Drug discovery and development: the final frontier

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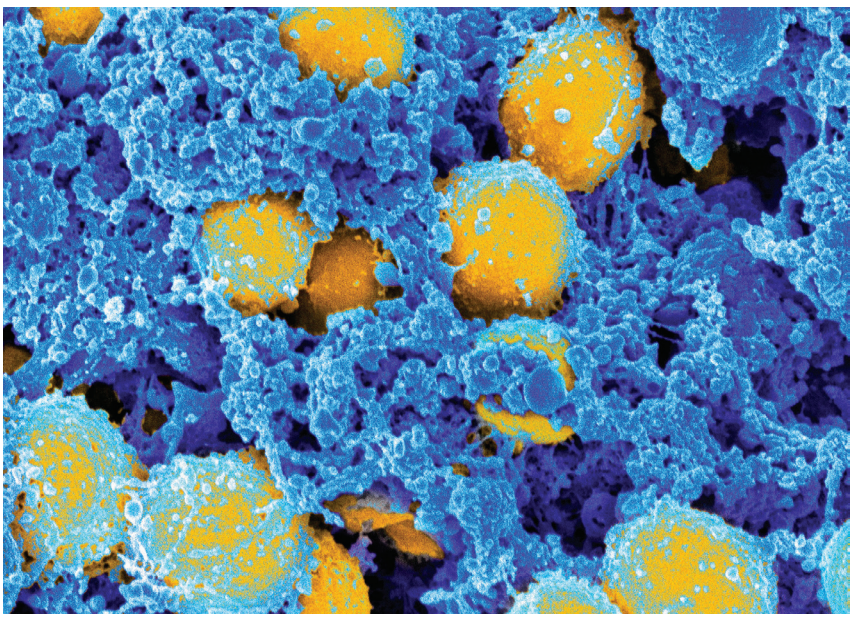
Space offers a unique environment for studying disease processes, as well as for drug discovery and development. Mark Greener discusses how research conducted in microgravity in space is allowing medicine to boldly go where it has never gone before.



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Certain bacteria such as *Staphylococcus aureus* (pictured) and *E. coli* seem to show increased resistance to several antibiotics when cultured in space

Since Yuri Gagarin made a single Earth orbit in 1961, about 570 humans have spent a total of more than 150 years in space.[1] We've also launched a menagerie into orbit and beyond. In 1968, the Russian Zond 5 mission sent bacteria, flies, mealworms and two tortoises around the moon and back. The tortoises lost 10% of their body weight, but otherwise seemed fine.[2] Nevertheless, space travel causes marked physiological changes: like the Russian tortoises, astronauts lose body weight, for example.

So, space offers the opportunity to investigate physiological pathways in a unique environment, which, in turn, raises the prospect of identifying new therapeutic targets for diseases as diverse as cancer, antibiotic-resistant infections, muscular atrophy and osteoporosis. Astronauts can also grow crystals of unprecedented quality in space – a first step in designing many drugs. Indeed, insights gained in space helped inform the development of several recently marketed drugs. "Research aboard the International Space Station [ISS] improves life on Earth, supports the development of a space economy and enables human exploration beyond the Earth, including the Moon and Mars," says David Brady, NASA's ISS Associate Program Scientist.

Into orbit

Gravity has been a fact of life on Earth ever since the first organisms emerged, probably near undersea hydrothermal vents, 3.77 billion to 4.29 billion years ago.[3] Indeed, every cell seems able to sense changes in gravity.[4]

The first tetrapods to tentatively explore land 370 million years ago evolved numerous adaptations to support body weight, regulate fluid and move.[4] One early tetrapod (*Ichthyostega*), for example, evolved bony projections that interlocked

its vertebrae to help support its weight as it walked through swamps.[5]

"Water offers buoyancy," says Martin Braddock from Sherwood Observatory, Sutton-in-Ashfield, Nottinghamshire. "So, terrestrial animals needed to develop a strong skeleton. However, although the effect of Earth gravity has remained the same since life emerged, that will clearly change if we establish long-term colonies on Mars or the Moon. We already know that reduced gravity can cause dramatic physiological changes."

