UPWARD

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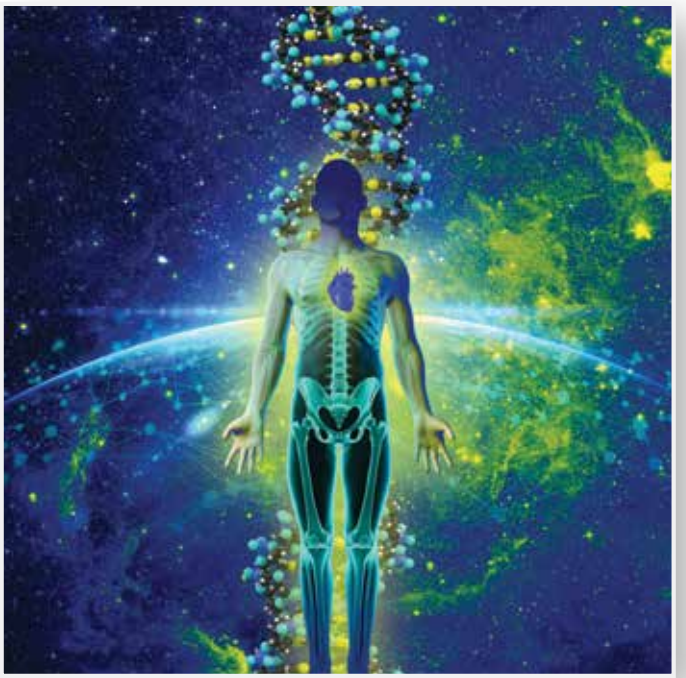
SPECIAL ISSUE
INDUSTRIALIZED BIOMEDICINE

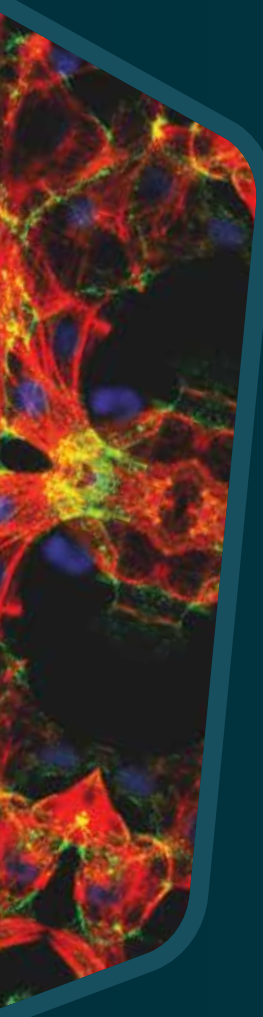


VOLUME 4
ISSUE 2

A COLLECTION OF PREVIOUSLY PUBLISHED ARTICLES DETAILING RESEARCH SUCCESSES

ISS U.S. NATIONAL LABORATORY

CONTENTS



INDUSTRIALIZED BIOMEDICINE PROGRAM:
Projects enabling biomedical advancements with a defined pathway for translation from scientific research to industrial or clinical applications, such as new therapeutics, medical procedures, or diagnostic devices.

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THE VIEW FROM THE CUPOLA

BY LUCIE LOW, *National Center for Advancing Translational Sciences*

Lucie Low is a Scientific Program Manager at the National Center for Advancing Translational Sciences.

The opportunity to do biomedical research on the International Space Station (ISS) is so far outside the realm of possibility for most researchers in the life sciences that it doesn't even appear on their radar. The fact that there are actually biomedical research capabilities on the space station—and part of a U.S. National Laboratory at that—is extremely exciting! And, increasingly, the availability of the extreme and unique environment on the ISS National Lab offers researchers remarkable opportunities to ask scientific questions that are difficult or impossible to answer on Earth. The resulting outcomes could potentially have transformative impact on how we understand molecules, cells, and whole organisms both in space and on Earth.

This special issue of *Upward* will revisit some of the most groundbreaking life sciences research that has been done on the ISS National Lab over the past few years, with articles highlighting work focused on how heart and bone cells react to microgravity, how studying crystallization of antibodies could affect drug manufacturing, and how model organisms such as worms and rodents can inform Earth-based research.

While the benefits of space-based research are plentiful, the mechanics of research in microgravity are an ongoing and evolving process, and this kind of research is intrinsically more complicated than terrestrial lab-based work. For example, in the standard laboratory, the Petri dishes won't float away if you neglect to stick them down! From an experimental design aspect, how can your system be designed so it's robust enough to survive sitting on a launch pad for a variable amount of time before being subject to the rigorous physical and gravitational experience of launch and later splashdown upon return? How will you keep your tissues alive and healthy for the duration of the experiment? How will the ISS crew (your lab technicians) perform the needed experimental interventions, or will the system be automated? And what are your contingency plans for the unexpected events that inevitably occur with spaceflight research (such as launch delays, hardware glitches, or technical mishaps) when these events throw a wrench in your carefully planned experiments? When working in an environment where even basic techniques such as pipetting and mixing of reagents are inherently more involved and complex, the detailed planning for every eventuality has to be considered in ways that would never cross your mind ordinarily, and with preparation to anticipate any and every possible thing that could go wrong.

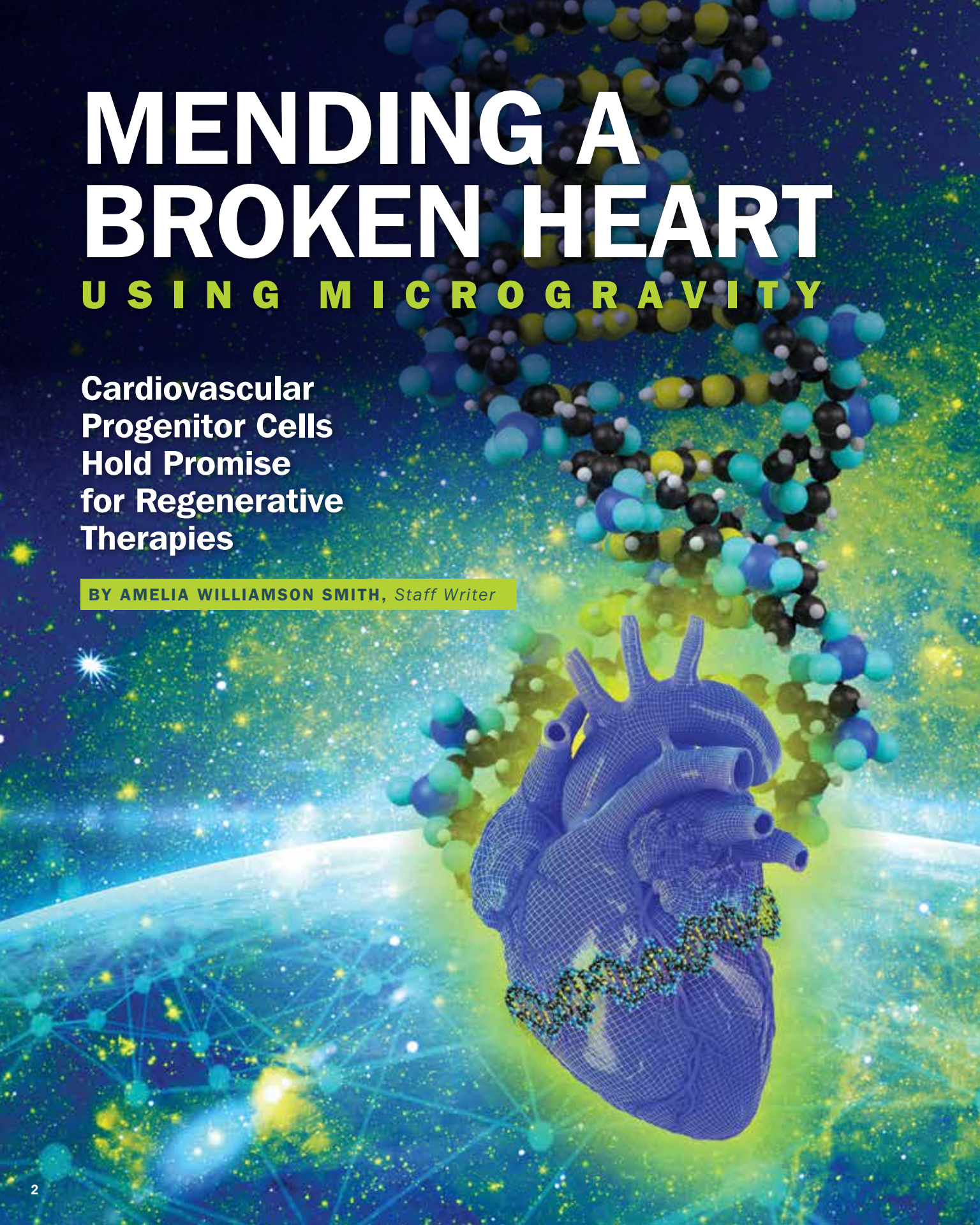
For these reasons, science research in orbit can be exponentially more difficult to plan and conduct than standard laboratory work. Luckily, the ISS National Lab and their Implementation Partners are experts in advising researchers on how to translate their bench-side ideas into fully flight-ready payloads that will yield insight into a wide range of biological aspects. For the National Center for Advancing Translational Sciences (NCATS, at the National Institutes of Health), partnering with the ISS National Lab has made our Tissue Chips in Space program possible. Our researchers have been taking part in an extraordinary endeavor to create "organs-on-chips" that are being launched to space to use the microgravity environment of the ISS to understand health and disease here on Earth. NCATS, and the broader NIH, are excited to partner with both the ISS National Lab and NASA as we continue to explore our biological, technical, and astronomical frontiers. We hope that this issue of *Upward* may inspire you to reach (your biology) for the stars! ■

MENDING A BROKEN HEART

USING MICROGRAVITY

Cardiovascular Progenitor Cells Hold Promise for Regenerative Therapies

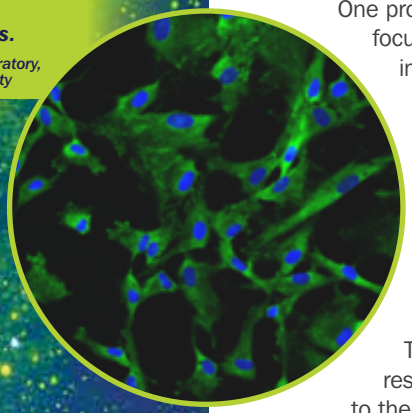
BY AMELIA WILLIAMSON SMITH, *Staff Writer*



The human heart is truly amazing. Each day, this small muscular organ beats approximately 100,000 times and pumps around 2,000 gallons of blood, bringing life-sustaining oxygen and nutrients to all parts of the body.

The heart continuously pumps blood—unless a coronary artery, which supplies blood to the heart, suddenly becomes blocked and the blood flow is severely restricted or stops. Without oxygen, the heart tissue rapidly begins to die. Even after blood flow is restored, the damaged tissue is unable to pump blood as well as healthy tissue. According to the Centers for Disease Control and Prevention, about 735,000 Americans have a heart attack and around 610,000 die from heart disease yearly, accounting for one out of every four deaths in the United States.

But what if there were a therapy that could regenerate heart tissue and help restore cardiac function? The microgravity environment of the ISS National Lab allows scientists to study cells in ways not possible on the ground, and research being conducted on the orbiting laboratory could help lead to the development of cell-based regenerative therapies for people with heart disease back on Earth.



Human neonatal cardiovascular progenitor cells.

Kearns-Jonker Laboratory, Loma Linda University

One promising area of research into cell-based regenerative therapies is focused on human cardiovascular progenitor cells (CPCs). These cells are immature but in a beneficial way. CPCs are at a very early developmental stage along the path to becoming cardiovascular cells. This means they can differentiate into several different types of cells, such as heart muscle cells or the endothelial cells that line blood vessels.

On Earth, scientists are studying how CPCs might play a role in regenerative treatments for heart disease—and as unexpected as it may seem, knowledge gained from studying CPCs in space could accelerate their development as a therapeutic tool.

To examine the effects of spaceflight on CPCs, Loma Linda University researcher Mary Kearns-Jonker and her team sent cultures of CPCs to the ISS National Lab. The team looked at the effects of microgravity on both neonatal CPCs as well as adult CPCs, as the age of the person from whom the cells are derived affects how well the cells function.

"Our goal was to identify the functional effects of the CPCs after they've been flown in the spaceflight environment," Kearns-Jonker said. "We found that microgravity does cause some very distinct changes that are unique to neonatal cells when comparing them to adult cells."

Mary Kearns-Jonker examining CPCs as they are being prepared for launch at Kennedy Space Center.

Stefanie Countryman, BioServe Space Technologies

TAKING CELLS TO SPACE

Previous research using ground-based simulated microgravity—achieved by placing the CPCs in a 2D rotating culture device—yielded several indicators suggesting microgravity may hold promise for adaptation of CPCs for human therapies on Earth, said Jonathan Baio, who worked as a doctoral student in Kearns-Jonker's lab at the time of the team's ISS National Lab investigation.

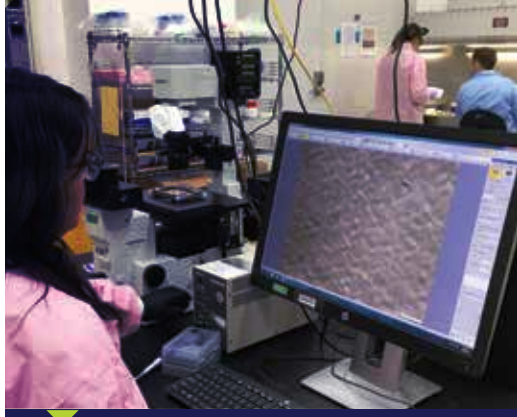
> ***"We looked at both neonatal and adult cells in microgravity to understand the differences that occur between the two and whether this information could ultimately be leveraged for regenerative therapies or to better understand how the heart develops," Baio said.***

The team worked with ISS National Lab commercial service provider BioServe Space Technologies and optimized conditions for the cells to grow in BioServe's BioCell cell culture system. Preflight optimization was challenging because Kearns-Jonker and her team had to find the ideal concentration of cells to put into the BioCell hardware. The team had to make sure the cell population was not too sparse initially but also did not become too crowded as the cells reproduced. Another challenge was to optimize the feeding schedules so that the neonatal and adult CPCs could be fed on the same day at the same time even though each type of cell grows at a slightly different rate.

Last-minute changes to the launch schedule also posed challenges, said Aida Martinez, a medical student at Frank H. Netter MD School of Medicine at Quinnipiac University, who worked as a research assistant in Kearns-Jonker's lab during the team's ISS National Lab investigation. The first launch date was canceled due to bad weather, bumping the launch back by two days.

"This heightened the anticipation and excitement, but we had to go back and rethink part of it too, because our experiment had very specific time points," Martinez said. "The cells had to be under specific conditions for a certain amount of time with little wiggle room, so we had to brainstorm beforehand and in real time how we would deal with a change in the schedule."

In preparing an investigation for launch, preflight validation studies and activities involve contingency planning driven by the ISS National Lab Operations team and the commercial service provider to help prepare researchers for circumstances such as launch slips that could lead to setbacks. This allows the research team to think ahead and develop a detailed mitigation plan to help ensure the success of their investigation.



The Kearns-Jonker team prepared the CPCs live for launch on SpaceX CRS-11 in the laboratory at NASA's Kennedy Space Center.

Stefanie Countryman, BioServe Space Technologies

PREPARING THE CELL SAMPLES

Kearns-Jonker and her team used CPCs from four neonatal patients (ranging in age from birth to 4 weeks) and four adult patients (ages 57 to 72 years), looking both at individual patient samples and samples pooled according to age group. The cells from each patient were clonal populations, which means they were derived from a single cell and expanded, making the cells identical.

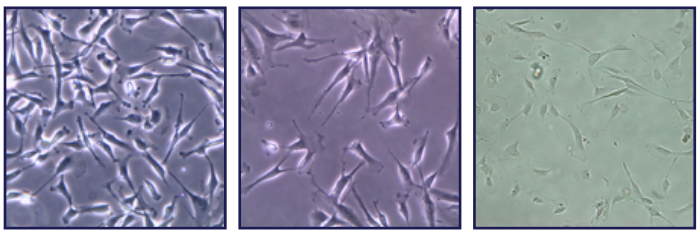
Aida Martinez and Jonathan Baio loading CPCs into the BioCell hardware at NASA's Kennedy Space Center in preparation for launch.

Stefanie Countryman, BioServe Space Technologies

The weather cooperated on the second launch date, and the team's investigation was launched on SpaceX's 11th commercial resupply services mission to the ISS, while the control cells, which were also grown in BioCell hardware, remained on the ground. After 12 days in space, some samples were placed in a fixative that stabilizes RNA, and the rest of the live cells were returned to Earth after 30 days in orbit.

"We took the samples from splashdown, drove them straight to the lab, and were very happy to find that the cells had excellent viability," said Kearns-Jonker. "We didn't let the cells recover in the lab and did all of the functional studies right then to minimize the recovery time."

In the lab, Kearns-Jonker and her team looked at the functional characteristics of the live cells—their ability to divide, move, and communicate via signaling. Such analyses had to be done immediately, before the cells had a chance to re-adapt to Earth's gravity. The team also analyzed the cells' gene expression and microRNA (noncoding RNA molecules that help regulate gene expression) profiles. The team compared results from the cells grown on Earth with those grown in microgravity, while also comparing differences between the neonatal and adult CPCs.



Neonatal CPCs cultured in the BioCell hardware.

Kearns-Jonker Laboratory, Loma Linda University

EXAMINING CHANGES FROM MICROGRAVITY

Kearns-Jonker and her team found that microgravity induced changes in the CPCs when compared with Earth-grown cells. Some changes were seen in both the neonatal and adult CPCs, while others were unique to the neonatal cells alone.

In the spaceflight samples, the team measured several markers of cardiac development. The neonatal CPCs were found to exhibit markers characteristic of a slightly earlier stage of development. This slight de-differentiation is associated with enhanced "stemness"—making the CPCs behave more like stem cells and enhancing their potential to develop into different types of cardiovascular cells. Interestingly, these changes were not found in the adult CPCs.

In the neonatal CPCs, calcium signaling and AKT signaling were both activated in response to spaceflight. This is significant because calcium signaling plays a prominent role in the early stages of heart development, Baio said. "Additionally, AKT is an important molecule in promoting pluripotency and stemness and the ability of a stem cell to continue to divide and expand and retain its stem-like state," he said.

The neonatal CPCs grown in microgravity were also found to have enhanced proliferation, meaning they were able to divide and increase in number more rapidly. In addition, both the neonatal and adult CPCs exposed to microgravity exhibited an enhanced ability to migrate. Migration is important, because once therapeutic cells are injected into the heart, you want them to be able to migrate and move to injured or damaged areas, Baio said.

These spaceflight results are significant because researchers could use this knowledge to recapitulate the effects on the ground in the context of advancing cell-based regenerative therapies.

Aida Martinez, Jonathan Baio, and Mary Kearns-Jonker at NASA's Kennedy Space Center.

Jonathan Baio

"There are multiple examples in the literature where a slight de-differentiation and activation of the specific transcription factors that we see elevated here have been associated with improved outcomes in a cell transplant setting," said Kearns-Jonker.

***A transcription factor** is a molecule that helps regulate gene expression by controlling whether a gene's DNA is transcribed into RNA.*

COMPELLING RESULTS AWARD

Kearns-Jonker was presented with a 2018 ISS Compelling Results Award in Biology and Medicine at the annual ISS Research and Development Conference. The award was given in recognition of her compelling research into microgravity's effects on CPCs and the potential to leverage these results to advance the development of regenerative therapies for cardiac repair on Earth.

