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ORBITAL ONCOLOGY

3D CELL CULTURES IN SPACE REVEAL THE SECRET TO KILLING CANCER CELLS ON EARTH

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ISSUE 3

VIEW FROM THE CUPOLA
AMELIA WILLIAMSON SMITH

FROM VIRAL VILLAIN TO GENE THERAPY HERO

ARMOR FOR ASTRONAUTS

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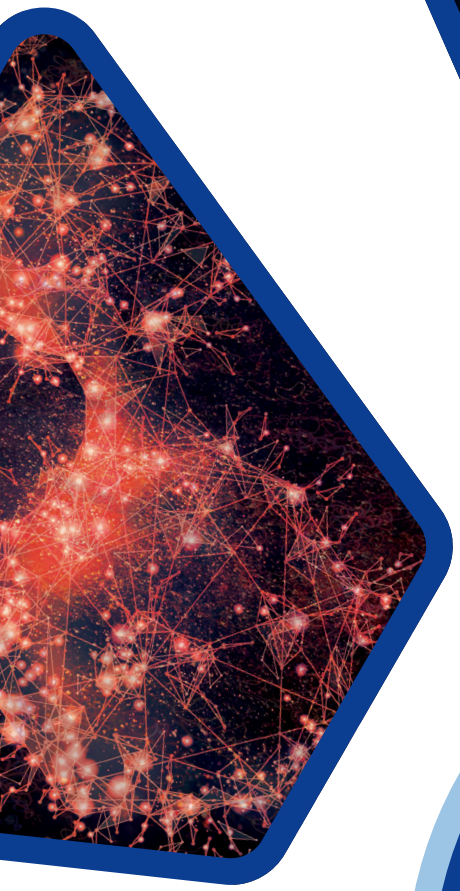

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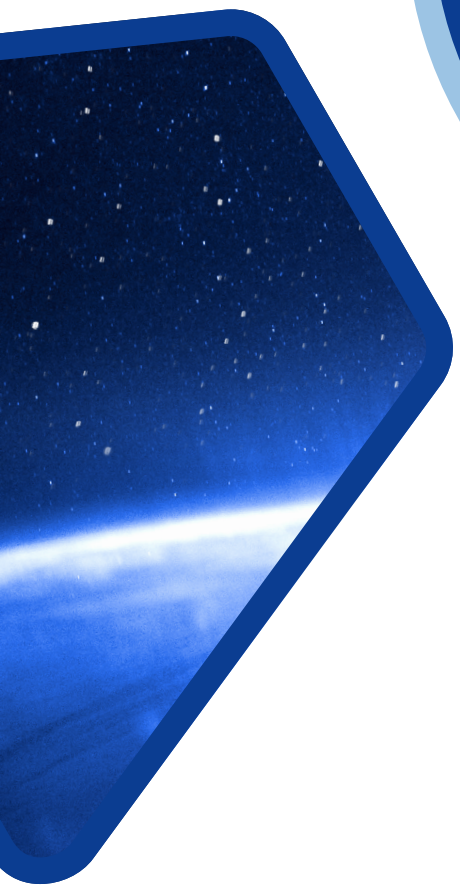

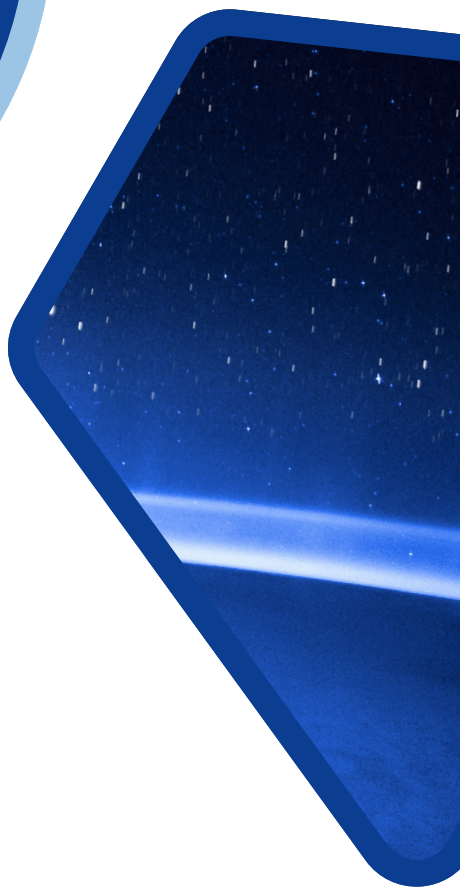

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VIEW FROM THE CUPOLA

By Amelia Williamson Smith,
***Upward* Managing Editor**

Amelia Williamson Smith is the managing editor of Upward and the science communications manager for the ISS National Lab

When I began my job with the ISS National Laboratory 10 years ago, I discovered how science in space benefits humanity on Earth. However, research on the space station became much more personal when I recently learned of results that could someday directly benefit me, along with the more than 2 million women around the world diagnosed with breast cancer each year.

Nearly three years ago, at age 37, I sat trembling in a dark ultrasound room. I will never forget the look on the doctor's face when she came in and told me I had breast cancer. I had no family history or risk factors and found the cancer accidentally. I was immediately struck with terror at the thought of my husband being left alone and our children growing up without me. As I struggled through surgery, chemotherapy, and hormone therapy, my dad lost his 21-year battle with brain cancer.

To say it was a difficult and frightening time is an understatement, but thankfully, my treatment was successful, and I am now in remission. However, others are not so lucky. When I learned about groundbreaking results from a biotechnology startup that leveraged microgravity to grow 3D cultures of breast and prostate cancer cells, the value of space-based research became real to me.

MicroQuin's investigation on the ISS revealed something astonishing—the survival of cancer cells depends on the regulation of environmental changes within the cells. This finding could lead to new treatments not just for breast and prostate cancers but for all types of cancer. The results can even be applied to other diseases. This issue's cover story takes readers through MicroQuin's journey to space to help patients on Earth.

The issue also showcases research from Axonis Therapeutics, a startup developing a gene therapy to restore neurological function in patients with currently uncurable conditions like Alzheimer's, Parkinson's, and spinal cord injury. The company designed a viral vector that targets neurons to deliver the gene therapy but needed to test it in a mature human brain model. Developing such a model takes months on the ground, and often, the cells don't mature enough to accurately recapitulate how an adult human brain functions.

When Axonis sent frozen vials of mature neurons and astrocytes to the ISS and cultured the cells together, something incredible happened. The mature cells rapidly self-assembled into 3D brain organoids in a matter of days—a feat impossible on Earth. The study allowed Axonis to validate its viral vector to help advance the therapeutic toward clinical trials and laid the foundation for more complex brain organoid studies in low Earth orbit.

As we continue to drive innovation and push exploration further into space, we must ensure astronauts are safe from cosmic radiation that can cause cancer and other health issues. The third story in this issue highlights the success of the AstroRad vest, designed to protect astronauts on long-duration missions. ISS crew members assessed the wearability of AstroRad, developed by StemRad in partnership with Lockheed Martin, and provided valuable feedback to improve its design.

In the future, AstroRad will serve another important purpose—increasing accessibility to space. Women are particularly susceptible to health issues from radiation exposure because female breast tissue and reproductive organs are more sensitive to radiation. The AstroRad vest tested in space was designed specifically with women in mind and includes selective shielding to protect these sensitive areas.

As someone who has dealt with cancer head-on, I know the fear and uncertainty that come with that terrifying diagnosis. I am forever grateful to researchers like those highlighted in this issue who go to the ends of Earth and beyond in search of new treatments. It's hard sometimes to imagine how science in space directly impacts people's lives back on the ground, but stories like these make that connection real. Discoveries in space aren't just achievements on a space station—they are breakthroughs that could lead to a world where families like mine are filled with hope instead of fear in the face of cancer and other devastating diseases.

Orbital Oncology

3D Cell Cultures in Space Reveal the Secret to Killing Cancer Cells on Earth

By Amelia Williamson Smith,
Managing Editor

At a fundamental level, science and magic are about as far apart as you can get. Science is focused on systematically studying the structure and behavior of the physical world through observation and experimentation. It is rooted in figuring out the rules of nature—how things work and why—and being able to make predictions based on those rules. Magic, on the other hand, is quite the opposite. Magic is the apparent ability to alter the world, not through the natural laws of science, but through supernatural forces that break those rules. Magic is unpredictable, and it evokes a sense of wonder and awe.

