Preflight image of the JAXA Cell Experiment Small Prefixation Apparatus for the ISS investigation Fish Scales from which researchers will examine regenerating scales collected from anesthetized goldfish in microgravity. NASA ID: jsc2010e089995.

Publication Highlights

Biology and Biotechnology

The ISS laboratory provides a platform for investigations in the biological sciences that explores the complex responses of living organisms to the microgravity environment. Lab facilities support the exploration of biological systems, from microorganisms and cellular biology to the integrated functions of multicellular plants and animals.

Researchers from the ASI investigation The Coenzyme Q10 as an Antiapoptotic Countermeasure for Retinal Lesions Induced by Radiation and Microgravity on the ISS: Experiment on Cultured Retinal Cells (CORM) are currently examining how the antioxidant Coenzyme Q10 can serve to protect against the damaging effects of microgravity and radiation in astronauts.

Microgravity and ionizing radiation, two of the most detrimental stress factors impacting astronaut health, have been found to cause cell death in the ocular tissue of mice as well as numerous alterations involving structures of the brain and the eyes (i.e., Spaceflight Associated Neuro-ocular Syndrome – SANS) in over 50% of astronauts.

In a recent study published in *Cellular and Molecular Life Sciences*, researchers set out to better understand the effect of spaceflight on reported ocular abnormalities to gain new knowledge for potential treatments. Methodologically, researchers exposed several human retinal cell cultures (ARPE-19) to space for 3 days in the cold stowage unit MELFI. Some cell cultures were treated with the well-known bioenergetic antioxidant coenzyme CoQ10 to prevent cell death. Control retinal cells were also cultured on Earth using comparable experimental hardware and following a temperature cycle similar to the one applied in space. Upon return to Earth, researchers examined the anatomy and RNA transcripts of the CoQ10-treated and untreated cells via a TUNEL assay, immunofluorescence, and transcriptomic analysis.

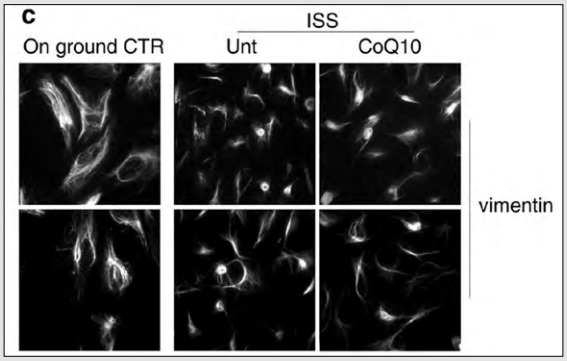

Figure 7. Immunoflourescent images of human retinal cells in different conditions. Image adopted from Cialdai, Cellular and Molecular Life Sciences.

Analyses showed that exposure to microgravity did not influence cell proliferation or cell death, regardless of the cell culture's CoQ10 treatment status. However, the vimentin network – critical for cytoskeleton cohesion – showed marked changes and disorganization that indicated disintegration. Additionally, numerous spaceflight adaptation gene expression pathways involved in metabolism, protein processing in the endoplasmic reticulum, and cell aging were affected compared to ground controls. Importantly, researchers noted that fewer pathways were impacted in cells treated with CoQ10, likely because CoQ10 induces the activation and deactivation of certain genes (i.e., TFRC and SLC7A11), increasing death resistance in iron-dependent cells. These results contribute to a better understanding of the cellular and genetic pathology of SANS and to the development of effective countermeasures.

Cialdai F, Bolognini D, Vignali L, Iannotti N, Cacchione S, et al. Effect of space flight on the behavior of human retinal pigment epithelial ARPE-19 cells and evaluation of coenzyme Q10 treatment. Cellular and Molecular Life Sciences. 2021 October 29; DOI: 10.1007/s00018-021-03989-2.

Publication Highlights

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The ROSCOSMOS investigation **Magnetic 3D Bioprinter** is a high-tech device aboard the ISS that uses a magnetic field to assemble bacteria. In a new study published in the *International Journal of Molecular Sciences*, researchers examined the additive effect of spaceflight and magnetic levitation on the structure, protein expression, and proliferation of the intestinal bacteria E. coli. The experiment was conducted during Expedition 58-59 (December 2018).