Loma Linda University

NASA astronaut Peggy Whitson exchanging the CPC growth media in the BioCell hardware onboard the ISS.
NASA

BRINGING BENEFITS BACK TO EARTH

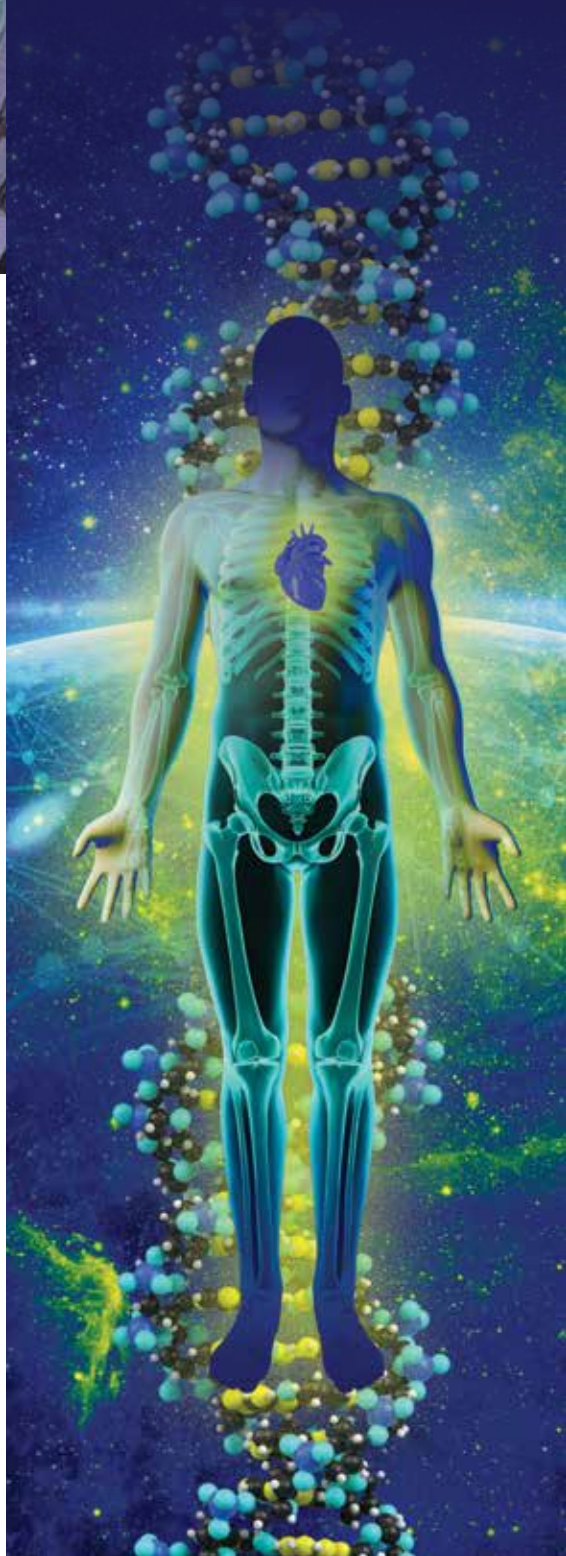
Cells with markers of early stages of development and enhanced stemness could enable a more effective integration of therapeutic cells into heart tissue and improve tissue regeneration after an injury such as a heart attack, Baio said. Thus, the microgravity-induced changes in the neonatal CPCs may be helpful in developing cell-based therapies on Earth that can improve outcomes for patients with heart disease.

The next step would be to explore whether the microgravity-induced changes observed in neonatal CPCs produce beneficial effects *in vivo*, that is, in living organisms, Kearns-Jonker said. "What we don't know is: How do the gene expression and microRNA changes noted after exposure to microgravity translate into effects that we can see *in vivo*? We actually need to test that to see whether or not what appears to be something that could be very good for regeneration is, in fact, beneficial."

It is also important to continue to study CPCs to more fully understand the mechanisms behind the microgravity-induced changes. Although ground-based simulated microgravity is not a perfect model for true microgravity, scientists may be able to use simulated microgravity as a method to adapt CPCs for use in cell-based therapies.

> ***"If one finds that microgravity-induced benefits can be recapitulated in simulated microgravity models on Earth, it's very reasonable to envision pretreating the cells with simulated microgravity for prospective therapeutic applications," Kearns-Jonker said.***

Looking to the future, there is great potential in how microgravity or simulated microgravity could be used to directly affect cardiac repair on Earth, Baio said. "Being able to do space-based research is critical to being able to provide a unique perspective into cellular physiology and what the impacts could be for human health and, potentially, new therapeutics that we would never otherwise consider."



SPOT LIGHT

Going to Space to Advance Regenerative Medicine on the Ground

BY AMELIA WILLIAMSON SMITH, *Staff Writer*

Microgravity has profound effects on the human body and on cells within the body, and insights gained from research conducted onboard the ISS National Lab are helping to advance the field of regenerative medicine back on Earth. In recognition of its leadership in enabling valuable stem cell and regenerative medicine research, the ISS National Lab was presented with a Leadership Award from the Regenerative Medicine Foundation at the 14th annual World Stem Cell Summit in January 2019.

The unique environment of the ISS National Lab allows researchers to study cells in ways not possible on the ground. Microgravity affects the way cells aggregate, allowing them to form into three-dimensional structures that more closely resemble tissues in the human body—providing improved models to study cell behavior and test drugs and accelerating advances in tissue engineering.

Microgravity may also enhance some properties of stem cells, such as their ability to survive, proliferate (increase in number), form 3D aggregates, and differentiate (develop from general-purpose cells into specialized cells)—which could provide significant benefits in advancing personalized medicine and developing stem cell-based regenerative therapies.

ISS National Lab Board of Directors member Dr. Gordana Vunjak-Novakovic (third from right) and Senior Associate Program Scientist Dr. Marc Giulianotti (far right) accept the Leadership Award at the World Stem Cell Summit.

Tissue chips, small chips containing cells grown on an artificial scaffold to model the detailed physical structure of human tissue, have the potential to revolutionize medicine and are now being leveraged on the ISS National Lab to enable improved disease modeling and higher-accuracy testing of potential new drugs.

Soon, scientists may be able to manufacture human organs and tissues on the ISS National Lab for use back on Earth using the 3D BioFabrication Facility, which was developed by Techshot and launched to the ISS on SpaceX's 18th commercial resupply services mission.

ISS National Lab-sponsored requests for proposals have provided grants that support use of the space station to advance stem cell and tissue chip research. In addition, the ISS National Lab has collaborated with the National Center for Advancing Translational Sciences and the National Institute of Biomedical Imaging and Bioengineering—both part of the National Institutes of Health—to support tissue chip research on the ISS National Lab centered on human physiology and disease. The ISS National Lab has also partnered with the National Science Foundation to support transformative tissue engineering research on the ISS.

The final plenary session of the 2019 World Stem Cell Summit focused on "How Space Technology and Microgravity Will Lead to Profound Benefits to Human Health on Earth," and several ISS National Lab investigators spoke about the opportunities they have had to leverage the ISS National Lab for valuable research aimed at advancing stem cell biology and regenerative medicine to benefit life on Earth.

This content was abridged and updated from an article that originally appeared on ISS360 at issnationallab.org/blog/going-to-space-to-advance-regenerative-medicine-on-the-ground.

SPACE WORMS

UNEXPECTED PIONEERS OF DISCOVERY & COMMERCIAL SERVICES

BY EMILY TOMLIN, *Staff Writer*

Three years ago, a two-headed worm returned from the International Space Station (ISS), and in the summer of 2017, the worm achieved internet and popular media fame when researchers from Tufts University published a paper describing the worm and other results from their ISS U.S. National Laboratory experiment.

The story behind this mutant worm, however, is even more rich than its delightfully deformed morphology. The payload that carried the famous worm and 77 of its relatives to the ISS and back inspired researchers, payload specialists, and even FedEx to take the first steps toward new scientific discoveries and processes that now have their own successes to share, all of which was pioneered by these first worms—planarian flatworms, that is—in space.

THE WONDER OF WORMS

Planarian flatworms have been studied since the 1800s as a model for genetics, body patterning, and neuroscience. The flatworms have a "true brain," unlike earthworms and other species in which the nervous system is more spread out and less well-developed.

Because of their brain's bilateral symmetry, their genomes, and other factors, planarian flatworms are more similar to our human ancestors than some of the more derived model systems such as flies or *Caenorhabditis elegans* (nematodes), said Michael Levin, director of the Allen Discovery Center at Tufts. This model system is being used to understand regeneration, stem cell regulation, and behavior. The worms are also popular for drug addiction research because they have most of the same neurotransmitters as humans, so they exhibit symptoms of withdrawal and addiction to the same drugs.

Levin's lab, however, uses the worms to study how physical forces influence body patterning, the process through which an organism ensures that cell types and body structures are properly shaped and placed during development. Planarian flatworms are a unique model for such studies because they are incredibly robust regenerators and yet they are mixaploid—their cells accumulate a plethora of mutations from constant regeneration and non-sexual reproduction.

"There's not even the same number of chromosomes in every cell of a single worm," said Levin. "How can your genome be such a mess and yet you retain this beautiful ability to create a perfect, correctly shaped planarian every time? Through our work over the last 15 years, the worms are telling us that patterning is controlled by very interesting biophysical dynamics."

An amputated flatworm fragment sent to space regenerated into a double-headed worm, which is a rare spontaneous occurrence.

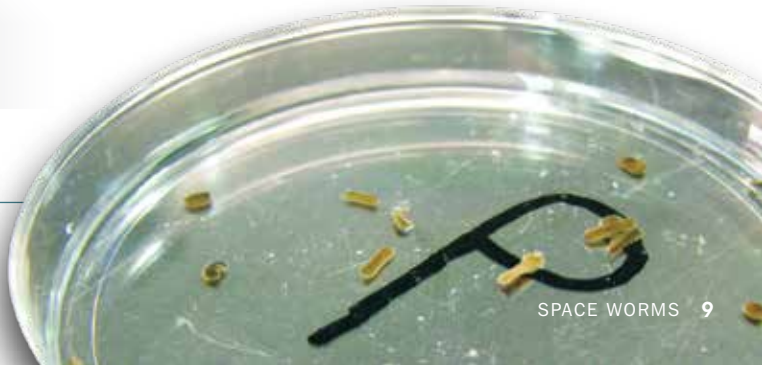
Allen Discovery Center at Tufts University

In other words, because the DNA of planarian flatworms can diverge widely and yet their anatomy remains intact, their cellular decision making is revealing novel aspects of how genetics and physics cooperate to control dynamic anatomy. Levin and his team studies the role of bioelectric signals—electrical communication between individual cells—in determining anatomy. They created the first-ever line of stable patterning mutants in planarians by editing their natural electrical signals, said Levin. In doing so, the team showed that it is possible to re-write electrical pattern memories to control anatomy.

Over the long term, understanding these processes may shed light on how biophysical dynamics are involved in the development, aging, and modification of body plans in higher organisms—and how we might use bioelectric and other signals to control cell behavior. Beyond myriad benefits in discovery science, the power to control cell fate with bioelectric signals could ultimately allow manipulation of regenerative or developmental processes involved in human health. For example, perhaps doctors could use this knowledge to accelerate wound healing, grow organs outside of the body for use in transplants, slow negative effects of aging, prevent certain birth defects, or improve treatments for neurodegenerative diseases.

Michael Levin, director of the Allen Discovery Center at Tufts

Tufts University



Planaria fragments in a Petri dish

Space Tango

WHY SPACE?

A notable player in biophysical dynamics is the geomagnetic field, or GMF. This natural characteristic of the Earth imposes a magnetic field of approximately 0.5 Gauss onto all living organisms. The GMF changes only over very large areas, so it may seem unintuitive that organisms sense and use it in their daily behaviors. However, experiments on plants, animals, and cells in culture show that they do sense and respond to changing magnetic fields, and some classic examples (for example, migrating fish and birds) demonstrate ways in which organisms perceive and use Earth's magnetic field lines. Despite these data, it is unknown *how* and *why* embryonic cells (like the stem cells of a regenerating planarian) sense the GMF.

In laboratory model organisms that are shielded from the GMF by metals and other techniques, "all kinds of things go wrong," Levin said. "Cells are counting on this field for something, and we have no idea what." The unique GMF conditions onboard the ISS are what prompted Levin to seriously consider an unexpected opportunity to send worms to space.

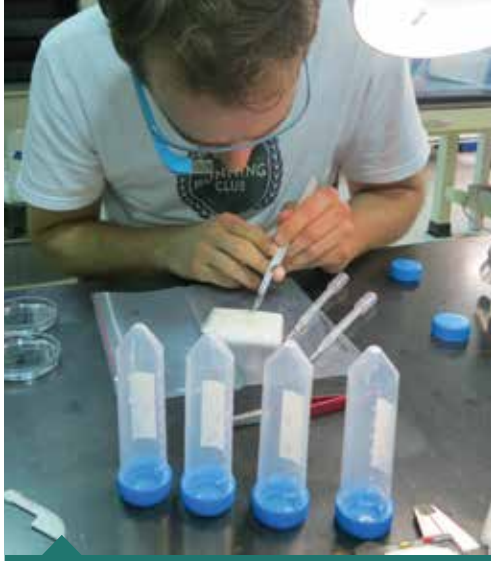
The opportunity was borne of a search for innovation. The nonprofit Kentucky Space, LLC was seeking white papers from new-to-space investigators interested in using microgravity to tackle big research questions—to spark their imagination in the early stages of the nonprofit learning how to enable research onboard the ISS.

"We heard there was this group from Kentucky that was doing some space stuff, and they were interested in sending worms to space," said Junji Morokuma, who joined the Levin lab in late 2004 and subsequently worked on the space worms project. "I thought 'oh wow,' but we weren't sure what to do or what we would find." There was some research on worms in simulated microgravity that had shown a strong increase in regenerative capacity, he said, but there had been no planarian flatworms in space to shed light on what the team should expect.

So the team kept it simple, said Levin, and set out to determine how the combined components of space travel might affect regeneration in the worms. These components included takeoff, acceleration, splashdown, vibration, microgravity, and, of course, the altered geomagnetic field. These physical forces perturb how the cells talk to each other and thus can influence the pattern formation within cells of a regenerating worm. "It's not zero GMF," Levin said, as it is in ground-based shielding experiments, "but it's different, and we didn't have any clue what we might find."

Canister developed by Space Tango to house vials of flatworms for launch to the ISS National Lab.

Space Tango



Space Tango CEO Twyman Clements cutting the flatworms into thirds prior to spaceflight.

Space Tango

BREAKING NEW GROUND

As the first-ever space-faring planarian flatworms unknowingly prepared for their launch onboard commercial resupply services (CRS) vehicle SpaceX CRS-5, Kentucky Space and the Levin lab tackled a variety of their own firsts.

> ***"Space business and research is exciting, but there's lots of money, paperwork, delays, and 'oops' involved," said Morokuma. "I've heard to launch a rocket, you need paperwork taller than the rocket," he laughed, "but luckily Kentucky Space handled all of that."***

Kris Kimel, founder of Kentucky Space and co-founder of its for-profit spinoff Space Tango, acknowledged that early experiments like the space worms, while simple in design, played a critical role in allowing his team to "learn the ropes" with respect to payload integrations, interfacing with NASA, and dealing with customers.

"This experiment was a really important one for us," said Kimel. "We learned a lot technically from the payload as well as how to protect the end user from some challenging processes. This experience laid the philosophical bed for what became TangoLab and our successful business ventures as Space Tango."

Supported by CASIS sponsorship, the worms launched on January 10, 2015, spent several weeks onboard the ISS National Lab, and returned exactly one month later.

Post flight vial of flatworms Space Tango

Prior to return, Kentucky Space also worked with Levin and new partner FedEx Space Solutions to arrange delivery services for the worms back to the lab following splashdown. "The logistics were really interesting," said Levin. "I've never weighed a flatworm. I understand why they needed that data, but it was kind of wild."

David Drees, who managed the flatworm delivery process for FedEx, said his primary concern was returning the worms as rapidly as possible after splashdown so the team could analyze the worms before they readjusted too much to Earth's conditions. Some of this depended on temperature. "We needed to keep them not warm enough for cell division but not cold enough to kill them," said Drees.

The rest was timing. "We have a great infrastructure at FedEx, but we run on schedules," said Drees. "The capsule coming down from the ISS is not on a schedule we can predict months in advance. It could come down any time of day or night, and we'll get maybe three hours' notice." To adjust to this uncertainty, resources from several FedEx operating companies were activated, and a FedEx Express courier was on standby.

Adding to the lists of firsts on the space worms' resume, this was the first payload that FedEx executed as a "rapid return," in which they intercept returning experiments during the hand-off from launch provider to NASA, in this case at an airport on the California coast.

"It was a new thing, so I actually went out there and personally worked with Kentucky Space as they took possession of the box with the worms from the NASA plane," said Drees. "I was a space geek growing up, so it was a really cool thing to be a part of." From there, the box was fitted with a "SenseAware" device that sends information on location, pressure, light, moisture, and temperature to a customer web portal—and then, since it was 11 p.m., it went to a FedEx station for the night.

"Our operations folks really stepped up to handle a nontraditional shipping situation," said Drees. "Everyone was really proud to play a small part in the experiment." The worms' proof-of-concept "rapid return" helped FedEx develop this service as a new product offering to its customers, with an entire system now developed for this purpose. "Space worms was the prototype!" said Drees.

A DARK AND MURKY JOURNEY

Meanwhile, back in Boston, there was a blizzard as the Tufts team waited for the new FedEx delivery process to return their box of worms from the ISS (via Long Beach, California).

> ***"For the weeks they were in space, we'd had no way of monitoring them, so we didn't have any clue what we would find," said Levin. "I mean, they were up there without any supervision. Our biggest concern was that when they got back they'd all be dead."***

Normally, the worms would routinely receive fresh water and fresh air as well as be exposed to light when they were fed and cleaned twice a week. But to keep the initial experiment as elegant and simple as possible, none of these elements were preserved in the spaceflight experiment or ground controls. Because the goal was to evaluate regeneration, the worms were merely cut into thirds, placed into the approved container and space-certified hardware, and loaded onto the SpaceX rocket.

"We basically shoved a lot of worms into a test tube, which went into a sealed coffee-can-sized container," said Morokuma. "Temperature was controlled to some degree, but that was it."

FedEx executes a "rapid return," intercepting the experiment for immediate delivery. Space Tango

FedEx delivers the experiment to Tufts University. Space Tango

Junji Morokuma gets the first glimpse of the returned flatworms. Space Tango

As promised, the post flight box arrived, and the team gathered to see whether their planarian pioneers had successfully re-grown and survived—and what stories they had to tell. "We got this box that came from space, and we were all very excited to open this box," said Levin, "and sure enough, they were alive!"

Immediately, the team witnessed the first interesting behavior of the space worms. When they took the worms out of the dark, stagnant water from their long journey and placed them in normal water, the worms' bodies curled. Normally, this type of curling response indicates that worms are unhappy with their environment, said Levin. "It was amazing. We put them into this beautiful, fresh Poland Springs water they normally love, they all curled up like they didn't like it," he said, "like they had gotten used to the stinky water—which is really strange."

Worms returning from space curled up and were immobile when transferred to petri dishes containing fresh spring water. By contrast, stay-at-home control worms moved rapidly and fully extended themselves.

Allen Discovery Center at Tufts University

Then of course, as the team examined the individual worms more closely, they noticed the now-famous two-headed worm—which later garnered acclaim in mainstream media outlets including Gizmodo, CNET, CBS News, Fox News, Engadget, Yahoo, and Smithsonian Magazine.

