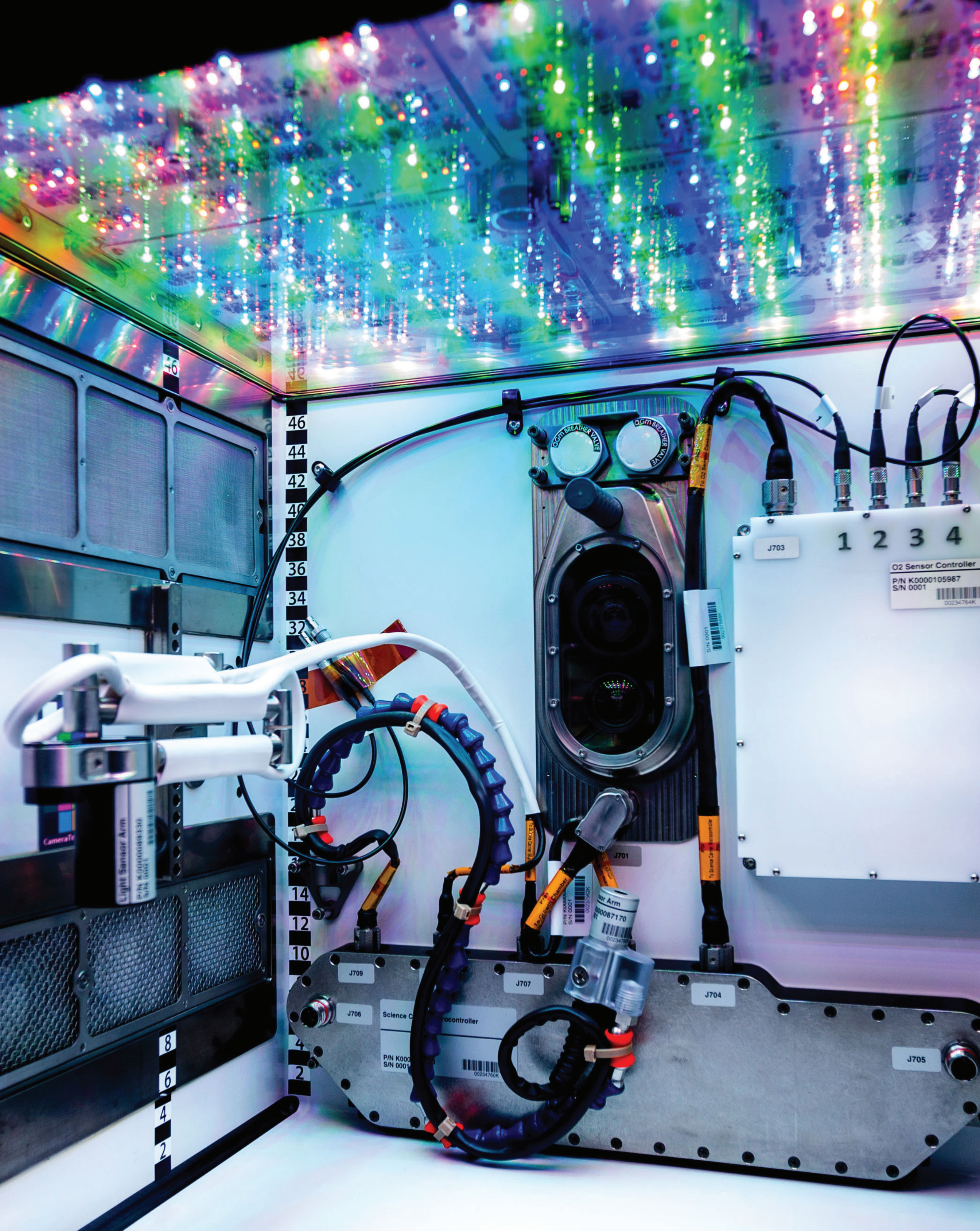

Advanced Plant Habitat (APH) flight unit at NASA's Kennedy Space Center in Florida. This is an exact replica of the unit that was delivered to space station for its first investigation, **Plant Habitat-01**. Developed by NASA and ORBITEC, the APH is the largest plant chamber built for NASA to conduct automated plant growth studies on station. NASA ID: jsc2020e003415.