Intestinal bacteria, which plays a role in many areas of astronaut health, is altered by spaceflight. Bacteria ranging from pathogens to tissue growth receptors are subject to changes in response to external stimuli and as a result, become more prolific or grow excessively when when compared to Earth conditions. To understand how bacteria respond to spaceflight and an external magnetic force, researchers observed how the common non-virulent bacterium E. coli strain M17 behaved under magnetic levitation on orbit and on the ground. Researchers inspected changes to bacteria morphology, protein activity, and used electron microscopy to evaluate bacteria proliferation. Overall, results suggest that magnetic forces strengthen the effects of microgravity on bacterial metabolism that eventually leads to bacterial self-assembly.

Magnetic levitation was used in microgravity as a force to induce clustering of bacterial cells. To achieve high magnetic force, the bacterial cells were treated with media containing Gadovist, an agent isolated from the rare element gadolinium that has a slightly attractive magnetic field.

Researchers grew bacteria cells under pure microgravity conditions for 144 hours and simultaneously studied the effects of magnetic levitation on Earth. When subjected to both microgravity and a magnetic field, some bacteria showed structural defects in their cell walls. There were also changes in the expression of 23 proteins, and bacteria congregated in the area of least magnetic field, which increased competition for oxygen as a primary resource. Additionally, bacterial enzymes involved in energy regulating processes were more active under magnetic field conditions than those without, both on Earth and in space. Researchers concluded that the morphology, physiology, and behavior of E. coli was affected by spaceflight and microgravity more significantly than by microgravity alone.

Interestingly, the ground magnetic force studies had similar results to the combined spaceflight and magnetic field. These results show that magnetic levitation of bacteria on Earth may be useful for spaceflight simulation because similar results were observed across spaceflight and

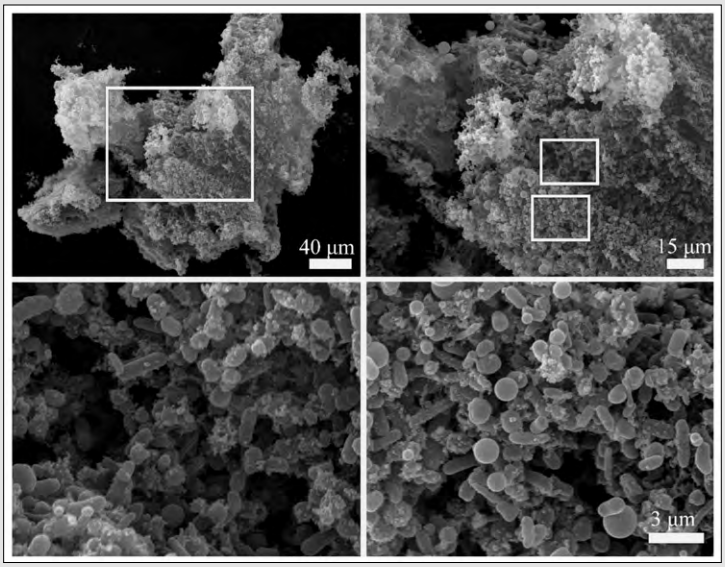

Figure 8. Scanning electron microscopy image of E. coli clusters. Image adopted from Patel, International Journal of Molecular Sciences.

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ground conditions. These results contribute to the growing understanding of how magnetic force affects bacteria, potentially improving future production of therapeutic bioproducts that could treat arthritis, wounds, and digestive issues.

Patel D, Parfenov V, Kononikhin A, Petrov S, Shevlyagina N, et al. Combined Impact of Magnetic Force and Spaceflight Conditions on Escherichia Coli Physiology. International Journal of Molecular Sciences. 23.3 (2022), 1837. DOI: 10.3390/ijms23031837.

The JAXA investigation The Effect of Space Environment on Embryonic Stem Cells and their Development (Stem Cells) observes how radiation impact the DNA of mouse embryonic stem cells. In the past, radiation measurements have been focused on the physical environment of microgravity through active or passive dosimeters. However, quantitative predictions by space radiation on biological tissue or cells have proven difficult due to various factors such as interactions between different radiation types (cosmic, solar, magnetic) in microgravity, continuous low doses of radiation on astronauts, individual differences in response to radiation, and reliance on ground simulation experiments.

In this study published in the journal *Heliyon*, researchers compared standard physical radiation estimates defined as dose equivalent by the International Commission on Radiological Protection (ICRP60) to direct biological measurement using chromosome aberration in frozen wild-type and genetically modified mouse embryonic stem cells preserved. The goal was to extrapolate the risks of radiation on humans during spaceflight through the use of an animal cell model. The genetically modified cells lacked the H2AX gene involved in DNA repair; therefore, these H2AX-deficient stem cells were rendered more sensitive to radiation damage.