Beyond being visually captivating to the public, this phenotype is statistically significant. "Planaria are robust, stable regenerators," said Levin. "You'd have to cut thousands of these kinds of worms on the ground to see two heads." This was the first indicator that something indeed was unique about the regenerative environment onboard the ISS.

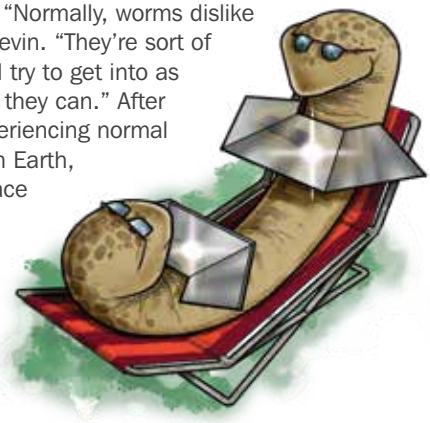
Yet as unexpected and interesting as these early findings were, the most astounding results from the space worms study would not be seen for more than a year, when the team examined the worms' behavior 18 months after readjusting to normal Earth conditions.

MORE THAN JUST A BOX OF WORMS

At first look after 18 months of reacclimation, the worms remarkably had a different complement of bacteria than their relatives who had remained on Earth. As with the human microbiome, the population of microorganisms in a worm's gut, on its surface, and in its surroundings intimately influence bodily functions and behaviors.

According to Levin, until this point, no one had really studied planarian flatworm microbiomes—though some studies on their immune systems and response to infection may yield insights into the role of the microbiome. Inspired by the space worm results, however, Levin and collaborators are currently finalizing the first study of the native planarian microbiome, in terms of what the complement of bacteria typically looks like in standard populations and why it matters.

The second curious and remarkable behavior of the retired space worms suggested an atypical fear of the dark. "Normally, worms dislike the light," said Levin. "They're sort of photophobic and try to get into as dark a corner as they can." After 1.5 years of experiencing normal lab conditions on Earth, however, the space worms showed a notable and unusual preference for light.



"Planarian flatworms are amazing in that they are, in effect, immortal—they don't age," said Levin. "They have stem cells that continuously repopulate the animal as somatic cells age and die off." Within approximately one month, most of a worm's cells have to be renewed. Yet 1.5 years after spaceflight, after 18 such turnovers, the space worms still showed an imprint of their unique experience, moving toward and sitting in the light far more frequently than control worms.

"This is of course conjecture at this stage, but one possibility is that they remember that long," said Levin. "Another possibility is that it's related to the microbiome change." Evidence of the microbiome changing organism behavior and acting as a cognitive modulator has been seen in other animals and even humans, according to Levin.

However, more studies are needed to answer these and other questions about the influence of the ISS environment on planarians.

> ***"The worms are telling us that the experience of going to space profoundly alters the mechanisms within the regenerative process—patterning, differentiation, and cell migration," said Levin. "But we need to understand how these effects are exerted if we are to work toward modulating regeneration for useful purposes on Earth."*** ■

The First American Space-Based Bioprinter

BY EMILY TOMLIN, *Staff Writer*

SPOT LIGHT

NASA astronaut Andrew Morgan works on setting up the BioFabrication Facility to test-print tissues as part of an investigation into whether human organs can be 3D printed in the weightless environment of space. NASA

Commercial service provider Techshot, in partnership with NASA and the ISS National Lab, launched the first American bioprinter to the ISS National Lab onboard SpaceX's 18th commercial resupply services mission. The 3D BioFabrication Facility (BFF) will enable cutting-edge biotechnology research is space.

While researchers on Earth have had limited success in printing human elements like bones and cartilage, the ability for them to manufacture soft human tissue, such as blood vessels, has proven to be much more difficult. Specifically, the bioinks used in 3D bioprinting have a low viscosity, and on Earth, scientists must use scaffolding to support bioprinted structures and prevent them from collapsing. However, this scaffolding makes it difficult to construct void spaces needed in tissues.

Bioprinting in microgravity could prove beneficial because scaffolding is not needed to keep printed structures from collapsing, allowing scientists to overcome what has been a significant hurdle in ground-based biomanufacturing of human organs.

To further assist in the printing of human elements on the space station through the BFF, Techshot partnered with nScrypt, widely regarded as one of the most reputable 3D-printing companies in the world. To address bioink-related constraints to bioprinting on Earth, nScrypt's patented SmartPump Micro-Dispensing tool head will enable precise placement of bioink, thereby enabling stronger formation of printed materials.

Techshot and nScrypt are first seeking to validate the BFF—initially by attempting simple engineering prints, with the expectation to progress to more complex cardiac-like tissue. Within Techshot's cell-culturing system, the printed tissue is expected to strengthen over time—and once it is viable and self-supporting, it will be sent back to Earth for further analysis.

According to the Centers for Disease Control and Prevention (CDC), on any given day in the United States, an estimated 75,000 people are on the active waiting list for organs, far exceeding the organs available for patients. While the road to assembling a human lung or other organs is likely still many years away, the space station is enabling researchers and engineers the ability to develop, test, and validate new facilities that could have dramatic impacts on patient care back on Earth.

More broadly, through the emergence of new space-based facilities such as the BFF, the ISS National Lab is evolving to meet the needs of the modern-day research community, providing more opportunities for groundbreaking, innovative science. ■

NASA astronaut Christina Koch works on the BioFabrication Facility onboard the ISS.
NASA

This content was abridged and updated from an article that originally appeared on ISS360 at issnationallab.org/blog/the-first-american-space-based-bioprinter-is-launching-soon.

PURE OF HEART:

How Microgravity is Improving Cardiac Cell Quality

BY EMILY TOMLIN, *Staff Writer*

It is old wisdom that life is a journey and not a destination. But when your destination is space, it might seem difficult to find excitement in the mere act of preparing for flight. Surprisingly, however, many researchers make exciting and novel discoveries during their preflight experiments—and these findings not only pave the way for greater success in flight but also immediately contribute to scientific knowledge on Earth.

A recent illustration of preflight science discovery can be seen in the work of Chunhui Xu, associate professor in the Emory University Department of Pediatrics. While preparing for a future flight experiment, Xu generated results in the fields of translational biology and regenerative medicine that could advance our ability to model heart defects, improve precision medicine, and even cure diseases.

Xu's journey toward flight began in late 2013 when she was awarded a grant for a ground validation study in response to a CASIS Request For Proposals titled "The Impact of Microgravity on Fundamental Stem Cell Properties." Four years later, Xu has authored six publications detailing results related to her simulated microgravity and stem cell research, and her team is now preparing for a follow-on flight project to take their research to the next, and much higher-altitude, level of discovery.

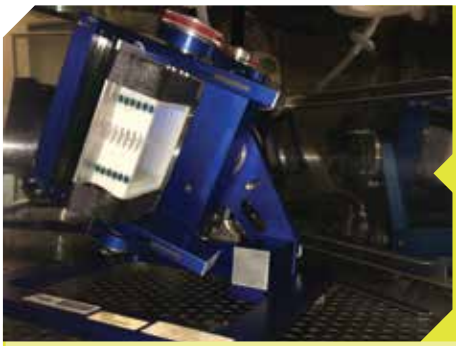
THE COMPLEXITY OF STEM CELLS IN MICROGRAVITY

Biological experiments have been conducted in space for more than 50 years—beginning with model organisms, expanding to include humans, and now encompassing all manner of cells and creatures, great and small. However, most of what is known about stem cell biology in microgravity is from ground analogs operated on Earth that simulate some aspects of a free-fall environment.

Moreover, results have been mixed. Although microgravity clearly alters gene expression of cells in culture and induces the aggregation of cells into tissue-like structures, stem cells appear to have cell-type-specific responses to microgravity. For example, some embryonic stem cells have shown increased growth and proliferation (associated with maintenance of their undifferentiated states), but precursors to liver and fat cells have shown increased rates of differentiation.

From a scientific perspective, both responses are interesting because they may reveal critical information about underlying molecular mechanisms of stem cell function. But it does leave some questions regarding what to expect from a stem cell experiment sent to space—hence the rationale for performing additional ground simulations.

"Ground-based research prior to flight is critical for every project," said Michael Roberts, deputy chief scientist for CASIS, "but some experiments are more critical than others. Analog validation helps us understand expected outcomes before moving to flight, improving the likelihood of scientific success and the likelihood of identifying and measuring the root cause." It is an added benefit, he noted, that many projects can inform and advance their field of research from these experiments alone.



Random positioning machine used to culture cells under simulated microgravity
Xu Lab

TAKING A LOAD OFF

There are several methods that can be used to simulate microgravity and test for 3D cultures. Two well-established methods involve use of a random positioning machine or a rotating wall vessel (developed by NASA). Such devices mimic true microgravity by providing a lack of sedimentation and improved 3D aggregation. Xu's experiments primarily used the random positioning machine, which rotates on multiple axes at varied speeds and angles, functionally removing the gravity vector by continuously shifting the orientation of cells. However, true removal of gravity is not achieved, and the effects of fluid movement on cells in spaceflight experiments are dramatically reduced compared with only modest reductions in simulators.

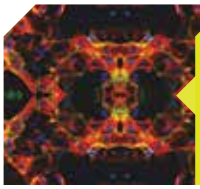
Chunhui Xu, Associate Professor, Emory University School of Medicine
Xu Lab

THE PROBLEM WITH HEART CELLS

Research in the Xu lab focuses on progenitor cells—cells that are partially differentiated (i.e., more specialized than a stem cell) but can still differentiate into multiple cell types. Specifically, the team is studying cardiac progenitors and their differentiation into cardiomyocytes, cells of the specialized muscle tissue of the heart.

"The problem in the field of cardiac medicine is that the heart has billions of cells, and any injury or defect can lead to serious illness," said Rajneesh Jha, a research associate at Emory University who worked on the Xu project as a postdoctoral fellow. "The bad cells need to be replaced, but cardiac cells have limited proliferative capacity."

In other words, cardiac cells can only regenerate, or reproduce, a certain amount. Moreover, although cardiomyocytes are a promising cell source for regenerative medicine, disease modeling, and drug discovery, practical use requires that the cells be enriched (isolated as a pure population) and mature (fully developed). Current methods for producing cells of such quality fall short, typically growing and differentiating cells in 2D environments that generate immature cardiomyocytes within mixed populations.



Microscopic image of cardiomyocytes derived from human induced pluripotent stem cells
Xu Lab

SUCCESS IN SIMULATION

Previous studies have established that producing cardiac cells in 3D liquid-batch cultures (such as a rotating or reciprocal motion shake flask) has an improved yield over 2D cultures (such as a petri dish) in their production of cardiomyocytes. Building on this logic, the Xu lab set out to examine whether their cells might have an even more robust yield if they combined a 3D culture with simulated microgravity.

Interestingly, they found that short exposure to simulated microgravity increased both survival and yield of the cardiomyocytes; specifically, three days of exposure was sufficient for maximum yield. Xu's team published their findings from this experiment, describing their success in producing highly enriched cardiomyocytes (99% purity) with expected functional properties—in other words, the cells appeared mature. Moreover, the cardiomyocytes were produced at up to a four-fold higher yield than in standard 3D cultures (and at an eight-fold higher yield than in 2D cultures).

Rajneesh Jha, Research Associate, Emory University School of Medicine

Xu Lab

"We need mature cells for clinical applications and testing," said Jha, "and microgravity yields these mature cells more effectively than standard cell cultures."

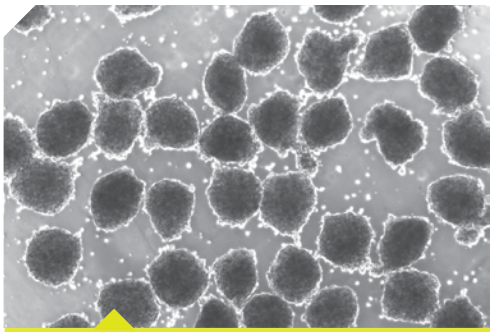
ECONOMIC AND COMMERCIAL APPLICATIONS

Beyond the obvious humanitarian benefit of improving cell characteristics for use in cardiac medicine, there is a commercial incentive to the success of Xu's research: Cardiomyocytes generated in the lab are for sale, and mature cells are in high demand for non-clinical use.

For example, mature cells can be used to develop organoids (micro-scale aggregates of cells that mimic the function of tissues or even whole organs). Organoid development is a blossoming industry in the realm of translational medicine because it offers an alternative to animal testing—they can be used to (1) study organ function and cell behavior in ways not before possible in standard cell cultures and (2) test the effectiveness and toxicity of potential therapeutics in ways more relevant to human biology because they are composed of human cells rather than those of non-human, model organisms. The Xu lab is experienced in such testing using early-stage "lab-on-a chip" technology (e.g., a microelectrode array chamber filled with organoids), on which high-throughput analyses can be performed.

"So many drugs pass safety testing in animals, but when they get to the clinic, they cause issues in humans," said Xu. "This could be, in part, because of electrophysiology specific to human cells." Thus, a pre-test in organoids could have both humanitarian and economic value by minimizing negative effects on patients in clinical trials and saving associated costs (each phase of a clinical trial typically costs millions of dollars). However, yield is again crucial to making this sort of test a reality.

"Large numbers of cardiomyocytes need to be generated in a more efficient way than currently possible," said Xu. "When we combined simulated microgravity with 3D culturing techniques, we saw promising steps toward reaching that goal."



Microscopic image of cardiac progenitor spheres generated by tissue engineering

Xu Lab

MAKING PERSONALIZED MEDICINE A REALITY

In addition to developing and testing treatments for damaged heart tissue, the Xu lab also uses cardiomyocytes to model the effects of congenital (present from birth) heart defects. To do this, the researchers collect skin cells from patients, manipulate those cells to acquire certain stem-cell-like features, and then re-differentiate them into cardiomyocytes for lab-on-a-chip studies. This allows the researchers to be certain that the genetic background of the test samples is identical to the patient.

The team uses the patient-specific chip to study disease characteristics and drug responses. The goal of such work is a future paradigm in which doctors could prescribe certain drugs that the chip shows will be most effective (and least toxic) to an individual, based on their unique genetic background. These studies are at the heart of personalized medicine, and the concept of treating heart defects or damage using such an approach is not as far removed from clinical reality as one might think.

THE BIGGER PICTURE

Heart disease is the leading cause of death in the U.S. and costs an estimated **$207 billion** each year in healthcare services, medications, and missed work. Accelerated drug testing, personalized medicine, stem cell therapies, and discovery science to open new solution pathways all hold promise to lessen the devastating effects of this common condition.

In fact, the Xu team recently worked with Peter Fischbach at Children's Healthcare of Atlanta to study patients with a genetic disorder that causes stress-induced arrhythmias (irregular heartbeat patterns). Arrhythmias caused by this genetic disorder are called catecholaminergic polymorphic ventricular tachycardia (CPVT). As described earlier, Xu's team collected patient cells, induced re-differentiation into cardiomyocytes, and analyzed the drug responses of the personalized organoids.

"In the clinical setting, we saw some children that did not respond to the commonly used beta-blockers, but they did respond to other specific drugs," said Xu. "We saw the same phenomena in a dish. Our results mirrored the clinical data." Beta-blockers are the first-line therapy to prevent arrhythmias in patients with CPVT; however, for unknown reasons, such therapy is unsuccessful in about 25% of patients. Xu's results, published in 2016, show proof of principle that their team's approach to personalized testing could potentially be used in these populations.

TAKING THE NEXT STEP

"The innovations in the Xu lab hold great promise for delivering more effective therapeutics in the future," said Roberts, "and microgravity is having a profound effect on their research."

The Xu team is excitedly preparing for their flight project, which will send cardiac progenitors derived from human skin cells into space and evaluate how microgravity affects their proliferative capacity. Simulated microgravity has allowed the team to make great strides in this area, according to Xu, and she hopes to uncover additional information by sending the cells to space.

Stem cells hold enormous potential for both curing disease (through replacement of damaged tissues) and advancing translational biology (through the growth of organs and tissues as disease analogs). "Dr. Xu's project has the potential to fill both applications," said Roberts. "A lot has been learned about her system from devices that simulate some aspects of microgravity, but true microgravity onboard the ISS National Lab may have advantages above and beyond these ground-based successes." ■

ADDITIONAL RESULTS

According to Xu, an additional exciting outcome of her simulated microgravity studies involved the upregulation of heat shock proteins in their samples. Heat shock proteins are a type of protein involved in stress response, and they are present in all living things. Though their name implies activation by heat stress, heat shock proteins actually respond to high or low temperatures as well as to a drop in vital nutrients such as oxygen and sugar. Although heat shock proteins have been implicated as having a role in cancer and other diseases, their activation in this case may actually be of benefit.

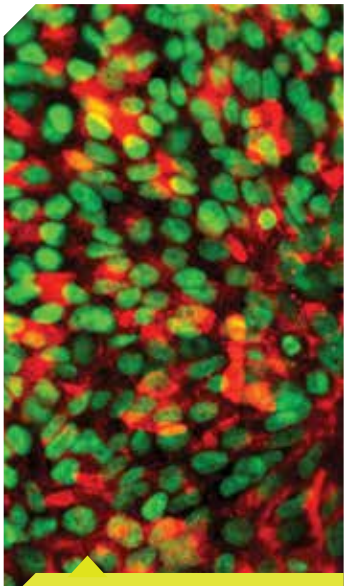
"We already know heat shock can improve survival, which may be part of why microgravity leads to increased viability," said Xu. "But heat shock cells are also more resistant to cell death, and they survive better when transplanted into animal models, so the microgravity upregulation of these proteins is very exciting."

The team is currently working on generating strategies to recapitulate this effect without microgravity.