Biology and Biotechnology

The space station 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. From the beginning of station to date, more than 900 articles have been published in the area of Biology and Biotechnology.

The NASA investigation **Biotube-Magnetophoretically Induced Curvature in Roots (Biotube-MICRO)** examines how plant amyloplasts, which are involved in the production of starch and plant root orientation, respond to magnetic gradients in microgravity. On Earth, amyloplasts are dense particles that sediment in the direction of gravity, inducing the downward growth of plant roots. The presence of magnetic gradients additionally displaces amyloplasts and induces curvature.

In a recent study published in *Scientific Reports*, researchers used *Brassica rapa* seeds to understand the effect of magnetic gradients on transcriptional responses of genes related to growth, metabolism, auxin (hormones), and stress in microgravity. A strong magnetic gradient was generated using ferromagnetic wedges that exceeded the magnetic force to which plants respond. Researchers expected the roots to curve away from the wedges.

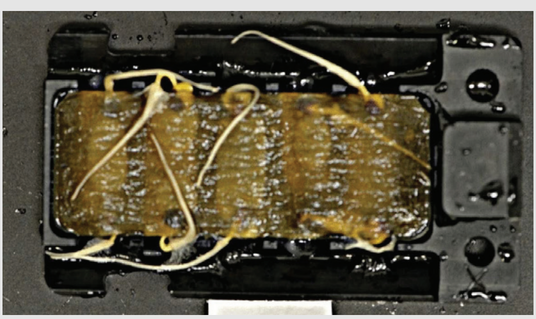

Figure 6. Germination and root curvature of *Brassica rapa* seeds in microgravity. *Image adopted from Hasenstein, Scientific Reports.*

Researchers compared the germination, root curvature, and transcription of 16 genes in four tissue types of seedlings grown in space versus seedlings grown on the ground in static and clinorotated conditions, taking into account the magnetic and non-magnetic fields in which the seeds were placed. Results showed that the presence of a magnetic field did not affect germination but did induce curvature (Figure 6). However, curvature also occurred in non-magnetic chambers, presumably in response to hydrotropism. Magnetic fields in space also did not affect transcription activity involved in metabolism, indicating that magnetic fields are not detrimental to plants.

However, the size of amyloplasts increased in microgravity but decreased during the clinorotation condition on the ground. This outcome indicates that plants perceive gravity, but ground control experimentation is not a suitable replacement for research conducted under weightlessness. These results call for more investigation on the role of starch metabolism to develop scientists' understanding of how space affects plant root growth in a weightless environment.

It is worth highlighting that the crew was not involved in the operation of this experiment because all procedures were controlled remotely from the ground. High-tech systems on-board station allow researchers to monitor their experiments remotely and obtain reliable results without involving crew time.

Hasenstein KH, Park MR, John SP, Ajala C. High-gradient magnetic fields and starch metabolism: Results from a space experiment. Scientific Reports. 2022 October 29; 12(1): 18256. DOI: 10.1038/s41598-022-22691-2.

Publication Highlights

Biology and Biotechnology

The Roscosmos and NASA investigation **Tissue Regeneration - Bone Defect (Rodent Research-4 CASIS)** examines the processes, changes, and outcomes of wound healing after exposure to microgravity. Effective tissue repair after injury could result in fewer health complications and reduced costs. While the main objective of the overall investigation is to learn about regeneration and healing of bone tissue, a recent study published in *The International Journal of Molecular Sciences* focuses on the healing of skin tissue.

In the new study, researchers analyzed the connective tissue from the thigh dermis of 40 mice (10 in microgravity and 30 in multiple control groups) after ~23 days of spaceflight. Connective tissue in the dermis plays a significant role in adaptation to different gravity conditions, with its fibers (type-I and type-III collagens) providing the structure, strength, and elasticity for the skin. Mast cells in connective tissue assist in fiber reconstruction of the dermis and other internal organs through the secretion of proteins, enzymes, and growth factors.

