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UNLOCKING THE SECRETS OF THE IMMUNE SYSTEM

HOW TISSUE CHIPS IN SPACE COULD HOLD THE KEY

ON PAGE 2

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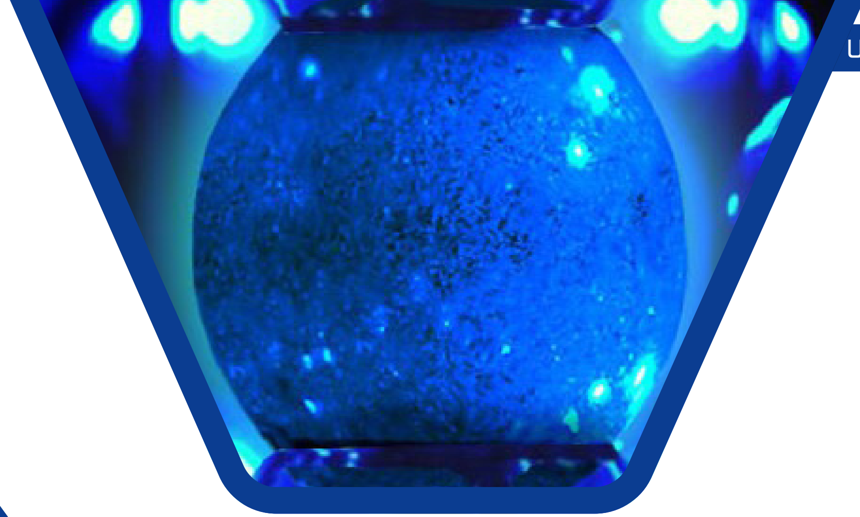
ISS NATIONAL LABORATORY®



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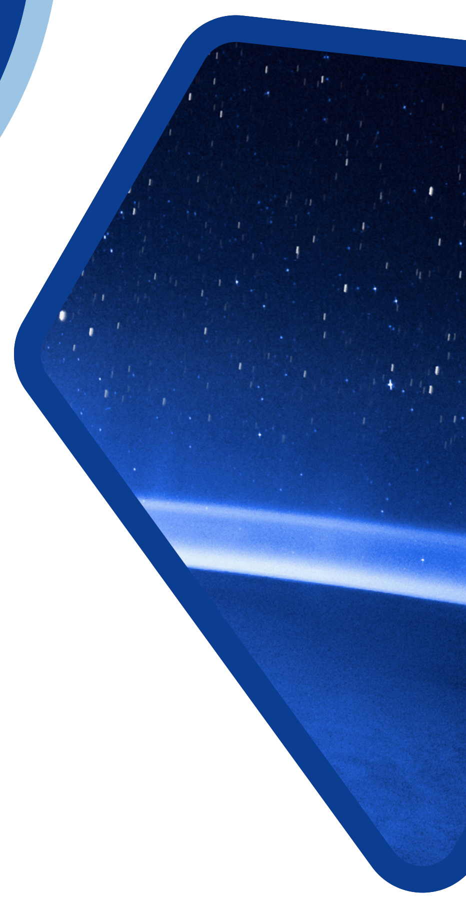


ISS NATIONAL LABORATORY®

VIEW FROM THE CUPOLA

BY SUSAN MARGULIES, Assistant Director for Engineering, U.S. National Science Foundation

Susan S. Margulies leads the U.S. National Science Foundation's Directorate for Engineering in its mission to transform our world for a better tomorrow by driving discovery, inspiring innovation, enriching education, and accelerating access.

What can we discover about phenomena on Earth when soaring approximately 250 miles above our planet's surface? As you will find in this issue of Upward, the answer lies in the unique insight gained from working at near-zero gravity. The U.S. National Science Foundation (NSF) is pleased to support the fundamental research onboard the ISS, as it enables us to solve questions that cannot easily be answered here on Earth.

The mission of NSF is to promote scientific progress and capacity by investing in research and education across the country. Since our partnership with the Center for the Advancement of Science in Space™, manager of the International Space Station (ISS) National Laboratory, began in 2016, NSF has invested more than $26 million in funding for 60 projects from institutions across the nation. To date, 23 projects have already been launched. Each of these funding opportunities is designed to support critical research that will lead to benefits for life worldwide.

For example, researchers from Arizona State University and Rensselaer Polytechnic Institute are leveraging the ISS National Lab to improve the mass production of protein-based pharmaceuticals. Funded by NSF, this investigation used the microgravity environment to learn how hydrodynamic forces can alter the structure of proteins in solution and lead to protein clumping, which is currently a significant hurdle in drug manufacturing. The findings of this fundamental research will have widespread implications, as most vaccines and many therapeutics we use today are protein-based. Predicting the complex motion of proteins in solution will help us find ways to mitigate negative effects and improve our capability to manufacture life-saving drugs.

Another great partnership featured in this issue includes an investigation into the effects of aging on our immune system. Funded by the National Institutes of Health (NIH), researchers at the University of California, San Francisco, set out to study physiological age-related changes using tissue chips designed and engineered to model human tissue. Because prolonged spaceflight can have many of the same effects as aging, the microgravity environment is a uniquely valuable platform for this research. Investigations such as this will not only lead to more effective treatments for age-related diseases but could also shed light on how we may be able to reverse the effects of aging itself someday.

A third featured article in this issue highlights another project that has wide-reaching benefits for humanity. A team from the University of Wisconsin-Madison utilized the ISS National Lab to investigate how cotton grows in microgravity. Targeting Improved Cotton through Orbital Cultivation (TIC-TOC) was funded by the Target Corporation and aims to determine how we can produce cotton more sustainably. Findings from this study could enable us to develop more resilient crops on Earth and one day grow plants during long-term space missions.

It is my pleasure to introduce this latest issue of Upward and the fascinating work being done onboard the space station. As we set our sights on the future, NSF eagerly anticipates its ongoing collaboration with the ISS National Lab and is committed to supporting essential research that promises to enhance the lives of people around the world.

Unlocking the Secrets of the Immune System:

How Tissue Chips in Space Could Hold the Key

BY AMY THOMPSON, *Staff Writer*

They say you're only as old as you feel, but in reality, you're only as old as your immune cells. Even though the calendar reminds us that we're another year older, not all people's immune systems age at the same rate. Aging is associated with a decline in immune response, leaving a person more susceptible to disease.

However, immune system dysfunction isn't just an issue for older adults. It's also something that affects people with chronic illnesses, such as cytomegalovirus (CMV). Like its relative, herpes zoster (the virus that causes chickenpox), CMV is incredibly common. However, unlike the chickenpox virus, most people don't realize they have CMV because their immune system keeps it in check. According to the Centers for Disease Control and Prevention, one in every three children has come in contact with CMV by age five, and more than half the adult population has been infected with it at some point in their lives. The virus, like its relatives, never goes away but stays dormant in the body, waiting to reactivate, and most people who are infected never know it because they don't have symptoms.

That's due to a healthy immune system, which is the first line of defense against invaders like bacteria and viruses. This protective system is made up of an army of cells that stop potential sickness in its tracks through a nifty trick called immunological memory. Your immune system remembers diseases and pathogens it battled in the past and helps fight subsequent infections through antibodies, which are generated by a type of white blood cell called lymphocytes. These cells come in two varieties, each with its own task: B-cells, which make antibodies that your body uses to fight off disease, and T-cells that target and kill infected cells. Like generals in an army, T-cells help lead the immune system's fight against disease. But for people with a latent viral infection like CMV, the army is always in attack mode.

"When we have a viral infection where the virus stays in the body for a long period of time, the immune system is constantly activated," said Sonja Schrepfer, a professor of surgery from the University of California, San Francisco. "This eventually exhausts the immune system, leading to what we call an 'aged immune system.'" Age-related immune system decline, known as immunosenescence, is often the driving force behind the increased risk of severe outcomes from diseases like COVID-19. It also makes it harder for the body to heal wounds.