OPPORTUNITIES FOR REGENERATIVE MEDICINE IN SPACE

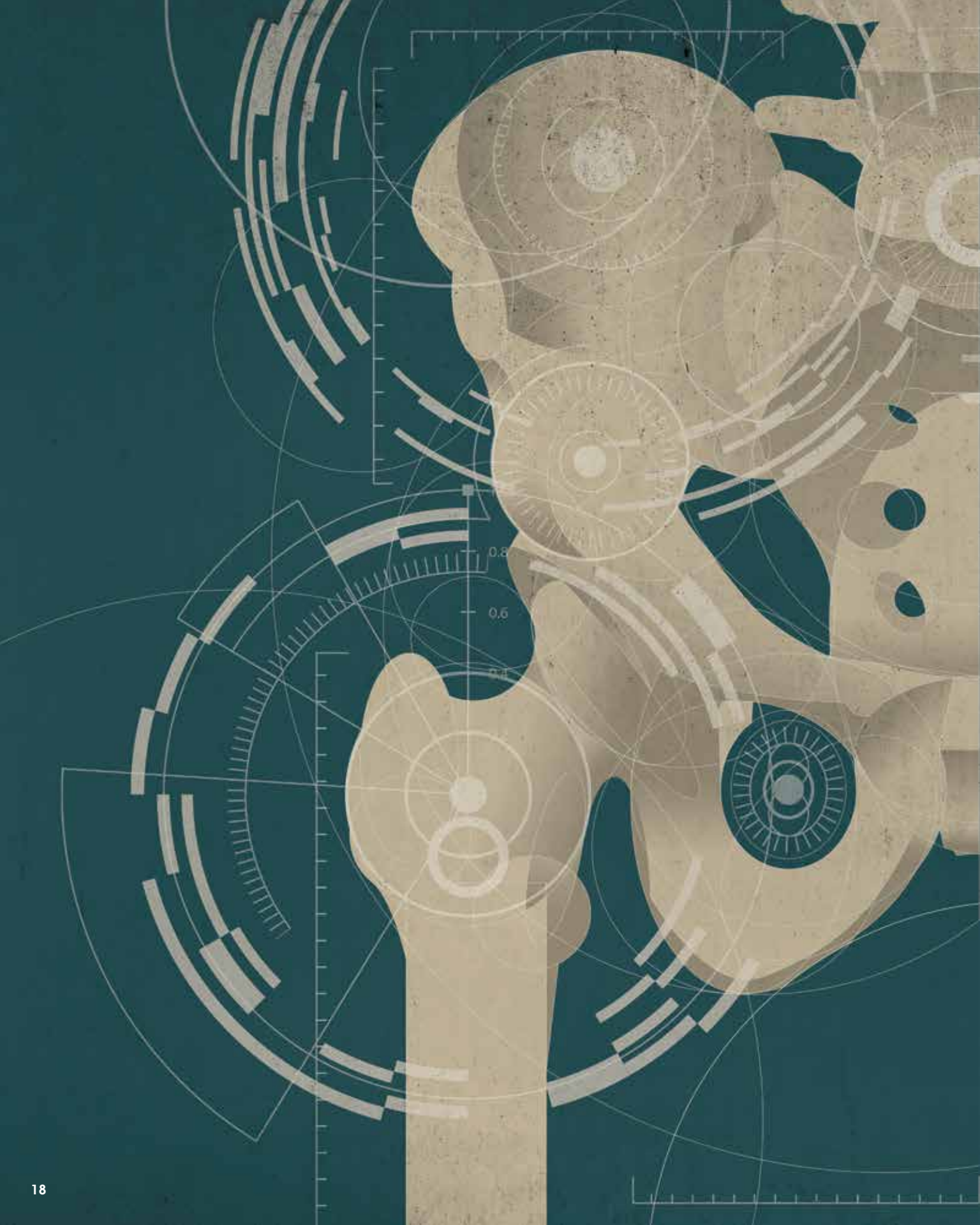
Xu's project is one of several cardiac stem cell projects in the ISS National Lab portfolio, and it complements a growing suite of bioscience projects geared toward tissue engineering, lab-on-a-chip technologies, and regenerative medicine in general. Many of these projects are powered through innovative public-private partnerships, including CASIS collaborations with government funding agencies. For example, in 2017, CASIS awarded five new projects in response to a research solicitation funded by the National Center for Advancing Translational Sciences (NCATS), part of the National Institutes of Health (NIH).

Another collaborative opportunity, this time in partnership with the National Science Foundation, is currently open and seeking proposals in transformative tissue engineering. This includes cellular engineering, tissue engineering, and modeling of physiological or pathophysiological systems in topic areas that include, but are not limited to: scaffolds and matrices, cell-cell and cell-matrix interactions, stem cell engineering and reprogramming, cellular immunotherapies, cellular biomanufacturing, system integration between biological components, and electromechanical assemblies.



Microscopic image of human induced pluripotent stem cells

Xu Lab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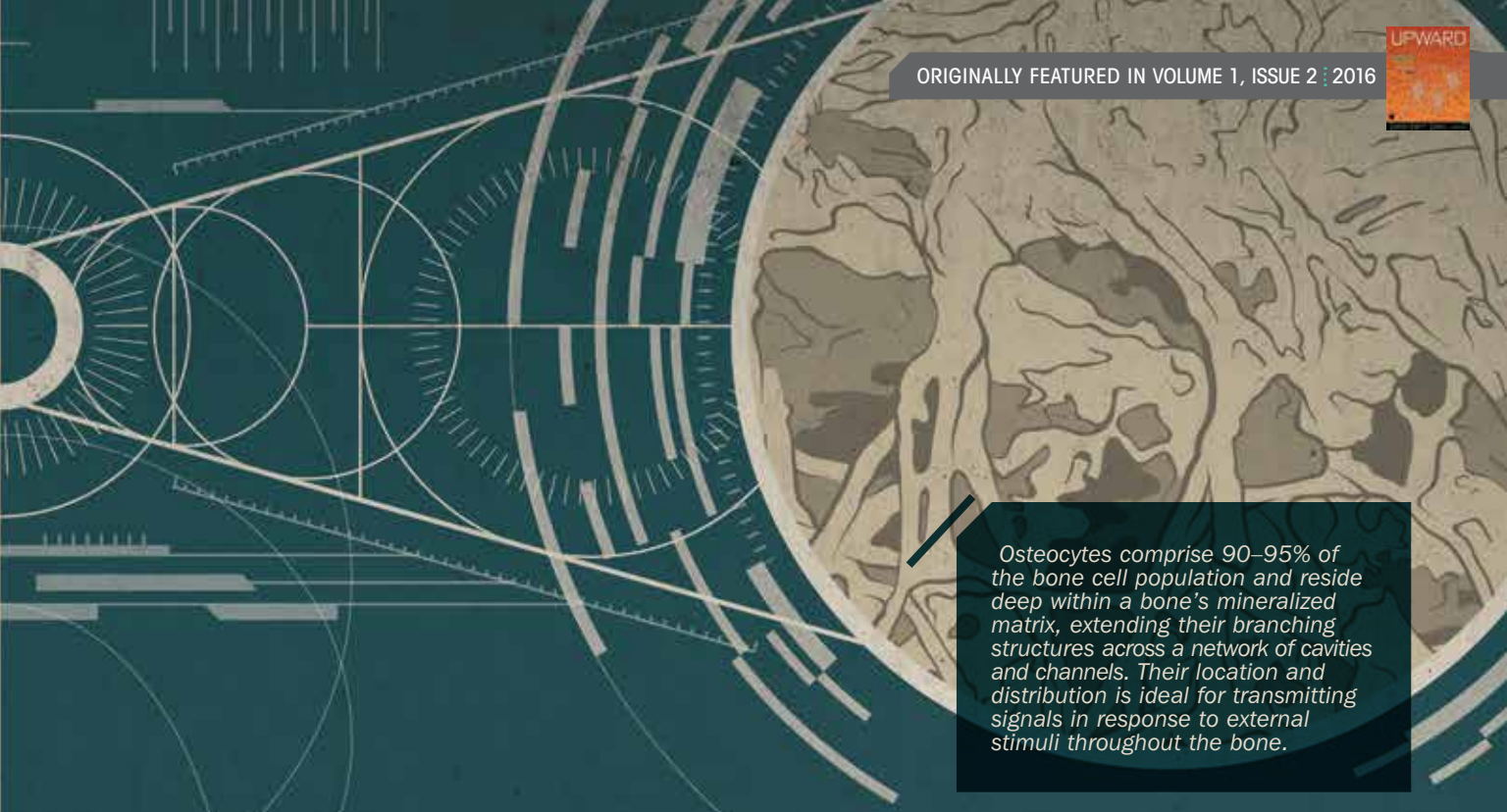


Osteocytes comprise 90–95% of the bone cell population and reside deep within a bone's mineralized matrix, extending their branching structures across a network of cavities and channels. Their location and distribution is ideal for transmitting signals in response to external stimuli throughout the bone.

GRAVITY INCARNATE:
BONE HEALTH AND PHYSICAL FORCE

BY JOHN CREECH, *Staff Writer*

The human body, while often glorified in artistry for its aesthetics, is nonetheless a type of organic machinery—and like all machines, it is governed by the physical laws of nature. In a recent experiment conducted on the ISS, Boston University researchers looked beneath the superficial layers of the human form to discover the intricate beauty of bone physiology in relation to these physical forces.

The human skeleton consists of 206 individual bones. While bones may be the frequent subject of forensic postmortem analysis, it is the living tissue that gives the organ form and "plasticity," the critical ability to change and adapt.

In the ISS experiment Osteocytes and Mechano-transduction, Paola Divieti Pajevic, associate professor of molecular and cellular biology at Boston University's Goldman School of Dental Medicine, keyed in on osteocytes (the most abundant cell type in bone) to identify their role in bone health. By deciphering which parts of the osteocyte's genetic code are activated under different conditions, Divieti Pajevic and her team discovered a potential new avenue for drug development aimed at treating diseases related to bone loss.

STUDYING OSTEOPOROSIS IN MICROGRAVITY

On Earth, gravity is a constant force that imparts mechanical resistance to the body's activities. This resistance is perceived by osteocytes and translated into cellular signals that regulate the balance between tissue formation (growth) and tissue resorption (breakdown), termed bone remodeling. Osteoporosis—a condition in which bones become fragile from excessive resorption—is the most common disease related to bone remodeling, affecting more than 200 million people worldwide.

The risk for osteoporosis increases with age, especially for women with declining estrogen (a hormone that limits bone loss by inhibiting resorption). The need for new and improved therapies is critical to help prevent age-related damage to bones. Researchers are using new methods to discover factors that contribute to bone loss and may serve as alternative targets for drug development. Other approaches to developing new therapies look at reduction in physical activity—or more technically, mechanical force—as models of bone loss.

For example, bone loss in bedridden patients, such as the elderly and those with paralysis or chronic diseases, results from greatly reduced levels of regular mechanical force applied to the bones. Microgravity simulates the lack of bone and muscle loading in an accelerated manner, making the ISS National Lab an ideal environment for studying the underlying biological mechanisms related to mechanical force and bone disease. To investigate the dynamics of osteocyte signaling, gravity, mechanical force, and bone health, Divieti Pajevic took advantage of this unique research platform.

"We need to understand how the system works to know how it doesn't work," said Divieti Pajevic. "This understanding will allow us to intervene and treat bone pathology."

THE MACHINERY OF BONE HEALTH

In context of the body's machinery, osteocytes are less like a linchpin locking the bone remodeling cycle statically in place and more like a transmission, able to move the cycle forward or backward. A decrease in mechanical force shifts the balance in the bone remodeling cycle in favor of resorption while an increase shifts the balance in favor of formation. This dynamic balance not only maintains bone health but also allows an organism to adjust to its environment.

One of the earliest observations regarding bone remodeling comes from Galileo Galilei, who observed that the bones of large animals (which experience increased weight-bearing mechanical force) are robustly greater in diameter relative to their length. In modern times, a similar effect is seen in professional athletes, especially those who experience unilateral mechanical force. For example, X-rays reveal the augmented growth of bone in a tennis player's dominant hand.

"Within the bone, the cells responsible for changing bone mass according to how much external load force is applied are osteocytes," said Divieti Pajevic. This much is known, but the mechanisms of *how* osteocytes sense and respond to mechanical force are unknown. To understand this "mechano-sensing" process and the resulting cellular signals ("mechano-transduction") at the molecular level, Divieti Pajevic set out to perform the first-ever analysis of osteocyte gene expression in space.

This analysis was made possible through a powerful collaboration between the National Institutes of Health (NIH) and NASA: ***the NIH Biomedical Research on the ISS*** (BioMed-ISS) program, which specifically supported experiments in space aimed at improving health on the ground.


CALM Technologies Inc.

PERSISTENCE: THE VIRTUE OF SCIENCE

Had it not been for the dogged persistence of one graduate student (Jordan Spatz) with an unorthodox idea and the help of an international team, the Osteocytes and Mechano-transduction project may never have come to fruition. In 2010, Spatz (now a medical student at University of California, San Francisco), kicked off the initial interest in using microgravity to investigate the bone's cellular connection with mechanical force.

Inspired by a funding opportunity from the *NIH Biomedical Research on the ISS* program, Spatz reached out to Calm Technologies, a Canadian hardware development company. The initial response was hesitance; the company's existing hardware (originally designed for the unmanned Russian Photon spacecraft) was not certified for use on ISS.

"However, the project stood out on the strength of the science," said Calm Technologies Vice President Chris Adamson, and this convinced the company to take on the upgrade. Iterations of the former hardware had supported three previous bone cell investigations in space. The new hardware, however, was the first to study the osteocyte cell type and the first to fly to the ISS.

"There were quite a few challenges to make the hardware 'station-worthy,' but it turned out to be a huge success," said Adamson. In fact, he noted that the success of this project has generated new interest in the hardware from other investigators.

"BioMed-ISS was developed to facilitate NIH mission-relevant research on the ISS," said Faye Chen of the NIH's National Institute of Arthritis and Musculoskeletal and Skin Diseases. "The program was intended to promote biomedical research that uses the unique microgravity and radiation environment of the ISS for the benefit of human health on Earth."

Through this unique program and later sponsorship by CASIS, the Osteocytes and Mechano-transduction experiment launched to the ISS National Lab aboard SpaceX-6 in 2015, allowing Divieti Pajevic to peer inside the osteocyte cell as it responded to the absence of mechanical force in microgravity. "There was a need to understand the mechanisms of mechano-transduction in osteocytes, the most abundant bone cells, at the cellular and molecular level," said Chen.

Italian Astronaut Samantha Cristoforetti carrying out the Osteocytes and Mechano-transduction experiment onboard the ISS.
NASA

HIDDEN AMONGST THE GENES

Genes and the environment are the ultimate determinants of our physical traits. Each cell in our body contains the same genetic material, but gene expression (which genes are turned on or off in any given cell) differs according to environment. GeneChip technology (see sidebar) enables analysis of which genes are turned on and off in a cell population at any given time. By comparing GeneChip data from osteocytes in spaceflight to those on Earth, changes in gene expression associated with the removal of mechanical force are highlighted.

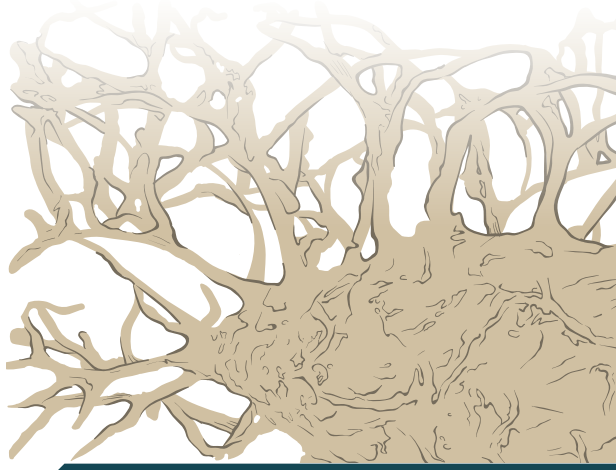
Through this approach, Divieti Pajevic discovered a surprising and exciting connection between bone cell response to mechanical force and genetic pathways not previously associated with bone disease. A significant portion of the gene expression changes in microgravity were part of the "hypoxic" pathway (a series of gene control mechanisms known for being induced by oxygen deprivation) and glycolysis (a form of glucose metabolism).

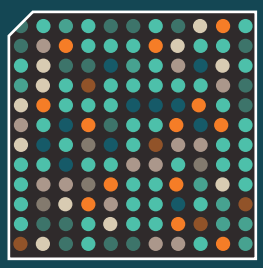
THE FUTURE OF DISCOVERY

With a new understanding of microgravity's effect on osteocyte gene expression, Divieti Pajevic can now design experiments on Earth to more deeply understand how the pathways of hypoxia and glycolysis relate to osteocyte behavior and, more specifically, disease. Because of their association with mechano-sensing and mechano-transduction, the genes in these pathways could represent new targets for osteoporosis treatments.

"These discoveries might not otherwise have been possible through ground-based means," said Chen. Moreover, not much has been done to target these pathways in the field of osteoporosis treatment, leaving the future open for Divieti Pajevic to further elucidate the osteocyte's genetic code. And the results gathered so far are only the beginning—analysis from the spaceflight samples is ongoing, with evaluation of protein levels and the expression of microRNAs (a recently discovered class of short RNA fragments involved in regulating gene expression) still to come.

"This was the first experiment on osteocytes in space, the first on the space station, and the first use of this hardware on the station," said Divieti Pajevic, "so it was quite interesting to have such success and discover two new pathways that might be important for bone metabolism. We are excited about reproducing this on the ground and performing additional testing." ■





MEASURING GENE EXPRESSION

Each spot on a GeneChip represents a specific gene. To measure which genes are being expressed (or not expressed) in space, total RNA was collected from cells in orbit and then processed on the ground to attach fluorescent labels. The fluorescently labeled RNA samples bound to their complementary gene sequence, and a laser scanner enabled a quantitative analysis of gene expression patterns. This gave the team the opportunity to see which genes were turned on or off in spaceflight compared with ground controls.

RESHAPING DRUG DELIVERY

MILLIONS OF CRYSTALS AT A TIME

BY AMELIA WILLIAMSON SMITH, *Staff Writer*

Many cancer therapies, such as Merck & Co.'s immunotherapy drug Keytruda®, are dilute solutions of large molecules called monoclonal antibodies in a saline solution that must be given intravenously (IV) through a slow infusion over the course of several hours in a doctor's office or hospital. However, if pharmaceutical companies like Merck discover a way to formulate these drugs as highly concentrated crystalline suspensions, the medicine may one day be given as a simple injection under the skin during a quick visit to a doctor's office.

A team of Merck researchers is utilizing the unique microgravity environment of the ISS National Lab as a first step in trying to achieve just this.

"The drug might provide tremendous benefit, but the route of administration isn't necessarily ideal," said Matthew Truppo, executive director and head of Chemical Biotechnologies and Global Structural Sciences at Merck. "If we could simplify the administration, it would be better for the patient and practitioner, and it would make the entire process a little easier to handle."

Merck's investigation, led by Principal Investigator Paul Reichert, associate principal scientist at Merck, launched on SpaceX CRS-10 in February 2017. The experiment sought to grow millions of highly ordered, uniform crystalline particles of the therapeutic monoclonal antibody Keytruda®. The team's research has applications aimed not only at improving drug formulation and delivery but also at improving the drug purification process. Additionally, results from this investigation could lead to improved drug stability and storage.

"Conducting experiments on the ISS National Lab gives us the opportunity to test unique preparations and make primary discoveries that we can then apply to drug development on the ground and onward to manufacturing," Reichert said. "It's a long process, but what we find in this investigation could be very important not only for the drug Keytruda® but also for therapeutic monoclonal antibodies in general."

CONDUCTING A DIFFERENT KIND OF PROTEIN CRYSTAL GROWTH EXPERIMENT

Many protein crystallization experiments on the ISS seek to grow large, high-quality crystals (or crystal complexes of proteins bound to small molecules) for structural analysis aimed at structure-based drug design. However, Merck's investigation is quite different. Instead, Reichert and his team were aiming to grow a crystalline suspension of millions of tiny uniform crystals.

Monoclonal antibodies are not very soluble, which is why it is difficult to get highly concentrated formulations of drugs like Keytruda®. If it were possible to produce high-quality crystalline suspensions of therapeutic monoclonal antibodies, it would enable pharmaceutical companies such as Merck to change the formulation of these drugs from an IV to an injection, which would greatly improve patients' quality of life. If such drugs could be given as a quick injection at a doctor's visit, it would save time and reduce costs.

This investigation followed from experiments in the space shuttle era, in which Merck researchers crystallized another biologic drug, alpha interferon, and instead of getting a single large crystal, they got a crystalline suspension of small crystals that were very uniform in size. "We're applying what we learned from the space shuttle era to a new type of biologics, monoclonal antibodies," Reichert said.

Monoclonal antibodies make up the majority of therapeutic biologic drugs. However, many monoclonal antibodies are difficult to crystallize on Earth, which is why Merck turned to the ISS National Lab for crystallization. Minimizing gravity significantly reduces physical forces that are dominant on the ground, contributing to the production of more ordered, high-quality crystals that often provide higher-resolution structures.

WHAT IS KEYTRUDA®?

