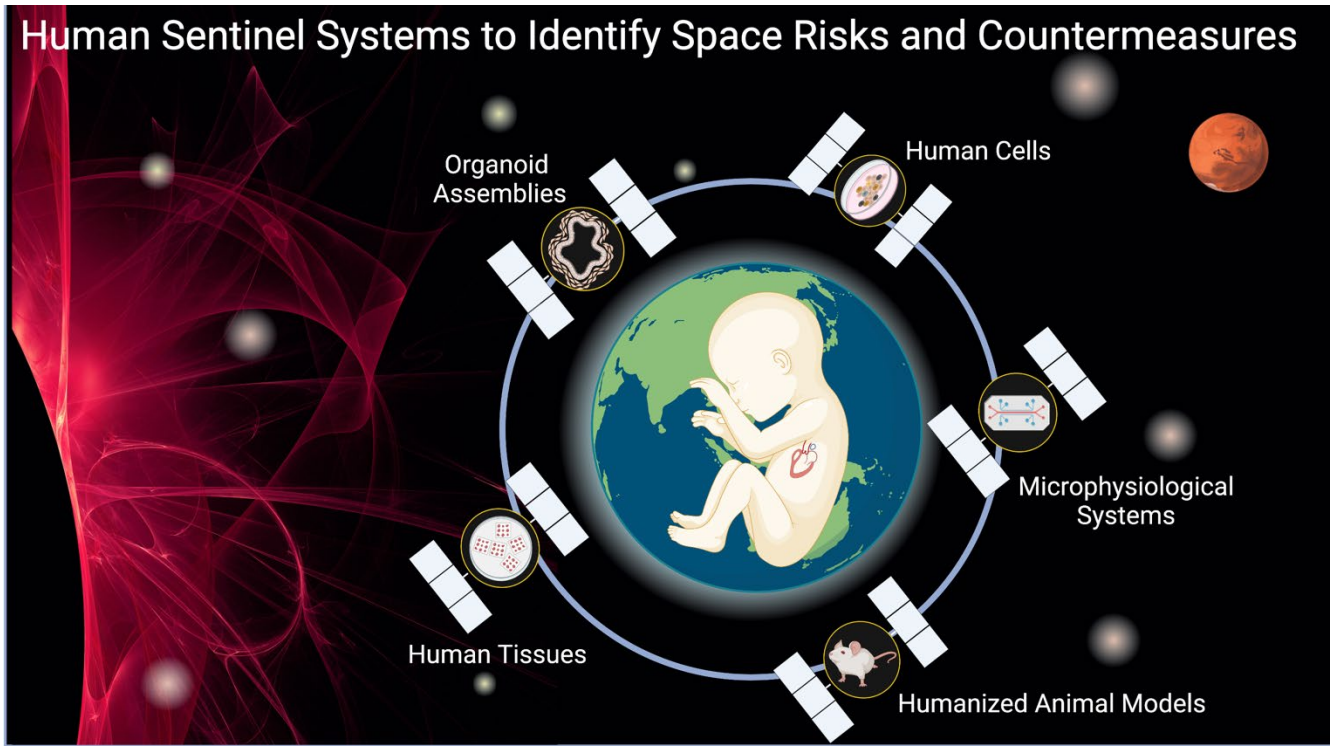
Topical: Engineered Human Tissues and Humanized Mice to Individualize Risk Calculations and Countermeasure Use



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Purpose

The purpose of this white paper is to highlight the potential of two important new biomedical research tools to improve our understanding of the adverse health effects from space radiation exposures encountered in long duration space missions and thus enable the development of countermeasures to mitigate these effects. Both tools, complex tissues engineered from human induced pluripotent stem cells and "humanized" mice, allow human tissues to be irradiated with simulated space radiation. Used in conjunction, they would allow long-term radiation studies to be run on viable tissues derived from individual astronauts. These tools are currently being used independently in ground-based studies but are readily adaptable to space flight. Indeed, cardiomyocytes derived from human induced pluripotent stem cells, multi-cellular engineered heart tissues (EHTs) and conventional laboratory mice have already been flown aboard the ISS.

Background

The Challenge

The spaceflight environment, including microgravity, radiation, confinement, and other stressors, presents a number of health risks. Mammalian model organisms, usually rodents, are studied to better understand the extent of these risks and to develop countermeasures against them. However, translating results obtained in rodents to humans is challenging due to species differences, including differences in physiology, size, and lifespan. Our broad proposition is that studies on engineered human tissues and human tissues engrafted into rodents can be used to extrapolate results obtained in rodent experiments to humans. Furthermore, these tools can be used to identify clinically relevant biomarkers of stress and injury from the space environment, assess risks at the level of individual astronauts and develop and test countermeasures.