But, sometimes, science reveals something so unexpected and wonderous that it almost feels magical. Research in space that uncovers a way to treat all types of cancer, killing cancer cells without harming healthy cells, sounds like magic—except it's not. Biotech startup MicroQuin leveraged the International Space Station (ISS) National Laboratory to grow 3D cultures of breast and prostate cancer cells to identify mechanisms behind the transformation of healthy cells into cancerous ones.

In labs on Earth, cancer cells grow in a single layer due to gravity. However, on the ISS, where gravity is significantly reduced, cells grow into 3D structures that more accurately represent how tumors grow and behave in the human body.

Representatives from the ISS National Lab and Boeing pose for a photo with the MicroQuin team, which was awarded a 2018 Technology in Space Prize for two investigations.

MassChallenge

Microgravity also causes changes in cancer cell signaling and gene expression, which dictates how cancer cells respond to their environment and communicate with other cells. By studying 3D cancer cell cultures in space, MicroQuin hoped to uncover critical pathways involved in the formation and growth of breast and prostate cancers. However, the company's space station investigation revealed much more than that.

Results led to what MicroQuin founder and CEO Scott Robinson says is a "massive paradigm shift" in the way scientists think about treatments not just for certain types of cancer but for all cancers. And it doesn't end there. The results apply to many other conditions as well—from neurodegenerative diseases like Alzheimer's and Parkinson's to traumatic brain injuries and even viral infection. It all comes down to changes in the environment inside cells caused by disease or injury and how these changes are regulated.

"One of the best things about science is that you can start off looking in one direction, and then your data starts to show you something really magical in a different direction," Robinson said. "What was so magical about our findings is that

they don't just apply to cancer. The new theories we came up with because of our ISS research allowed us to pivot to understand how our findings could be applied across many other disease areas as well."

The Path to Space Scientist

When Robinson was young, he dreamed of being an artist one day—possibly a cartoonist working for Disney. But, in high school, the wonder of space changed his life's direction and led him to his career as a scientist.

"It was space that showed me how fascinating science is," he said. "As soon as I had that first science lesson on space in high school, I was just enamored, and there was nothing else I wanted to do."

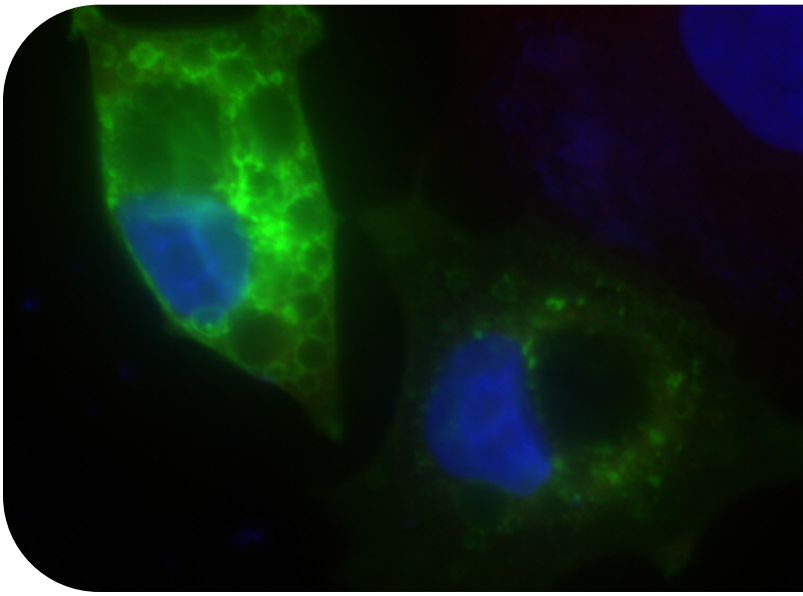
However, it wasn't until 2018 that Robinson found himself with an opportunity to work on research that brought him back to his inspiration. At the time, MicroQuin was participating in the MassChallenge startup accelerator program, and Robinson received an unexpected message. MassChallenge contacted him to say MicroQuin was identified as a startup whose research could benefit from leveraging the ISS National Lab.

Intrigued, Robinson attended a meeting on the Technology in Space Prize, which provides startups with grant funding and access to the space station for innovative research and technology development (R&D). Since its inception, the prize—funded by the Center for the Advancement of Science in Space™, which manages the ISS National Lab, and Boeing—has provided more than $10 million to startups identified through MassChallenge.

At the meeting, Robinson learned how other startups utilized the unique space environment to advance their R&D. He had never considered how microgravity could benefit his research, and his eyes were opened to exciting possibilities.

"Honestly, I think I walked out of that meeting with seven ideas immediately," he said. "I was blown away and knew I wanted to do this."

MicroQuin applied for the 2018 Technology in Space Prize and received awards for two spaceflight projects: one to crystallize TMBIM6, a protein that plays a key role in cancer development and growth, and the other to study 3D cell culture models of human breast and prostate tumors.



Immunofluorescence image of breast cancer cells (MCF-7) treated with a MicroQuin therapeutic taken at 100x magnification.

MicroQuin/Scott Robinson

Untangling Cancer Cell Signaling Pathways

To defeat an enemy army, you must first understand how its members communicate and coordinate operations. Similarly, to find a way to kill cancer cells, scientists must first understand how the cells communicate and coordinate actions that ensure their survival.

Cell behavior is governed by complex cell signaling. Signals from within a cell, the cell's environment, or other cells trigger cascades of actions in the cell. These chains of actions, called signaling pathways, serve many purposes, including regulating gene expression.

When specific genes are expressed, the cell produces proteins that carry out different tasks. For example, proteins can initiate cell growth or the production of other molecules necessary for cell survival. When cells become cancerous, these signaling pathways change, and genes are expressed differently to carry out actions that benefit cancer growth. If researchers could determine the pathways essential to cancer development and their role in cell survival, they could design drugs that target these pathways. However, this is difficult because cancer cells have many signaling pathways that play different roles in cancer development and growth.

So, what does space have to do with it? When you take cancer cells to space, microgravity acts as a unique stressor that triggers changes in cell signaling to keep the cells alive. Researchers have found that for many cancers, cell signaling pathways activated on Earth are suppressed in microgravity. The suppressed pathways are clearly not essential to cancer cell survival. So, the activated pathways must be critical, and researchers can focus on those.

"There are a lot of benefits you can get by doing work on the space station that really allow us to understand what the key pathways for cancer are," Robinson said. "We can then start to look at how to target the pathways to kill the cancer and how to manipulate the cancer microenvironment to enable life-saving technologies like immunotherapy to come through."

Launching to Space

To translate MicroQuin's research into spaceflight-ready investigations, the company worked with ISS National Lab Commercial Service Provider BioServe Space Technologies. "BioServe did a phenomenal job making sure we got our research launched," Robinson said. "I told them exactly what I wanted to do, and there were things we couldn't do and things we had to compromise on, but they came up with new ideas we could try."

BioServe provided support throughout the process—helping the team design investigations to operate in microgravity, conducting experiment verification testing before launch, and even communicating with the astronauts doing the research.

"We were present in real time on the console, talking to the crew, answering questions, and giving them cues," said BioServe research associate Sheila Nielsen. "It's really important that we know as much as possible about the science so we can understand how best to operate the experiment on station."

The team's first investigation to crystallize TMBIM6, a protein involved in cancer development, launched to the ISS on SpaceX's 18th Commercial Resupply Services mission.

BioServe's Brian Medaugh (forefront) and Luis Zea (background) preparing the cells for integration into the flight hardware ahead of the NG-17 launch.

Ivan Castro

TMBIM6 is difficult to purify and crystallize on Earth, and scientists had been unable to produce high-quality crystals to determine its structure. In microgravity, crystals often grow larger and more well-ordered, resulting in higher-quality crystals than those made on Earth. Robinson and his team hoped to leverage the space station to produce crystals of TMBIM6 with a high enough quality for structural analysis.

The BioServe team on console communicating with ISS crew members during in-orbit operations for MicroQuin's 3D cell culture experiment (right to left: Shela Nielsen, Shakini Doraisingam, and Matt Vellone).

BioServe Space Technologies

MicroQuin's second investigation to grow 3D cultures of breast and prostate cancer cells flew on Northrop Grumman's 17th Commercial Resupply Services mission. The team used BioServe's BioCell hardware for the cultures, and the cells were launched live. Once on the ISS, the cell cultures were allowed to acclimate to the microgravity environment for a week. Then, half the cultures were treated with a peptide-based drug developed by MicroQuin, and the cells were left to grow for another week, with the astronauts periodically checking on their progress. The cultures were then fixed, frozen, and returned to Earth for analysis.