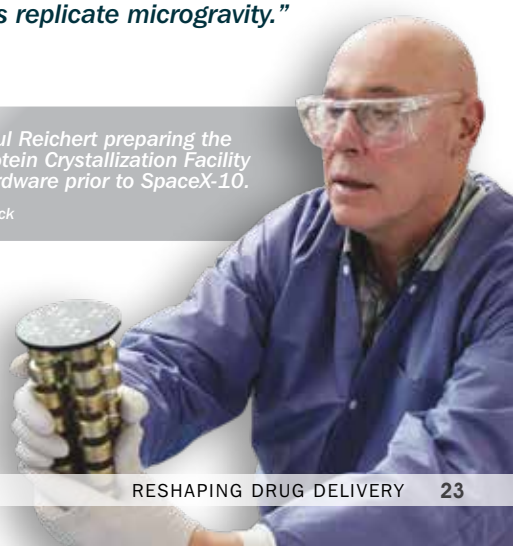
Keytruda® (pembrolizumab) is an immunotherapy drug approved by the U.S. Food and Drug Administration to treat melanoma, non-small cell lung cancer, head and neck cancer, Hodgkin lymphoma, bladder cancer, and tumors with MSI-H or dMMR.

The drug helps the body's own immune system detect and destroy cancer cells.

Some cancer cells have a high expression of a protein called PD-1 (programmed death receptor-1), which prevents the immune system's T-cells from detecting the cancer cells. Keytruda® binds to PD-1 on the cancer cells so the T-cells can recognize the cancer cells as mutants and orchestrate the immune system to fight and destroy them.

WHAT ARE BIOLOGIC DRUGS? *Biologic drugs are unlike normal drugs in that they are not manufactured through a chemical process in a lab. Instead, biologics are a class of drugs made from large complex molecules derived from living organisms, such as microorganisms or human or animal cells.*

"The ISS represents a truly unique laboratory platform where you can access conditions that you just can't access here on Earth," Truppo said. "There are many things you can replicate on Earth that are conditions you might find in space, but what you can't do is replicate microgravity."



Paul Reichert preparing the Protein Crystallization Facility hardware prior to SpaceX-10.

Merck

USING MICROGRAVITY TO GROW HIGH-QUALITY CRYSTALS

To grow high-quality crystals, Reichert and his team took advantage of the decreased fluid motion in microgravity and improved conditions for the formation of ordered crystal lattices. These benefits of space-based crystallization are due to several physical forces being reduced in microgravity.

"When you're trying to get very high quality and very uniform crystals, it's important to have a really slow and orderly process by which those molecules come together to form a crystal," Truppo said. "The more you minimize movement within the solution and rely solely on the ability of the molecules to one by one come together and build the crystal lattice, the more likely you'll get a highly ordered, pure crystal."

By crystallizing therapeutic monoclonal antibodies on the ISS National Lab, Merck researchers hope to learn more about the key variables affecting crystal growth that could then be applied to pharmaceutical applications of interest back on Earth.

"To be able to take away a fundamental force for an extended period of time and see how that affects processes we are used to running on Earth could potentially lead to incredible insights into the fundamentals of how those processes work," Truppo said. "And then we can start thinking about how to exploit that information back on the ground and change the way we do things."

IMPROVING DRUG STORAGE AND PURIFICATION

The production of high-quality crystalline suspensions of monoclonal antibodies could allow for improvements in drug formulation (changing from an IV formulation to an injection), and it could also lead to improvements in drug storage and purification.

"Currently, most monoclonal antibody preparations have a limited shelf life and must be stored under refrigerated conditions in large, cumbersome bags," Reichert said. "It would be advantageous to develop a concentrated drug substance that is stable at room temperature that could be moved to formulation sites around the world in small containers."

Merck

HOW DO REDUCED PHYSICAL FORCES IMPROVE CRYSTALLIZATION?

In microgravity, several physical forces are minimized, which improves the conditions for ordered crystal growth.

- ***Sedimentation:*** *On the ground, as crystals grow in a vial, gravity causes crystals to fall to the bottom and sediment. However, in microgravity, the crystals remain suspended in the solution. This results in less turbulence around the crystal as it is growing, which leads to higher-ordered crystals.*
- ***Convection:*** *On Earth, temperature differences in a poorly mixed solution lead to a density difference, which gravity drives into two currents (the warmer lower-density fluid moves up, and the cooler higher-density fluid moves down). In microgravity, however, temperature differences do not lead to convection currents, resulting in less bulk fluid movement around the crystals and the growth of more uniform crystals. Additionally, the absence of convection currents results in a more uniform temperature gradient that can benefit crystal growth because precise temperature control is needed.*
- ***Rate of molecular diffusion:*** *Microgravity reduces the rate at which molecules move in a solution. This allows the molecules to enter the crystal lattice more slowly and in a more organized, orderly way.*

Merck is currently trying to demonstrate that crystalline suspensions of monoclonal antibodies are stable in long-term storage at room temperature, which would be a tremendous advantage. It would eliminate the need for and costs of refrigerated transportation and would enable distribution of the drug in areas of the world that lack refrigeration. Additionally, lowering the costs of production and transport could ultimately lead to lower costs for patients.

ISS crew member Thomas Pesquet removing the Protein Crystallization Facility hardware from the incubator on the ISS.

NASA

Merck is also hoping to improve the purification process involved in manufacturing therapeutic monoclonal antibodies. Currently, monoclonal antibodies are produced using cell culture fermentation, followed by multiple steps to purify and isolate the active ingredient from the fermentation broth using a technique called chromatography. This purification process is effective, but it is also very time consuming and expensive. Merck is trying to see whether crystallization could be used to purify monoclonal antibodies directly from the fermentation broth, thus significantly reducing or eliminating the need for extensive chromatography and, in turn, lowering production costs.

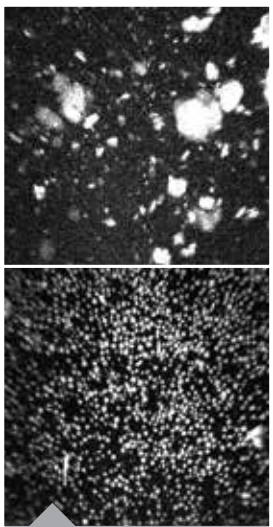
In their SpaceX-10 investigation, Reichert and his team sent to the ISS samples of the monoclonal antibody at different steps in the purification process, including some that were very crude extracts from the fermentation broth. The researchers wanted to see whether crystals would form out of the crude broth and what level of purity could be achieved.

"The question is—can we directly crystallize out of the fermentation broth?" Truppo said. "Can we produce crystals that potentially match the quality and purity of our entire production process, in which we do multiple chromatography steps until we get to a very pure antibody?"

ANALYZING SAMPLES AND APPLYING RESULTS

Reichert and his team are now in the process of analyzing their samples. The team first performed microscopic analyses to determine the particle size distribution of the flight samples versus ground samples in a blind study. Reichert said preliminary results are successful, and the differences between the ground and spaceflight samples are clear.

"The samples crystallized in microgravity contain highly ordered, uniform crystalline suspensions similar to the crystalline suspensions of alpha interferon from our previous space shuttle experiments," Reichert said. "This is exciting because it demonstrates the results are reproducible under these conditions."



UV imaging of a ground control sample (top) and spaceflight sample (bottom) from Merck's investigation, clearly showing the much more uniform size and distribution of crystals grown in microgravity.

Merck

The team is also using a battery of biophysical characterization methods to analyze the samples. If the space-grown crystals meet the desired property parameters, researchers can apply that information to experiments back on the ground. "It would give us a lead that tells us we should invest the extra time and resources to identify alternate process conditions on Earth that could be used to recreate the crystals we observed in space," Truppo said.

The team's findings could help researchers better understand the crystallization process of not only Keytruda® but also therapeutic monoclonal antibodies in general. Such knowledge could lead to important advances in monoclonal antibody drugs that could one day translate into significant improvements in quality of life for patients with cancer and autoimmune disorders.

WHAT HARDWARE WAS USED IN THE EXPERIMENT?

There are many hardware options for protein crystallization that support various methods of crystal growth. Merck researchers used two types of hardware in their investigation.

Merck

⊕ ***Protein Crystal Growth (PCG) Box:***
The PCG box can hold five six-packs of samples. This hardware enables a process called vapor diffusion, which is usually used to grow crystals for structural analysis. The PCG box is easy for ISS crew members to operate—they simply use a wrench hooked on the side of the hand-held box to turn a valve that exposes the protein samples to solvent.

Merck

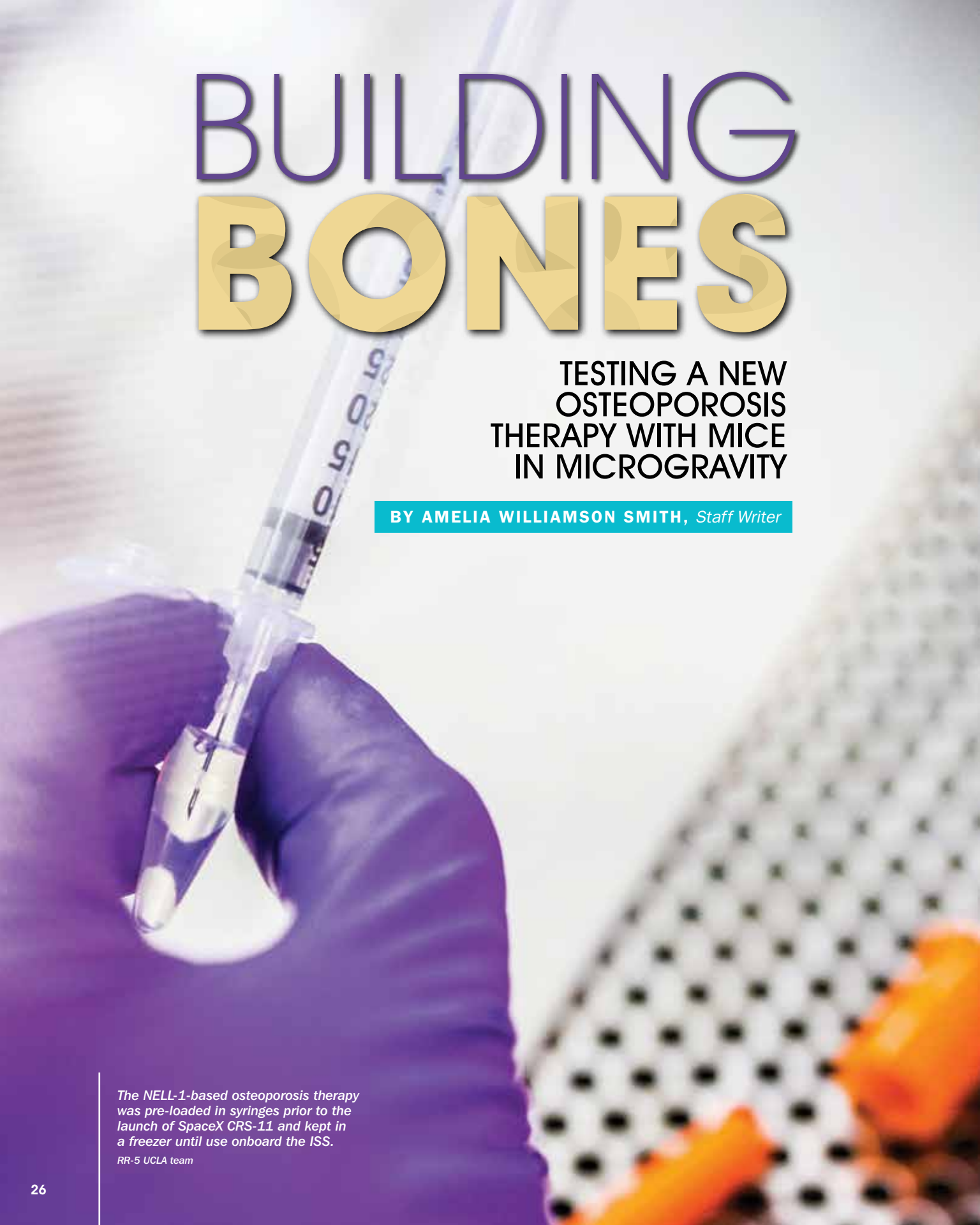
⊕ ***Protein Crystallization Facility:***
This hardware consists of a module that holds up to five stacks of seven 1-mL bottles. The module can be kept at a very precise temperature, and researchers can vary the conditions within the module. To operate the hardware, ISS crew members place the module into an incubator and ramp up the temperature to induce crystallization.

"This experiment really has boundless potential applications," Truppo said. "And I can't think of any better example of boundless research than conducting experiments in space onboard the ISS National Lab." ■

BUILDING BONES

TESTING A NEW OSTEOPOROSIS THERAPY WITH MICE IN MICROGRAVITY

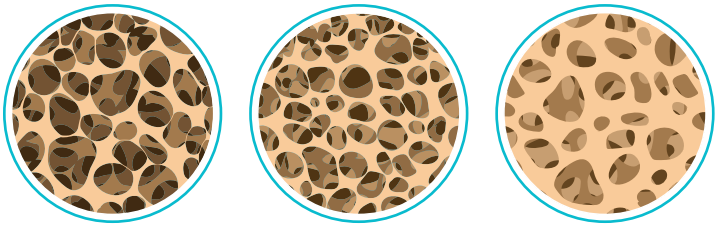
BY AMELIA WILLIAMSON SMITH, *Staff Writer*



The NELL-1-based osteoporosis therapy was pre-loaded in syringes prior to the launch of SpaceX CRS-11 and kept in a freezer until use onboard the ISS.

RR-5 UCLA team

Every three seconds, a person somewhere in the world breaks a bone due to osteoporosis—a progressive disease that decreases bone density, making bones weak and fragile. Osteoporotic fractures greatly reduce quality of life, and immobilization following a fracture can lead to further bone loss, which puts these patients at risk for breaking another bone.



When SpaceX CRS-11 launched to the space station last June, it carried 40 mice to the ISS National Lab for a mission aimed at improving treatment for the millions of people with osteoporosis back on Earth. The Rodent Research (RR)-5 mission successfully proved the robustness of a new potential osteoporosis therapy based on a naturally produced protein, NELL-1, and also led to significant improvements in the delivery of the therapy.

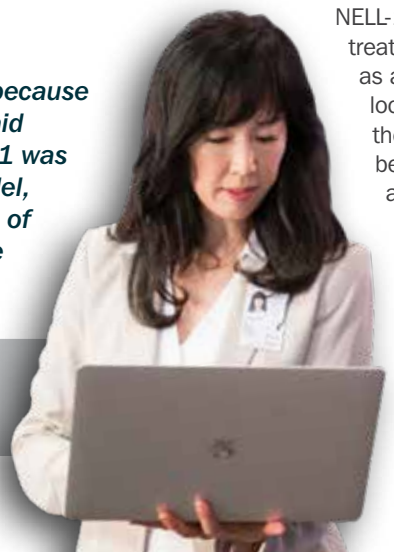
Most current osteoporosis drugs only work to slow bone breakdown, not form new bone. A therapeutic approach using NELL-1 being developed by a group of researchers at the University of California, Los Angeles (UCLA) works both ways. NELL-1 has been shown to not only help prevent further bone loss but also build new bone to replace what was lost. Such a therapy would be of tremendous benefit to patients with severe osteoporosis.

The Rodent Research Hardware System

NASA/Techshot

To further evaluate the effectiveness of NELL-1, the UCLA team, led by principal investigator Chia Soo, tested its therapeutic use in mice onboard the ISS National Lab. Microgravity has been shown to induce accelerated bone loss in mice at rates that exceed those caused by either post-menopausal or disuse osteoporosis on Earth, making space an ideal environment to study osteoporosis therapies.

"Testing in microgravity is a huge deal because it induces an extreme state of bone loss," said RR-5 co-investigator Jin Hee Kwak. "If NELL-1 was found to be successful in a microgravity model, then it may work for the most extreme cases of bone loss on Earth, and that would prove the rigorousness of our therapy."



Principal investigator Chia Soo assures data sync prior to RR-5 mission launch.

RR-5 UCLA team

LINKING NELL-1 AND BONE FORMATION

NELL-1 is a protein made by the body, and its bone-forming effects were originally discovered more than 20 years ago by craniofacial orthodontist and RR-5 co-investigator Kang Ting. He had been examining children with a condition that causes an overgrowth of bone in the skull and wondered whether looking at gene expression in these patients could reveal a protein involved in bone growth.

Ting said, "The question was, can we find a way to see what induces bone formation in patients with too much bone? Can we find the protein and use it to help people who need bone?"

RR-5 UCLA team

After screening millions of genes, Ting found one protein—NELL-1—that was overexpressed in children with skull bone overgrowth, leading him to begin investigating NELL-1 as a bone-forming agent. Following results from an independent research group that linked the underexpression of NELL-1 in patients to low bone density, Ting and his UCLA colleagues found that mice lacking NELL-1 exhibit symptoms of osteoporosis as they age.

In several animal models, the UCLA team was able to demonstrate the use of NELL-1 as a successful osteoporosis treatment; however, use of the protein as a therapy was possible only via local administration. In other words, the NELL-1 protein would have to be injected directly into a patient's affected bone during surgery.

To make NELL-1 more useful for patients with osteoporosis, the team needed to modify NELL-1 to administer it systemically. This type of therapy could be given as a quick injection under the skin to build bone throughout the body.

Fluorochrome bone labels were injected in the mice three times throughout the study to measure the rate of bone formation. Because fluorochromes are susceptible to light, the pre-loaded syringes were stored in opaque protective bags.

RR-5 UCLA team

As the UCLA researchers began working to modify NELL-1 for systemic use, they submitted a proposal to test the therapy in the microgravity environment on the ISS National Lab and were awarded a CASIS grant, which supported the RR-5 mission. Once modification of the molecule was complete, the team could launch their investigation to the space station.

MODIFYING THE MOLECULE

Developing NELL-1 into a systemic therapy that could be given as an injection every couple of weeks to build bone would greatly benefit patients; however, modifying the molecule to achieve this was no easy task. The team needed to find a way to keep the therapeutic protein molecules circulating in the blood long enough for NELL-1 to induce bone formation. The molecules also needed to be able to successfully and exclusively attach to bone tissue to be effective and safe.

Additionally, ISS crew members have typically only accessed mice on the space station once every 14 days, so the interval between injections had to be at least that long. Keeping NELL-1 in the bloodstream for such a long period was a tremendous challenge. Moreover, with such a long circulation time, the team needed to reduce any toxicity so that the therapy would be safe.

With the help of Benjamin Wu, RR-5 co-investigator and former chairman of the UCLA Department of Bioengineering, the team modified NELL-1 using a method called PEGylation, which slows the rate at which molecules are degraded by the liver. This would keep NELL-1 circulating in the bloodstream longer. Then, to help NELL-1 find bone tissue, the team attached the protein to an inactive form of a bone-seeking molecule called bisphosphonate.

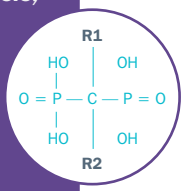
RR-5 UCLA team

> ***"We tried to modify the molecule so instead of just blindly circulating in the blood, it became kind of a targeted molecule that seeks bones and attaches to them," Ting said. "This increases efficacy in the bones and reduces toxicity in organs where we do not want the molecule."***

The team tested the modified molecule, called BP-NELL-PEG, in a ground-based experiment using female mice whose ovaries had been surgically removed to induce osteoporosis. The team found that injection of the therapy into the abdomen of the mice once every 14 days was successful.

WHAT IS BISPHOSPHONATE?

Bisphosphonate used to be a common drug to treat osteoporosis; however, it is no longer used as often because of a serious potential side effect involving bone deterioration in the jaw. By inactivating bisphosphonate, the molecule no longer has a therapeutic effect (nor does it cause side effects), but it retains its bone-seeking function.



Developing the therapy with a 14-day injection interval was one of the greatest technical challenges the team faced, but successfully extending the dosing interval will also be a substantial benefit to patients, Kwak said. Patients would need fewer injections of BP-NELL-PEG compared with most current osteoporosis drugs. This would not only save extra trips to the doctor's office, but also make the therapy more affordable.