High-charge, high-energy (HZE) ions are a component of space radiation. Their health effects on humans are largely unknown because life on Earth is shielded from HZE ions by the Earth's magnetic field. However, beyond low Earth orbit that protection is lost. Since HZE ions or their fragmentation products can penetrate any practical level of spacecraft shielding astronauts on lunar or interplanetary missions will be exposed to significant levels of this type of radiation. To date, NASA's only human experience with HZE ions at levels found beyond low Earth orbit comes from astronauts who flew Apollo lunar missions. However, those flights were of very short duration, so the exposure doses were only a small fraction of what will be experienced by astronauts on currently envisioned missions.

To determine the likely extent of adverse health effects from space radiation NASA employs the strategy of extrapolating the extensive epidemiologic data available on humans exposed to terrestrial radiations, mostly γ-rays. The adverse effects include increased cancer risk, cardiovascular injury and neurocognitive decrements. However, the HZE ions present in space radiation differ from γ-rays in their spatial pattern of energy deposition and extent and clustering of the resultant molecular lesions. Consequently, they have unique biological effects not captured by existing epidemiological data that must be characterized empirically. Essentially, what we know about the unique health effects of HZE ion exposures comes almost entirely from rodents experimentally exposed to them here on Earth. These studies indicate that HZE ions are extremely effective in causing cancer and neurocognitive decrements. However, there are considerable uncertainties involved in translating that knowledge to future human exposures.

Cultured human cells have been irradiated with HZE ions at the NASA Space Radiation Laboratory (NSRL), a ground-based facility with the capability to simulate space radiation and then assayed for a variety of endpoints thought to ultimately be associated with tissue injury, neoplastic transformation or neurodegeneration. Efforts have been made to move from monolayer cultures to 3D cultures to better simulate tissues, but ideally the effects of HZE ion exposures would be studied in human tissues irradiated *in vivo*. This could be accomplished by constructing human tissues *ex vivo*, implanting the tissues in immunodeficient mice and irradiating them in the mice.

Several factors dictate individual predisposition to the adverse effects of radiation, including genetic factors. This is evidenced by genetic epidemiologic studies of cohorts exposed on Earth. There are also data on chromosomal and mitochondrial aberrations measured in blood from astronauts on long-duration missions (Luxton et al 2020a, Luxon et al 2020b), though other stressors likely play a role. Both DNA and mitochondria are major targets for HZE ions causing an extended state of systemic inflammation. Accrual of such slow changes may remain undetected and manifest as a disease in a delayed manner. Experimental human derived model systems can be used as organ-specific 'sentinels' with limited repair capacity and tolerance to unravel cascades that fail early in the pathological sequence.

Engineered human tissues

Human induced pluripotent stem cells (iPSCs) provide an unlimited and unique source of normal human cells for radiation studies. The application of iPSC-derived cells for developing drug screens based on cell-autonomous and disease-relevant phenotypes is the first step to improving translation of preclinical studies to the clinic. Accurate modeling of cell-cell communications and 3D structure is critical for recapitulating important features of tissue physiology. More complex iPSC models are warranted to recapitulate disease-relevant cell-cell interactions, such as engineered co-cultures, microphysiological systems, organoids, or human-rodent chimeric models. Biopharmaceutical companies are now using human iPSC-derived organoids as a basis for drug discovery or safety and efficacy testing.

In addition to their uses in medical research and drug development, iPSC derived systems are being used in NASA funded research. For example, over the past several years, great strides have been made in understanding effects of space flight on human heart using iPSCs. Dr. Joseph Wu and his team were the first to study the effects of spaceflight on iPSC-derived cardiomyocytes (iPSC-CMs) which they flew in a 5-week mission aboard the International Space Station (ISS). Ca^{2+}-handling capacities were altered in space flown iPSC-CMs following return to normal gravity, perhaps due to impairment of CM energetics (Wnorowski et al. 2019). They also developed a scaffold-based engineered heart tissue (EHT) platform composed of iPSC-CMs, endothelial cells (iPSC-ECs), and cardiac fibroblasts (iPSC-CFs) that mimic the extracellular tissue-like environment of the heart and its multi-cellular composition (Oscar et al. 2018). Incorporation of supportive cell types such as ECs and CFs offers a unique platform for direct exposure and quantitative assessment of cellular stress and injury leading to activation of inflammatory pathways linked to endothelial damage and cardiac fibrosis. These assessments include the detection of biomarkers relevant to clinical disease. The 3D platform enables the screening of countermeasures in a semi high-throughput manner with functional readouts. In addition, iPSCs can be obtained from multiple individuals to account for human genetic diversity. Recently, the 'Cardinal Heart' study funded by the National Institutes of Health (NIH) and National Center for Advances in Translational Sciences (NCATS) enabled Dr. Wu's team to send EHTs to ISS to further investigate the effects of spaceflight on heart muscle function. 216 EHTs from 6 different patient lines were generated and launched to the ISS on the SpaceX-21 cargo resupply mission (on December 6th, 2020). After a successful launch, the EHTs were maintained at the ISS for 4 weeks before returning to earth. Both in-flight and post-flight analyses revealed line-specific differences in contractility of the EHTs driven by molecular changes during early and later timepoints. These studies provide a unique opportunity to study the effects of space travel on human cardiac tissues.