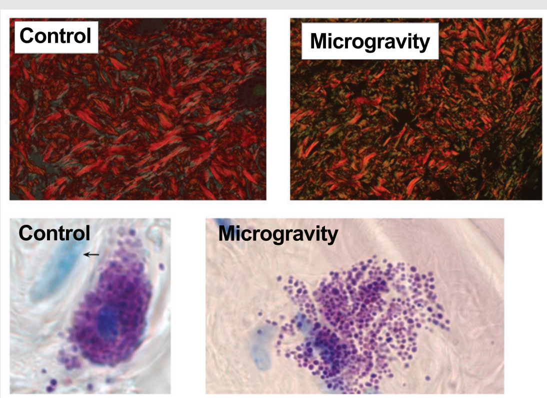

Figure 7. Connective tissue (top row) and mast cell (bottom row) differences between control and microgravity samples. *Image adopted from Shishkina, International Journal of Mofecular Life Sciences.*

Compared to control mice, experiment mice sent to space showed increased interfascicular spaces in the connective tissue, shorter and tangled fibers forming a cellular web, and more type-III fibers than type-I fibers (Figure 7). Disappearance of reticular fibers around the mast cells and more degranulation were also observed. Although the size of mast cells in the spaceflight group remained the same as that in controls, the mast cells showed protuberances as well as excess secretion of small granules after microgravity exposure. According to the researchers, these changes to mast cells may indicate a more active granule metabolism or an adaptation to gravity changes.

This pioneering study characterizes the structure of supporting connective tissue in mice after spaceflight. Its results are an initial step toward discovering the mechanisms of tissue remodeling, which could lead to the treatment of degenerated tissue through the development of pharmaceuticals that assist the renewal of connective tissue in diseased organs.

In 2021, the ESA investigation **Space Omics Analysis of the Skin Microbiome of Diabetic Foot Ulcers (Ice Cubes #9 – Project Maleth)** from the Mediterranean country Malta, conducted its first biomedical science experiment in space to examine the effects of spaceflight and microgravity on human skin tissue microbiome.

Malta has a high prevalence of diabetes. Patients suffering from this disease have reduced blood flow to their extremities and develop nerve damage that results in weakness and numbness of the hands and feet. Consequently, up to 25 percent of patients develop diabetic foot ulcers (DFU) that can become infected. The microbiome present in

Shishkina VV, Kostin A, Volodkin A, Samoilova V, Buchwalow I, et al. The remodeling of dermal collagen fibrous structures in mice under zero gravity: The role of mast cells. International Journal of Molecular Sciences. 2023 January 18; 24(3): 1939. DOI: 10.3390/ijms24031939.

these ulcers forms a biofilm that makes the host's immune system and systemic antibiotics unable to penetrate the ulcer, making it very difficult to treat. Infected and untreated DFUs may progress into bone infections (i.e., osteomyelitis) that would require amputation to save the patient.

Astronauts, like diabetics, develop thinner and drier skin susceptible to damage while their compromised immune system further delays wound recovery. The similarities in skin profiles between astronauts and diabetics allow researchers to understand bacterial diversity in DFUs under two environmental conditions, Earth and microgravity.

Figure 8. Mixed bacteria culture from a diabetic foot ulcer obtained before spaceflight. *NASA ID: jsc2021e0933545*

In the most recent study published in *Heliyon*, researchers obtained six skin tissue samples (Figure 8) along with its microbiome from Type-2 diabetes patients with DFUs with the goal of identifying differences in the microorganisms using 16S rRNA gene sequencing. Analyses revealed different sample structures between tissue types, more similarity and clustering in the Earth control samples, and more diversity of bacterial species without clustering in the microgravity samples. Additional analyses showed that certain bacteria survive, adapt, and thrive more in station's DFU tissue samples. In particular, a high abundance of *Pseudomonas* and *Morganella* was identified.

The data collected through this investigation is now part of the GeneLab repository. Subjecting skin tissue to microgravity helps researchers understand the adaptation and survival of disease-causing bacteria to improve wound healing in diabetic patients and astronauts under the stresses of disease or space.

Through multiple JAXA investigations on station – **Transcriptome analysis and germ-cell development analysis of mice in the space (MHU-1)**, **Mouse Habitat Unit Technical Verification (MHU-4)**, **and** **Mouse Habitat Unit-5 (MHU-5)** – researchers are examining physiological and gene expression changes in mice after exposure to partial and full microgravity.

