INTRODUCTION

The past 18 months have made clear the value of scientific collaboration on a massive scale. Within 12 months of the first reported cases of COVID-19, a global community of researchers had not only sequenced and published the genome of the SARS-CoV-2 virus, but distributed clinical tests to aid in virus detection, and even – with unprecedented speed – developed vaccines against the virus. While the COVID-19 pandemic is not yet over, these efforts have together saved countless lives.

But before COVID, the space program – and specifically the work that happens on the International Space Station (ISS) – was perhaps the best and biggest example of international scientific cooperation on record. Since it was established as a permanently crewed, orbiting research outpost in the year 2000, approximately 3,000 scientific investigations have been carried out aboard the ISS, in fields ranging from physical science to biology to Earth science. Nineteen countries have sent crewmembers to contribute to this work, making the ISS a shining example of international cooperation that has even been nominated for the Nobel Peace Prize.

If we are to continue to work on multidisciplinary teams to tackle big, global scientific challenges, we need to ensure the next generation of scientists is equipped with the skills they will need to do so. And fortunately, there are opportunities for future innovators to start flexing those scientific muscles before they even graduate high school.

Genes in Space is one such opportunity. Genes in Space is a competition for middle and high school students who want to get involved in the biology research happening on the ISS. Each year, we invite students to design DNA analysis experiments that will help us understand how life is affected by the unique conditions of space. Each year, one winning experiment is selected to fly to the ISS, where it is carried out by astronauts.

Since Genes in Space was founded in 2015, the contest has inspired proposals from 7,400 students and launched eight winning experiments to the ISS. Each student-led investigation has pioneered the use of new biotechnology on orbit, informed our nascent understanding of how life is affected by cosmic conditions, and will ultimately inform the development of safeguards against the risks of spaceflight.

On the following pages, the 2021 Genes in Space finalists publish their award-winning proposals in hopes of seeding inspiration for future innovators. We hope you find their ideas as compelling as we did.
ABSTRACT

Previous research suggests that spaceflight makes plants more vulnerable to pathogens. However, it is not known which molecular and genetic pathways cause this increased vulnerability. We hypothesize that microgravity causes depressed immunity in plants and that ETI (Effector-Triggered Immunity) is more affected than PTI (PAMP-Triggered Immunity). We propose to test our hypothesis with parallel experiments conducted on the ISS and on Earth. Immune response in Arabidopsis thaliana, a small flowering plant, will be measured through the production of Pathogenesis-Related gene1 (PR1), a gene that is known to be turned on in response to pathogens. We will turn on PTI in the Arabidopsis by applying flagellin, a component of bacteria and potent immune stimulus. The ETI pathway will be turned on through AvrRpt2, a different type of bacterial component and immune stimulus. We plan to use this system in a variety of Arabidopsis mutants that have specific genes knocked out: MPK3, ACA4, ADR1, and NDR1, to determine which genetic pathways in plant immunity are more affected by spaceflight. We will also be testing whether overexpression mutants of CRK5 and ELP2, genes associated with plant defense, can reproduce immune responses in spaceflight. In order to measure PR1 production, the PR1 gene will be fused with green fluorescent protein (GFP) and a housekeeping gene, EF-1α, will be fused with (red fluorescent protein) RFP, allowing us to measure protein production by visualizing the ratio of green to red fluorescence. When PTI is induced, we expect a decreased induction of PR1, measured by reduced GFP, in plants grown on the ISS compared to Earth. When ETI is induced, we expect an even larger decrease in induction of PR1 on plants grown on the ISS compared to Earth. Our proposed experiment will yield a mechanistic understanding of plant immunity in spaceflight, establish a proof of concept for on-site, on-demand gene expression analysis, and open possibilities for creating pathogen-resistant plants in Space and on Earth.
ABSTRACT

Of the 570 total astronauts that have traveled to space only 65 were female, leaving a gap in knowledge on how the female body functions in space. Estrogen is a major sex hormone integral to female reproduction and organs like the brain, kidneys, liver, and bones. Estrogen mis-signaling can result in breast cancer, Alzheimer's, Parkinson's cardiovascular disease, and one of the most common challenges for astronauts' health, osteoporosis. Despite the link between estrogen depletion and osteoporosis on Earth, the impact of space on the estrogen signaling axis and its concomitant impact on astronauts' bone health are unknown.

We will investigate the impact of spaceflight on estrogen signaling by focusing on the most common form of estrogen, estradiol-17β. Using young female mice, we will compare estradiol synthesis, transport and signaling in space and on Earth. First, we will adapt the BioBits cell-free protein expression system to measure estradiol synthesis and transport in mouse serum by inserting a green fluorescent protein (GFP) gene into an estrogen response element promoter. Estradiol will activate the estrogen response element promoters, stimulating GFP transcription and providing a direct correlation between serum estradiol levels and GFP expression. To analyze spaceflight's impact on estradiol synthesis, ovarian samples will be collected and assessed with RT-qPCR for the presence of aromatase, an enzyme essential for estradiol production. Finally, we will examine estrogen receptor alpha and beta expressions in bone and breast cells using RT-qPCR. Bone and breast tissue require very high levels of estradiol, so receptor expression in these cells could indicate potential estradiol signaling complications like osteoporosis.