Low Earth orbit (less than 2000km above sea level) exposes astronauts to 'microgravity'. The ISS orbits some 350km above the earth: about 0.1% of the distance to the moon, where gravity is about 92% of that at ground level. But the ISS orbits at about 28,000km per hour, so its acceleration matches the Earth's curvature. This counterbalances gravity, thereby creating 'weightlessness'.[4,6,7]

Numerous studies show that microgravity produces profound physiological changes in plants, microbes and mammals. Astronauts, for instance, show a rapid decline in bone mineral density (BMD), especially at sites supporting body weight, such as lumbar spine, femoral neck and trochanter, pelvis, calcaneus and leg.[4] "Astronauts tend to lose 1% of their BMD a month despite being ultra-fit, working out and taking anti-resorptive drugs such as bisphosphonates,"[8] says Dr Braddock, who also leads respiratory drug development projects for a major pharmaceutical company. Broadly, the rate of BMD loss during a six-month stay on the ISS is similar to that occurring between the 5th and 6th decade of life on Earth.[4]

Indeed, cardiovascular, musculoskeletal, nervous, gastrointestinal and immune responses to microgravity are similar to changes associated with ageing, but seem to occur about 10 times faster in space than on Earth.[4] So, microgravity offers a unique test bed for musculoskeletal drug discovery and development.[9] "Medications have been studied as a countermeasure to bone loss that occurs due to extended stays in microgravity," Mr Brady says. "This will be needed to ensure that humans are able to perform surface tasks when they arrive at distant destinations like Mars. In addition, the understanding gained may benefit the lives of the hundreds of millions of people who suffer from bone degenerative diseases, like osteoporosis."

Microgravity produces numerous changes in the musculoskeletal, visual and immune systems.[17] Although astronauts undergo rigorous screening, they still show marked inter-individual differences. So, the NASA Twins Study compared numerous aspects of biology in a pair of male monozygotic twins. The first twin spent 180 days in space over 12 years followed by 340 days on the International Space Station (ISS). His earthbound twin had spent 54 days in space. Neither had been in space for about four years before the study.[20]

The study confirmed that spaceflight produces numerous changes including:

- Decreased body mass
- Elongation of telomeres (lengths of DNA at the end each chromosome that ensures correct replication)
- Genome instability
- Distension of the carotid artery and increased intima-media thickness
- Altered ocular structure and shape
- Transcriptional and metabolic changes
- Changes in DNA methylation (an epigenetic regulator) in pathways related to immune and oxidative stress
- Altered gastrointestinal microbiota
- Some cognitive decline.[20]

Average telomere length, global gene expression and microbiome changes returned to near pre-flight levels within six months after return to Earth. But the astronaut still had increased numbers of short telomeres and disrupted expression of some genes.[20]

"It's an n of 1," Dr Braddock remarks. "But the NASA Twin study helps characterise stress responses and adaptation, which may help us better understand chronic diseases. We need to apply big data approaches to mine this data and the often conflicting results from microgravity genome studies."

Currently, physiological changes in microgravity are problems for only those with, in Tom Wolfe's words, the Right Stuff. But, Dr Braddock notes, space tourism could mean the physiological changes become issues for people with co-morbidities who want to reach low Earth orbit.

Box 1. The NASA Twins Study

Osteoprotegerin, for instance, inhibits receptor activator of nuclear factor kappa-B ligand (RANKL), a protein that promotes formation of osteoclasts (cells that absorb bone). Mice flown on the Space Shuttle and treated with an experimental RANKL inhibitor had a greater BMD than untreated mice on the flight and controls on Earth. A related RANKL inhibitor – denosumab, which shows better pharmacokinetics – is approved for osteoporosis and bone metastases. Luis Zea remarks that the "experiment demonstrated the use and value the microgravity environment can have for testing molecules for drug development".[10]

Sclerostin inhibits osteoblasts (cells that make bone) while stimulating osteoclasts, partly in response to mechanical stress. So, inhibiting sclerostin stimulates osteoblasts and reduces resorption.[11] Mice on a Space Shuttle flight treated with romosozumab – an antibody targeting sclerostin – showed greater BMD than untreated mice and the Earth controls.[10] The European Commission granted a marketing authorisation for romosozumab in December 2019 for severe osteoporosis in postmenopausal women at high fracture risk.