After 4 years of space exposure in MELFI, stem cells were returned to Earth, thawed, and cultured before conducting an analysis of chromosomal abnormalities. Results showed no differences in chromosomal abnormalities between wild-type stem cells and ground controls. Therefore, length of exposure to space radiation did not play a role in genetic changes. However, the genetically modified stem cells with an increased sensitivity to radiation showed more abnormalities in DNA translocations compared to modified stem cells on the ground. This result suggests that H2AX-deficient stem cells would not be able to completely repair DNA damage accumulated in space, but wild-type cells could. By using a biologically compromised sample of frozen stem cells, researchers were able to detect accumulated radiation damage quantitatively in mouse cells once thawed and cultured on Earth.

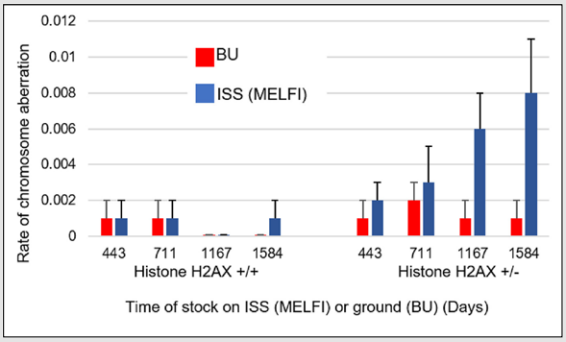

Figure 9. Rates of chromosomal abnormalities in mouse embryonic stem cells. Genetically modified cells more sensitive to radiation (H2AX-deficient) showed more damage after exposure to space. Image adopted from Yoshida, Heliyon.

Irradiation of the genetically modified stem cells to proton beam by an accelerator on Earth allowed researchers to identify the referential effect of radiation resulting in chromosome translocations. An analysis showed that the biological effect of space radiation in MELFI was

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1.54-fold of proton, while the estimated dose equivalent in MELFI using ICRP60 formulas and data of absorbed doses obtained through the PADLES physical dosimeter on ISS was 1.48-fold of proton, indicating that they were almost equal. This comparison led researchers to conclude that current predictions of space radiation effects calculated from dosimeters does not over- or underestimate the effects of space radiation on animal cells. These results enhance the understanding of radiation effects on human cancer and increase the confidence in risk assessments for long-duration missions to the Moon and Mars.

Yoshida K, Hada M, Kizu A, Kitada K, Eguchi-Kasai K, et al. Comparison of biological measurement and physical estimates of space radiation in the International Space Station. Heliyon. 2022 August 1; 8(8): e10266. DOI: 10.1016/j.heliyon.2022.e10266.

The NASA investigation Microbial Observatory-1 examines the diversity of microbes living on the surfaces of the ISS to understand their adaptive changes, their effect on crew health, and contamination of food and air.

In a new study published in *Frontiers in Microbiology*, researchers sampled ISS surfaces from various locations and identified three novel strains belonging to the *Agrobacterium* genomospecies 3 (G3) through ribosomal RNA gene sequencing.

Hundreds of microbial species have been previously isolated from the ISS for a full mapping of microbial diversity. *Agrobacterium* is particularly known for its disease-causing characteristics, and it has been isolated from a variety of inhospitable environments and locations on Earth, including a cave, a hospital, a tobacco plant, and in human cerebrospinal fluid.

The objectives of the study were to describe the phylogenomic novelty and characterize

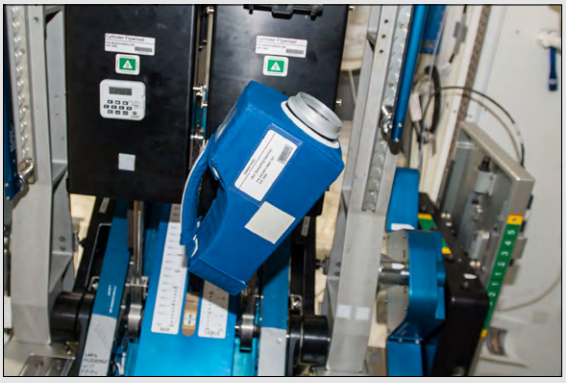

Figure 10. Microbial Observatory-1 aboard the ISS. NASA ID: iss043e198394

the taxonomic affiliations of the strains, perform comparative genomic analysis with strains from other *Agrobacterium* species, and run a pangenome analysis to identify core homologous gene clusters.