This study will further the field of space travel by ensuring the safety of all astronauts. Our BioBits cell-free assay for estrogen detection could also pave the way for new hormonal detection methods to address concerns like estrogen signaling and make long-term missions a more inclusive reality.
Mitigating cancer risk in space: Chemoprevention of space-induced carcinogenesis

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ABSTRACT

In 2020, researchers at NASA released a report identifying radiation-induced cancer as one of the highest priority space challenges. Recommendations have been made to limit the amount of radiation exposure for astronauts, guided by a 3% increase in cancer mortality risk over their entire career. Startlingly, a single mission to Mars can increase cancer mortality risk by 5%, far exceeding established guidelines.

Chemoprevention methods have been developed to mitigate the potential of developing cancer in high-risk individuals. However, few studies have translated these findings in the space environment, which can promote DNA damage via inflammation and oxidative stress, and increase cancer risk. We hypothesize that chemoprevention drugs that increase DNA repair rate and minimize inflammation will successfully lower cancer risk.

To test this hypothesis, we will culture genetically modified UACC-257 cells aboard the International Space Station (ISS) and on earth, with or without the application of chemopreventive drugs. Cells will receive nicotinamide, sulindac, dimethyl sulfoxide (DMSO), or no treatment for 31 days. Nicotinamide is a precursor of NAD+, a necessary cofactor for ATP-synthesis that is required for energy-intensive processes like DNA repair. Sulindac inhibits cyclooxygenase function and has antioxidant properties, reducing inflammation. DMSO-treated samples act as the vehicle control. The eight samples will be subjected to three experimental readouts: (1) PCR-based DNA Lesion Assay to quantify DNA lesion frequency, (2) H2AX-GFP Visualization to determine DNA damage and repair, and (3) Caspase-3 Biosensor to measure cellular apoptosis.

The findings of this study will advance our understanding of the molecular pathways underlying space-induced carcinogenesis and the efficacy of chemoprevention in mitigating cancer risk. Successful development and implementation of these therapeutics will enable longer-duration space travel and exploration.

[Diagram of experimental setup]
Muscle atrophy, or the deterioration of muscle tissue, is one of the most debilitating issues astronauts face daily. In microgravity, astronauts can lose up to 20% of their muscle tissue and up to 50% of their functional strength, making important tasks more difficult to perform. To slow muscle deterioration, astronauts must exercise for an average of two hours a day.

A potential treatment for muscle atrophy is stem cell therapy, in which stem cells are used to heal or repair tissues. Stem cells can mature into nearly any type of cell in the human body, and are a powerful potential tool to help regenerate damaged tissues. On Earth, stem cell therapy has effectively treated numerous illnesses such as chronic myeloid leukemia, strokes, and multiple sclerosis. But most importantly, injecting just 900 muscle stem cells effectively restored muscle function in atrophied mice. Additionally, stem cells grown in microgravity have enhanced therapeutic effects when used at normal gravity.

We hypothesized that mice could demonstrate that stem cell therapy effectively reverses muscle atrophy in space. Initial grip strength measurements will help us gauge the impact of microgravity on muscle strength in the mice. Two weeks later, the mice will receive injections of muscle progenitor stem cells into their soleus muscle. Over the next six weeks, grip strength tests will demonstrate whether the mice gain back any muscle strength. The treated muscles will also be removed and weighed, and the expression of muscle atrophy-related genes will be assessed using RT-qPCR. The results of these tests will be compared with results from vehicle-injected control groups on Earth and aboard the ISS.

This experiment may provide a potential treatment for muscle atrophy in space and could also lead to enhanced therapeutics on Earth for a variety of illnesses.
ABSTRACT

A pathogen-free water supply is of utmost importance to the future of safe long-duration space travel. Despite multiple occurrences of water contamination by pathogenic bacteria during the Apollo program and on the International Space Station (ISS), detecting and monitoring contaminants in spaceflight settings remains a challenge. Currently, water samples are prepared in space and then returned to Earth where bacteria are identified via rRNA sequencing. However, this procedure creates a strong dependence on ground-based testing facilities and will not be able to effectively support long-term space expeditions. This study thus optimizes a cell-free biosensor for in-flight detection of *Pseudomonas aeruginosa* in drinking water, breaking the reliance on ground-based pathogen testing.

*P. aeruginosa* is a multidrug-resistant pathogen that has been isolated multiple times from spacecraft. Analyses of water samples returned from the Apollo 8 mission and the ISS have repeatedly detected this pathogen in drinking water. Following the Apollo 13 mission, *P. aeruginosa* was even identified as the causative agent of a severe urinary tract infection in a crew member. Given the frequent occurrences of and health threats posed by this pathogen, the development of a rapid means of detection may significantly reduce its risk toward astronauts.

To control population density, *P. aeruginosa* utilizes quorum sensing, a form of cell-to-cell communication mediated through the exchange of metabolites such as OdDHL. This biosensor uses a genetic circuit engineered to detect OdDHL, producing a red fluorescent protein if the metabolite is present. Expression of this red fluorescent protein, which can be observed under the Genes in Space fluorescence viewer, is indicative of contamination by *P. aeruginosa*. Ultimately, this biosensor offers an efficient, user-friendly, and portable water quality test while also opening avenues toward broader applications of cell-free technology in space.