To better understand the relationship between immune aging and how the body heals itself, a team of researchers led by Schrepfer leveraged the International Space Station (ISS)

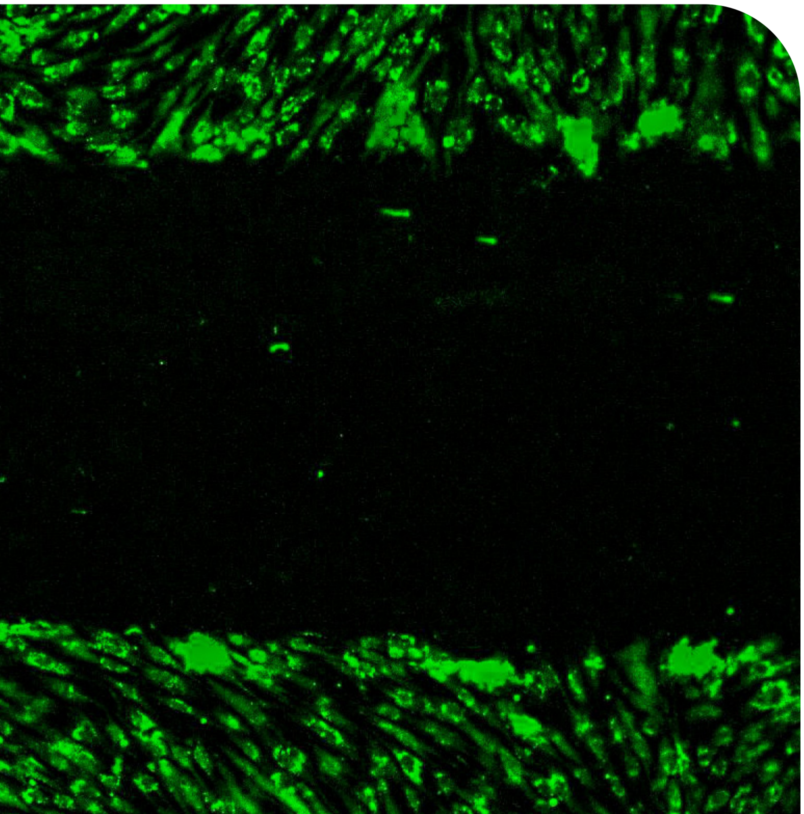
National Laboratory to study the human immune system in microgravity. Using tissue chips, which are small devices engineered to model the function of human tissue, the team studied how key immune cells behave in microgravity and how that behavior affects immune cell aging.

"By sending immune cells into space, we were able to simulate the aging process of the immune system and better understand how it affects our body's ability to repair itself as we grow older," Schrepfer said. "We were able to use space conditions to imitate real health problems on Earth, and without a research platform like the ISS, our understanding of the human body, especially in the area of the immune system, would be limited."

Turning to Space to Understand Immune Aging

Schrepfer became interested in the immune system during her extensive career as a surgeon specializing in organ transplants. "If a patient has an aged immune system, that can have a negative impact on the overall outcome of many different surgeries and procedures," she said. "For instance, if you have an aged immune system in patients with bone fractures, they would heal more slowly or incompletely."

This is what led Schrepfer to begin studying the immune system. But the idea to take her studies to space was inspired by another researcher at UCSF. Millie Hughes-Fulford was a molecular biologist at UCSF and the first female scientist to fly to space as a NASA payload specialist. In 1991, onboard the Space Shuttle Columbia, she carried out the first mission fully devoted to life sciences. Over the course of the nine-day mission, she worked on more than 18 experiments that provided a treasure trove of medical data.



Hughes-Fulford returned to Earth with a new passion for studying what happens to the human body in space, particularly how microgravity affects the immune system. Following her flight, she returned to UCSF, where she opened her research laboratory focused on studying immunosuppression in space.

Millie Hughes-Fulford speaking with Thomas Lang in her San Francisco VA Medical Center laboratory in 2016.

UCSF

Over the next two decades, Hughes-Fulford carried out multiple spaceflight experiments. In 2011, she conducted her first ISS National Lab-sponsored investigation and launched a second in 2015. These experiments focused on T-cell function and compared the natural reduction in immune function that occurs over time in the elderly with the immune suppression in astronauts during spaceflight. Her research revealed that microgravity inhibits the activation of T-cells, which was an important clue to understanding immune system dysfunction in healthy astronauts. These findings piqued Schrepfer's interest, and she decided to send her own immune function experiments to the orbiting laboratory.

Sending Tissue Chips to Space

Research has shown that the same types of immune changes in patients with aged immune systems on Earth are seen in healthy astronauts during spaceflight, only on a much faster time scale. This makes the conditions on the space station ideal for developing an accelerated model of immune aging.

A wound healing assay demonstrates how mesenchymal stromal cells (green) are negatively affected by immune cells in space and are slower to migrate into the black area, which simulates a wound.

Grigol Tediashvili of the TSI Laboratory at UCSF

Schrepfer and her team took advantage of this unique research platform to conduct a space-based tissue chip investigation funded by the National Institutes of Health's National Center for Advancing Translational Sciences (NCATS). BioServe Technologies, a Colorado-based organization that focuses on supporting space life science research, provided the tissue chip hardware and support for the project.

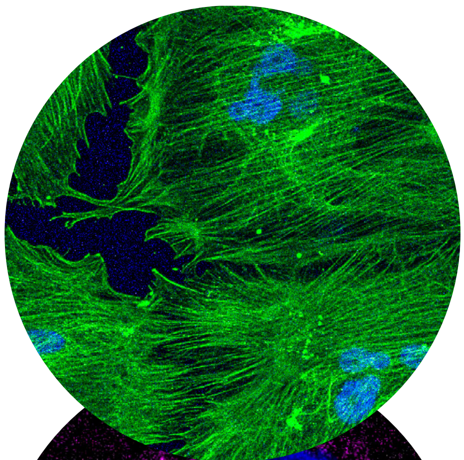


Image from the investigation of healthy mesenchymal stromal cells, which are essential for bone, wound, and cartilage repair.

Dr. Dong Wang, Transplant and Stem Cell Immunobiology (TSI) Laboratory at UCSF.

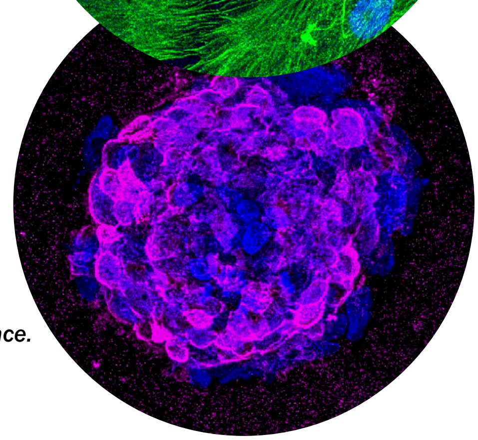


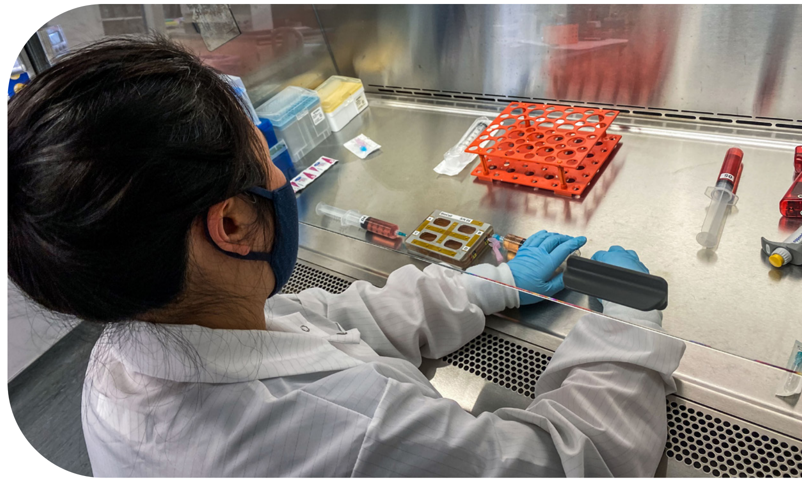
Image from the experiment showing a T-cell with impaired wound healing due to immunosenescence.

Prof. Sonja Schrepfer, USCF

By using tissue chips, Schrepfer and her team could study immune system aging in a model that mimics human tissue function much better than rodent models. "You can compare human and rodent data on a really high level, but you can't go into more detail because the two immune systems are so different," Schrepfer said. "The tissue chips really give us better, more accurate data."

