The role of study of cell senescence in organoid bioprinting for Artemis Missions and sustainability of Human Lunar Exploration.

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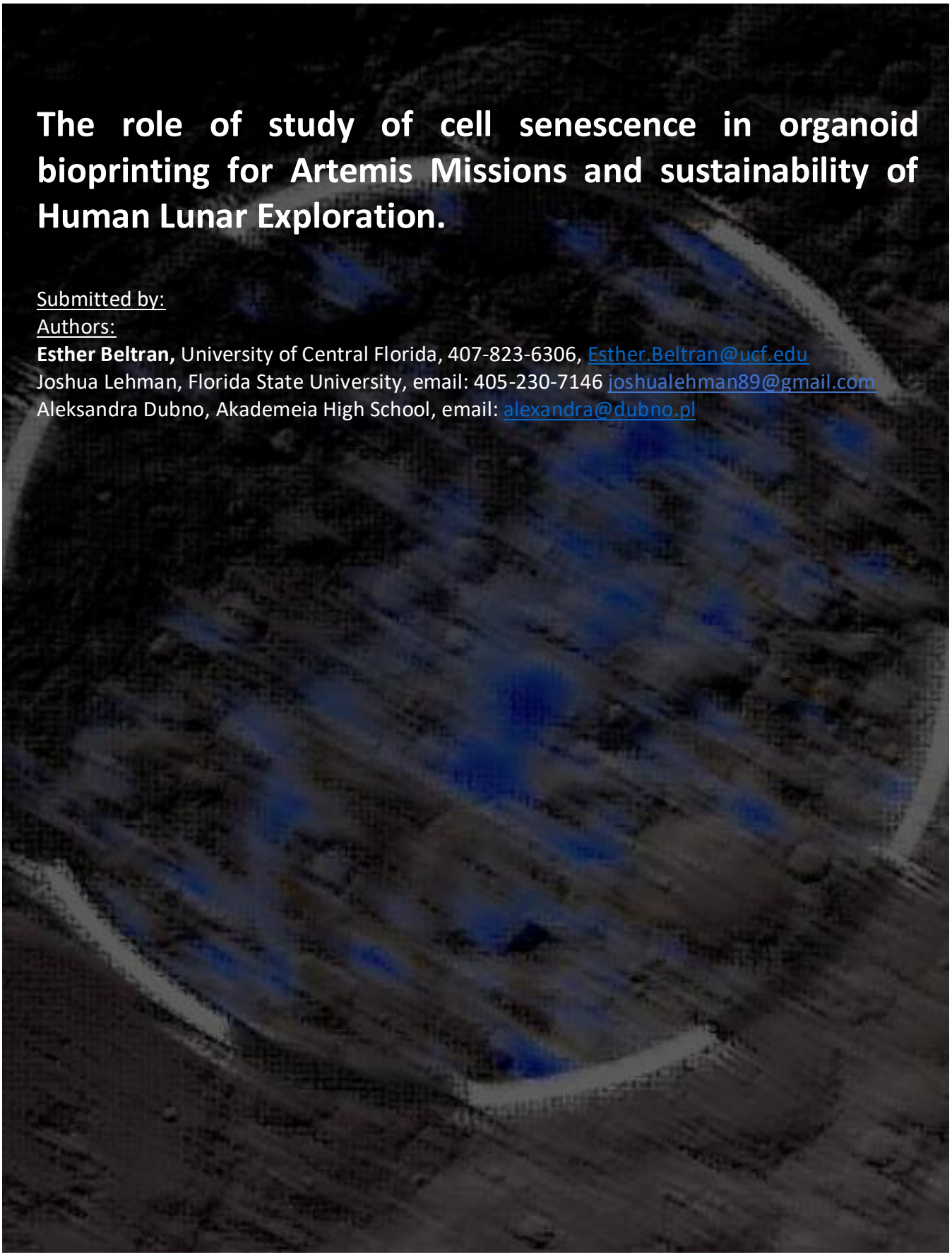