When Wei Luo, MicroQuin investor and long-time advisor to the company, first heard about MicroQuin's opportunity to send research to space, he didn't know what to make of it. Was it realistic? Could it really be beneficial? He wasn't sure. But as the team worked with the ISS National Lab and BioServe to design the experiments and prepare them for flight, any doubt he had turned to excitement.

"Seeing the rocket go up on the livestream was incredible—even though I knew it was happening, it didn't feel real," he said. "I never imagined that some of MicroQuin's research would go into space and then come back with amazing results, so it has been a fantastic journey."

It All Boils Down to ICE

When MicroQuin examined the 3D cancer cell cultures from space, the team made a remarkable discovery. “We identified some key pathways that are essential in what seems to be all cancers,” Robinson said. “Although we’ve looked at two cancer types on the ISS, we have taken that research and started to test more on Earth.”

These pathways are related to the intracellular environment, or ICE, and the proteins that regulate it, like TMBIM6. The team knew TMBIM6 was important in cancer growth and that its functions were related to the intracellular environment. But they didn’t realize the critical role ICE regulators like TMBIM6 play in cancer cell survival, drug resistance, and cancer spread.

By its nature, cancer produces a toxic environment within tumor cells. Cancer begins when key genes that regulate cell growth mutate, causing cells to replicate uncontrollably, which puts a lot of stress on the cells, Robinson explained.

“Imagine you were working 24 hours a day and were pushing really hard and had to constantly eat and drink and run on a treadmill—you would find that ridiculously stressful,” he said.

These stressful conditions lead to problems within the cells. For example, for cancer to grow continuously, cells must constantly work to reproduce, which requires a significant increase in metabolism. A byproduct of metabolism is lactic acid, so the increased metabolism causes a buildup of acid within the cell. Acidification is detrimental to cellular function, and if the cell doesn’t do something about the toxic environment, it will die.

This is where a family of proteins called ICE regulators come in. These proteins perform functions to correct a toxic intracellular environment, such as pushing excess acid out of the cell. The genes that produce ICE regulators aren’t activated unless the cell’s environment becomes so toxic the cell cannot survive.

“ICE regulators are like the military,” Robinson said. “On a day-to-day basis, you may need the police to help keep things in check, but you shouldn’t really need the military unless things get extreme, like in a disease or injured state.”

MicroQuin found that when you take cancer cells to space, microgravity causes significant changes in the intracellular environment, which triggers a massive upregulation of genes that produce ICE regulators. The ICE regulators then perform whatever functions are necessary to keep the cells alive. So, if you can design a drug that binds to ICE regulators and changes how they function, you can kill the cancer cells, Robinson explained.

(Above) A BioCell Return Bag from the ISS containing plates used to grow cells. The cells are being analyzed to identify differences between cancer cells cultured in space versus those cultured on Earth.

MicroQuin/Scott Robinson

(Below) MicroQuin founder and CSO Scott Robinson, holding a specialized 12-well tissue culture plate containing breast cancer cells grown on the ISS.

MicroQuin/Scott Robinson

NASA astronaut Christina Koch working on MicroQuin's protein crystallization investigation on the ISS.

NASA

"It was our discoveries on the space station that really led us to identifying these key pathways and understanding why they are so important," he said. "This is a very poorly characterized form of cell death, but it's one of the most fascinating, and our work on the ISS will hopefully allow us to enrich an area that we really don't know much about on Earth."

Applying Discovery to Drug Development

MicroQuin's ISS National Lab-sponsored research led to another exciting result—albeit with a twist. The team determined the structure of TMBIM6, but not from the space-grown crystals. Due to a series of unfortunate issues, the ISS experiment did not return crystals that could be used for structural analysis. However, sometimes the journey is more important than the destination.

Knowing What to do When

Through MicroQuin's ISS National Lab-sponsored research, the team gained valuable insight into how ICE regulators work to balance changes in the intracellular environment.

"TMBIM6 has 15 or 20 functions in the cell, and that's always bugged me," Robinson said. "It's great to do so many things, and they're all so important, but how do you know what to do and when to do it?"

As part of MicroQuin's first investigation, the team crystallized the TMBIM6 protein in several different growth conditions. The team found that the environment around the protein triggers a change in its conformation, or 3D shape, which determines its function. So, if the intracellular environment is too acidic, the high acidity changes the shape of TMBIM6 so it performs an action to regulate the amount of acid in the cell.

In preparing for their spaceflight investigation, Robinson and the team worked hard to optimize their protein generation and purification methods for the highest chance of successful crystallization. When they analyzed the crystals produced on Earth as the ground control for their spaceflight experiment, they were thrilled to find several high-quality crystals. Analysis of these crystals provided valuable data on TMBIM6's structure and revealed several binding sites for drug design.

Equipped with the new knowledge of TMBIM6 as a critical ICE regulator and its structure, MicroQuin was able to improve its current therapeutic and develop a new small molecule drug. The drug binds with TMBIM6 inside cancer cells and alters how it regulates the intracellular environment.

"We refocused our efforts on small molecules and have had phenomenal results; if you know the structure of the protein and how to interact with it, you know how to get the effect you want," Robinson said.

In the case of cancer, the therapeutic directs TMBIM6 to carry out actions that recruit enzymes to the cells' endoplasmic reticulum. The enzymes eat away at the endoplasmic reticulum, eventually causing the cells to die. Because ICE regulators are only activated in diseased cells, the therapeutic can target and kill only cancer cells, leaving healthy cells unharmed.

"It didn't matter which cancer cell line we tested," Robinson said. "It didn't matter if it was breast, lung, prostate, or ovarian cancer—using our therapeutic, by 24 hours, we would literally get floating dead cancer cells."

It all comes back to MicroQuin's theory around ICE regulation, uncovered through its space station research. And even more amazing—the theory is not limited to cancer. The company now has a large portfolio of disease areas and new therapeutics it is working on.

"What we're seeing is that at the heart of every disease or injury, you have an intracellular environment change," Robinson said. "And if you can alter how a disease changes the cell's intracellular environment, you can either fully stop the disease or slow its progression."

Cleaning Up the Microenvironment

While MicroQuin's small molecule therapeutic is highly effective at killing cancer cells, it may not provide a complete cure for all cancers, Robinson said. However, the therapeutic could be revolutionary in complementing immunotherapy drugs. A key obstacle for immunotherapies is the toxic microenvironment around tumors.

Everything that gets pushed out of cancer cells to enable their survival pollutes the environment immediately surrounding the tumor, killing healthy cells in that area. Immunotherapies rely on immune cells to attack tumors, but the immune cells cannot penetrate the toxic microenvironment.

If MicroQuin's therapeutic can alter the way intracellular changes are regulated in cancer cells, limiting the toxic pollutants that are pushed out into the microenvironment, it will be easier for immunotherapies to get through and successfully kill the cancer.

From Space to the Future

Following its ISS National Lab-sponsored research, MicroQuin has been awarded several grants to continue research on ICE regulators and therapeutic development, including multiple grants from the U.S. Department of Defense.