For the investigation, the team used tissue chips to grow three-dimensional cultures of three types of cells. These included mesenchymal stromal cells (MSCs), multipotent stem cells found in bone marrow that help make and repair a variety of skeletal tissues; endothelial progenitor cells (EPCs), stem cells that develop into vascular cells; and the immune system's T-cells. The cells used in this investigation came from three donors—one with a healthy immune system and two with aged immune systems.

With this tissue chip model, the team was able to examine how well the immune cells in space worked to trigger stem cells to multiply and migrate for tissue repair. However, the use of the three cell types made the investigation complex.

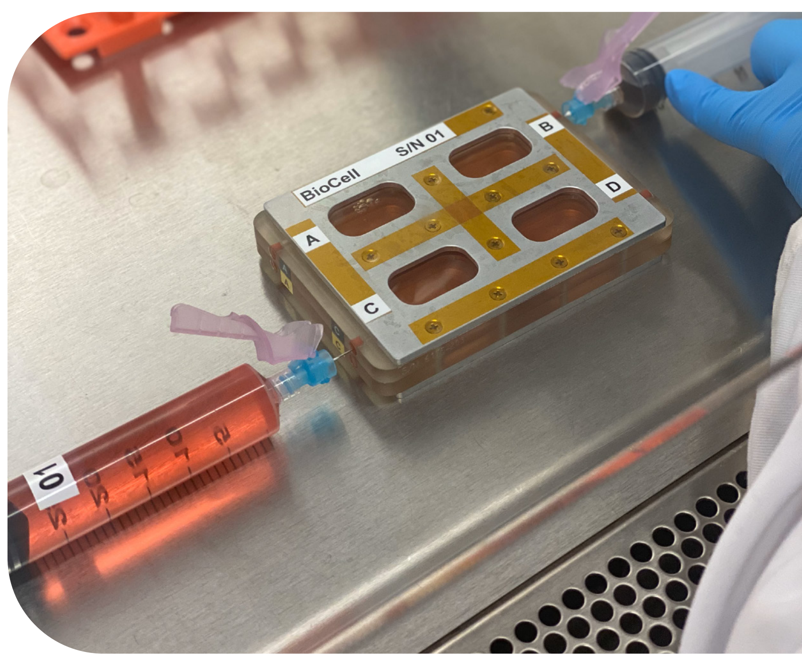


Xiaomeng Hu conducts an Experiment Verification Test (EVT) in preparation for the Microgravity as a Model for Immunological Senescense and its Impact on Tissue Stem Cells and Regeneration (Immunosenescence) investigation.

Prof. Sonja Schrepfer, USCF

That's because MSCs and EPCs are adherent cells, meaning they stick to surfaces like blood vessels in the body, while immune cells are floating cells. To accommodate both adherent and floating cells, the team needed specific tissue chip hardware, and BioServe was able to provide it.

"As an organization, we help researchers choose the hardware that is best suited for their experiments," said Stefanie Countryman, director of BioServe. "For this investigation, we needed to make sure the astronauts doing the experiment could change the media without sucking out all the immune cells floating in it."



Tissue chips for the immune cell aging investigation are prepared for launch to the space station.

Prof. Sonja Schrepfer, USCF

Grigol Tediashvili conducts an Experiment Verification Test for the immunosenescence investigation, which studied the effects of microgravity on cells involved in tissue regeneration.

USCF

"We've seen evidence of decreased wound healing in space, which means the immune system is not functioning like it should be," Lindgren said. "The research that we do on station correlates to disease processes in the human body on Earth, and to be able to work on an experiment that has a direct benefit for people on Earth is really exciting."

Understanding Spaceflight-Induced Changes

Results from the team's first spaceflight experiment confirmed that microgravity induces a decline in immune function similar to that caused by the aging process on Earth but on a much quicker time scale. "In microgravity, we learned that we could imitate the aging of immune cells, and our data from the first flight showed that it happens as fast as within three days," Schrepfer said.

During that initial spaceflight, the team also learned that stem cells thrive in space, but once they come in contact

Schrepfer said the tissue chip design was critical to the investigation because it allowed the cells to be fed and sustained for long-duration culture in space. The design also enabled the return of live tissue samples to Earth for further culture, providing the opportunity to observe immune cell function in space and the cells' process of reacclimating to gravity back on the ground. This allowed the research team not only to study the process of immune aging in space but also to investigate whether there may be a way to reverse it.

Under a Doctor's Care

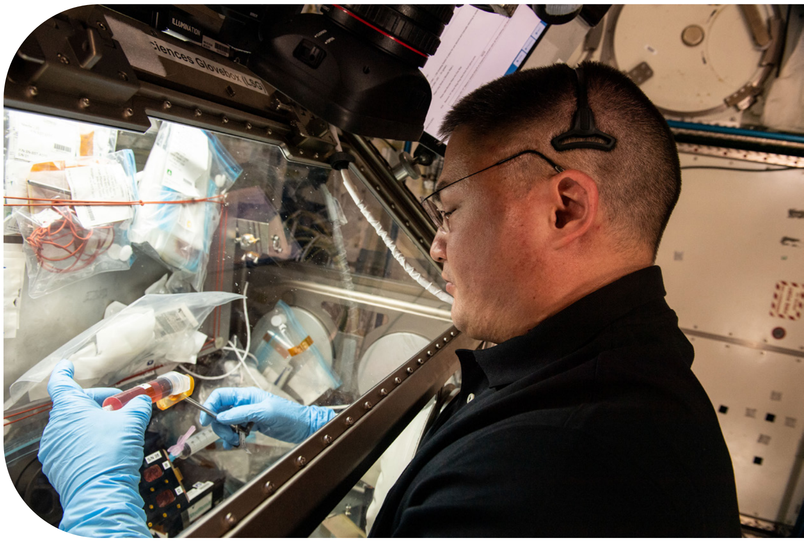
The investigation had two parts. The first, which launched on SpaceX's 16th Commercial Resupply Services (CRS) mission, aimed to understand how long it takes immune cells to age in space by studying them over the course of two weeks. And the second, which flew on SpaceX CRS-25, sought to determine whether the aging process could be reversed once the cells were back on the ground.

For Schrepfer's second spaceflight experiment, the tissue chips remained on station for about a month, where they were cared for by NASA astronaut Kjell Lindgren, who is also a medical doctor. Lindgren and other crew members would feed the cells in the tissue chips by changing the nutrient media and would photograph the chips so the research team could track how the experiment was progressing.

Years before becoming an astronaut, Lindgren attended Colorado State University, where he studied the effects of weightlessness on the cardiovascular system. His interest in spaceflight and how it impacts the human body motivated him to pursue a career not just in medicine but, more specifically, in aerospace medicine. So, when Lindgren learned that he would get to work on Schrepfer's immune cell aging experiment while in space, he was thrilled.

NASA astronaut Kjell Lindgren processes samples inside the Life Science Glovebox for the Immunosenescence space biology study.

NASA



with immune cells, that all changes. “The immune cells have a drastic effect on stem cells,” Schrepfer said. “They are not like normal stem cells we see on Earth; they’re not even functional at all.”

To probe deeper into this finding, the team designed the second spaceflight experiment to better understand what happens to stem cells in space. Results revealed that microgravity significantly increases the growth of TEMRA cells, which are terminally differentiated T-cells that help eliminate infected cells. TEMRA cells produce proteins called cytokines that kickstart the immune system and help the body regulate inflammation.