1. **Motivation**

The Artemis program has rekindled a great deal of enthusiasm for researchers across the board. For many reasons the Moon is still a sanctuary for scientific discoveries (Moriarty et al, 2020). Scientists from many disciplines have submitted white papers to Decadal Surveys in the recent past and now is the time for the field of Biological sciences to guide the way forward in important research which advances our knowledge not only for astronauts, but for life on Earth as well. Although observational, robotic missions are critical in closing scientific gaps, the scientific advances accomplished through human exploration are wide and cannot be underestimated. Human cognition, mobility, dexterity, and a wide range of other factors are all dynamic variables important to success of the mission. This paper aims at highlighting areas of research that are important to maintain optimal crew performance and achieve mission goals. In light of NASA's plans for crewed exploration of the Lunar South Pole, this paper includes recommendations to the Decadal survey that would enhance human space exploration while furthering our understanding of neurobiology.

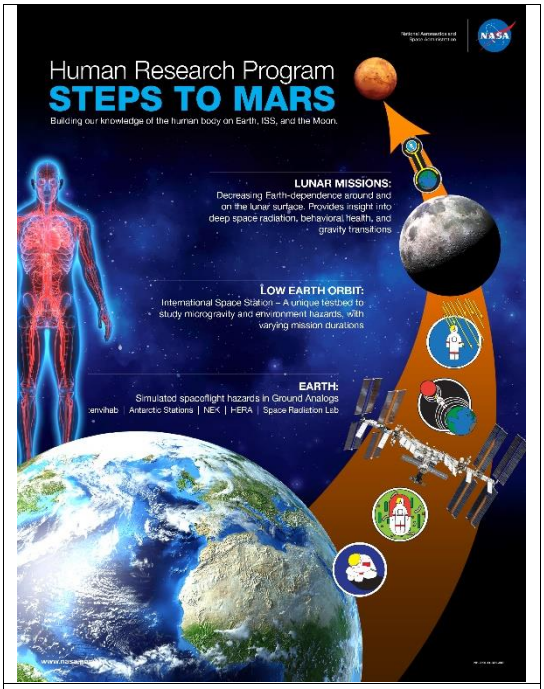

Figure 1- HRP roadmap Path to Mars. Courtesy of NASA.

1.1 **Space is an extreme environment for the human body.** Astronaut studies, including the recent NASA Twin Study conducted during Scott Kelly's Year in Space, have demonstrated impacts to multiple organ systems and changes in gene expression from exposure to microgravity [1]. The pathophysiologic changes noted in astronauts after their missions include cellular/molecular changes inducing early "aging" at the cellular level with all organ systems ultimately affected. Such effects manifest over longer duration exposures to microgravity and more importantly can be reduced through various countermeasures. Thankfully, these effects seem to be reversible after return to Earth. Based on these considerations, it is increasingly accepted that spaceflight might provide a mechanistic insight for better understanding of certain pathophysiological processes [2].

1.2 **Significance:** There is a need for more research specific to the risks of spaceflight including high G-forces associated with ascent and descent, microgravity, and radiation. Radiation is considered one of the main risks, particularly when planning for Artemis missions with notably longer extra-vehicular missions outside the protection of Earth's magnetosphere, as longer exposure inevitably leads to more damage to genetic material at the cellular level. Therefore, we must quantify the magnitude of risk, use established technology for radiation detection, and determine more accurate Permissible Exposure Limits (PEL) for various mission types based on their inherent level of exposure and risk.

One method to help risk-stratify missions based on level of exposure to background space radiation, including Galactic Cosmic Rays mixed fields, are approved NSRL Simulations which we can be used to predict exposure based on mission location, length, and duration. These simulations provide valuable information to create effective mitigation protocols prior to crewed missions, but these simulations need to be supported by *in-vivo* research evaluating the effectiveness of mitigation strategies along with continued monitoring of dose of radiation exposure and the inevitable downstream effects on the crew.