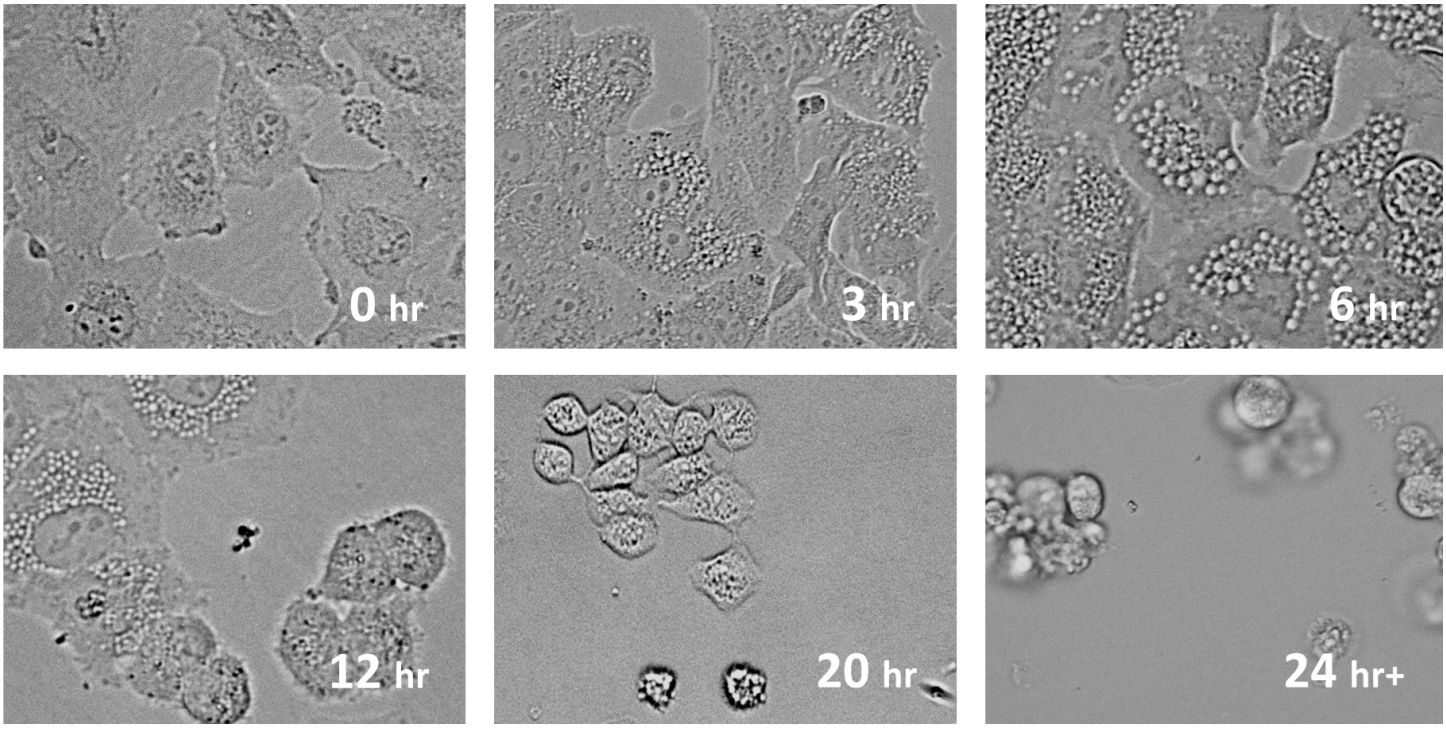
"The work we've done on the ISS has been instrumental in the grant application process to showcase what we have achieved that nobody else has to be able to achieve," Luo said. "In addition to the funding we received to do our experiments on the ISS, the validation it provided is paramount for startups and really opens doors."

MicroQuin's vision is to eventually work with a larger pharmaceutical company to take its therapeutics to market.

"We want to make an impact, and we're hoping this will be a revolutionary treatment to help people with cancer," Luo said.

As Robinson looks to the future, he is excited by the idea of MicroQuin's therapeutics one day helping people find hope in the face of a devastating cancer diagnosis. Now, he is inspired not only by the wonder of space but also by the power of space-based R&D to enable breakthroughs that benefit people on Earth.

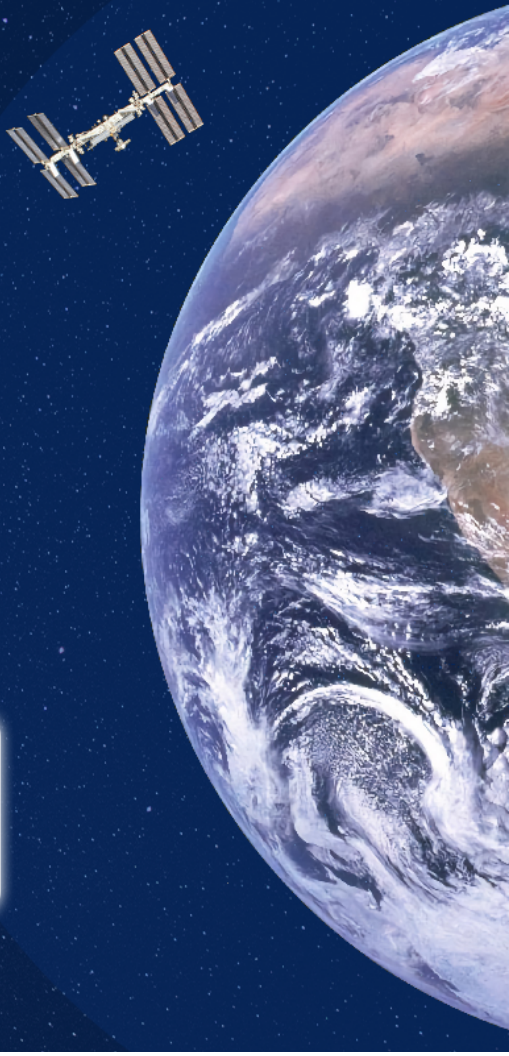
"I think it's such a strange concept for people that you can do something on the space station and that it has any applicability to your life," he said. "But the results from research in space can be transforming—it transformed our company for sure."



This 24-hour time progression of lung cancer cells treated with MicroQuin's small molecule drug shows holes forming in the endoplasmic reticulum of the cells, with floating dead cancer cells in the final frame.

MicroQuin

SPACE STATION SPOTLIGHT



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From Viral Villain to Gene Therapy Hero

Assembling 3D Brain Organoids in Space to Advance Precision Neurological Treatments

By Amelia Williamson Smith,
Managing Editor

In the classic dichotomy of hero versus villain, everything is black and white. Heroes are brave, noble, and the epitome of good. Villains are selfish, cruel, and downright evil. But, in reality, our world is much more complex, and everything is varying shades of gray—heroes have flaws, and villains can do good.

Most people think of viruses as villains. Viruses infect people, leading to bothersome symptoms like congestion, coughing, sneezing, and fever. In some cases, viruses can be more dangerous, such as HIV, the virus that attacks the immune system and causes AIDS. Other viruses, like the coronavirus that causes COVID-19, can spread quickly, causing severe illness and leading to a global pandemic. In these cases, viruses certainly act like villains.

However, sometimes viruses can be good—even heroic. Viruses have evolved to become very efficient at infecting cells in a host and inserting genetic material into the cells. In viruses that cause disease, this genetic material hijacks and damages cells. But scientists can reprogram some viruses to carry helpful, not harmful, genetic material. These modified viruses serve as messengers to carry beneficial gene therapies into cells to treat disease and injury.

One promising area is the use of gene therapies to treat currently incurable neurological conditions, such as Alzheimer's, Parkinson's, and spinal cord injuries. The problem is that gene therapies that work in rodent models often fail to translate into effective treatments for humans. So, there is a critical need for more accurate models of the human central nervous system (CNS)—which includes the brain and spinal cord—to test new therapies.

On Earth, researchers can generate human CNS cells from induced pluripotent stem cells (iPSCs), which typically are human blood or skin cells that have been reprogrammed. However, it is difficult to culture more than one neuron subtype together, and cell growth is limited to 2D monolayers because of gravity. This makes it challenging to create models that accurately mimic the diversity of CNS cells that function together in the human CNS.

Scientists at biotechnology startup Axonis Therapeutics wondered, "What if you take the human CNS cells to space, where gravity is removed?" Axonis had developed a viral vector designed to target neurons and deliver a novel gene therapy for neurological disorders. This therapy was designed to not only prevent neuron degeneration but also spur regeneration. The viral vector worked in mice, but would it work in a mature human brain model? Axonis needed to develop such a model to find out, and to do this, the company turned to the International Space Station (ISS) National Laboratory.

Axonis researchers Tia Dey and Shane Hegarty preparing for the team's ISS National Lab-sponsored investigation.

Axonis Therapeutics and BioServe

"Doing research in space is not something you'd ever think about normally, but the opportunity to leverage microgravity conditions can unlock a lot of untouched potential by pushing the boundaries of science in a unique environment," said Shane Hegarty, Axonis Therapeutics co-founder and chief scientific officer. "We wanted to see if we could overcome the limitations of ground-based iPSC-derived CNS models by co-culturing mature neurons and astrocytes in orbit and having them rapidly self-assemble into a 3D model of the human brain."

Regeneration Rundown

The CNS is the most complex organ system in the human body. It allows us to think, feel, and take in the world around us through our senses. It keeps our lungs breathing and heart beating and allows us to move, communicate, learn, and remember. It's how we solve complex problems and create beautiful things.

The CNS plays such a critical role in how we live and function that injury or disease involving the brain or spinal cord is often devastating and is currently irreversible.