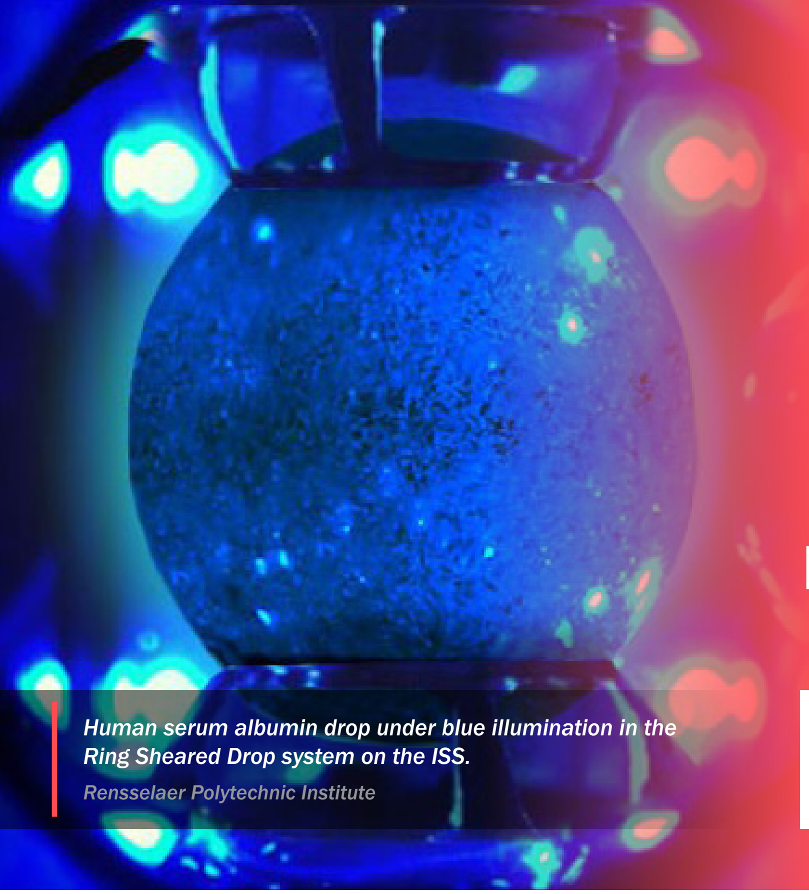
When the immune system functions normally, cytokines trigger an inflammatory response that helps fight disease and repair tissue. But in microgravity, the team observed increased growth of TEMRA cells resulting in an excessive number of cytokines. And Schrepfer’s team observed that this excessive cytokine activity has a negative impact on stem cell function, which can, in turn, hinder the body’s ability to heal wounds and get rid of invaders like viruses.

Schrepfer and her team also found that immune dysfunction in the spaceflight cells remained once the cells were back on Earth, which unfortunately meant they could not identify a way to reverse immune aging. However, results provided new insights into the mechanisms behind immune system function, which could lead to the discovery of novel targets to develop therapeutics to treat immune aging.

“We’re currently looking for ways to eliminate senescent immune cells, much like the way we use immune cells as cancer therapy—we want to teach the immune cells to look at senescent cells like they would look at tumors and help eliminate them,” Schrepfer said. “Then, we could replace the exhausted immune cells with healthy ones, restoring immune function in patients.”

Such a therapy would allow the body to better fight off disease. It could also help patients respond better to vaccines and help prevent the resurgence of latent viruses, all of which are controlled by the immune system. Although nothing can prevent aging, these types of therapies could help improve the quality of life for an ever-growing elderly population and patients with immune system dysfunction.

“Unfortunately, we learned that we cannot reverse immune cell aging, but we may be able to treat it,” Schrepfer said. “That’s something we are looking into and are excited to explore more.”



Human serum albumin drop under blue illumination in the Ring Sheared Drop system on the ISS.

Rensselaer Polytechnic Institute

A Small Drop With a Big Impact:

Fundamental Science in Space to Improve Medicine on Earth

BY AMELIA WILLIAMSON SMITH,
Managing Editor

It's 10 p.m., and researchers and students excitedly gather inside a lab at Rensselaer Polytechnic Institute (RPI) in New York. All are focused on the computer screen, looking with fascination as a colorful one-inch sphere of protein solution is deployed into the Ring Sheared Drop system on the International Space Station (ISS). The liquid sphere, pinned between two rings, looks like a beautiful spinning marble and is mesmerizing to watch. Even more intriguing is that studying this drop of protein solution in space could be key to improving pharmaceutical manufacturing on Earth.

The first protein-based therapeutic, insulin, was approved in 1982. Since then, this new class of drugs has become critical in treating and preventing diseases that range from cancer to HIV. There are hundreds of protein therapeutics on the market, with many more in clinical trials. In 2020, the global protein therapeutics market was valued at more than $280 billion and is estimated to reach more than $565 billion by 2030, according to Allied Market Research.

However, there is an Achilles' heel in protein therapeutic manufacturing, explained Amir Hirsa, professor of mechanical, aerospace and nuclear engineering at RPI. During manufacturing, proteins can aggregate (clump together), negatively affecting drug quality and reducing the amount of viable medication or vaccine produced.

If scientists better understood the complex motion of proteins in solution and what leads to aggregation, they could look for ways to avoid or reverse it, which would be invaluable to the field of medicine. An added complexity to ground-based studies is that protein solutions must be put into a beaker or other container, and the container walls alter how the proteins behave. This makes observing various aspects of protein flow and behavior challenging.

To get past this obstacle, a team of researchers from Arizona State University and RPI turned to the ISS National Laboratory®. In an investigation funded by the U.S. National Science Foundation, the team leveraged microgravity to do something that cannot be done in ground-based labs—study a drop of liquid protein without a container. In space, liquids don't fall. Instead, they form into self-contained, floating spheres, making containers unnecessary.

"In microgravity, the experiment's uniqueness is the fact that it's essentially containerless, so the interaction of protein with solid walls is not a dominant effect as it would be on Earth," Hirsa said. "Having a containerless biochemical reactor at the 10-milliliter scale is the selling point. Without microgravity, this wouldn't be possible."

RPI researchers Amir Hirsa (right) and Joe Adam (left).

Rensselaer Polytechnic Institute

The Problem With Proteins

Proteins are large, complex molecules made of long chains of amino acids, and a protein's structure (how it folds) determines its function. But proteins are flexible and can change how they fold depending on their environment, complicating pharmaceutical manufacturing. For example, some proteins change structure when they come into contact with air at the surface of a liquid. This is because some sections of proteins are attracted to water, while others are repelled by water, explained Juan Lopez, a professor at Arizona State University.

"When a protein is in solution completely, the part that doesn't like water gets folded in and protected by the parts that do like water," Lopez said. "Then, when the protein comes to the surface, the part that doesn't like being in water can pop out, and once it does that, it can start linking up with other proteins."

As the proteins link together, they form a film on the surface of the fluid that behaves differently than the protein solution below. As the film interacts with the rest of the fluid, it changes the behavior of the whole solution, which is problematic for therapeutics. During pharmaceutical manufacturing, protein solutions are exposed to air at several points, so understanding how proteins react when they meet air is crucial.

Another environmental factor that can change a protein's structure is stress from shear, which arises from the force produced when fluids slide past each other. During pharmaceutical manufacturing, protein solutions are mixed in bioreactors that contain hundreds or thousands of liters of solution. The mixing process induces shear in the liquid, and manufacturers must be careful not to mix the solution too quickly or too hard because the stress can alter protein structure, Hirsa explained.

Shear stress can also affect flow in protein therapeutics. The reason has to do with the fact that the protein solutions are highly concentrated. It is impractical to put a large amount of fluid into a patient to provide the right dose. So, the protein solution is concentrated into a smaller amount of fluid that can be given as an injection or through an IV. But when there is a high concentration of proteins, it alters the fluid properties of the solution.

Normally, shear stress does not change the viscosity (resistance to flow) of a fluid. Think of stirring a glass of iced tea. The viscosity, or thickness, of the tea remains the same no matter how hard you stir it. This is called "Newtonian" fluid behavior because the fluid follows Newton's law of viscosity. But concentrated protein solutions do not behave this way. In such "non-Newtonian" fluids, shear stress changes the fluid's viscosity, and it thins or thickens, affecting its flow.

ESA astronaut Lt. Samantha Cristoforetti installing the Ring Sheared Drop system into the Microgravity Science Glovebox on the ISS.

NASA

"Ketchup is a great example of a non-Newtonian fluid," Hirsa said. "If you put ketchup on your turkey burger, you want it to stay there and not run off, but you also want the ketchup to come out of the bottle easily when you squeeze it."

When you apply shear stress to the ketchup by squeezing the bottle, the ketchup becomes less viscous and flows out more easily. When the shearing stops, the ketchup becomes more viscous again. That's an example of shear thinning, and some proteins react similarly. But other proteins do the opposite, Hirsa explained.