"Our work on the RR-5 mission really helped us make the delivery more effective and targeted, and it significantly increased the eventual clinical feasibility of the therapy," said RR-5 principal investigator Chia Soo. "If you have an easier way to deliver the therapy and if you don't have to deliver it as frequently, it has a much greater human application."

Grip strength testing was done to measure the muscle strength of the mice.

RR-5 UCLA team

Having successfully optimized the molecule and achieved the 14-day injection interval necessary for rodent research on the space station, the team was now ready to test the systemic therapy in the extreme case of microgravity-induced bone loss.

SENDING MICE TO THE SPACE STATION

SpaceX CRS-11 launched to the space station last June with the RR-5 mission, carrying 40 female mice aged to 8 months, which is when they reach skeletal development. An additional 40 mice remained on the ground at NASA's Kennedy Space Center as a control group. In both the flight and ground groups, half the mice received BP-NELL-PEG treatment and the other half did not.

Rodent habitat onboard the ISS National Lab

NASA

The RR-5 mission was unique in that halfway through the nine-week investigation, 20 mice in the flight group (10 receiving treatment and 10 not) were returned to Earth alive to complete the rest of the experiment on the ground at UCLA. The other 20 mice remained on the space station until the end of the investigation when they were sacrificed, and the frozen specimens were returned on SpaceX CRS-12.

Many analyses can only be performed on live tissues and cells, so live return was important to the RR-5 team. When the SpaceX Dragon capsule splashed into the Pacific Ocean last July with the RR-5 live-return mice, the team was elated to find that all 20 mice were alive and healthy. The team also noticed that the fur of the mice was shiny within a day of live return to UCLA, meaning they had been grooming—an indication of contentment.

"Our live return involved full survival of the animals, and they were safe, healthy, and happy," Kwak said. "This is a huge milestone, and the exciting part for our science team is that all of our data is going to be very reliable."

ANALYZING DATA AND LOOKING TO THE FUTURE

The RR-5 team is still in the process of analyzing all the data, but preliminary results indicate the investigation was a success. Data from the hind limbs and vertebrae of the spaceflight mice showed significant bone loss from microgravity and a remarkable recovery by BP-NELL-PEG treatment.

> ***"We can unequivocally say that NELL-1 increases bone density in microgravity conditions, which is very exciting," Soo said. "This success demonstrates the robustness of the therapy to treat extreme bone loss."***

From here, the team plans to probe deeper into the molecular biology of the NELL-1 protein to gain a more detailed understanding of how the molecule works, while continuing to focus on the practical translational aspects of the therapy.

"We want to look at how we can make this a better osteoporosis treatment for eventual clinical application," Soo said. "Not only for the millions of osteoporosis patients on Earth but also, in thinking about future space travel and a mission to Mars, we want to see how we can prevent the detrimental effects of microgravity on bones during spaceflight."

The RR-5 team: Ben Wu, Chia Soo, Kang Ting, Jin Hee Kwak.

RR-5 UCLA team

Although modification and use of NELL-1 as a therapy has come far since its discovery more than 20 years ago, there's still a long journey ahead before this treatment approach can be applied to humans, Ting said.

COLLABORATION FOR DATA COLLECTION

Bone scans and behavioral tests were done on all the mice before launch, at the time of live return, and again at the experiment's conclusion. Blood tests were also done before launch and at the conclusion.

Collecting these data on the ground and in orbit would not have been possible without close collaboration among the science teams from CASIS, NASA's Ames Research Center, UCLA, and implementation partner BioServe Space Technologies, as well as NASA crew members onboard the ISS. During the mission, the teams collaborated in three distant locations: NASA's Kennedy Space Center, where the ground control mice were housed; the ISS National Lab, where the flight groups were housed; and UCLA, where the live-return mice were housed.

"Everything had to be done at the same time point, with the same exact methodology, using the same type of injections and animal care, and doing the same analyses—it was a tremendous effort to make that happen," Kwak said. "I was always on standby and always had my phone on next to me when I was asleep because anyone could call me at any time to ask questions about the experiment."

Additional testing included oral swabs to analyze changes in the oral microbiome of the mice. Fecal samples were also analyzed to correlate oral microbiome changes with changes in the digestive tract microbiome.

RR-5 UCLA team

"But that's what research is about—you have persistence and tenacity, and you never give up," he said. "Everyone involved in the RR-5 mission was so devoted and committed to making the project successful, and it shows that if we all have the same goal and push forward, we can achieve anything. The sky is not the limit anymore!" ■

RODENT ROCKET RESEARCH

BIOMEDICAL DISCOVERY IN SPACE

BY MARC GIULIANOTTI, *Staff Writer*

Rodents have been traveling to space since the 1950s, helping to pave the way for humans to safely venture off our planet. Since these early voyages of discovery to determine whether animals could survive spaceflight, mice and rats have continued to play an important role in space research. Rodents provide critical insight into not only the effects of spaceflight that impact astronaut health but also effects that mimic human disease on Earth, including those targeting the musculoskeletal and cardiovascular systems, immune function, wound healing, and metabolism.

Rodents flew on 27 U.S. space shuttle missions, and thanks to the work of scientists and engineers at NASA's Ames Research Center, rodents now have a new role in space research onboard the ISS National Lab. Previous shuttle missions housed rodents in microgravity for about 10 days. The new Rodent Research Hardware System is designed to allow rodents to spend up to 180 days in space, greatly improving our ability to use this powerful animal model to study the effects of spaceflight on human health.

MODELING HUMAN DISEASE

Rodents are surrogates for studying human disease because they share many similarities to humans in terms of anatomy, physiology, and genetics. Rodents and humans share virtually the same set of genes, and there is great similarity in genetic sequence and protein function.

Due to the similar genomes but much shorter lifespan of rodents—typically around two years—mice and rats experience many of the same diseases and effects of aging as humans, but on a vastly accelerated timescale, said Ruth Globus, ISS rodent research project scientist and co-director of the Bone and Signaling Lab at Ames.

> ***"Rodents are similar to humans in that as they age, they undergo detrimental changes such as bone mineral loss and muscle wasting," Globus said. "However, because rodents age much more quickly than humans, studies that would take years to conduct in humans can be done in weeks using rodents."***

This accelerated path to results is even further amplified by spaceflight, which induces rapid physiological changes in humans and animals, including accelerated bone loss and muscle wasting. By housing rodents onboard the ISS, experiments can be designed to better understand the mechanisms of these adverse effects and potentially develop solutions. The results from rodent studies on the ISS could not only enable longer space missions for humans in the future but also translate into new targets for the diagnosis, treatment, and prevention of human disease on Earth.

VALIDATING THE RODENT RESEARCH FACILITY

Rodent Research-1 (RR-1), which launched in September 2014, was the inaugural mission to validate the new ISS Rodent Research Facility. The facility includes modules for transporting rodents to and from the ISS as well as units to handle and house the rodents with ample food, water, and environmental control during the mission (see *The Rodent Research System* below).

The RR-1 mission delivered 20 mice to the ISS—10 as part of NASA-sponsored research and 10 supporting a commercial CASIS-sponsored investigation. The mission also delivered key hardware components to the ISS, including the Rodent Research Hardware System and the bone densitometer, a dual-energy x-ray device designed by Techshot, Inc.

The bone densitometer, an enhanced form of X-ray technology, has for the first time allowed in-orbit measurements of bone loss, soft-tissue density, lean/fat muscle mass, and total animal mass of live mice. A new addition to spaceflight research, the bone densitometer has worked very well onboard the ISS, enabling investigators to successfully monitor the effects of microgravity on rodent bone and muscle, said Rich Boling, Techshot vice president of corporate advancement.

THE RODENT RESEARCH SYSTEM

The Rodent Research Hardware System consists of three main components: the Transporter Unit, the Animal Access Unit, and the Habitat Unit. The Transporter Unit houses rodents on their voyage from Earth to the ISS. The unit can house 20 mice or 12 rats for up to 10 days and contains all the necessary consumables for the rodents.

Once the rodents arrive at the ISS, the Animal Access Unit is utilized to transfer the rodents to their long-term housing unit, the Habitat Unit. The Animal Access Unit allows crew members to insert their hands into the unit through a glove interface and transfer the rodents from one container to another (e.g., to the bone densitometer) to conduct experiments.

Each individual Habitat Unit can house 10 mice or 6 rats for up to 90 days, supplying the rodents with food, water, fresh air, and lighting systems that simulate day and night conditions. The units contain infrared and visible-light cameras as well as other sensors that allow constant monitoring.

1

TRANSPORTER UNIT

2

ANIMAL ACCESS UNIT

3

HABITAT UNIT

NASA/ Ames Research Center/ Dominic Hart

"The scans from the control studies performed onboard the ISS match perfectly with the ground-based studies," Boling said. "This means that researchers now have access to the same bone densitometer equipment onboard the ISS that they do in their ground-based labs."

Additionally, data obtained from the bone densitometer is downlinked from the ISS to researchers on the ground, providing data in near real time.

STUDYING RODENTS IN SPACE

While the primary goal of RR-1 was to validate the performance of the Rodent Research Facility, the translational research experiments performed in this inaugural mission also sought to elucidate microgravity's effects on organisms at a molecular and organ-system level.

For example, tissue samples from the 10 mice flown for the NASA-sponsored study were collected and analyzed for markers of gene expression and tissue responses as well as protein content. These analyses both validated crew procedures for future spaceflight research and provided investigators with detailed information on the biological effects of long-term spaceflight on healthy mice.

Moreover, RR-1 included research designed by investigators at the Novartis Institute of Biomedical Research, a major pharmaceutical company and CASIS commercial partner. The Novartis experiment included five wild-type ("normal") mice and five transgenic ("knock-out") mice that lack a specific gene, MuRF1, reported to trigger muscle wasting by degrading muscle protein. By comparing the effect of microgravity on the two sets of mice, the Novartis team hopes to reveal novel molecular targets for treating skeletal muscle atrophy, a debilitating condition associated with aging and many systemic diseases (e.g., diabetes, cancer, and renal failure).

A NEW PLATFORM FOR INNOVATION AND TRANSLATIONAL BIOMEDICAL RESEARCH

Robust success of the RR-1 mission paved the way for the Rodent Research-2 (RR-2) mission, which included an expanded investigation by the Novartis group. RR-2 built on the results from RR-1 by assessing changes in mouse hind-limb muscles, including skeletal muscle mass and fiber size. Mice in RR-2 were also exposed to

spaceflight for varying periods of time—ranging from 2 to 8 weeks—to determine the progression of muscle wasting over time in the mice.

The ultimate goal of these Novartis rodent research studies is to aid in the development of new therapeutics to prevent or ameliorate the devastating effects of muscle wasting diseases. CASIS continues to facilitate such use of the Rodent Research Facility for research aimed at truly improving human health on Earth. Future Rodent Research missions 3, 4, and 5 (see *Upcoming Rodent Research Missions* below) are all currently slated to take on this grand challenge.

MAKING AN IMPACT

The ability to collect and analyze spaceflight data on individual mice by repeated measures of their bones and muscles accelerates the pace of medical discovery. Such research provides insight into the effectiveness of therapeutics designed to delay medical conditions such as osteoporosis (loss of bone density) and sarcopenia (loss of muscle mass and strength).

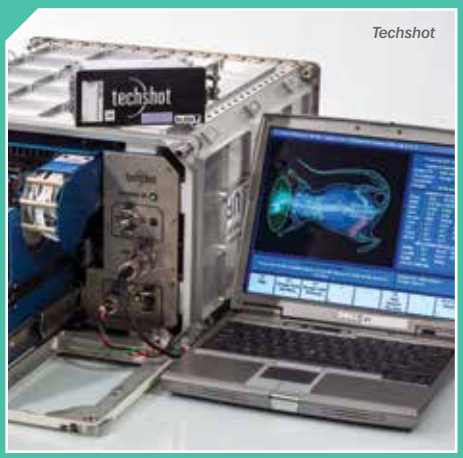
The ISS National Lab is thus a critical platform for research that may improve the quality of life for those of us here on Earth—and the new ISS Rodent Research Facility is a huge step toward augmenting our ability to realize that potential.

Both CASIS and NASA are reaching out to the scientific community to identify disease areas that could benefit from rodent research in a microgravity environment, said Janet Beegle, project manager at NASA's Ames Research Center. This may result in not only new planned missions but also tissue- and data-sharing collaborations (see *Tissue Sharing and the GeneLab Campaign* to the right).

"We want to inform the experts in these disease areas about the capabilities onboard the ISS National Lab," Beegle said. "We then want to work with them to design and implement research missions that will have a significant impact on human health."

UPCOMING RODENT RESEARCH MISSIONS

RR-3	**When:** Scheduled for SpaceX-8 (estimated launch early 2016) **Who:** Eli Lilly & Company, a major pharmaceutical company and CASIS commercial partner
	Aim: Utilize microgravity-induced muscle wasting to determine the impact of an antimyostatin antibody on rodent muscles. **Impact:** No Earth-based mouse models currently exist to study muscle wasting associated with whole-body immobilization, such as being bed-ridden. On the ISS, researchers are able to study predictable muscle atrophy in an otherwise healthy animal.
RR-4	**When:** Scheduled for SpaceX-10 (estimated launch late 2016) **Who:** Indiana University School of Medicine researchers Rasha Hammamieh and Melissa Kacena (supported by the U.S. Department of Defense)
	Aim: Validate bone regeneration studies in spaceflight by evaluating whether new therapies for fracture healing and bone regeneration are more effective than current options. **Impact:** This work aims to improve clinical outcomes for bone healing in injuries resulting from trauma, surgery, or infection. Positive results would increase the breadth of rodent research on the ISS National Lab.
RR-5	**When:** Scheduled for SpaceX-12 (estimated launch 2017) **Who:** University of California Los Angeles researcher Chia Soo
	Aim: Utilize microgravity-induced bone loss to test a novel osteoporosis therapeutic based on the protein NELL-1. **Impact:** Unlike most current therapies for osteoporosis that work by preventing bone loss, this novel treatment also promotes bone formation—potentially combating disease effects on two fronts. Critically, this mission will mark the first attempt by NASA to return live rodents to Earth from the ISS National Lab using the NASA Rodent Research Facility.



Techshot

TISSUE SHARING AND THE GENELAB CAMPAIGN

GeneLab is a NASA initiative to create a centralized bioinformatics system that consists of a sample repository and a database for depositing, accessing, analyzing, and modeling datasets from model organisms exposed to the microgravity environment. GeneLab is being designed to integrate with existing databases containing results from Earth-based studies, and it will facilitate sharing of unique life science data obtained in spaceflight for comparison against carefully matched ground controls.

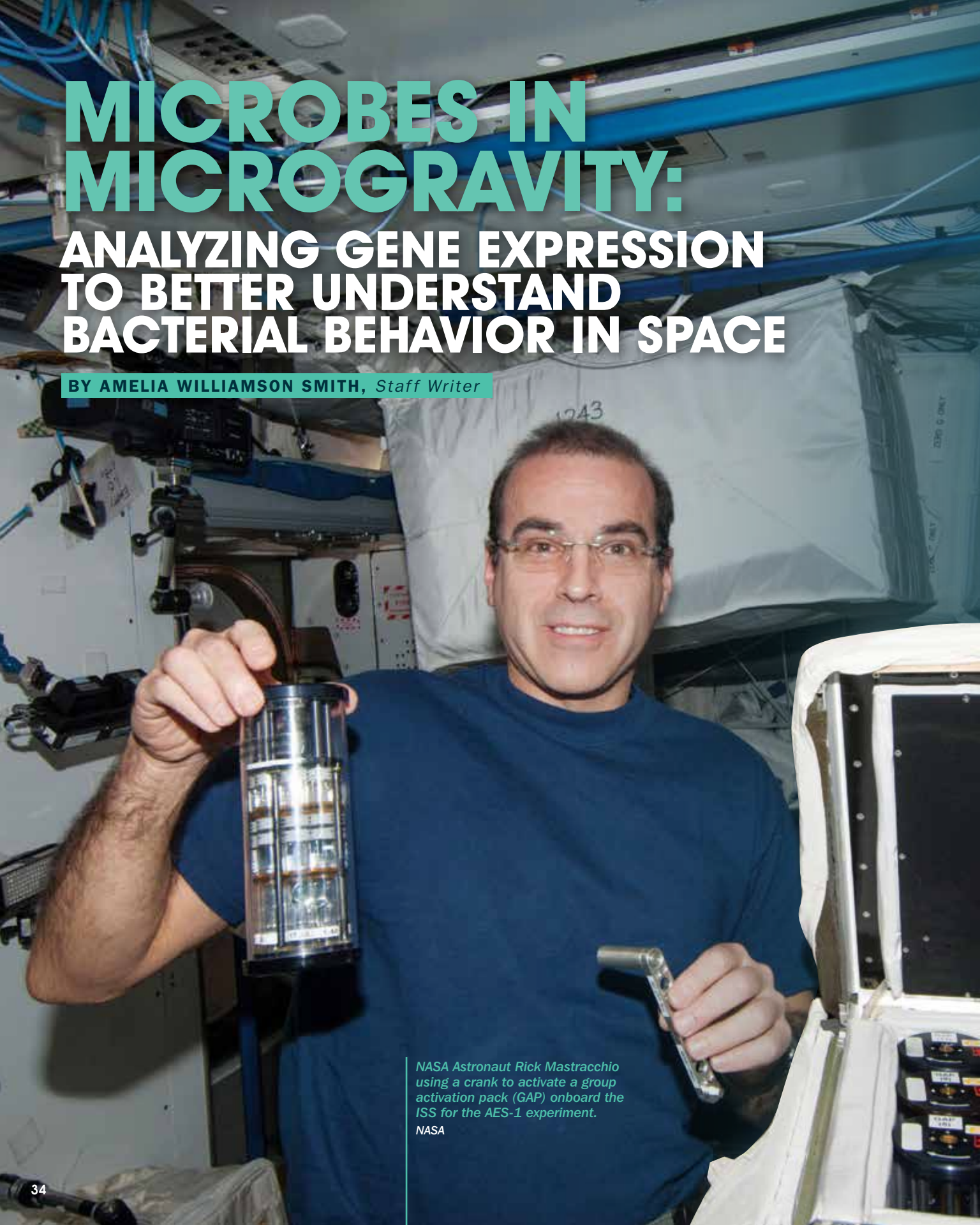
As the open-access, online searchable data repository for data from select rodent research missions and associated tissue sharing initiatives, GeneLab will benefit basic and translational research as the primary gateway to integrated genomics, transcriptomics, proteomics, and metabolomics data for health research comparing disease models in space to those on Earth.

genelab.nasa.gov

MICROBES IN MICROGRAVITY:

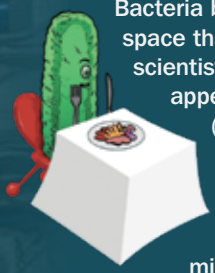
ANALYZING GENE EXPRESSION TO BETTER UNDERSTAND BACTERIAL BEHAVIOR IN SPACE

BY AMELIA WILLIAMSON SMITH, *Staff Writer*



NASA Astronaut Rick Mastracchio using a crank to activate a group activation pack (GAP) onboard the ISS for the AES-1 experiment.
NASA

By observing the health of astronauts that travel into space, scientists have learned that microgravity has important effects on the human body, causing substantial changes to our bones and muscles. However, scientists have also found that microgravity has dramatic effects on far smaller living organisms, such as bacteria.



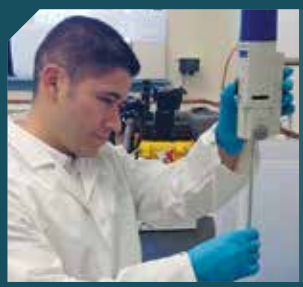
Bacteria behave differently in the microgravity environment of space than they do in a 1-g ground environment. For example, scientists have observed that in space, some strains of bacteria appear to exhibit enhanced growth and increased virulence (ability to cause disease). Additionally, higher doses of antibiotics are needed to kill some bacteria in space.