Although more than 40 percent of the human body's total mass is comprised of skeletal muscle, little is known about how different levels of mechanical loading and unloading impact the cellular and molecular processes of muscle fibers. Given that muscle wasting conditions are associated with loss of strength, deformity, disability, and increased mortality, this new station research could greatly benefit the development of countermeasures for

Gatt C, Tierney BT, Madrigal P, Mason CE, Beheshti A, et al. The Maleth program: Malta's first space mission discoveries on the microbiome of diabetic foot ulcers. Heliyon. 2022 December; 8(12): e12075. DOI: 10.1016/j.heliyon.2022.e12075.

astronauts and treatments for patients on Earth.

In a study recently published in *Communications Biology*, researchers sought to investigate how skeletal muscle myofibers (slow-twitch and fast-twitch) in calf muscles responded to different levels of gravity (Earth artificial gravity, lunar gravity, and microgravity) to better understand protein regulation and degradation. In particular, researchers wanted to learn whether lunar gravity (i.e., one-sixth of Earth gravity) was enough to retain healthy muscle fibers.

These results demonstrated that lunar gravity meets the minimum gravitational threshold needed to prevent full muscle atrophy. However, the transcriptome of mice in lunar gravity resembled that of mice in microgravity more than mice in artificial Earth gravity or ground controls. This result suggested that the expression of some genes is better regulated by a gravitational load higher than one-sixth gravity. Finally, researchers found that lunar gravity, like microgravity, induces slow-to-fast myofiber transitions. That is, there was downregulation of genes associated with

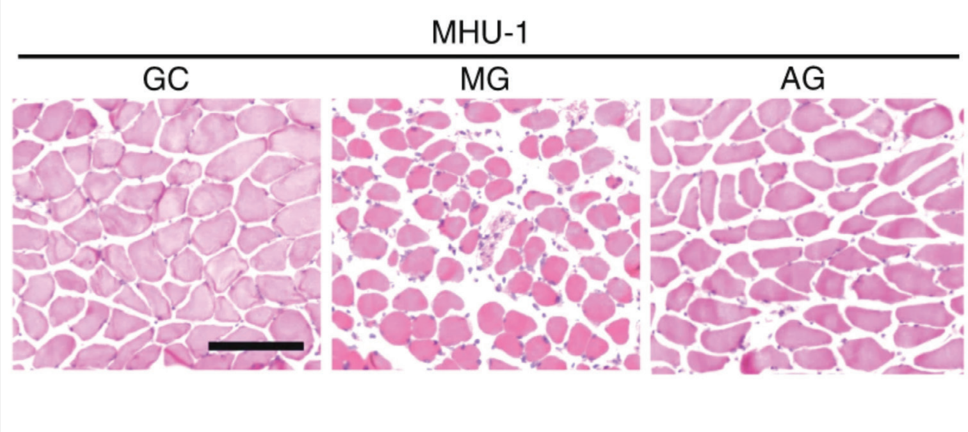

Figure 9. Cross-section of calf muscle in mice. Muscle fiber sfrom ground control (GC) and lunar gravity (AG) were more alike than to muscle fibers exposed to microgravity (MG). *Image adopted from Hayashi, Communications Biology*.

Researchers used the Multiple Artificial-gravity Research System (MARS) on the space station to manipulate the level of gravity and examine skeletal muscle changes in 24 young male mice after one month of exposure to the different gravitational loads.

Researchers conducted histological and RNA sequencing analyses approximately two days after landing and found a 40 percent reduction in myofiber size in the MG group but not in the AG or GC groups (Figure 9).

mitochondrial function and ATP production in mice exposed to microgravity and lunar gravity, consequently affecting muscle metabolism to support contractions.

Through this study, researchers demonstrated that an artificial gravity system is critical to understanding the response of muscle fibers and improve therapies for skeletal muscle health in astronauts.

Hayashi T, Fujita R, Okada R, Hamada M, Suzuki R, et al. Lunar gravity prevents skeletal muscle atrophy but not myofiber type shifts in mice. Communications Biology. 2023 April 21; 6(1): 424. DOI: 10.1038/s42003-023-04769-3.