"Shearing can make the protein solution more viscous and turn it into a gel and eventually a solid," he said, "and these complications are headaches in pharmaceutical manufacturing."

To alleviate the headache, scientists need to understand how protein behavior changes at air-liquid interfaces and how the altered proteins interact with other non-Newtonian flow phenomena in the fluid below the surface. With this information, researchers could create robust models to predict protein behavior in specific situations, which would be extremely beneficial to the field of medicine.

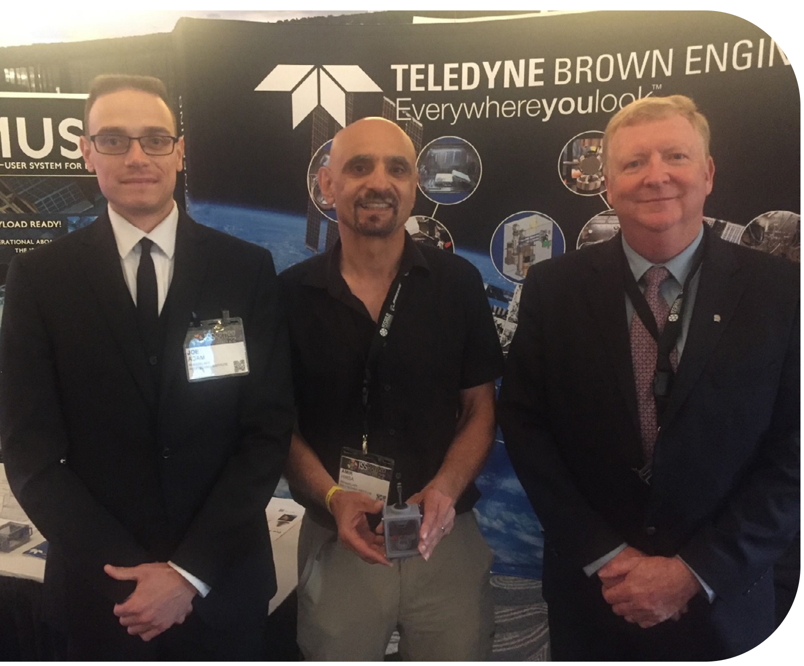
"Trying to understand what's happening on the surface of liquids is becoming more and more relevant to pharmaceutical applications—trying to figure out at what stage non-Newtonian behavior kicks in at the interface and in the bulk fluid, as well as the two-way coupling between the two," Hirsa said. "They're not independent and are touching each other, so their interaction needs to be understood, and we expect our results to contribute to that."

A drop deployment test cell for the Ring Sheared Drop system on the ISS.

NASA

Going Containerless in Space

To study protein solution interactions without effects from solid walls getting in the way, Hirsa and the team launched an investigation to the ISS. Here, they could conduct experiments using the containerless Ring Sheared Drop system designed, developed, and operated by ISS National Lab Implementation Partner Teledyne Brown Engineering in tandem with the RPI research team.



Paul Galloway from Teledyne Brown Engineering (right) with RPI researchers Joe Adam (left) and Amir Hirsa (middle), who is holding a Ring Sheared Drop system test cell, at the 2022 ISS Research and Development Conference.

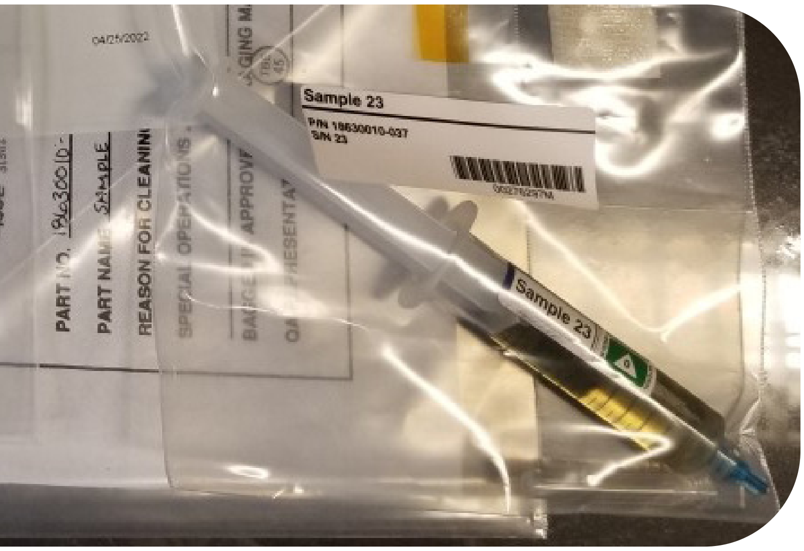
Paul Galloway

The system consists of a small apparatus that houses a test cell containing an injection tube and two rings. Syringes of protein solution are installed into the system, and drops are deployed through the injection tube that extends into the center of one of the rings. As the self-contained drop grows in diameter to a size of one inch, it eventually contacts the second ring on the other side and is pinned between the two rings. Then, one ring can be rotated while the other remains stationary to create rotation and shear within the drop. Video and still cameras in the system capture imagery of the drop, and a microscope allows researchers to get a closer look at the drop's interior. The system can also visualize the drops with red, blue, or white LED lighting, enabling the measurement of different fluid properties in the drop.

"The critical part of the Ring Sheared Drop system is the ability to observe these deployed drops in real time and induce shear into the drop by spinning the lower ring," said Paul Galloway, who recently retired from his position as senior systems engineer at Teledyne Brown Engineering. "The shear induces flow within the drop, and we use the cameras to view the flow field."

For this investigation, the team used two proteins: human serum albumin (HSA) and bovine serum albumin (BSA), which make up the majority of protein in the blood of humans and cattle, respectively. The team chose these proteins because they are very accessible and well-studied. Also, HSA and BSA are similar in structure and behavior to many other proteins, explained Joe Adam, a postdoctoral researcher at RPI who was on the research team.

"The idea is to use very approachable proteins like HSA and BSA and determine their overall function and motion methodologies in microgravity and on Earth, and then try to extend findings to other proteins," Adam said. "If you have a model that's working for a very well-known and well-studied protein, you can start to test your theory, hypotheses, and rules governing equations for motion with other proteins and refine these methods for whatever application you're looking for."



Protein solution sample for the investigation loaded into a syringe for launch to the ISS.

Teledyne Brown Engineering

Teledyne Brown Engineering sent the research team a syringe kit with approved NASA labels to prepare the experiment. All the researchers had to do was fill the syringes with the HSA and BSA protein solutions—which they had prepared and tested for quality assurance—and freeze them. Then, Teledyne Brown Engineering took care of all the logistics to keep the samples frozen and ensure they arrived at Kennedy Space Center and got through the preparation process for launch, said Galloway, who has performed the systems engineering and system safety function for the Ring Sheared Drop system since it was in the early concept phase.

"The real selling point of the Ring Sheared Drop system is the simple and fast integration process that we can use to get your science onboard the space station," Galloway said. "The research team just provides the flight-approved liquid protein solution along with a plan and timeline to complete their investigation on station. They don't have to build anything at all, and that's the real beauty of Ring Sheared Drop as a system for science users."

Interactions In Orbit

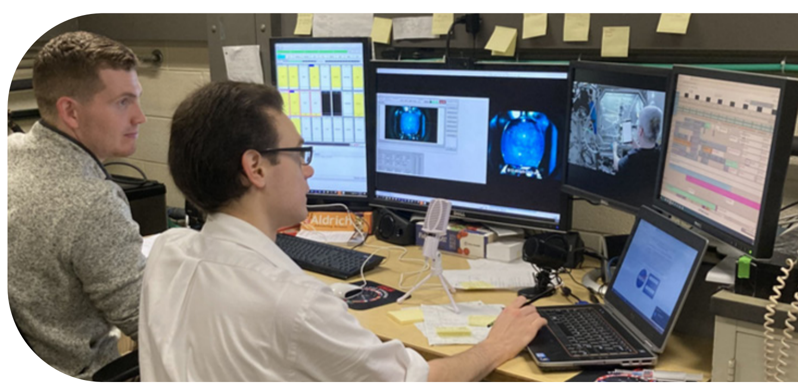
Once the experiment was on station, the team deployed drops of the two proteins at three concentration levels. One was the level naturally found in the body (40 milligrams per milliliter). Another was a highly concentrated solution like that of pharmaceutical applications (10 times the level found in the body). The last was a diluted solution like that used in biotechnology sensing applications (one-tenth the body's level).