Humanized rodents

Human tumors and normal tissues can be engrafted into immunodeficient mice and rats. The original immunodeficient murine strains arose from spontaneous mutations in the *Foxn1* and *Prkdc* genes, but newer strains of mice and rats that are more profoundly immunodeficient have been engineered through genetic manipulations. Immunodeficient rodents engrafted with normal human tissues are referred to as being "humanized." Examples are mice or rats with humanized hematopoietic systems, livers, skin, and astrocytes. Most work to date has been done in mice and the remainder of this white paper will focus on humanized mice, but it should be noted that for some potential studies humanized rats might be the preferred species.

Humanized mice offer two advantages over conventional tissue culture for the study of radiation effects on human cells or tissues. The first is that the cells or tissues are irradiated *in vivo* which provides a more complete picture of the complex interplay of various physiological systems which are not represented in conventional tissue culture. This can extend to immune system interactions since mice can be doubly humanized with components of the human immune system along with another tissue of interest. The second advantage is that presently human tissues can be maintained longer in mice than in culture and even serially transplanted. This is important in studies of low dose rate effects in which it is desirable to deliver radiation continuously over weeks or months. It is also important for those acute exposure studies in which the biological effect of interest occurs long after irradiation.

Several radiation effects studies have employed humanized mice. These include studies of the genetic events leading to radiation-induced thyroid cancer (Mizuno et al 2000, Nomura et al 2008) and the effects of radiation on the hematopoietic system (Rodman et al 2017, Lee et al 2018, Hoehn et al 2019). The Lee et al study was designed to identify biomarkers of radiation exposure that could be used for biodosimetry. The Hoehn et al and Rodman et al studies are of particular interest because they were NASA funded. Hoehn and colleagues exposed mice with humanized hematopoietic systems to high- and low-LET radiation and monitored changes in human hematopoietic stem-cell counts and blood cell subset frequencies at time points ranging from one week to seven months after exposures. The Rodman et al study involved irradiating human hematopoietic stem cells with γ-rays, protons, or HZE ions *ex vivo* and then transplanting them into immunodeficient mice. The effects of radiation quality on extent of engraftment and spectrum of lineage commitment were followed. Irradiation for both studies was performed at NSRL.

Extending the use of engineered tissues and humanized mice for to individualize risk calculations and countermeasure usage

As mentioned above, human tumors can be engrafted in immunodeficient mice. "Patient derived xenografts" are a more recent development of this technology. Minced tumor tissue from an individual patient is directly transplanted into immunodeficient mice where it behaves very much as it would in the patient. This enables clinicians to determine the metastatic potential of a specific tumor and to compare the efficacy of different therapies against it in mice. Findings in these "avatars" can inform the selection of treatment options for the human patient, thus personalizing therapy. The next logical advance would be to extend this approach to mice

humanized with normal tissues with the goal of determining an individual patient's risk of normal tissue toxicity from various treatment options. Along these lines, it should be possible to use the same approach to generate an individualized risk profile for an astronaut prior to being exposed to space radiation. Such a profile would allow an astronaut and his or her flight surgeon to make informed decisions on what countermeasures would be worthwhile and what types of post-flight surveillance would be prudent.

There is an obvious potential synergy between the two technologies – engineered human tissues and humanized mice – for broad scope studies of radiation effects and focused risk studies for individual astronauts. For comprehensive studies exploring radiation effects on specific tissues, engineered tissues of interest could be generated from available human iPSC derived from diverse donors and used in experiments. Much of this work could be done completely *in vitro* with short term cultures. However, when longer time courses or results in the context of an intact organism are needed the engineered tissues could be transplanted (or serially transplanted) into immunodeficient mice. For studies of radiation risks faced by individual astronauts, iPSC derived from them could be used to generate engineered tissues. These tissues could be engrafted into mice, irradiated with simulated space radiation and monitored for biomarkers of tissue injury or neoplastic transformation.

Conclusions

1. Engineered human tissues and humanized mice can be used to facilitate extrapolation of results obtained in rodents to humans.

2. "Avatars" of individual crew members can be generated by combining these two methodologies.

3. Engineered human tissues and humanized mice can be powerful platforms for the discovery of biomarkers of injury, countermeasure development and assessing spaceflight risks for individual spaceflight crew members.

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