"It is well known that peripheral nerves, which go from your spinal cord to the rest of your body, can regenerate," said Axonis founder and scientific advisor Lisa McKerracher, who is also the founder and CEO of BioAxone BioSciences. "But for most of the 20th century, it was thought that any damage in the central nervous system would be permanent, and there was no chance to replace neurons or have them regenerate damaged axons."

However, in the 1980s, McGill University neurologist Albert Aguayo, who would later become McKerracher's postdoc mentor, was the first to demonstrate this is untrue. Aguayo showed that if you put a peripheral nerve graft into damaged CNS neurons, new axons will grow into the graft. He hypothesized that CNS neurons do not regenerate their axons because the environment around them isn't conducive to regeneration.

The mission patch for the team's ISS National Lab-sponsored investigation that was designed by Axonis team members Eilah Berlow, Tia Dey, and Shane Hegarty.

Axonis Therapeutics

The Nitty Gritty of Neurons

The brain comprises two types of cells: neurons, which transfer messages, and glial cells such as astrocytes that support neurons and provide structure in the brain.

For a neuron to send a message to other neurons, an electrical signal travels down the neuron's long axon. The axon branches off into axon terminals that pass an electrochemical signal to other neurons, and the process continues.

These messages travel down the CNS axons in the spinal cord to peripheral nerves, which are bundles of axons from different neurons grouped together. These nerves act like electrical wires that transmit messages throughout the body to control functions and sense our environment.

After joining McGill University as a professor herself, McKerracher grew intrigued by a hypothesis from Swiss researcher Martin Schwab, who suggested it wasn't just the cells' environment that was a problem. Schwab proposed the existence of inhibitory proteins in the CNS whose purpose is to block cell regeneration. This hypothesis proved accurate, and McKerracher's group was the first to biochemically isolate one such growth inhibitory protein. Since then, other inhibitory proteins have been discovered.

"The idea is that inhibitory proteins prevent sprouting of axon branches where you don't want it to occur," McKerracher said. "They keep the axons on their highway and don't allow them to get distracted by going off on some other trajectory."

However, this becomes problematic when a person has a spinal cord injury or a neurodegenerative disease that damages axons. The proteins prevent injured axons from regenerating, and damage is permanent.

Developing a Targeted Therapy

Axonis advisor Zhigang He at Harvard University discovered there are also intrinsic mechanisms within CNS neurons that play a key role in preventing axon regeneration. During brain development, CNS neurons grow their axons relatively long distances to form connections with other neurons in the CNS (for example, from the brain to spinal cord). However, in adults, expression of a gene called PTEN in CNS neurons acts as a master inhibitory protein within the neuron that suppresses the ability of axons to regrow after an injury.

Zhigang He was the first to show that a single gene manipulation, PTEN deletion, can directly reprogram CNS neurons back into a growth state that allows them to regrow their axons after injury. This groundbreaking discovery came from He's Harvard Medical School and Boston Children's Hospital lab and was spun off into Axonis.

To begin working toward a novel therapeutic to treat neurological conditions, Axonis developed a gene therapy that silences PTEN expression. But PTEN isn't only expressed in neurons. The PTEN gene plays an important role in growth suppression in non-neuronal cells in the brain and in many cell types throughout the body. So, Axonis needed a way to target only CNS neurons with its neuroregeneration gene therapy.

The company used an adeno-associated virus (AAV), which does not cause human disease, to create a viral vector that infects cells and inserts the gene therapy in them. When the therapeutic gene is expressed in the cells, it turns off the PTEN gene.

Although the viral vector infects many different cell types in the body, Axonis found a way to limit the therapeutic gene's expression to neurons by tailoring the vector's gene promoter. A gene promoter is the DNA segment that comes just before a gene in a DNA strand and dictates the type of cells in which the gene can be expressed.

"What we did was design a vector that has a gene promoter only for CNS neurons," Hegarty said. "Even though we knew the AAV would get into astrocytes and other cells, the therapeutic payload would not be expressed because that promoter is only active in neurons."

When the team tested its neuron-specific AAV vector in rodent models, it successfully targeted only CNS neurons. The next step was testing it in a human brain model with both neurons and astrocytes.

Shane Hegarty of Axonis (second from left) participates in a panel discussion on startups in space at the 2024 ISS Research and Development Conference.
ISS National Lab

Building a Better Model

When Axonis was just getting started in 2019, its CEO and co-founder, Joanna Stanicka, applied to the MassChallenge startup accelerator program. There, she learned about the Technology in Space Prize, which provides startups with grant funding and the opportunity to conduct innovative research on the space station. The prize is funded by the Center for the Advancement of Science in Space™, which manages the ISS National Lab, and Boeing.

"When the Technology in Space Prize was announced, our first thought was that we're not a space company, so it's not suitable for us," Hegarty said. "But the opportunity encouraged our founding team to sit down and think, what could we do in space that would advance our company's goals in terms of making new therapeutics for neurological disorders?"

Hegarty and his team came across a report from a research group that sent mature liver cells to space. The cells spontaneously assembled into organoids at a rapid pace in microgravity. This made them wonder if mature CNS cells would do the same.

Producing brain organoids on Earth is possible, but it is difficult and takes months. McKerracher explained that the process involves maturing iPSC-derived neurons and other CNS cells by using an artificial matrix substrate and growth factors. Even if you make brain organoids, the cells may not mature enough to accurately model adult CNS conditions and test drugs.

"You can take iPSCs and let them proliferate and differentiate into organoids, and these are powerful culture systems because you get 3D CNS models," Hegarty said. "However, there's a limitation on the maturity and on what types of cells you get—usually, if you only generate excitatory neurons, you won't get any inhibitory neurons, so you don't model that essential excitatory and inhibitory CNS neuron circuit. It is also a very lengthy culture process, which limits its scalability for drug screening."

If Axonis could send fully mature neurons and astrocytes to space and have them rapidly self-assemble into a 3D organoid, it would provide a robust model for the company to demonstrate that its neuron-specific AAV gene therapy vector works in a human brain model. It would also significantly advance human brain modeling for future space-based research and therapeutic development.

"For studies in space, we wouldn't need to send culture boxes with pre-grown organoids, which are impacted by launch and the flight to the ISS," Hegarty said. "We could send various subtypes of cryo-preserved mature neurons and glia to the space station and then rapidly produce mature brain organoids in orbit, which would be extremely valuable."

Adapting Science for Spaceflight

After submitting a proposal and being awarded the Technology in Space Prize, the Axonis team began working to develop their scientific concept into a spaceflight-ready project. To do this, the team partnered with ISS National Lab Commercial Service Provider BioServe Space Technologies.

(Above) Through MassChallenge, Axonis was awarded a 2019 Technology in Space Prize from the ISS National Lab and Boeing. Axonis CEO, President, and Founder Joanna Stanicka (center) is pictured here receiving the award.

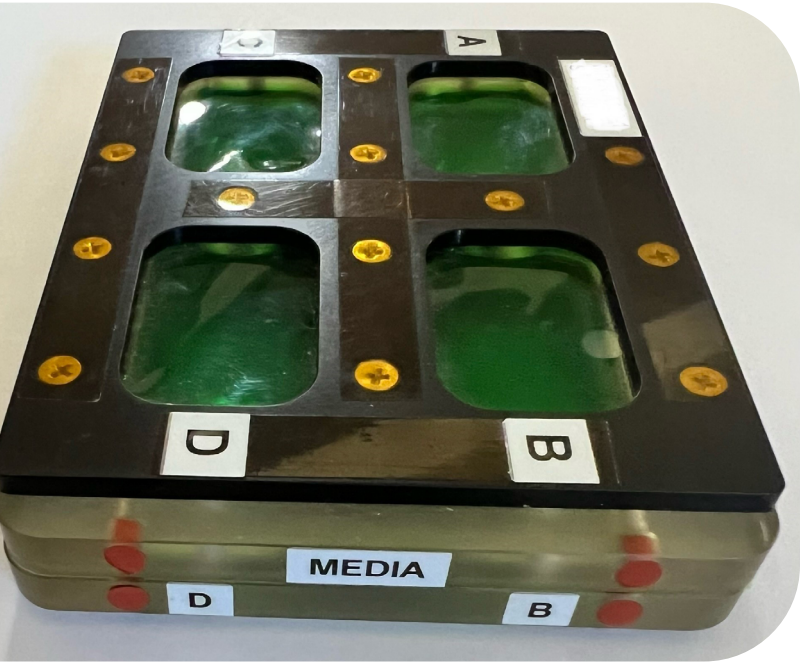
MassChallenge

"We work with research teams to understand their science, identify or develop hardware to support the science, and then help adapt their project to the hardware and the microgravity environment," said BioServe research associate Sheila Nielsen.