NASA- Human Research Program roadmap path to risk reduction (2021):

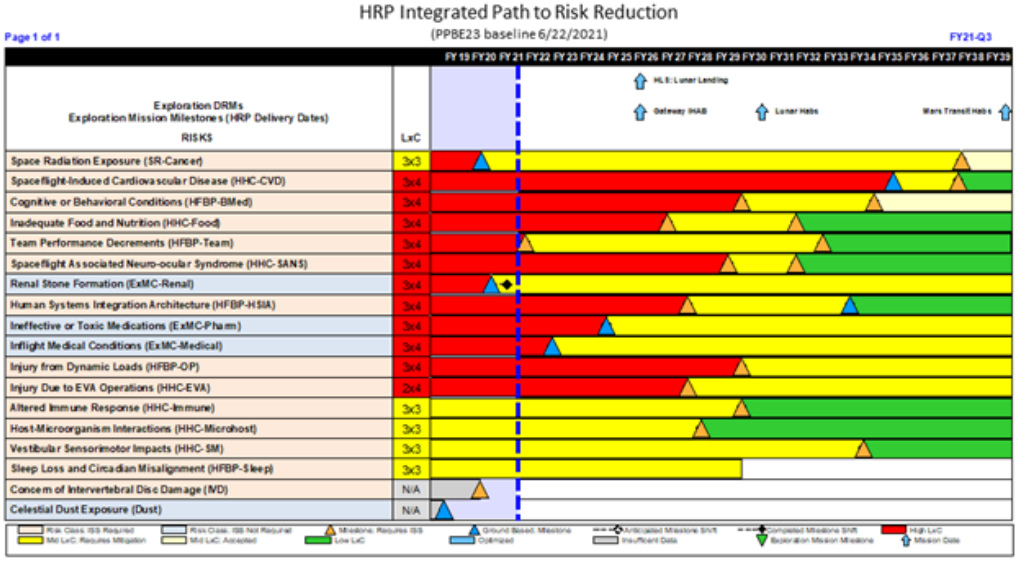

Table 1: The development of the HRP content of its risks research has been formulated around the management architecture of the HRP roadmap that describes supports the existence of the human system risk, gaps in knowledge and pathways to characterize or mitigate the risks with tasks that can be carried out to produce the deliverables ser up for the Agency. The graph also depicts the estimate deadlines, which tend to be variable and change according to budgets and policy priorities.
https://humanresearchroadmap.nasa.gov/intro/.

2.0 Research Area:

As the Artemis era calls for longer missions outside the safety of the vehicle, there will be an increased risk of exposure to space radiation for astronauts, including direct damage to cells and epigenetic modifications that promote cell senescence (CS), a form of cellular aging, defined by extensive changes in gene expression that ultimately lead to cessation of cellular proliferation. The enhanced exposure to space radiation can amplify the cascade reactions contributing to CS. Our focus is on the Central Nervous System (CNS), a pivotal organ of concern for potential adverse effects due to exposure to microgravity and space radiation and identifying specific biomarkers and cell characteristics that can be used to recognize senescence in the CNS.

Furthermore, age-related inflammation, known also as inflammaging, plays a key role in macro- and microvascular pathologies including atherosclerosis, vascular aneurysms, meningitis, and has been implicated in neurodegenerative disorders such as Alzheimer disease. Previous studies indicate that vascular aging is associated with a shift in the expression levels of inflammatory cytokines, chemokines and adhesion molecules, promoting a pro-inflammatory environment in the vascular endothelium. The secretion of inflammatory molecules, specifically by senescent cells, plays a significant role in this process. We believe new advances in space research aimed at reducing these pathologic changes associated with radiation exposure along with enhanced biomarker data for earlier detection of pathology, will benefit all humans, terrestrial and spacefaring, and are essential for furthering our exploration both in Artemis and missions beyond.