Endothelial cell function

Microgravity allows nuanced investigations of cellular structure and function, which could yield new treatments.

"Microgravity can shed light on the role of small-scale forces from gravity and fluid shear stress on cellular processes, such as the remodelling of the cytoskeleton during cell movement or proliferation. We've found dramatic differences in endothelial cell behaviour in microgravity, perhaps because gravity helps orient actin filament and microtubule reorganisation in moving endothelial cells," says Shou-Ching Jaminet, Chief Scientific Officer at the American biotech company Angiex. "We believe that microgravity offers great opportunities to evaluate drugs that affect the cytoskeleton and cell movement by influencing actin filaments or microtubules."

For instance, mutations and abnormal expression of cytoskeletal proteins seem to contribute to cancer invasion, metastases and resistance to chemotherapy.[12] For example, the anticancer drugs paclitaxel and docetaxel bind to proteins that form part of the cytoskeleton, which stops the cell cycle and, in turn, prevents cell division.[13]

In addition, dysfunctions in the layer of endothelial cells that covers the inner surface of blood and lymphatic vessels contribute to several cardiovascular diseases, including atherosclerosis, hypertension and thrombosis.[4] Microgravity produces important changes in endothelial cell function, including impaired wound healing. "This naturally raises concerns about the health of astronauts and the feasibility of human exploration of space," Dr Jaminet says. "The ramifications of this discovery for drug development remain to be seen. Microgravity may provide a model for certain vascular pathologies and pathways of cellular ageing. What remains to be determined is the overlap between the pathologies observed in cells in space, the health challenges astronauts experience in space and the diseases humans develop on Earth."

Angiex is developing a targeted therapy that destroys blood vessels in solid tumours without affecting normal vasculature. "Our goal in microgravity research was to find a model system for the normal endothelium to better evaluate toxicity pathways," Dr Jaminet explains. "We found that our drug is less toxic to endothelial cells in space than on earth. Our drug acts over several weeks and it is impossible to maintain a realistic low gravity simulation for such a long time on the ground."

In early findings, Angiex found "substantial differences" between endothelial cells cultured in microgravity and those cultured on Earth. "Endothelial cells seemed to partially adapt to microgravity conditions by three weeks after launch, but cell morphology was still altered compared to ground control cells," Dr Jaminet adds. "These abnormalities persisted for months after the cells were returned to ground. So, it appears that significant and enduring epigenetic changes occur in microgravity." (Essentially, epigenetics refers to changes arising from modified gene expression rather than alterations to the DNA code.)

"The next step is to determine whether this pathological state resembles the condition of endothelial cells in any extant human disease or condition, such as ageing," Dr Jaminet says. "If so, cells could be cultured in space and returned to Earth for study, since they retain the microgravity phenotype for many months. The endothelium is a key player in humanity's most deadly diseases: heart attack, stroke, cancer. Figuring out why endothelial cells lack resilience to the challenges of microgravity might enable us to endow them with greater resilience against the ravages of ageing. That, at least, is our hope."

Crystal clear

Chains of amino acids fold into three-dimensional structures. Scientists study these 'tertiary' structures by looking at the diffraction pattern when X-rays pass through a crystal of the protein. The size and quality of the crystals determine the method's accuracy: larger, higher-quality crystals give better images of the protein's tertiary structure.[10] "The microgravity environment on the ISS enables the growth of protein crystals to a size and quality that provides an otherwise unattainable understanding of the biological processes associated with those proteins. This may enable new drugs to be developed that otherwise would not be available," says Mr Brady.