The team used BioServe's BioCell hardware, which has four wells per plate. The BioCell also has a large media chamber separated by a porous membrane that allows media to flow in but prevents cells from flowing out.

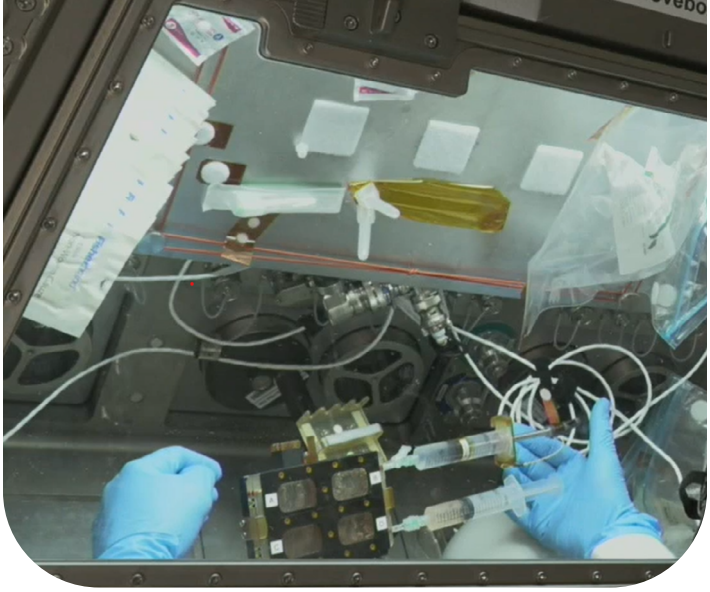
"The nice thing about our BioCell is that it provided a large environment for the cells to grow without attaching to the hardware, and it held enough media to feed the cells without having to do frequent disruptive media changes," Nielsen said.

Axonis' investigation launched to the ISS on Northrop Grumman's 19th Commercial Resupply Services mission in August 2023. The team sent the BioCell hardware prefilled with media, frozen vials of mature neurons and astrocytes, and frozen vials of the AAV vector. On station, the crew thawed the mature cells, injected them into the BioCell hardware, and then added in the thawed AAV vector.



BioServe's four-well dual chamber BioCell hardware
BioServe Space Technologies

Instead of loading the vector with Axonis' gene therapy, the team loaded it with a fluorescent protein gene to visualize the cells. If the vector was successful and the fluorescent protein gene was only expressed in neurons, the neurons would glow green when the astronauts viewed the live human brain organoid cultures under a microscope.



In-orbit operations for Axonis' investigation
NASA

After 72 hours of letting the cells grow, the time had come to check on the cultures. BioServe communicated with the astronauts as they peered into the microscope, and anticipation ran high.

"The pivotal day was when the crew was doing the microscopy, and we saw big aggregates of green, glowing 3D tissue," Nielsen said. "Shane and I were sending images of the organoids back and forth to each other, and we were pretty ecstatic because this is not something they had ever seen on the ground."

Redefining Future Neurological Treatments

Over the course of 72 hours, the mature neurons and astrocytes self-assembled into 3D brain organoids. This was incredible, Hegarty said, because on Earth, mature neurons and astrocytes would die unless grown as a monolayer on a matrix substrate. Furthermore, the team could clearly see the bright green neurons, demonstrating that Axonis' neuron-specific AAV gene therapy vector had worked.

"It was very exciting—we were able to both validate the gene therapy targeting ability and show that we indeed rapidly formed 3D assembloid models of the human brain," Hegarty said. "The major breakthrough is that you can self-assemble mature neurons and astrocytes rapidly into a model of the brain in microgravity conditions, which you can't do on the ground."

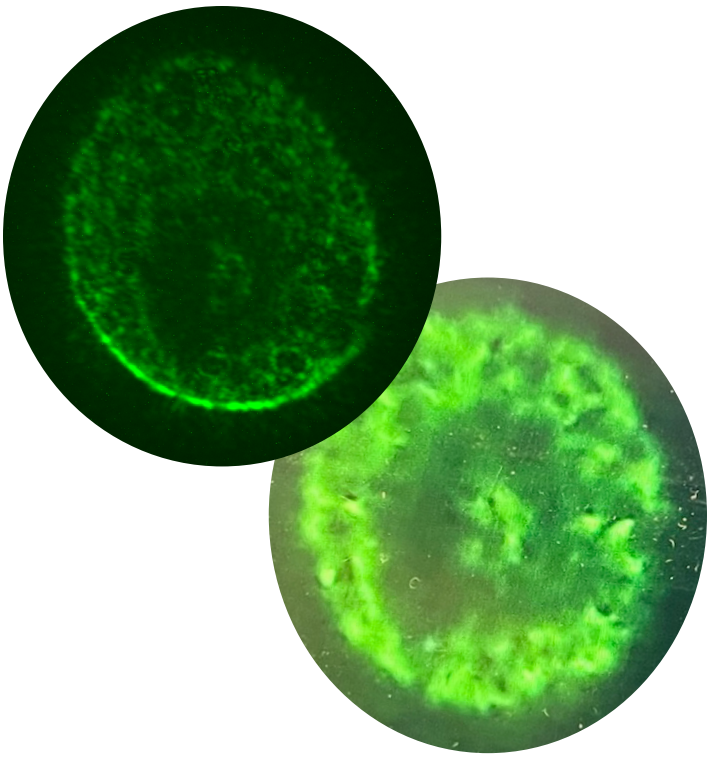
By successfully demonstrating the AAV gene therapy vector works in a human brain model, Axonis was able to significantly de-risk its therapeutic, helping to move it closer to clinical trials. Hegarty said the Technology in Space Prize also helped put Axonis on the map as a company. The grant made it easier for the startup to attract seed investors because it had its own non-dilutive capital and could hire its first research assistant.

Axonis' results also have exciting implications for future space-based research. Hegarty says his team could take blood or skin cells from any patient and reprogram them to make the CNS cells relevant to that patient's neurological disorder. The team could then combine these disease-relevant CNS cell types in microgravity cultures to rapidly develop a 3D model of the patient's particular disorder.

"This would allow us to take a personalized medicine approach to developing novel, precision CNS therapeutics for individual patients and overcome current modeling limitations on Earth," he said. "That's the type of pioneering biotechnology research that can be done on the ISS now."

Reflecting on his team's success, Hegarty thinks all scientists should spend time thinking about space as a valuable research platform and how they could drive innovation by leveraging the transformational environment of the ISS.

"We never imagined it would be possible to advance our research in space, but the Technology in Space Prize gave us the opportunity to explore this possibility more deeply," he said. "We now fully appreciate the amazing potential of performing research on the space station and have shown how you can leverage microgravity conditions to push your science beyond what's currently possible on Earth."



Images captured during in-orbit microscopy showing a human brain organoid model of iSPC-derived neurons and astrocytes expressing a neuron-specific AAV vector-delivered gene.

Axonis Therapeutics

Armor for Astronauts

Developing High-Tech Wearable Radiation Shields

By Stephenie Livingston,
Staff Writer

A view of the AstroRad vest in the Cupola module onboard the space station.
NASA

Drifting weightlessly onboard the International Space Station (ISS), NASA astronaut Kayla Barron carefully pulls on an unfamiliar garment. It feels snug, a bit bulky—like an over-engineered life vest. Only this isn't for water. It's the AstroRad vest, a cutting-edge wearable technology designed to protect astronauts from one of space's invisible and more relentless dangers: solar radiation.

Developed by StemRad in collaboration with Lockheed Martin, the vest might soon protect astronauts on missions that push far beyond the safety of Earth's protective bubble. Barron, who tested an early version during routine tasks and sleep on the space station, said, at times, it felt "like a gravity blanket in space."

"It wasn't ideally sized for my anthropometric dimensions… but, you know, it's prototype hardware," she said. She also pointed out the vest's main challenge: "They're trying to cover all of these critical parts of your body, so they want to maximize the coverage without really interfering with your mobility."

But at the time, the vest wasn't quite there. Comfort and flexibility are essential when floating in microgravity. Barron and other astronauts' feedback sparked a slew of new tests and improvements to refine the vest's design and make it more customizable.