The team observed the drops at several time points: during drop formation and pinning, as shearing began, during steady shearing, and as shearing stopped and the drop returned to an equilibrium state. The researchers also examined the fluid's turbidity, or cloudiness, and used small hollow glass tracer particles in the drop to track the velocity of the flow.

"These particles move, and their motion can be detected on a large scale in imagery," Adam said. "That can tell us a lot about the motion of the fluid and its behavior."

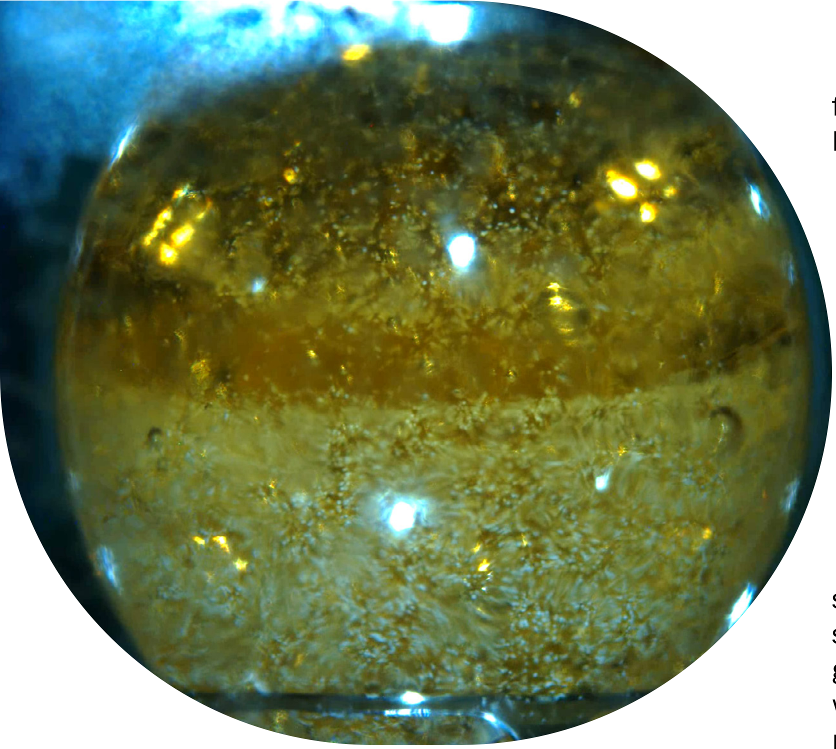
While the experiment was being done on station, the team watched on their computers in real time, with a view of the astronaut performing the test cell installation (and eventual removal) and a view of the drops inside the Ring Sheared Drop system. If a protocol was not working as planned and a change needed to be made, the researchers could alert the astronaut. At one point, the system's motor was malfunctioning, and the team helped determine that a screw had come loose and put together a protocol for the astronaut to repair the system.

"It's not just watching our experiment work. It's being able to step in and say, 'don't turn it that way,' or 'try this if that's not working,'" Hirsa said. "Having the ability to watch in real time what's going on and be involved in designing the protocol to fix the issue is priceless."



RPI postdoctoral researchers Joe Adam (right) and Patrick McMackin (left) commanding the investigation on station from their lab at RPI.

Rensselaer Polytechnic Institute



The Ring Sheared Drop system on the ISS containing a concentrated solution of human serum albumin, the main protein constituent of blood. Small white dots are glass tracer particles that allow researchers to study fluid flow of biofluids in space.

Joe Adam

From Fundamental Science to Application

Now that the experiment on station is complete, the team is analyzing the data collected, and several non-Newtonian flow models are being implemented and tested against the data. "All the heavy lifting on the analysis and modeling will happen in the coming months," Hirsa said. "We're beginning to digest the data and expect to have a publication before the end of the year."

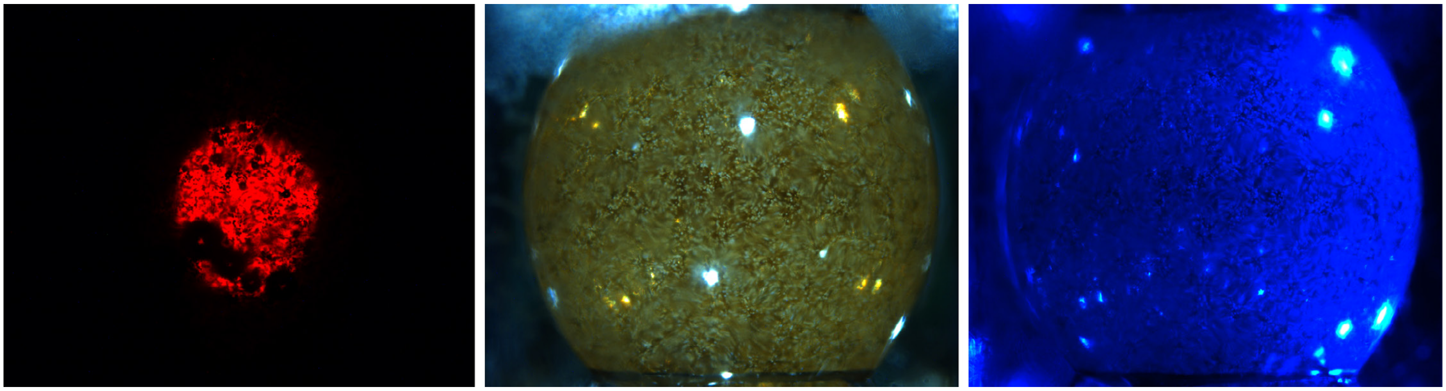
The team hopes to use fundamental knowledge gained from the investigation to improve predictive models and better understand the factors that lead to protein aggregation in highly concentrated solutions. "At the fundamental level, understanding how proteins denature at an interface, their general flow, and mechanics is required for developing applied knowledge," Adam said. "We can then determine, is this reversible? Can you use another fluid mixing process to reverse the state of the proteins and correct them?" Being able to control or reverse protein aggregation in therapeutic manufacturing would be a game-changer in the medical industry.

Thinking back to the lab full of students watching in captivation as the ISS crew worked on the experiment, Hirsa says there is a second aspect to the project's success—the impact it is having on the future science, technology, engineering, and mathematics (STEM) workforce. Throughout the project, the team alerted students when drop deployment was happening on station so they could come to the lab to watch and get involved. The students of today will become the workforce of tomorrow, and engaging students in meaningful, exciting science is key to inspiring them to pursue careers in STEM fields.

NASA-MSFC and Teledyne Brown Engineering staff visit the RPI lab to train students on how to command the Ring Sheared Drop system on station.

Rensselaer Polytechnic Institute

"I can draw top-notch students to my lab because they come and see the excitement as undergrads and stay to get a graduate degree and go on as a postdoc," Hirsa said. "That excitement, the draw of space—especially watching an experiment on the ISS—is fantastic."



View of red, white, and blue LED lighting of a concentrated solution of human serum albumin in the Ring Sheared Drop system on the ISS. Different illumination allows measurement of different properties such as turbidity, drop profile, and particle velocity.

Joe Adam

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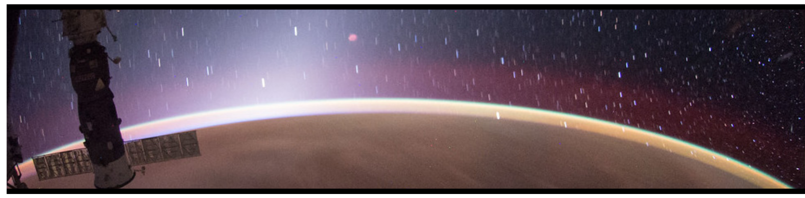

Research in space is happening now on the ISS, but how do we chart this new frontier? Our leaders at the ISS National Laboratory share insights and knowledge as we drive toward a new era of commerce and discovery in low Earth orbit.