Analysis showed unique phenotypic and genotypic characteristics of *Agrobacterium* genomospecies 3 (G3), demonstrating that the ISS isolate is a separate and authentic bacterial species. Researchers proposed naming the new bacteria *Agrobacterium tomkonis* in honor of David Tomko, a well-known NASA Space Biology scientist who advanced space research in the United States.

The results suggest that *Agrobacterium tomkonis* novel strains can colonize in habitats with fewer nutrients. This capability is advantageous because it eliminates competition for survival with other agrobacteria that grow better in richer environments. The study of microbes offers insight into maintaining the safety of the environment of living spaces for astronauts in spaceflight and for humans on Earth.

Singh NK, Lavire C, Nesme J, Vial L, Nesme X, et al. Comparative genomics of novel Agrobacterium G3 strains isolated from the International Space Station and description of Agrobacterium tomkonis sp. nov. Frontiers in Microbiology. 2021 December 6; 12: 765943. DOI: 10.3389/fmicb.2021.765943.

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The ROSCOSMOS investigation Identifying the Genetic Features Determining Individual differences in the Resilience of Biological Objects to Long-term Spaceflight Factors Studies with the Fruit Fly Drosophila Melanogaster (Poligen) examines chromosomal abnormalities and repair in the fruit fly as an animal model.

Preliminary intergenerational studies of gametogenesis and embryogenesis in fruit fly *Drosophila Melanogaster* have shown reduced development after spaceflight. Researchers think that changes in the expression of genes encoding for cytoskeletal proteins could impact stem cells that eventually become differentiated as eggs and sperm cells.

In the present study published in the *International Journal of Molecular Sciences*, scientists examined reproductive health (respiration, cytoskeletal gene expression, and motility of sperm cells) in the fruit fly *Drosophila Melanogaster* after 12 days in microgravity. Because the fruit fly's lifespan is only about 15 days, young maggots were sent to space so that a full cycle of development, including the development of sperm cells, was achieved during spaceflight. Upon return of adult fruit flies, researchers removed the testes of the males and froze them for subsequent analysis. Samples of *Drosophila Melanogaster* kept on Earth for control purposes were part of a simulation study that replicated the G-forces of launch and landing as well as weightlessness in a random positioning machine.

Results showed that the sperm motility of ISS fruit flies decreased by 35% compared to the fruit flies in the control group on Earth. Adding the protein synthesis inhibitor Ser/Thr phosphate restored motility speed in ground controls but not in the flight sample. However, adding a broad-spectrum protein kinase inhibitor did not restore motility in ground controls but it did do so for the flight sample. These results suggest that sperm motility is associated with the content of the proteins present in the organism. Because the simulation study that manipulated the gravity forces of launch, orbit, and landing showed significant motility decreases during landing, researchers believe that hypergravity during descent could be the cause of reduced motility in sperm cells.

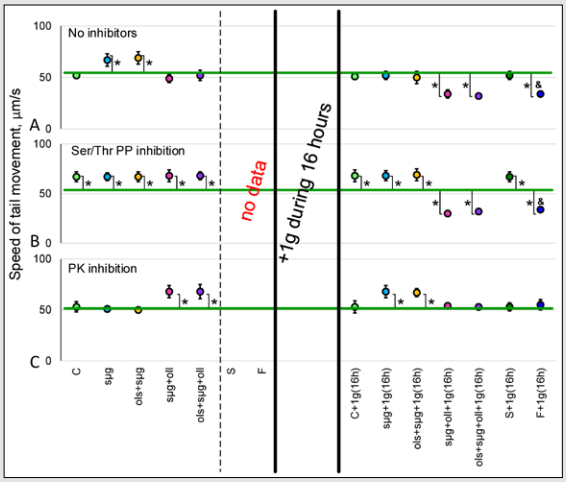

Figure 11. Sperm tail speed across different control conditions and spaceflight. Image adopted from Ogneva, International Journal of Molecular Sciences.

As an animal model for the understanding of reproductive health in astronauts, this study demonstrates how spaceflight affects sperm cell motility so that future investigations can develop countermeasures to protect reproductive potential in space.

Ogneva IV, Zhdankina YS, Kotov OV. Sperm of fruit fly Drosophila melanogaster under space flight. International Journal of Molecular Sciences. 2022 July 6; 23(14): 7498. DOI: 10.3390/ijms23147498.