Scientists believe that these behavioral changes are not necessarily a direct effect of microgravity acting on the bacteria themselves—so what causes these changes in bacterial behavior?

ALTERING THE ENVIRONMENT AROUND BACTERIA

One model—the altered extracellular environment model—hypothesizes that the changes in bacterial behavior are actually an indirect effect of the microgravity environment, said Luis Zea, a researcher at BioServe Space Technologies at the University of Colorado, Boulder. The model postulates that microgravity alters the immediate environment around the bacterial cells, which then leads to changes in bacterial behavior.

"On Earth, there are different flows and forces, such as sedimentation, buoyancy, and convection, that don't exist in space because they are gravity dependent," Zea said. "The model states that it is the lack of these forces and flows that creates a different environment around the bacteria."



Dr. Luis Zea preparing the AES-1 experiment at Kennedy Space Center prior to the launch of Orbital ATK CRS-1.

BioServe Space Technologies

Zea and his team conducted an experiment called Antibiotic Effectiveness in Space-1 (AES-1), in which they compared gene expression data from a nonpathogenic (not disease-causing) strain of *Escherichia coli* bacteria grown onboard the ISS National Lab with cultures grown on the ground. AES-1 was launched to the ISS on Orbital ATK CRS-1 and returned to Earth on SpaceX CRS-3 and SpaceX CRS-4. Results from AES-1—published in the journal *PLoS ONE* in November 2016—strongly support the altered extracellular environment model.

"Through AES-1, we were able to corroborate, for the first time, this altered extracellular environment model that has been hypothesized for decades and could not be proven empirically or computationally," Zea said. Although the AES-1 data only shows correlation and not yet causation, the results shed light on why bacteria behave the way they do in microgravity and open the door to further research on unicellular organisms.

PEEKING INSIDE BACTERIAL CELLS

On Earth, the movement of bacterial cells through their media is influenced by the physical properties of the medium, including gravity-driven forces like buoyancy and sedimentation, as well as other forces, such as the viscosity of the medium. As the cells move, they interact with fresh media and absorb molecules of nutrients. The cells also excrete waste products that may sediment down, float up, or trail behind the cells if they move, while simultaneously diffusing away.

However, in microgravity, these gravity-driven forces are absent, and the transportation of nutrients to cells and waste products away from cells are limited to diffusion-only transport. The altered extracellular environment model hypothesizes that the resulting reduction in the movement of molecules leads to less interaction of the cells with fresh media and thus reduced availability of nutrient molecules for absorption. Additionally, less movement causes waste products to accumulate around the cells, resulting in higher concentrations of potentially toxic compounds. The model postulates that these changes in the immediate environment around the cells are what lead, at least in part, to the changes in bacterial behavior observed in microgravity.

Researchers had tried to confirm the altered extracellular environment model using both physical measurement techniques and computational modeling; however, both methods fell short. Gene expression analysis gave researchers a new way to look at bacteria and test the model. If the model is correct, one would expect to see specific differences in gene expression in the bacteria grown in space versus ground controls, said AES-1 principal investigator David Klaus, professor at the University of Colorado, Boulder and faculty affiliate of BioServe Space Technologies.

"The gene expression data gives us a little peek inside the cell, which we have not had before," Klaus said. "It is another layer that we've peeled back as we continue to try to figure out how bacteria respond to microgravity."

HANDLING TEST TUBES IN SPACE

For the AES-1 experiment, the research team prepared 128 bacterial culture samples to send to the ISS National Lab. The samples were each contained in a fluid processing apparatus (FPA), a test tube specially designed by BioServe Space Technologies for use in microgravity. Each FPA has four separate chambers—the first containing the growth medium with nutrients (glucose molecules), the second containing the *E. coli* bacteria, the third containing an antibiotic, and the fourth containing a fixative to preserve the sample for analysis back on the ground. Two different antibiotics were each tested under 16 conditions (different drug concentrations and fixatives), and all were tested in quadruplicate.

BioServe Space Technologies

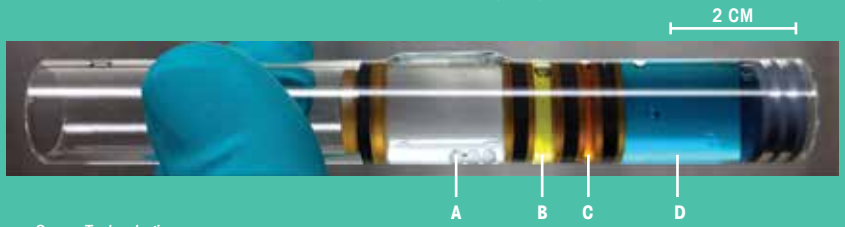
BioServe Space Technologies

Groups of eight FPAs were loaded into 16 BioServe Space Technologies group activation packs (GAP), which are cylinders that hold the FPAs and control the release of the solutions from the different compartments. Once the samples were onboard the ISS, a crew member used a crank to rotate the top of each GAP, introducing the bacteria in each FPA into the growth medium to start the experiment. The bacteria were left to grow, and then the antibiotics of varying concentrations were introduced into the samples using the GAP.

"In space, you can't just take test tubes and transfer the contents from one to another," Klaus said. "The BioServe Space Technologies hardware allows us to first isolate the fluids and then sequentially mix them to start and stop the experiment within a single container without introducing the possibility of fluid leakage in the cabin."

Once the AES-1 flight samples were returned to Earth, the research team performed gene expression analysis on the flight samples and ground controls at the HudsonAlpha Institute for Biotechnology in Huntsville, Alabama. This allowed the research team to examine—at a molecular level—the responses of the bacterial cells to their environment. The team compared the gene expression data from the microgravity-grown bacteria to the ground controls to determine if the flight results matched those predicted by the altered extracellular environment model.

BIOSERVE'S FLUID PROCESSING APPARATUS (FPA)



BioServe Space Technologies

A: 2.75 mL of sterile growth medium with glucose

B: 0.50 mL of *E. Coli* bacteria in minimal medium without glucose

C: 0.25 mL of antibiotic solution

D: 2.10 mL of fixative

CORRELATING OBSERVATIONS WITH THE MODEL

If the model is correct, the microgravity-grown bacterial cells would have reduced interaction with fresh media and thus less nutrients available (even though the same amount of nutrients was provided for the flight samples and ground controls). Therefore, the researchers expected to see signs that the bacterial cells grown in microgravity were experiencing starvation conditions—and, indeed, they did.

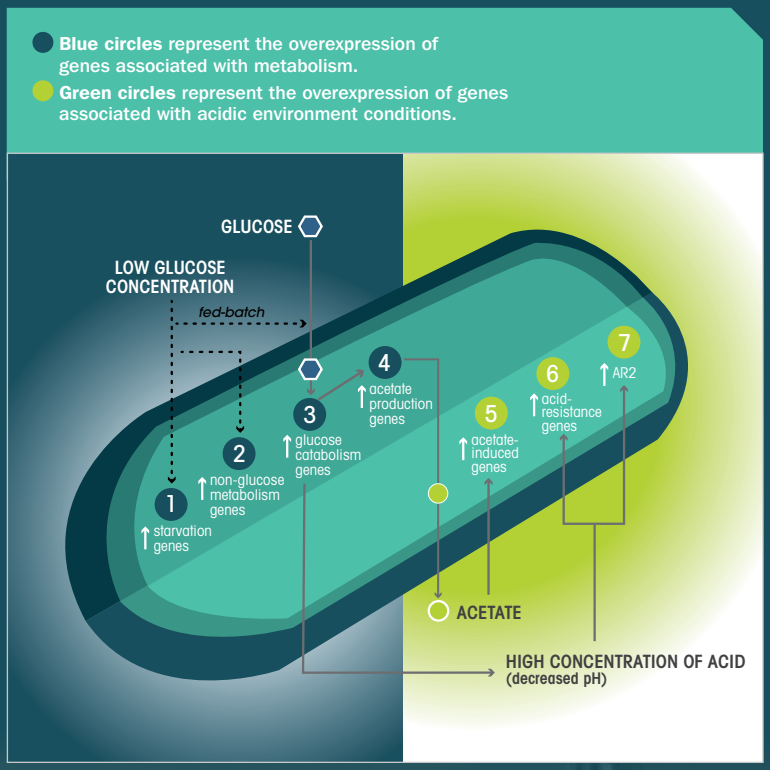
The researchers found an overexpression of genes in the microgravity-grown cells, relative to the ground controls, that indicate the cells grown in space experienced a lack of available nutrients. They also observed an overexpression of genes associated with the metabolism of other carbon sources (non-glucose nutrient molecules) that were not present in the growth medium. "It is believed bacteria do this to be able to change their metabolic pathways as soon as another carbon source becomes available," Zea said. "This can be compared to the risk-prone foraging strategy that some animals use, in which they start engaging in high-risk behaviors looking for food."

Analysis of the microgravity-grown bacteria also revealed an overexpression of genes associated with the metabolism of glucose, which may explain why some types of bacteria exhibit enhanced growth in space. Related to this, the team also found an increased expression of genes associated with acid production—acetic acid is a waste product of glucose metabolism.

It may seem counterintuitive that there was an increase in cell growth, given that the cells were under starvation conditions; however, this same phenomenon has been observed to happen on Earth under a very specific growth condition called fed-batch processing, Zea said. Scientists have found that higher bacterial cell counts occur when nutrients are absorbed incrementally (such as under starvation conditions), as opposed to when there is a steady absorption of nutrients (under normal batch conditions).

If the altered extracellular environment model is correct, the researchers also expected to see signs of higher concentrations of waste products, such as acetic acid, around the microgravity-grown cells due to the reduced mass transport of chemical compounds around the cells—and, again, they did.

ALTERED EXTRACELLULAR ENVIRONMENT MODEL



Zea L, Prasad N, Levy SE, Stodieck L, Jones A, Shrestha S, et al. (2016) A Molecular Genetic Basis Explaining Altered Bacterial Behavior in Space. PLoS ONE 11(11): e0164359.

BioServe Space Technologies

In the microgravity-grown bacteria, the team found an overexpression of genes associated with acid resistance, suggesting increased acidity in the environment around the cells. However, the team did not observe differences in acidity of the bulk fluid around the cells in microgravity versus ground controls, suggesting the acidic environment in microgravity is limited to the immediate area around the cells. The buildup of acid may also explain the observed increase in virulence of some bacteria in space, as researchers have seen a correlation between an increase in acidity and an increase in virulence in certain bacterial strains, Zea said.

Together, these results provide strong support for the altered extracellular environment model. "I think the gene expression data was a real breakthrough," Klaus said, "but it doesn't prove cause and effect; it only shows correlation. Moving forward, we can begin to ask more definitive questions to get a much better understanding of what's going on."

Additional research is needed to confirm why higher concentrations of antibiotics are needed to kill bacteria in space. However, the altered extracellular environment model suggests that it may not be that bacteria have increased resistance to antibiotics in space, but instead, encounter less antibiotic due to the reduced concentration of antibiotic drug molecules around the cells.

NASA

"We're trying to differentiate between bacterial drug resistance and bacterial susceptibility to drugs," Zea said. "Is it that fewer drug molecules are reaching the cell due to the altered extracellular environment, thus physically reducing susceptibility, or is it that the cells are turning on resistance mechanisms more effectively? Or, it could be a mixture of the two, or something else altogether—that question is still open."

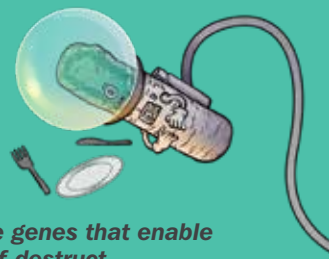
ADVANCING KNOWLEDGE OF CELLS IN SPACE

The microgravity environment on the ISS National Lab allows researchers to probe the interactions between bacterial cells and their environment, and the resulting influences on bacterial behavior, in unique ways. "We're trying to understand what's going on at the interface of a cell and its environment," Klaus said. "And in the absence of gravity, you can tease out some of these relationships in ways that are not really possible to recreate on Earth."

Understanding changes in bacterial behavior in space is important not only because it helps better protect astronauts from infection during future long-duration spaceflight missions, but it also illuminates the mechanisms of bacterial behavior in our bodies on Earth. Additionally, an understanding of the mechanisms by which the altered extracellular environment in space triggers behavioral changes in microorganisms and our own cells informs research aimed at developing new vaccines, uncovering novel molecular targets against drug-resistant bacteria, and developing new antibiotics.

"There's always an interplay between basic research and applied research, and they build on each other," Klaus said. "So the more we understand the fundamental mechanisms, the more we can then use that knowledge to go after the real end goal—the development of application-oriented products." ■

MAKING BACTERIA SELF-DESTRUCT



While analyzing the AES-1 gene expression data, the research team found something interesting about the genes that enable bacterial cells to self-destruct.

It is believed bacteria self-destruct under certain types of stress to ensure the survival of the colony. Scientists have long wondered if this mechanism could be exploited to kill bacteria that have become resistant to antibiotics by activating the genes that control self-destruction, often called "suicide" genes.

"The problem is that if you turn on the known activator for the suicide genes, then you're also activating 156 other genes that actually make the bacteria worse," Zea said.

The suicide genes are actually a pair of genes—one is a toxin, which triggers self-destruction, and the other is an antidote, which keeps the toxin gene under regulation. The AES-1 data showed a 24-fold increase in the expression of the toxin gene and a 40-fold underexpression of the antidote gene in one of the 16 tested conditions. And the interesting part, Zea said, is that the known activator for the genes was not turned on—which means that perhaps there is a second unknown activator.

"In space, the antidote gene was turned off and the toxin gene was overexpressed by 24 times, which is rather remarkable," Zea said. "Those out-of-the-roof numbers combined with the fact that the known activator was not differentially expressed indicates that there may be another activator that we could look into as a potential target for novel drugs."

For AES-1, each condition was tested in quadruplicate in microgravity and in ground controls. The research team found this overexpression of the toxin gene and underexpression of the antidote gene in all four microgravity replicates of the tested condition, but not in any of the ground samples.

An indication of a possible second activator has never before been observed, and the team hopes to probe this further in future microgravity research. If a novel activator is discovered, it could eventually lead to the development of a new class of antibiotics.

Expanding Horizons for Microbiome Research on the ISS

SPOT LIGHT

BY HEATHER ANDERSON, *Contributing Author*

Microorganisms, whether fungi, archaea, bacteria, or viruses, are often disparaged as germs with nothing better to do but cause disease. However, scientific advances to better understand the form and function of the human microbiome (the collection of microorganisms that live in and on people) have revealed that the blind eradication of microorganisms is not the answer even if it were possible. In fact, each of us has a unique microbiome that is a vital part of human health, and healthy microbiomes play an active role in protecting us from disease. Healthy microbes (and their interactions with each other as part of the human microbiome) need to be identified and fostered to sustain health.

NASA astronaut Ricky Arnold swabbed surfaces in the ISS to collect microbe samples and then processed the microbial DNA using the Biomolecule Sequencer, a device that enables DNA sequencing in microgravity, to identify microbes able to survive in microgravity.

NASA

The ISS National Lab workshop "Exploring the Microbiome/Immunome and Disease on the International Space Station" focused on how the spaceflight environment might accelerate research to better understand the microbiome and its role in maintaining the balance between health and disease. The workshop brought together more than 40 thought leaders from academia, government, and the private sector to discuss ways in which they could work together to improve human health on Earth through spaceflight microbiome research.

Because the microbiome involves complex community interactions of numerous species, many of which are known only by their DNA signatures, it is difficult to fully characterize how the microbes on skin or in the gut contribute to health and disease or how the balance within microbial communities changes in response to the environment. However, research conducted on Earth has shown that our microbiome performs many functions essential to human health, such as making otherwise inaccessible nutrients digestible, providing essential vitamins and nutrients, and protecting us from pathogens.

For example, healthy microbiomes transplanted into individuals with recurrent gut infections are an effective clinical treatment. Moreover, the gut microbiomes of obese people are different from those of lean people, and the microbiomes of people with autism are different from those of people without autism. Because the microbiome may be involved in such diverse conditions, it is critical that researchers find ways to more fully understand its behavior and function—which is where the ISS National Lab may provide an advantage by offering an alternative platform for studying the microbiome.

Spaceflight induces sudden but persistent and profound effects on the human body. Studying the microbiomes of humans and animal models onboard the ISS provides a window into how microbial communities associated with different environments or locations on the body respond to stimuli such as stress, dietary changes, and immune dysfunction—all of which are known to impact the microbiome.

Thus, characterizing the microbiome's response to spaceflight may yield insights into the complex community interactions that underlie the microbiome's beneficial—or detrimental—effects on human health. Better understanding these interactions will help medical professionals devise new approaches to leverage those beneficial effects and combat detrimental effects here on Earth.

Participants of the ISS National Lab workshop provided recommendations aimed at maximizing the impact of microbiome research on the ISS, facilitating collaborations and public-private partnerships in support of such initiatives, and standardizing research approaches. These recommendations, which are detailed in a report released in July 2019, are helping to define the path forward in developing a sustainable microbiome research program on the ISS National Lab.

> *"The workshop was a great opportunity to hear from a wide range of experts interested in using the ISS to study human health from a microbiology and immunology perspective," said Alexander Voorhies, staff scientist at the J. Craig Venter Institute, who attended the workshop. "The ISS is a collaborative endeavor by its nature, and bringing diverse scientists together will hopefully inspire collaborative investigations into making space more habitable for humans."* ■

This content originally appeared in Upward at upward.issnationallab.org/expanding-horizons-for-microbiome-research-on-the-iss.

Chips in Space Improve Treatment Options for Osteoarthritis

SPOT LIGHT

BY EMILY TOMLIN, *Staff Writer*

In an era of escalating death and damage from the opioid epidemic, innovative solutions to prevent the more than 130 daily U.S. deaths from opioid overdose are a national priority. Within this context, a major focus area for the healthcare industry is to better understand the underlying causes of chronic pain and establish pain management strategies that avoid the risks of opioid addiction—and a research team from the Massachusetts Institute of Technology (MIT) recently took this quest to space.

Of the more than 50 million Americans living with chronic pain, nearly 6 million suffer from long-term osteoarthritis that results from a previous joint injury. Vehicle accidents, sports injuries, and other sources of joint trauma can lead to post-traumatic osteoarthritis (PTOA), even in young adults. Approximately 50% of individuals with a knee injury develop PTOA within 10 years, and 30% of military personnel develop PTOA from combat injuries.

Sadly, restoring joint stability via anterior cruciate ligament (ACL) reconstruction in the knee, for example, does not reduce the risk of developing PTOA. In fact, there is currently no approved therapy to prevent the lifelong pain and dysfunction of PTOA, which is a leading cause of disability worldwide. The MIT investigation, led by Alan Grodzinsky, launched to the ISS National Lab on SpaceX's 17th commercial resupply services mission and seeks to develop a new approach to preventive care for PTOA.

Little is known about the molecular mechanisms underlying PTOA initiation and progression, but inflammation occurring immediately after joint injury appears to play a key role in the onset of chronic PTOA. The inflammation will resolve for some, but for others, the silent damage continues despite the absence of symptoms and apparent progress toward recovery. Previous PTOA research on trauma to cartilage has largely ignored the complex biomechanics involved in inflammation—some of which may be key to early medical intervention.