2.1 Research goal and specific objectives: One objective of this white paper is to incite more research on the effects of radiation exposure and promotion of CS on the CNS via enhanced methods with modern technology. Current research at ISS-NL mainly involves two-dimensional (2D) cell culture studies. It is anticipated that 3D cell culture models will provide significant advantages over their 2D counterparts, including better characterization of cell morphology, proliferation, response to chemical/drug exposure, alterations in gene expression, and even tissue structure. Manually producing these 3D culture models has proven difficult and expensive for many researchers, often resulting in high scaffold-to-scaffold variability. A 3D human culture model generated via bioprinting is critical for providing a more natural 3-dimensional tissue model. In addition, these methods facilitate the ability for enhanced understanding of the extracellular matrix (ECM), the cells external environment, and the ECMs role in tissue architecture and cell-cell communication which is pivotal for cellular events such as proliferation, migration, differentiation/specialization, and even apoptosis (programmed cellular death). 3D-bioprinted models via a tissue-on-a-chip bioreactor system are a far more realistic and logistically feasible tool to conduct adequate research studying tissue structure and the pathophysiologic processes associated with space travel.

We propose 3D-bioprinted models can enhance our understanding of the negative effects on the CNS caused by radiation and microgravity exposure by focusing on a specialized group of CNS cells, the "glial cells". Glial cells are comprised of oligodendrocytes, microglia, and astrocytes which, in the CNS, are ten times more abundant than neurons. The glia function as CNS housekeeping cells with activities including rapid uptake of ions and neurotransmitters, provision of substrates for neuron function, clearance of waste, immunologic surveillance, and maintenance of the blood-brain barrier (BBB). The BBB is a unique property of the CNS that depends on the interactions between glial cells and blood vessels via tight-junctions, which serve as a protective filter for the brain, by allowing the passage of critical substrates for energy production and protein synthesis while preventing the passage of disease-causing microbes, environmental toxins, and damage-inducing free radicals [4].

Of the glial cells, astrocytes serve as a good benchmark for CNS function are involved in substrate sensing, neurotransmitter uptake and release, regulation of ion concentration, synaptic transmission, or myelination of neurons via activating oligodendrocytes. In addition, Astrocytes have been linked to cerebral blood flow when measured by functional MRI, fMRI, and therefore can serve as a good surrogate marker of overall CNS health [6].

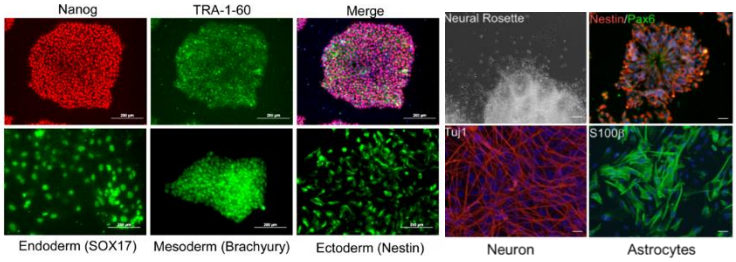

Figure 2. Generation of human iPSCs and differentiation into brain cell types.

Dr. Kanekiyo's group at Mayo Clinic have successfully generated iPSCs by electroporation of three plasmids encoding OCT3/4, SOX2, KLF4, L-MYC, LIN28 and p53-shRNA as shown in Figure 2. To differentiate human iPSCs to neurons or astrocytes, iPSCs were transferred into petri dishes with complete mTeSR1 medium for 2-3 days to allow for spherical embryoid bodies to develop in suspension cultures.

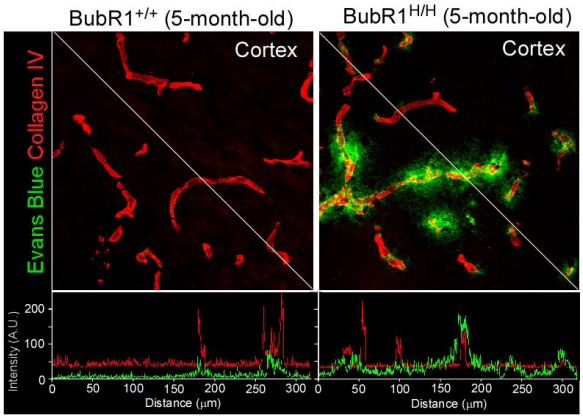

Figure 3: Impaired BBB integrity in the accelerated aging model BubR1$^{H/H}$

3.0: Specific value, Path forward:

3.1 Specific value: As future crews will ultimately be on longer missions outside Earth's protection, there is the possibility for increased morbidity and mortality, including increased risk of cancer. The increased risk of cancer is ultimately due to prolonged exposure to chronic, low-dose, high-energy particles that make up the background space radiation. Due to a lack of resource availability and constraints in space design, the shielding of crews from high-energy particles, remains imperfect, leaving crew members exposed to space radiation, particularly during longer duration deep space missions. While operational risk assessment for the astronaut population is limited to estimates of excess cancer mortality, to mitigate the risk of radiation carcinogenesis, NASA implemented a research strategy pathway. Ground-based analog studies are mainly conducted at the NASA Space Radiation Laboratory in Brook Haven National Lab (NSRL). These are used to better characterize the role of radiation quality and dose-rate in space radiation-induced carcinogenesis. Recent establishment of the Galactic Cosmic Ray (GCR) simulation beams allow researchers to study and understand the potential impacts of a mixed field exposure. Nonetheless, there is a notable interaction of other spaceflight stress factors that can exacerbate this risk not possible in a simulated environment.

Several research groups were tasked to find ways to characterize the different mechanisms of tissue specific carcinogenesis which led to the development of tissue-specific research models to support the identification of tissue-specific risk factors and improved understanding of physiologic biomarkers.

3.2 Path forward: Since their initial discovery, the understanding of biomarkers and cell phenotypes associated with cellular senescence have expanded beyond growth arrest to include better appreciation for alterations in cellular metabolism, secreted cytokines/chemokines, epigenetic modifications, and changes in protein expression. With the existing knowledge on CS and improved understanding of associated biomarkers, we propose future research study CS in relation to the CNS via focusing on the glia and whether CS in CNS glial cells can lead to alterations in tissue structure associated with nervous system disorders.

Based on available evidence, we suggest that there is some degree of similarity between the physiologic and anatomic changes that occur in neurodegenerative disorders and those changes observed in cellular senescence which leads to the proposition that neurons and glia can exhibit hallmarks of senescence previously demonstrated in peripheral tissues and, more importantly, that these changes may provide further insight into existing disease states. New studies conducted with computational models evaluating these phenomena are under current investigation, but computational models are limited and can account for all confounders. Therefore, these studies

should be supported by additional real-world models for more precise understanding of CS in relation to the nervous system along with what effects are associated with prolonged microgravity and radiation exposure.

4.0 Conclusions:

Emerging evidence indicates that cellular senescence (CS) takes place in the aging brain and may occur in post-mitotic neurons. However, further investigation of senescent biomarkers and related cell characteristics are needed to determine which CNS cell types undergo CS and to better understand the effects of senescence on the CNS aging process. Experiments aimed at addressing these critical questions will shed light on the contribution of CS to CNS aging and neurodegenerative disease. Furthermore, gleaning a better understanding of the impacts of microgravity and space radiation exposure on the nervous system is essential for future manned space exploration as we continue to advance our understand and capabilities beyond Earth.

5.0 Recommendations

We propose future research to understand cellular senescence and its role in the central nervous system via 3D-bioprinted tissue models which can provide better insight into how these phenomena occur in-vivo and what effects they have at both micro and macrostructural levels. Furthermore, we propose studying what effects prolonged exposure to microgravity and cosmic radiation has on normal tissue structure and whether these exposures can lead to upregulation in factors and cell phenotypes associated with CS which could serve as a framework to better understand normal nervous system aging versus pathology. Understanding these processes further could not only improve our protocols for future missions, but could also improve our understanding of existing disease states.

5. 0. References:

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[1]Garret et al, Science, 12 April 2019, Vol 364, issue 6436, doi.10.1126/science.aau8650,
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