Behind the scenes, Kat Coderre, an aerospace engineer at Lockheed Martin whose resume includes designing the Orion spacecraft for the Artemis I Moon mission, played a pivotal role in developing the vest. She refers to the long, intricate design and testing process involving various partners and experts as the "vest saga." "What's cool about this whole thing," Coderre says, pulling up data from the vest's recent tests on her computer, "is how multiple linked projects have come together. Lockheed Martin and StemRad are at the core, but we've also brought in other partners to push the innovation further. It's really a story about how all these partnerships came together to keep our astronauts safe."

The team partnered with the ISS National Laboratory® to test run the vest in microgravity, evaluating how it fit, moved, and functioned in real space conditions. Sure, Earth's magnetic field provides some shielding in low Earth orbit (LEO), but astronauts on the ISS can still experience more than 100 times more radiation than people on Earth.

Radiation becomes a more ruthless adversary during missions beyond Earth's protective magnetic shield to the Moon or Mars. Galactic cosmic rays, solar particle events, and radiation trapped in the Van Allen Belts can result in exposure levels up to the equivalent of 6,000 chest X-rays—and cause illnesses like cancer and radiation sickness. The vest, with layers of high-density polymers, aims to take the brunt of that punch.

Barron, who also served as a submarine warfare officer in the U.S. Navy, knows firsthand the challenges of working in radiation-heavy environments. "Radiation shielding is a really hard thing to do," she said, recalling her military experiences working in nuclear environments during her "previous life." On Earth, traditional solutions like lead walls work well, but mass and volume constraints on spacecraft make such methods impractical in space. This is where innovations like AstroRad become essential.

The idea of wearable, customized protection for astronauts offers a more feasible solution. “The idea that you could have wearable technology customized for each crew member, it’s kind of like an aha moment,” Barron remarked. “It’s an elegant solution to a really challenging engineering problem.”

From Fukushima to the Final Frontier

Speaking from his home in Tampa, Florida, Oren Milstein, founder and CEO of StemRad, is animated as he talks about AstroRad’s origins—how it all began with a disaster on Earth. “It started after the Fukushima nuclear disaster in Japan,” he recalls, leaning forward in his chair. “We developed the first gamma radiation personal protective equipment (PPE) for nuclear disaster first responders, so there’s a lot of heritage in protecting first responders.” The company developed a solution called selective shielding, which focuses on the body’s most vulnerable parts, such as bone marrow, the tissue most sensitive to radiation.

The idea to develop PPE for astronauts came in 2015 after NASA launched Orion with Mars ambitions in mind, followed by the Artemis program in 2017 to return astronauts to the Moon for the first time in more than 50 years. “We saw NASA’s ambitions and thought, ‘We can help,’” Milstein said.

He discussed the severity of cosmic radiation with an earnestness that underscored the urgency of his technology. “Space radiation can kill,” he said bluntly. “To travel to Mars without adequate protection is to gamble with your life, and that’s unacceptable. Radiation protection is critical because radiation is unavoidable, and that’s what AstroRad is about.”

Solar particle events, for instance, are like being swarmed by a hive of bees—if the bees were charged particles, Milstein says.

And it’s not just about short-term protection from intense solar events, he explained. “We’re talking about long-term protection to reduce the risk of cancer and other radiation-induced health issues for astronauts on long-duration missions.”

During a meeting in Tel Aviv, Milstein presented AstroRad’s concept to a room of aerospace executives. John Karas, a vice president at Lockheed Martin at the time, was in the audience. As Milstein recounts this moment, he leans back and grins. “John stood up, extended his hand, and said, ‘You’ve got a partner for this.’ It was that simple. Suddenly, we had Lockheed Martin on board.”

The ISS National Lab’s sponsorship and Lockheed’s involvement accelerated the project’s momentum. Speaking from her office in Denver, Coderre recounted the moment she was brought onto the project. “At first, we were like, ‘A

NASA astronaut Kayla Barron in the Blue Flight Control Room at NASA’s Johnson Space Center in Houston.

NASA/Bill Ingalls

radiation vest? This is different from what we’re used to.’ But the idea was intriguing. We knew we’d need to test how well it performs in the actual environment, and the best place to do that was the ISS.”

“There’s a lot of complexity when designing something that must protect against space radiation. It must be comfortable enough to wear for long periods and not add too much mass,” she explained, gesturing toward mockups of the vest on her laptop. “Space is always mass-constrained, so every decision comes down to balancing those factors.”

As Coderre speaks, it becomes clear that the project is not just about protecting astronauts in space—it’s also about making sure anyone can explore it, regardless of biology. The risk of radiation-related illnesses, for example, is particularly concerning for female astronauts. Research has shown that females are more susceptible, primarily because certain tissues, such as breast tissue and reproductive organs, are more prone to radiation-induced cell mutations. In fact, women have up to twice the risk of developing cancer from radiation exposure compared to their male counterparts.

“Women’s access to space was the whole impetus behind this,” said Coderre.

International Atomic Energy Agency (IAEA) team leader Mike Weightman examines Reactor Unit 3 at the Fukushima Daiichi Nuclear Power Plant in 2011 to assess tsunami damage.

Greg Webb/IAEA

Historically, NASA imposed stricter radiation exposure limits for female astronauts, though recent adjustments have aimed at equalizing these limits. In addition to those changes, protective measures like AstroRad remain crucial for mitigating the risks in customizable ways, ensuring that deep space exploration is safe and accessible for everyone.

This is even more relevant as the commercial space industry opens doors to space travel for more people from various walks of life. Projects like AstroRad are increasingly personal, says Coderre. It's no longer just a select few heroic figures going to space who seem distant from our everyday lives. "Now, they're our colleagues, scientists, neighbors, and friends," she noted. In other words: space is for everyone.

Trial by Space

In late 2019, a version of AstroRad designed for female astronauts, with extra protection for breast tissue and ovaries, flew to the ISS for its first real test in the environment where it must perform. The goal was to assess whether astronauts could wear the vest comfortably during their normal routines: sleeping, eating, exercising, and performing experiments in microgravity.

Jordan Houri, StemRad's lead scientist, discussed the experiment over Zoom from the Tampa office. "The ISS tests were a game-changer for us," he said as he played a video of NASA astronaut Jessica Watkins discussing the vest onboard the ISS. "We needed real feedback from astronauts in space—how the vest fit, whether it restricted movement, and if they could wear it for extended periods without discomfort. Without that data, we couldn't move forward."

After a 25-day lunar mission, Artemis I Orion manikins Helga and Zohar undergo post-flight inspections at Kennedy Space Center on Jan. 11, 2023. Equipped with radiation detectors, Zohar tested the AstroRad vest to assess its effectiveness for future astronaut protection.

NASA

Teams at NASA's Kennedy Space Center in Florida prepare two manikin "phantoms," Helga and Zohar, for the Artemis I mission to the Moon.

NASA

The vest is made from high-density polymer rich in hydrogen, which is particularly effective at shielding against cosmic radiation. "It's perfect for space because it's lightweight and highly effective at blocking the kind of charged particle radiation astronauts encounter," Houri explained. The vest's design incorporates a series of hexagonal blocks carefully arranged in layers to maximize protection while maintaining flexibility. These blocks are sandwiched between durable yet flexible fabric layers, allowing the vest to bend and move with the astronaut's body.

There's selective shielding, too: thicker protection for the bone marrow concentration in the hips, chest, stomach, and ovaries and thinner layers for areas where protection is not as critical and mobility is key. The result? A vest that blocks cosmic rays without transforming the astronaut into the Michelin Man. Afterall, an astronaut needs a full range of motion to perform even during a solar storm. However, according to Milstein, some limitations are fine compared to the alternative of being stuck in a cramped storm shelter during an event.

Four female astronauts tested the vest on the ISS. However, the team also tested its cross-gender applicability when a curious male astronaut tried it out. Surveys and real-time video assessed the vest's comfort and ergonomics. Feedback from astronauts is being used to refine the final design before future missions.

To test how the vest performed in different scenarios, Barron wore it for two distinct situations: during weekly housekeeping tasks and for a sleep period. The arm openings weren't in the perfect spot for her, so she had trouble controlling her body in microgravity, especially while holding things. Due to the

mobility restrictions and the size, "it was kind of hard to get into my sleeping quarters. And then once I was in my sleeping bag, I couldn't zip it up all the way," she said.

"I felt a little weird with my arms out of the vest, but I tucked them in, loosened the vest a little, and cocooned into it," she continued, "which was actually a little bit comforting, like a gravity blanket in space."