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Cultivating the Cosmos:

Decoding Crop Resilience Through Space-Grown Cotton

BY STEPHENIE LIVINGSTON, *Staff Writer*

NASA astronaut Megan McArthur tends to cotton growth chambers during the TIC-TOC experiment.

NASA

Plants don't have bones or muscles or brains, but they're always on the move. Driven by their genetics, some are hardwired to flower in a freeze. Others figuratively hold their breath to survive floods. Still, many of us know others die if you look at them funny.

Like Charles Darwin, botanist Simon Gilroy is obsessed with the mysterious behaviors of plants. Darwin, the "father of evolution," spent decades meticulously observing plant movement, from vines that can circumnavigate almost anything to carnivorous plants that trap unfortunate insects in their leaves. He even watched them sleep, folding their leaves up and down. Winding the clock forward more than 140 years, interest in how plants react to their environment, especially a changing one, is still palpable among scientists who study plants today.

Gilroy, a professor of botany at the University of Wisconsin-Madison (UW), continues Darwin's work 250 miles above Earth's surface on the International Space Station (ISS). He is interested in how plants react to a changing environment at the cellular and genetic levels.

"Plants are these awesome, dominant pieces of biology that solve the same problems humans solve to survive," Gilroy said, his white hair reaching a bright red Hawaiian shirt and matching a white mustache, "but they do it in totally different ways."

The Gilroy Lab research team at the Kennedy Space Center as they hand over the TIC-TOC experiment for launch: (left to right) Sarah Swanson, Simon Gilroy, Richard Barker and Arkadipta Bakshi.

Deb Wells

Gilroy has tested whether plants feel stressed out in microgravity and explored how they might adapt to thrive in space. Most recently, in 2021, his team launched cotton plants to the space station on SpaceX's 22nd Commercial Resupply Services (CRS) mission. The ISS National Laboratory®-sponsored project called Targeting Improved Cotton Through Orbital Cultivation (TIC-TOC) was funded by the Target Corporation to investigate cotton root growth in microgravity.

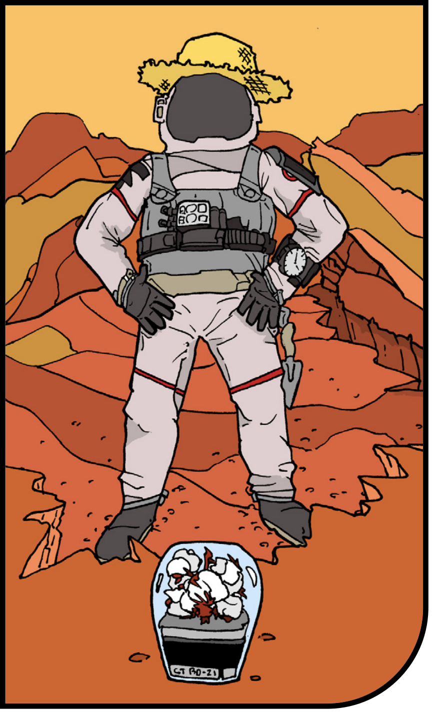


Illustration by Kair Rasmussen

The choice of cotton as the project's focus is far from arbitrary. Cottom is one of the world's most vital agricultural commodities, finding its way into myriad products, from clothing to medical supplies. But like many crops, it's also a thristy, hungry plant that drains resources at a time when we desperately need to conserve them. By removing the masking effects of gravity, Gilroy's team hoped TIC-TOC would reveal genetic clues that could lead to the production of resilient cotton plants that use resources more efficiently on Earth, said Sarah Swanson, directore of the Newcomb Imaging Center at UW and a microscopy and cell biology expert for Gilroy's lab.

"If we can understand a little bit more about how cotton can grow in places that are weird and different and challenging, like the space station, it could help us make crops more sustainable and grow better under enironmental stress," Swanson said.

Roots are how plants take up water, but on the space station, plant roots grow very differently because they lack gravity as a directional cue to grow downward toward the water in the soil. Plus, there's no gravity to pull water downward. For the experiment, Gilroy's team explored how ordinary cotton grew in space compared with cotton genetically modified to thrive under drought conditions.

"We took cotton to a truly alien realm, where things are happening that have never happened to its biology before," Gilroy said.

Defying the team's expectations and contrasting many previous ISS plant experiments, the modified and regular cotton grew better in space than the cotton in a control experiment back on Earth. The modified cotton grew the largest roots and experienced the least stress in spaceflight.

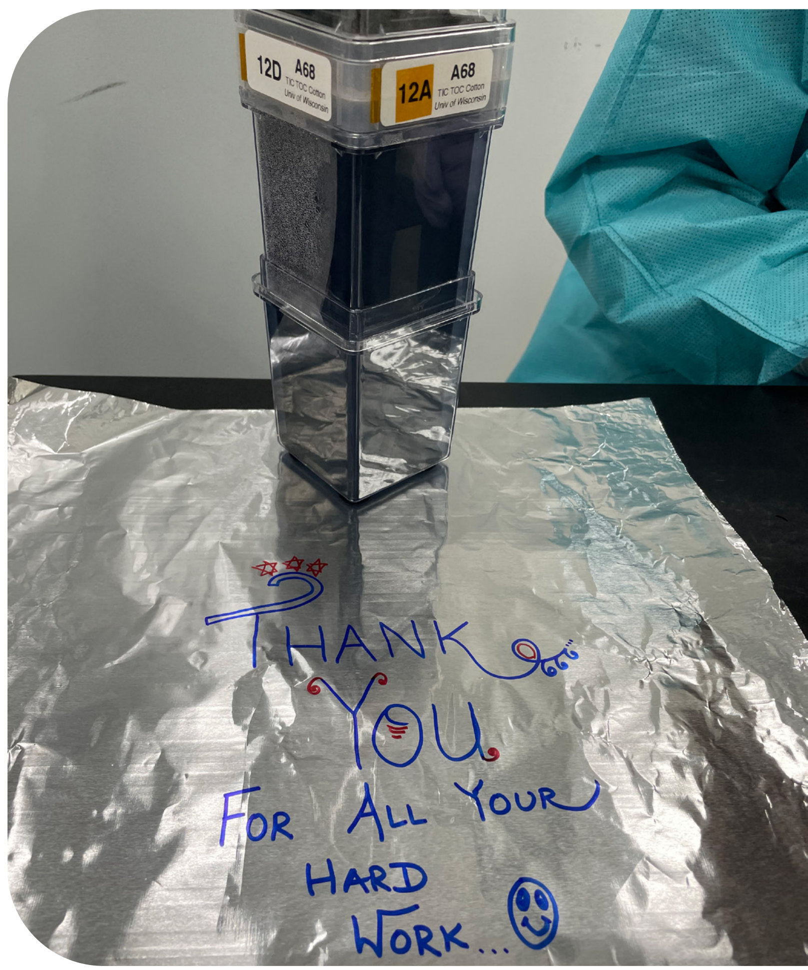
It grew so well that Gilroy says it presents a new challenge: figuring out why.

Sprouting Seeds of Resilience

While simultaneously dealing with pandemic-related delays before launch, the team faced another hurdle: figuring out how to grow such a large plant on the space station. Across the Cotton Belt in the United States, cotton can reach over peoples' heads. How would they grow it on an orbiting outpost with limited space?

"It was obvious very quickly that we needed a larger growth chamber, which didn't exist then," said Gilroy.

The researchers utilized expertise from the ISS National Lab and AECOM to design 12 cotton-custom growth chambers for the Vegetable Production System (Veggie) on the ISS. The clear plastic chambers contained the plants' growth within a 10-inch long and three-inch wide space and included a translucent gel substance in place of soil that enriched the plants as they grew. "We needed to see the roots to monitor their growth," said Swanson.



One of the custom cotton growth chambers sits on a message for the ISS crew written on foil used to pack the experiment.

Gilroy Lab

Once the cotton plants reached the ISS, the crew carefully unpacked the chambers and placed them into Veggie. Picture this: a carefully designed apparatus nurturing cotton plants as they dig deep into their genes to navigate the microgravity environment. The plants are observed by astronauts who document their growth and responses by taking high-resolution images and gathering data on how spaceflight influences the cotton's growth and overall health.