Chemists crystallise most proteins from solutions. On Earth, forces and flows driven by gravity mix the molecules and mean most crystals accumulate impurities.[10] "For a while, protein chemists and crystallographers felt that experiments in microgravity would be a panacea to the problems of growing crystals on Earth," Dr Braddock says. "This isn't the case. But microgravity studies have been helpful."

The investigational drug TAS-205, for instance, inhibits hematopoietic-type prostaglandin D synthase (HPGDS) and is currently in development for Duchenne muscular dystrophy (DMD).[14] HPGDS produces prostaglandin D_2, which seems to be involved in the inflammation that contributes to DMD.[14] NASA collaborated with a pharmaceutical company to grow larger, higher-quality crystals in microgravity. This allowed scientists to design TAS-205 to fit into a specific location on HPGDS. The implications may go beyond DMD: HPGDS may be involved in other allergic and inflammatory responses.[15,16]

Growing crystals in space might also markedly change a drug's pharmacokinetics. For instance, crystallising interferon alpha in space produced crystals that were 2.4 times longer and wider than those grown on earth. In an animal model, the crystals increased serum half-life to 12 hours compared with two to three hours for the normal non-crystalline form.[7]

Spaceflight also seems to influence several physiological processes that drive pharmacokinetics and pharmacodynamics, including delaying gastric emptying, changing fluid distribution and blood flow to the liver and kidneys, and altering metabolism. In some animal studies, spaceflight seems to change the activity of cytochrome P450 enzymes. The results are, however, inconsistent, perhaps reflecting methodological differences.[17]

However, such pharmacokinetic and pharmacodynamic changes mean that there is no guarantee that a drug developed on Earth will remain safe and effective in space. "We won't even have the luxury of a small clinical trial in space," says Dr Braddock. "We'll need to consider cell and animal studies conducted in microgravity as well as the clinical experience. But it's difficult to extrapolate to space. Ultimately, it'll be a judgement call on the benefits and risks for maintaining astronaut health."

Killer bugs from space

Few astrobiologists will, I suspect, be that surprised if there's life on Mars or another solar system body – microbes at least. NASA plans to launch a mission to Titan, one of Saturn's moons, in 2026 in part to look for chemical signatures of life. (Titan seems very similar chemically to the early Earth.) Of course, extra-terrestrial microbes could pose new infectious threats; a reverse of the demise of the invaders in *War of the Worlds*.

But terrestrial micro-organisms are also remarkably resilient. *Escherichia coli* and another Gram-negative bacterium *Paracoccus denitrificans* can proliferate even at 403,627 times the force of gravity. The Gram-positive bacterium *Lactobacillus delbrueckii* (used to make yoghurt) and the unicellular fungus *Saccharomyces cerevisiae* (Baker's yeast) proliferated at 22,505 times the force of gravity.[18] Hypergravity reduced proliferation. But it's remarkable they proliferated at all. "Bacterial species are remarkably resistant to the stresses of space," Dr Braddock says. "Despite the threat from resistance, many pharmaceutical companies have reduced investment in anti-infectives. But microgravity generates different approaches and may help reignite research."

For instance, *Staphylococcus aureus* and *E. coli* cultured in space seem to show increased resistance to several antibiotics. But some other organisms show increased susceptibility. Biofilms grown in space show a different structure and are more complex than those on Earth.[17] Experiments in microgravity help identify the regulatory pathways and genes driving changes in virulence, potentially offering new targets for treatments and vaccines against, for example, *Salmonella* gastroenteritis, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Streptococcus pneumonia*.[10]

"We've seen that microgravity can affect the characteristics of microbes, including their virulence," says Mr Brady. "This research will be useful toward obtaining the full picture on microbial processes, including how we can better control those that have gained resistance to our existing methods. Since the human body also changes in microgravity, microbiology studies remain a priority for our space exploration goals. Humanity will benefit as we bring this knowledge home to Earth to apply here." NASA's Biomolecule Extraction and Sequencing Technology (BEST) investigation is developing techniques to identify unknown microbial organisms on the ISS and to improve our understanding of how humans, plants and microbes adapt to microgravity.