However, she added that the design limitations noted during testing weren't enough to prevent her from wearing the vest to protect herself during a high-radiation event.

Houri shared other stories about how astronauts—known to have a penchant for practicality—interacted with the vest. "One of the astronauts suggested that we add pockets, like a hoodie," he says, chuckling. "They said it would give them a place to rest their arms." Others requested more flexibility around the shoulders and legs, attachments to keep tools like iPads from floating away (during testing, astronauts stuck Velcro to the vest), and more adjustability—all feedback the team is using to modify key vest elements. They've already developed a system in which the width or height of a standard vest can be made larger using modular add-ons to the side and front and back panels.

Barron explained, "with any crew-worn hardware, how it fits you is really, really important."

Artemis I Phantom Test and Ziplock Bags

According to Milstein, the ISS project delivered proof that the vest, with several adjustments, could perform in microgravity during a solar storm lasting days. "The ISS National Lab really opened the door for us," Milstein said. "It allowed us to test the vest in a real space environment and laid the groundwork for other collaborations and tests, where we could take the concept even further."

Simultaneously, StemRad tested the vest outside Earth's protective shields during NASA's Artemis I mission in late 2022. The test subjects? Two manikins, or "phantoms," designed to mimic human anatomy. One, named Zohar, was outfitted with the AstroRad vest. The other, Helga, flew without it. Both manikins were equipped with thousands of radiation detectors to measure the levels of radiation exposure during their trip through Van Allen Belts and around the Moon.

The radiation detection results for Helga, the unshielded manikin onboard the heavily shielded Orion spacecraft, were recently published in Nature. According to study authors, including Houri and Milstein, Helga's dosimeters absorbed approximately 30.7 millisieverts of radiation during the mission—an amount vastly exceeding the radiation we experience on Earth. To put this into perspective, Helga's dose was equivalent to more than 13 years of natural radiation exposure on Earth. This exposure rate was still 60 percent less inside Orion than in previous missions where radiation was documented.

Houri pulled up data from the mission. "This is what we were waiting for," he said, displaying radiation exposure charts for Helga and Zohar. "The results exceeded our original predictions of how much protection it would provide by around 33 percent." Based on the data collected during the Artemis I experiment, if a major solar particle event had occurred during the mission, such as the historically powerful solar particle storms of August 1972, Houri says the vest would have reduced Zohar's exposure by around 60 percent.

Tests during Artemis and on the space station raised questions about reducing the vest's mass for long-duration missions, where mass is especially tight due to the challenge of exiting Earth's gravity well. Enter Redwire, with its expertise in in-space manufacturing.

At the 2024 International Space Station Research and Development Conference, Ken Savin, Redwire's chief scientist, detailed how the company is repurposing Ziploc bags into filaments for 3D printing parts of AstroRad. Yes, Ziploc bags—a prevalent plastic waste onboard the station. Using the Braskem Recycler, a device designed to convert plastic waste into usable materials on the ISS, Redwire

has transformed these bags into hexagonal pieces that snap together to form the shielding components for the vest. “We’re going to try to convert plastic waste into a recycled protective vest and solve two problems with this one solution,” Savin explained.

Humanity’s Next Giant Leap…Done Safely

The work on AstroRad isn’t over. Additional tests funded by Space Florida and redesigns to the vest are in motion, fueled by data gathered from the ISS National Lab-sponsored experiment. The team continues to refine the vest, focusing on mobility and fit and reducing its mass while maintaining the protection astronauts need for deep space missions.

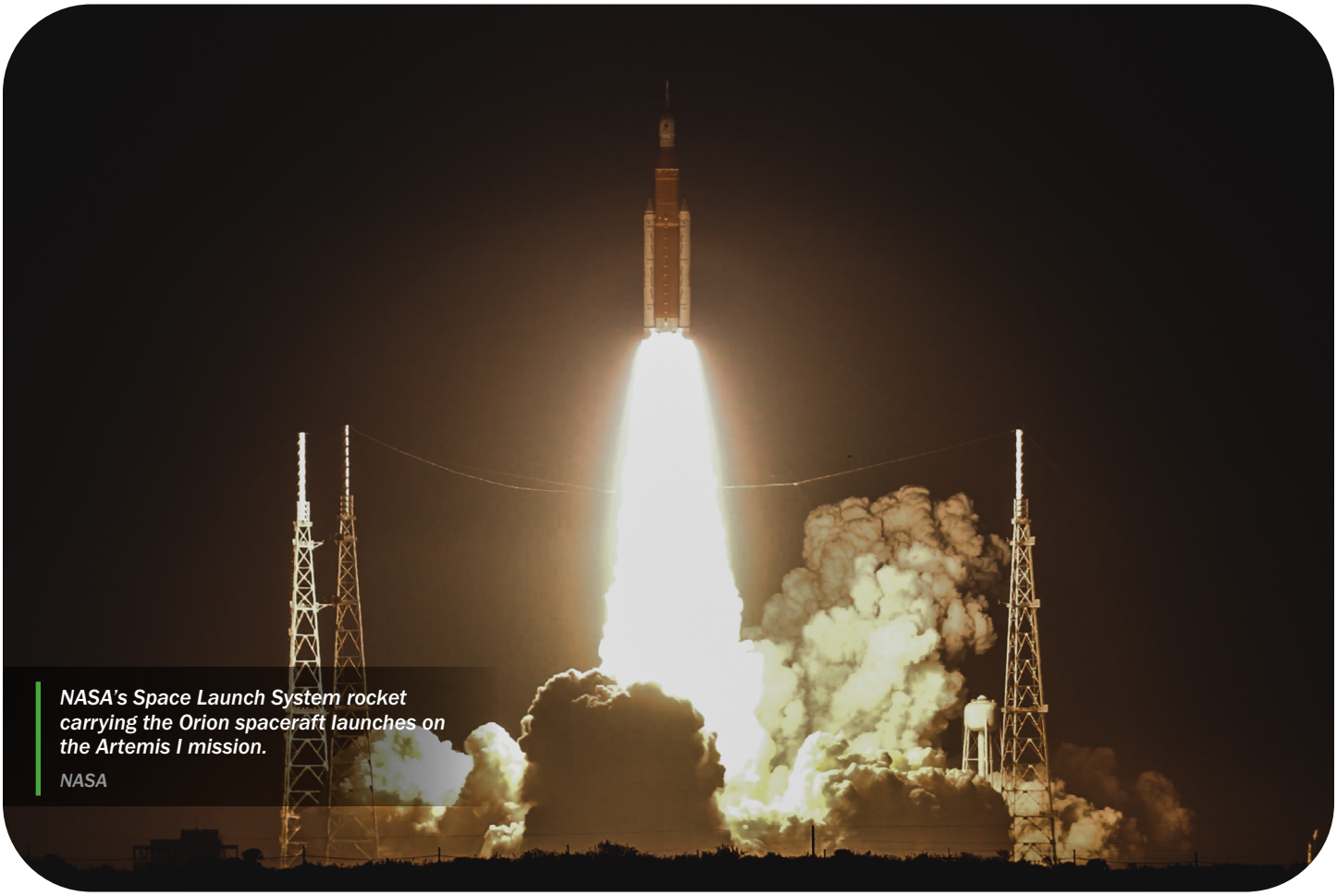
“Radiation is one of the biggest barriers to deep space exploration,” Coderre said. “If we can solve this, we can send people farther for longer. That’s what makes the vest so important. It’s not just about one mission—it’s about the future of space exploration.”

When you consider the complexity of going from LEO to the Moon and from the Moon to places like Mars, “it just goes to show the power of creative ideas and collaboration with people who come up with different ways to solve problems like this,” Barron said.

The ripple effects of AstroRad’s space tests stretched to StemRad’s Earth-bound medical and first responder protection. “Even though we’ve tried to make our wearables as comfortable as possible, everyone has different limitations,” Houri explained. “AstroRad testing in space has shown us the importance of customization—of designing protection that works for various body types while remaining effective.”

StemRad’s flagship product, the 360 Gamma, developed to protect first responders during nuclear disasters, has benefited indirectly from these insights. “The idea is the same,” Milstein said, “whether it’s for space or Earth. We focus on protecting the most vulnerable areas of the body.”

“Space pushes the limits of what’s possible,” Houri added, “and simply by going there, it’s inspired us to create better solutions for those facing radiation exposure on Earth.”



NASA’s Space Launch System rocket carrying the Orion spacecraft launches on the Artemis I mission.

NASA

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