Canadian Space Agency astronaut David Saint-Jacques works on the MIT tissue chip experiment onboard the ISS

NASA

Grodzinsky's team is using a different approach: a tissue chip containing multiple cell types to mimic the fundamental biomechanics of how bone and cartilage move and interact with the synovial membrane (the joint-lining tissue that produces a lubricating fluid). The team's ISS National Lab experiment validated this tissue chip model both on the ground and in space, where bone and cartilage damage may be accelerated.

In orbit, the team exposed the tissue chip to pro-inflammatory molecules, simulated an impact injury, tested several therapeutics, and preserved samples of cells and secreted molecules at several experimental stages for postflight analysis. They used tissues from different donor individuals in these studies to characterize complex tissue chip responses to both "injury" and response to therapy.

The study holds the potential for new biomarker discovery that will enable early diagnosis and reveal the mechanisms that underlie individual variability in response to both injury and treatment. Successful preventive treatment following injury, during the 10 to 20 years of asymptomatic damage in at-risk individuals, could potentially drastically reduce the number of patients who develop chronic pain.

This type of early intervention is common in the management of diabetes, heart disease, and osteoporosis, but for those individuals suffering from chronic pain, preventive medicine is still beyond reach. Creative and advanced biomedical approaches to studying disease—on Earth and in space—can hopefully provide nonopioid relief to the military personnel, student athletes, and millions of others afflicted by PTOA or other causes of chronic pain.

Grodzinsky's ISS National Lab project is one of several tissue chip studies co-sponsored by the National Institutes of Health's National Center for Advancing Translational Sciences (NIH NCATS), complementing other spaceflight tissue chip experiments co-sponsored by NIH's National Institute of Biomedical Imaging and Bioengineering (NIBIB) and the National Science Foundation (NSF).

This content was abridged from an article that originally appeared on ISS360 at issnationallab.org/blog/joint-jeopardy-mit-researchers-use-space-to-solve-the-chronic-pain-of-osteoarthritis.

Rethinking Rodent Research in Space: Concept & Design

SPOT LIGHT

BY AMY ELKAVICH, *Staff Writer*

Building on the scientific success of previous missions using animal models, the Rodent Research Reference (RRR) Missions adapt the standard rodent research format to maximize science return and resource utilization via tissue sharing—providing multiple investigators access to biospecimens from a single mission. Following the successful first RRR Mission, which launched to the ISS on SpaceX's 16th commercial resupply services mission in December 2018, the ISS National Lab announced its second RRR Mission for investigators seeking access to biological specimens to support fundamental biomedical inquiries.

NASA astronauts Scott Kelly and Terry Virts conduct rodent research investigations within the Microgravity Science Glovebox and the Rodent Habitat Module onboard the ISS.

NASA

The persistent microgravity environment on the ISS National Lab has profound effects on living organisms that can mimic the onset and progression of disease here on Earth, providing researchers with valuable information about the mechanisms behind diseases such as cancer and possible new methods of treatment. Mice and rats, which share many of the same genes and physiological characteristics as humans, have served as exceptional translational models in space-based research since the early 1980s.

The RRR Missions provide opportunities for investigators to obtain biospecimens from animal tissues that have been exposed to the spaceflight environment for a wide variety of research purposes. Insight gained from the RRR Missions may help advance research on diseases and aging effects involving muscle, bone, and other organ systems.

Each RRR Mission is a partnership between the ISS National Lab, NASA, and Taconic Biosciences. Taconic provides, at no cost to the mission, selectively bred and genetically engineered mice and rats for research use to advance our understanding of human disease. The objective of each mission is to generate data that validate the rodent research model in space for the benefit of medical science on Earth.

The significance of the RRR Missions rests in the adoption of an innovative and customizable mission concept using a standardized approach to operations and habitat configuration that both benefits researchers and maximizes ISS National Lab resources. Through the use of a simple and reproducible mission architecture based on use of the most commonly used genetic strains of mice, it is possible for RRR Missions to be quickly integrated for spaceflight to take advantage of missions of opportunity. In this way, the new RRR Mission concept has the potential to improve resource allocation by optimizing use of available space onboard flight vehicles.

Additionally, the RRR Mission concept significantly reduces the need for extensive feasibility assessment of individual rodent research investigations, enabling the ISS National Lab to rapidly implement RRR Missions, often reducing the time from mission concept to flight.

The RRR Missions join the existing Rodent Research Program Missions led by single investigators as a supplemental yet crucial element of a pathway for new partnerships across disciplines and industries. Partnerships with funders and investors, commercial service providers, and commercial suppliers introduced through this experimental design have the potential to expand access to invaluable biomedical research specimens, data, and knowledge. Additionally, cooperative partnerships such as these enable the costs of mission planning to be reduced while expanding access to specimens and data.

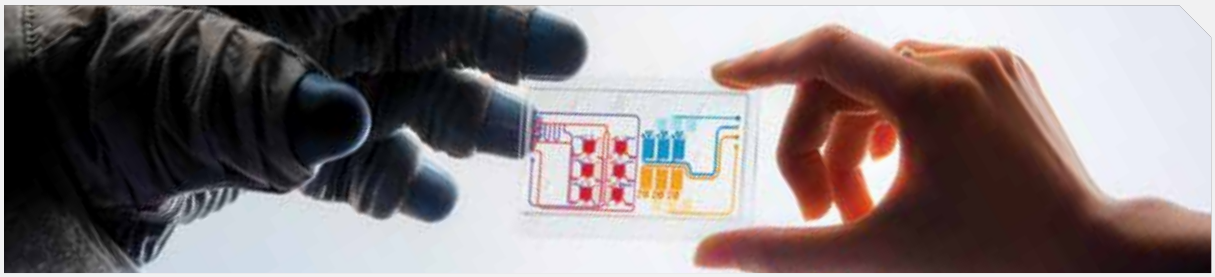
The introduction of the RRR Missions, conducted alongside the existing Rodent Research Program missions, may advance scientific knowledge to benefit human health here on Earth and reflects the ISS National Lab's commitment to delivering meaningful scientific advancement back to the U.S. taxpayer.

This content was abridged from an article that originally appeared on ISS360 at issnationallab.org/blog/rethinking-rodent-research-concept-design.

Collaborating with NIH on Tissue Chips in Space

BY AMELIA WILLIAMSON SMITH, *Staff Writer*

SPOT LIGHT



John Carlano; courtesy University of Pennsylvania School of Engineering and Applied Science, and Children's Hospital of Philadelphia

The ISS National Lab enables a wide variety of research aimed at advancing the field of regenerative medicine—from cell-based studies to organoid growth investigations, tissue chip research, and even the development of facilities capable of 3D bioprinting human tissues.

The ISS National Lab released its first research solicitation in the field of regenerative medicine in 2013, focused on stem cell research. Since then, the ISS National Lab has continued to support important projects and activities in the area of regenerative medicine, with 19 payloads in this research area flown to the ISS and 25 publications resulting from ISS National Lab research and development (R&D). Currently, there are 46 regenerative medicine projects in the ISS National Lab portfolio, supported by eight in-orbit commercial service providers.

Additionally, the ISS National Lab has partnered with the National Institutes of Health (NIH) and the National Science Foundation (NSF) on multi-year programs that support regenerative medicine research on the ISS National Lab. These collaborations have resulted in grant commitments from NIH and NSF totaling more than $26 million.

Advances in bioengineering have led to the development of tissue chips—systems containing human cells grown on artificial scaffolding to represent functional units of an organ. Such tissue chips mimic the 3D structure and function of human tissue, enabling accelerated and higher-accuracy drug screening. Tissue chips also provide improved models of disease that could help scientists uncover the molecular mechanisms behind a wide range of conditions affecting people on Earth.

In 2016, the National Center for Advancing Translational Sciences (NCATS), part of NIH, announced a four-year collaboration with the ISS National Lab to support the use of tissue chip technology for translational research onboard the ISS to benefit human health on Earth. In June 2017, five initial projects were awarded grants through the NCATS Tissue Chips in Space initiative. In December 2017, the ISS National Lab, NCATS, and the National Institute of Biomedical Imaging and Bioengineering (NIBIB)—also part of NIH—announced a second funding opportunity supporting tissue chip research in space, and four awardees were announced in October 2018.

The five initial Tissue Chips in Space investigations have already launched to the ISS National Lab:

- *A team of researchers at the University of California, San Francisco is using tissue chip technology to examine immune function in microgravity to better understand changes in the immune system that occur as people age.*
- *A project from Massachusetts Institute of Technology aims to study the effects of spaceflight on musculoskeletal disease using a cartilage-bone-synovium joint tissue chip model.*
- *Emulate, Inc. seeks to study microgravity's effects on blood-brain barrier physiology to develop and validate a proprietary tissue chip platform for experiments using human cells.*
- *The Children's Hospital of Philadelphia seeks to test tissue chip systems of the human airway and bone marrow to model how the respiratory and immune systems interact to fight infection.*
- *A University of Washington investigation will study tissue chip systems that model the human kidney to better understand proteinuria (a condition in which a person's urine contains an abnormal level of protein), kidney stone formation, and the body's use of Vitamin D.*

NIH Director Francis Collins shared his thoughts on sending human tissue chips to space in the NIH Director's Blog, and NCATS Director Dr. Christopher Austin discussed the importance of tissue chip research onboard the ISS National Lab in a *Scientific American* "Observations" article. For more information about the Tissue Chips in Space initiative, visit https://ncats.nih.gov/tissuechip/projects/space. ■

This content was abridged and updated from an article that originally appeared on ISS360 at issnationallab.org/blog/collaborating-with-nih-on-tissue-chips-in-space.

Designing Better Drugs: Piecing Together Protein Function Through Structure

SPOT LIGHT

BY AMELIA WILLIAMSON SMITH, *Staff Writer*

Proteins—large molecules made up of hundreds to thousands of amino acids—are vital to the proper function of tissues and organs in our body. Each protein has a unique three-dimensional folded structure based on the sequence of amino acids, and this structure determines the protein's function. The more scientists know about a protein's structure, the better they can understand its function and design drugs to work with the protein and treat disease.

To determine a protein's structure, scientists can crystallize the protein and use methods such as X-ray crystallography or neutron crystallography to figure out the position of the atoms within the protein. Neutron crystallography provides greater structural detail than X-ray crystallography because it allows researchers to determine the position of hydrogens within the protein's structure. However, neutron crystallography requires large, high-quality crystals of the protein that are difficult to obtain on Earth.

The ISS National Lab is a valuable platform for molecular crystal growth because crystals grown in microgravity are often larger and more well-ordered than Earth-grown crystals. The reduction of gravity-driven forces such as convection and sedimentation allows for a slower and more orderly incorporation of molecules into the crystalline lattice, and analysis of higher-quality protein crystals enables better structure determination. Two recent ISS National Lab investigations are seeking to use microgravity to grow high-quality crystals of medically important proteins for improved structure determination.

An investigation from Oak Ridge National Laboratory that launched on SpaceX's 14th commercial resupply services (CRS) mission is focused on crystallizing an enzyme called AChE that is affected by nerve gas and pesticide poisoning. Organophosphates are a type of chemical compound used in nerve gas and pesticides that target the AChE enzyme and cause it to malfunction, leading to paralysis and death. Organophosphate poisoning is responsible for more than 200,000 deaths worldwide each year.

The research team aims to use microgravity to grow AChE crystals that are large enough for analysis using neutron crystallography. Knowing the position of hydrogen atoms in AChE's structure is key to understanding how the enzyme functions and how it might be affected by organophosphates. This information could lead to the development of faster-acting antidotes to pesticide and nerve gas poisoning that could save more lives.

An investigation from Frederick National Laboratory for Cancer Research that launched on SpaceX CRS-16 is aiming to leverage the ISS National Lab to crystallize KRAS proteins. KRAS is the most frequently mutated member of the RAS family of genes—mutations of which account for more than 30 percent of all human cancers. Mutations in the KRAS gene are responsible for 95 percent of pancreatic ductal adenocarcinoma (the most common type of pancreatic cancer), one-third of non-small cell lung cancer (the most common type of lung cancer), and up to half of colorectal tumors.

European Space Agency astronaut Alexander Gerst working on the KRAS protein crystallization experiment onboard the ISS.

NASA

Lung, colorectal, and pancreatic cancers are among the deadliest and most costly types of cancer to treat. According to the World Health Organization, cancer caused an estimated 9.6 million deaths in 2018. The economic burden of cancer is also significant, and the World Health Organization reports that in 2010, the total annual economic cost of cancer was estimated to be $1.16 trillion.

Even after decades of research, there are currently no inhibitors that target RAS genes such as KRAS. It is difficult to obtain high-quality crystals of full-length KRAS proteins on Earth, and crystallization in microgravity may yield improved crystals that could aid in structural determination and lead to the development of inhibitors to treat cancers associated with the KRAS gene. In this ISS National Lab investigation, the research team sought to crystallize full-length unmodified KRAS proteins, cancer-causing KRAS mutants, and KRAS proteins in complex with various small molecule inhibitors. ■

This content was abridged from an article that originally appeared on ISS360 at issnationallab.org/blog/designing-better-drugs-piecing-together-protein-function-through-structure/.

Gluing Bones and Speeding New Bone Growth

BY ANNE WAINSCOTT-SARGENT, *Contributing Author*

SPOT LIGHT

Since 2013, the ISS National Lab has partnered with MassChallenge, the largest startup accelerator to award nondiluted funds to early-stage entrepreneurs. Founded in Boston, Massachusetts, MassChallenge has grown to become part of a movement to support entrepreneurs through grant competitions worldwide.

The ISS National Lab has also partnered with The Boeing Company since 2014 to award grants through a MassChallenge "Technology in Space Prize." Annually, this prize is awarded to several promising companies to support projects to be conducted onboard the ISS National Lab. Together, Boeing and the ISS National Lab have allocated more than $4.5 million in funding toward this prize since its inception.

A MassChallenge-funded project from LaunchPad Medical used the ISS National Lab to test a new biomaterial that can glue bones together. The injectable glue, Tetranite®, has the potential to speed new bone growth while reducing the recovery time and pain of patients, particularly in the orthopedic world, said Brian Hess, LaunchPad Medical CEO and an engineer by trade. Located in Lowell, Massachusetts, LaunchPad Medical was founded in 2014 by Hess and a team of entrepreneurs with extensive expertise in startup medical device development and commercialization.

The company's experiment, which launched on SpaceX CRS-13 in December 2017 and returned with SpaceX CRS-13 in January 2018, examined how osteoblasts—the cells responsible for bone formation—reacted in the presence of Tetranite®.

"These cells typically slow down when in microgravity, because the space environment accelerates osteoporosis," Hess said, citing the well-known statistic that without intervention, astronauts can lose, on average, up to 2% of their bone mass for every month they spend in space.

In LaunchPad Medical's ISS National Lab investigation, the research team examined how the osteoblasts responded to Tetranite® in comparison with osteoblasts alone and osteoblasts in the presence of an existing commercially available bone-graft product. ISS crew members captured photos of the cells to show their health and growth while also collecting cell culture media every five days to study secreted molecules that relate to bone-cell behavior. Finally, cellular RNAs from the beginning and end of the experiment were compared to evaluate changes in the expression of genes related to bone growth.

Analyses revealed how the spaceflight osteoblasts responded to Tetranite® by growing and exhibiting bone-formation behaviors. "We saw favorable gene-expression changes marking bone growth that will help advance our studies," Hess said. "To see this response in the extreme and rapid osteoporosis-inducing space environment motivates us to study Tetranite® for use in patients with osteoporosis, who are susceptible to a bone injury because of their poor bone quality and whose recovery from injury is quite challenging."

NASA astronaut Joe Acaba working on the LaunchPad Medical investigation onboard the ISS National Lab.

NASA

Hess anticipates beginning human trials within the next year and will initially target the dental industry by providing glue to immediately stabilize tooth implants, as opposed to the current treatment plan, which often requires staged surgeries. However, Tetranite® has the most significant potential impact for older people who suffer from osteoporosis. According to the International Osteoporosis Foundation, more than half of the U.S. population ages 50 years and above have either osteoporosis or low bone density. Studies suggest that approximately one in two women and up to one in four men in this age range will break a bone because of osteoporosis.

"Our first program will focus on dentistry and then orthopedics, targeting specific parts of the body, including the spine, cranium, joints, and extremities," Hess said.

Having an injectable glue that naturally resorbs and is replaced with bone would eliminate or reduce the need to make large incisions in order to drill holes to implant metal screws or other hardware devices to repair damaged bone. Moreover, patients would not have to return for later surgeries to have hardware removed.

"Tetranite® could change the landscape of how clinicians treat bone problems and improve patients' experience by reducing pain and accelerating healing," Hess said. "It potentially could be a real revolution to the surgical field of orthopedics.

This content was abridged and updated from an article that originally appeared in Upward at upward.issnationallab.org/masschallenge-grantees-move-early-stage-innovations-forward.

Going Beyond Earth's Limitations to Understand Parkinson's Disease

SPOT LIGHT

BY SARA CARNEY, *Contributing Author*

NASA astronaut Anne McClain works on The Michael J. Fox Foundation protein crystallization experiment onboard the ISS. NASA

We know that the symptoms of Parkinson's disease originate from a decrease in dopamine production due to the loss of neurons. But why are the neurons lost? Is there a genetic cause?

According to more than a decade of research, a mutation in a gene known as LRRK2 (leucine-rich repeat kinase 2) appears to be linked to Parkinson's disease in some patients. However, despite the knowledge that this gene may be involved in some cases of the disease, researchers have been unable to unlock the full potential of this genetic research because of the difficulty in studying its gene product, LRRK2 protein.

The Michael J. Fox Foundation, which is dedicated to finding improved therapies and ultimately a cure for Parkinson's disease, has partnered with the ISS National Lab to investigate the structure of the LRRK2 protein by crystallizing it in microgravity. For some proteins, crystallization in space produces larger and higher-quality crystals than on Earth.

"Therapies targeting LRRK2 are already advancing, even though we don't know its atomic structure," said Marco Baptista, Director of Research Programs at The Michael J. Fox Foundation. "But we can't yet perform structure-based drug discovery that fully leverages the insight of the drug's binding pocket. To get an accurate picture of the structure, researchers need LRRK2 protein crystals that are large and have few defects. However, LRRK2 crystals grown on Earth suffer from limitations in these areas."

Protein crystals grown onboard the ISS National Lab don't grow like they do on Earth—with less pull from gravity, crystals can grow larger and in a more uniform and ordered pattern. Once the large, well-ordered crystals return to Earth, they are then easier to observe using high-resolution imaging techniques. Thus, crystallization of LRRK2 on the ISS could lead to improved structure determination of LRRK2 and help advance structure-based drug design.

The Michael J. Fox Foundation's first investigation to crystallize LRRK2 on the ISS National Lab launched on SpaceX's 12th commercial resupply services (CRS) mission. Although the resulting crystals were of high quality, they were not large enough to improve structure determination of LRRK2.

The Michael J. Fox Foundation sent a second investigation to the ISS National Lab on Northrop Grumman CRS-10 to crystallize LRRK2 using a different type of hardware to enable larger crystal growth. This time, the team, which also includes researchers from Merck & Co. and Goethe University Frankfurt, was also able to monitor the crystals in orbit and make any necessary adjustments to improve crystal growth. The results from this experiment could help researchers gain new knowledge in the fight against Parkinson's and other diseases.

Interestingly, the knowledge gained from this and related studies could also be beneficial for those who have Parkinson's disease but do not have the LRRK2 mutation. Even when mutations in other genes may be at fault, many of the symptoms of Parkinson's disease are the same. Thus, treatments developed using the knowledge gained from studying LRRK2 could be broadly effective for the one million people in the U.S., and 6 million worldwide, currently living with Parkinson's disease.

This content was abridged from an article that originally appeared on ISS360 at issnationallab.org/blog/going-beyond-earths-limitations-to-understand-parkinsons-disease.

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