Within just a few days, the cotton plants were already growing roots, albeit strange ones. Instead of gravity forcing the roots to grow downward, they spiraled longitudinally in search of nutrients—bunching like spaghetti in a bowl. These root systems developed even bigger than the control experiment back on Earth in the Veggie hardware inside NASA's ISS Environmental Simulator at Kennedy Space Center, which mimics factors like temperature, carbon dioxide, and oxygen levels found on the ISS.

Plants, like all life on Earth, evolved to thrive under gravity. Without it, those grown on the space station are often slow-growing and appear stressed. "I expected our plants in space would look pretty unhappy," said Gilroy. But that's not what happened.

Swanson remembers when NASA astronaut Shane Kimbrough first pulled a cotton chamber from Veggie and removed the lid after the six-day experiment.

"It was beautiful," she said. "The leaves came out of the top, and the growth was amazing. We couldn't have been happier."

According to Gilroy, it was one of the first times that engineered plants grew better in space than regular plants. Images and data collected during the experiment and frozen cotton samples made their way home in a Cargo Dragon capsule.

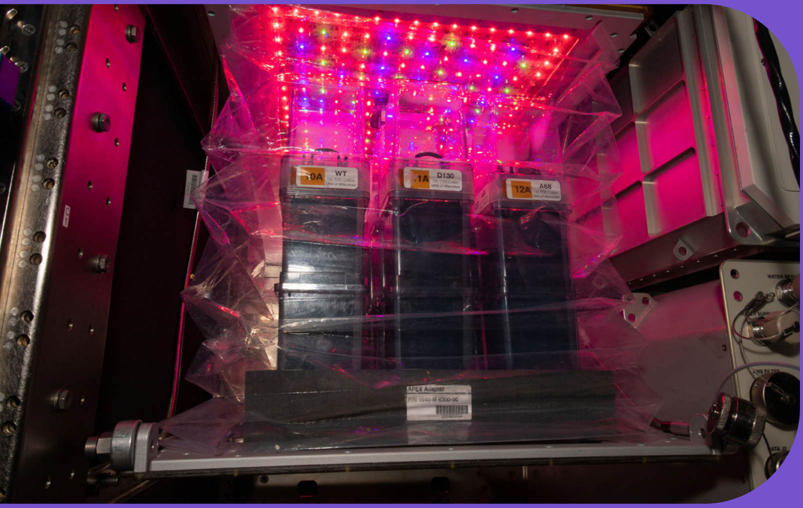
When the team began analysis on the ground, the puzzle deepened.

A Puzzling Outcome

Space-grown cotton may have big roots and healthy leaves because of something intrinsic about cotton that makes it respond well to the stresses of spaceflight. "Or, perhaps, it's something about how we grew it," Gilroy said.

The search for an answer is revealing genetic insights that science can harness to transfer these effects to plants on Earth and improve agriculture. If the research team finds that reduced gravity in space triggered enhanced growth, Gilroy said that would provide molecular targets to genetically engineer equivalent outcomes into crops on the ground and future plants grown in space.

As the human population exploded in the 20th century, midcentury agronomist Norman Borlaug tinkered with plant genetics using mutations to create modified varieties of crop plants that grow bigger and feed more people.



Cotton growth chambers are shown inside Veggie, a plant-growth facility on the space station.
NASA

NASA astronaut Shane Kimbrough shows off bright green cotton leaves during the experiment.
NASA

Nowadays, the need to feed billions of people is paired with harsh and increasingly devastating setbacks due to the climate crisis. In fact, the researchers in Gilroy's lab simultaneously experience extreme weather at home as they seek solutions. "This summer was a challenge," said Swanson, who lives in Wisconsin, which experienced one of the worst droughts in its history.

But one crop that didn't wilt under the pressure was Swanson's backyard onions. One weighing in at three pounds won her a first-place ribbon at the Wisconsin State Fair in August. "The judge used it as a barbell," she laughed. Whether you're picking a type of onion to grow for a state fair, a crop to feed the planet, or plants for astronauts' salads, she said, you want to pick the ones with a genetic code designed—naturally or through genetic modification—for what you need, such as drought resistance and size.

Scientists today have powerful genetic editing tools that allow them to tweak a plant's natural genes with surgical precision, opening doors to improve methods for designing safer genetically modified organisms (GMOs). While GMOs have a bad reputation among some people, Gilroy says they are not harmful when backed by solid science. "You need to do your homework to ensure it's safe before it's released into the environment," he explains. Turns out, the potential for homework is vast.

"There's a tremendous number of genetic traits that we can tweak now that are going to play out as important components of agriculture," he said.

One of these genes is AVP1, which produces an enzyme important to plant growth and stress response. Plants expressing AVP1 also have larger shoots and roots in normal conditions compared to those without it, while plants genetically modified to over-express the gene have shown increased plant growth under extreme stress, like drought. On Earth, cotton genetically modified to over-express AVP1 shows drought resistance and a 20 percent increase in fiber yield—the stuff we use to make clothes.

In a lab at Kennedy Space Center, Simon Gilroy holds one of several cotton growth chambers used for the team's experiment.

NASA

When the gene is expressed at higher levels, it triggers the plant's roots to reach deeper into the ground to find water and nutrients. However, researchers are still trying to understand precisely why AVP1 triggers this response.

"We want to know exactly how this enzyme makes the cotton more resilient because that knowledge could help us improve its use as a countermeasure to plant stress," said Swanson.

Peculiar Findings That Defy Gravity

Gravity directs roots and affects their growth, like an anchoring force in soil. By removing gravity's influences on plant roots, TIC-TOC sought to study how AVP1 alone affects root systems and the plant's resilience under stress. Using software to analyze images taken by astronauts in orbit, the research team conducted a quantitative analysis that showed that the AVP1 over-expressed cotton grew larger in space than the regular cotton. The space-grown genetically modified cotton also had more than four times as many roots as those grown on Earth. Genetic analysis is ongoing to determine why the cotton plants responded so well to space conditions. The team thinks the answer may relate to AVP1 influencing the cotton to regulate pH levels that help plants stay healthy.

Patch design by Sarah Friedrich

Genetic analysis of the unmodified cotton samples from space indicates these plants were more stressed out than their genetically modified counterparts. Ordinary cotton grown in space experienced increased protein degradation and showed various biochemical markers that signal stress not found in the genetically modified cotton.

Swanson opened a photo on her desktop of a green cotton field with a reddish-brown spot in the middle of it the size of a swimming pool. “What do you think it is? Strange, right? It’s the scene of a lightning strike,” she said, explaining that the unmodified cotton grown in space showed similar signs of increased pigment under stress. In contrast, the genetically modified cotton did not.

The genetically modified cotton grown in space also showed other changes in genes related to processes not observed before in regular cotton and generally not reported as spaceflight-related responses. Further investigation may show that these changes are related to the plants’ resilience. But, overall, genetic analysis of the space-grown cotton found plenty of responses typical of plants grown in space, especially the plant Arabidopsis thaliana, a relative of cabbage commonly used in plant biology as a model organism.

“That is a big plus for us, as it says that even though cotton is only distantly related to these other plants that have been grown on the space station, they are all showing similar responses,” Gilroy said. “That means the results all look to be pretty robust indicators of what plants can do in space.”

According to Gilroy, the findings show that AVP1 is a vital gene to explore as a countermeasure to the stresses of spaceflight. It’s a promising new tool for growing plants during long-term space missions to keep astronauts healthy and for growing food on a nearby planet one day.

Because both regular and modified cotton grew better in space than back on Earth, Gilroy says this must mean the growth effect is also directly related to the space environment and how they grew it. It’s possible, he said, that once the cotton-customized hardware mitigated the stressful effects of spaceflight, other features of microgravity may have encouraged the plants’ growth. Or maybe it’s something to do with cotton itself.

By decoding why cotton grows better in space than on Earth, Gilroy’s team may be able to write a new chapter in farming, one that enhances our crops on the ground and in space.

At this point, though, the story is unfinished. “What is magic about cotton that isn’t seen in other plants grown in space?” asked Gilroy. “At the moment, we don’t know.”

It’s a mystery beyond Darwin’s wildest dreams.

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