The lack of organs for transplant stimulated research into tissue engineering, often by growing cells over a scaffold. Gravity makes growing three-dimensional tissue structures difficult. Recent microgravity experiments, for instance, found that human thyroid cancer cells and human endothelial cells form multicellular spheroids rather than the flat single-cell layers typical on Earth. In microgravity, spheroids can develop into tubular structures resembling the intima of rudimentary blood vessels, even without scaffolds. The process could produce blood vessels for transplantation and bypass surgery.[7]

Three-dimensional bioprinting deposits layers of cells on a biocompatible scaffold to create a tissue or organ. On the International Space Station (ISS), researchers used bioprinting to create spheroids of mouse thyrocytes, to imitate a thyroid gland, and chondrocytes to produce cartilage. Both constructs showed living cells with a normal morphology. These are likely to be the first steps in using microgravity studies to create other tissues for transplant.[7] "The technique has very clear applications in space and on Earth," Dr Braddock comments.

Box 2. Growing transplant organs in microgravity

And so to bed…

When we're upright, gravity creates a fluid gradient ranging from 200mmHg in the feet to 100mmHg in the heart and 70mmHg in the head.[4] Body fluid distribution changes in microgravity and among Earth-bound people using bed rest. So, microgravity offers a model of the ways 'disuse' affects muscle, bones and lungs.

Tidal volume, for instance, falls significantly in space, possibly because changes in fluid distribution increase blood flow to the lungs. On Earth, alveoli at the bottom of the lungs deflate more easily than those higher up, which 'traps' air, reflected in the residual volume. This distribution alters in space, so residual volume falls. The lungs take several weeks to adapt to microgravity. This may suggest, Demontis *et al*. remark, that "adaptation occurs through anatomical rather than functional changes, a finding relevant to the impact of prolonged bed rest on elderly subjects or chronically ill patients".[4]

Obviously, spaceflight, normal ageing and prolonged bed rest differ. But each offers insights into the molecular and cellular effects of external and internal mechanical forces. So, studying physiological changes in space and other extreme environments may deepen our understanding of ageing and medical disorders.[4]

Pharmacological conundrums

Microgravity studies may solve basic pharmacological conundrums. For example, microgravity seems to produce changes in corticosteroid responsiveness. Investigating the underlying mechanisms, Dr Braddock speculates, might help clarify the pathways leading to steroid resistance, which remains poorly understood and difficult to manage.

Lidocaine directly binds to neuronal sodium channels and enters cell membranes, which changes their fluidity. The relative importance of its direct and indirect mechanisms is a long-standing moot point. So, researchers used experiments loaded on a rocket to assess lidocaine's integration into lipid vesicles. The increase in membrane fluidity produced by lidocaine was less in microgravity than on Earth. This supports a membrane-mediated effect for at least part of lidocaine's action. The effect on anti-arrhythmic and local anaesthetic actions is not known, but further implies that pharmacodynamics may change in microgravity.[19]

We've made tentative forays across the final frontier. But only 24 people – astronauts in the Apollo programme – have left low Earth orbit. "There are many

unanswered questions about the biology of space travel and some of these could be showstoppers," Dr Braddock says. "If we are serious about reaching Mars in 2030, we need to speed up these studies. A decade isn't that long. In the meantime, I'm optimistic that insights from space will help improve our understanding of disease and result in new treatments here on Earth."

The pace of drug development is set to continue on the ISS as well as on unmanned rockets and satellites. "In addition to the human researcher capability unique to the ISS, the space station serves as a technological and economic proving ground for attached and free-flying research, and technology development test beds, including hundreds of small satellites," Mr Brady concludes. "The low Earth orbit economy is just getting started and the future is bright, from a research as well as an economic standpoint. It excites me to think what we may enable as we venture out beyond the Earth's vicinity!"

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Declaration of interests

Mark Greener is a full-time medical writer and journalist and, as such, regularly provides editorial and consultancy services to numerous pharmaceutical, biotechnology and device companies and their agencies. He has no shares or financial interests relevant to this article.

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