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PERSPECTIVE

Tissue Chips in Space: Modeling Human Diseases in Microgravity

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ABSTRACT

Purpose Microphysiological systems (MPS), also known as "organs-on-chips" or "tissue chips," leverage recent advances in cell biology, tissue engineering, and microfabrication to create *in vitro* models of human organs and tissues. These systems offer promising solutions for modeling human physiology and disease *in vitro* and have multiple applications in areas where traditional cell culture and animal models fall short. Recently, the National Center for Advancing Translational Sciences (NCATS) at the National Institutes of Health (NIH) and the International Space Station (ISS) U.S. National Laboratory have coordinated efforts to facilitate the launch and use of these MPS platforms onboard the ISS. Here, we provide an introduction to the NIH Tissue Chips in Space initiative and an overview of the coordinated efforts between NIH and the ISS National Laboratory. We also highlight the current progress in addressing the scientific and technical challenges encountered in the development of these ambitious projects. Finally, we describe the potential impact of the Tissue Chips in Space program for the MPS field as well as the wider biomedical and health research communities.

KEYWORDS Microphysiological systems · Organs on chips · Tissue chips · Microgravity · International Space Station · Disease modeling

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INTRODUCTION

The process of drug development is lengthy and expensive and has extremely high attrition rates. Much of this attrition is due to issues with the safety and toxicity of promising compounds or to a lack of efficacy for therapeutic targets despite promising preclinical studies in 2D cell culture and animal models (1). Some attrition arises due to species differences when moving from animal models to human cells and models, and some arises from difficulties in accurately representing the human in an *ex vivo* system. Microphysiological systems (MPS), also known as "organs-on-chips" or "tissue chips," are novel tools to explore alternative approaches to drug development by providing early indications and potentially more reliable readouts of compound toxicity and efficacy. MPS development over the last decade has led to bioengineered models of a wide array of human organs and tissues modeled on-chip, and the advent of induced pluripotent stem cell (iPSC) and genetic engineering technologies has allowed modeling of human diseases and genetic disorders in new and promising ways (2).

As part of the larger NIH Tissue Chips for Drug Screening Program, NCATS and the ISS National Laboratory have partnered to take advantage of the unique microgravity environment of the ISS to model human diseases and test potential therapeutics in low Earth orbit. Physiological changes seen in astronauts seem to replicate certain disease and aging pathologies and occur rapidly in microgravity (3). The vast majority of these changes reverse when the astronauts return to the ground (4). These rapid changes and reversals can be modeled *in vitro* on MPS platforms, which could accelerate the discovery of molecular mechanisms that underlie a range of common human disorders on Earth. Additionally, modeling diseases-on-a-chip in microgravity could advance our understanding of therapeutic targets and treatments in a reduced fluid-shear environment that recapitulates some of the *in vivo* cellular and tissue interactions seen in the body on Earth.

Through this collaboration, the ISS National Laboratory and NCATS funded five projects under RFA-TR-16-019. In 2018, the National Institute for Biomedical Imaging and Bioengineering (NIBIB) joined the program, and the reissued joint solicitation RFA-TR-18-001 funded an additional four projects.

The changes seen in cell function in microgravity provide strong hypotheses for the projects funded through the Tissue Chips in Space initiative. In microgravity, cells experience a unique environment that has profound effects on cell signaling (communication between cells) (5–8), aggregation (physical contact between cells leading to their 3D structural organization into tissues) (9–11), and differentiation (the process through which a cell assumes a specific phenotype and specialized functions) (12–16). These biological changes arise from physical changes such as altered fluid movement and from specific stressors of the space environment. These aspects of microgravity provide opportunities for discoveries that cannot be made on Earth in a standard *ex vivo* model.

In addition, the interactive and cumulative effects of microgravity exposure on genes, cells, and organisms results in changes similar to those seen on Earth that lead to the onset and progression of diseases (17,18). After years of study on the effects of spaceflight on biology, many of these changes are predictable. The effects may be accelerated during spaceflight and can be reversed following return to Earth (3,4), providing novel access to accelerated disease mechanism pathways. Using MPS platforms to study these changes opens avenues for better understanding human health and disease and developing and testing new modalities to prevent and treat disease (e.g., cardiovascular deconditioning, immune system dysfunction, aging, bone loss, and muscle wasting).

The perspective provided here will highlight the disease-relevance of the projects funded through the Tissue Chips in Space initiative and will illustrate how some of the challenges faced by these projects will advance the development of MPS technology and facilitate broader uptake of MPS use.

MODELING HUMAN PHYSIOLOGY AND DISEASE IN MICROGRAVITY

The nine projects funded through the Tissue Chips in Space initiative are all biphasic, with the opportunity for two spaceflight experiments onboard the ISS (Fig. 1). The goals of the first phase are development and validation of the biological systems and adaptation of the hardware for spaceflight, ending with a launch and an approximate month-long experiment on the ISS. In the second phase, projects will test therapeutics in the disease model systems, with the aim to uncover novel disease or therapeutic pathways with direct clinical relevance on Earth.

Immune Alterations

During spaceflight, both the innate and the adaptive immune responses are dysregulated (4,19). Understanding the immune response in general is an ongoing area of intensive study due to its critical implications in all aspects of human injury and disease. Additionally, an understanding of the immune response to spaceflight is important for future manned long-duration spaceflight missions.

Three projects awarded through the Tissue Chips in Space initiative address the effects of microgravity on the immune response under a variety of conditions. In one, researchers at the University of California San Francisco (UCSF) are studying immunosenescence, or dysregulation of the immune system due to disease or aging (Schrepfer, 1UG3TR002192). Immunosenescence is a critical clinical issue on Earth, as common diseases such as influenza can disproportionately affect older populations. Using microgravity as an analog for accelerated physiological processes, the team at UCSF is studying the terminal differentiation of CD8+ effector memory T cells (TEMRA cells), which increase significantly with age and are implicated in dysregulated adaptive immune responses. Using a tissue chip to study cell culture and differentiation of TEMRA cells at 1G and in simulated and real microgravity, the team is examining the effects of bone healing (by inclusion of mesenchymal stromal cells) and vascular regeneration (by inclusion of endothelial progenitor cells) to gain a better understanding of the effects of immunosenescence on the regenerative capacity of specific stem cells (Fig. 2c).

In another project, researchers from the Children's Hospital of Philadelphia are leveraging microgravity to investigate immune responses to lung infection using a lung-bone marrow tissue chip to examine how neutrophils from the bone marrow are mobilized in response to bacterial infection of the lung by *Pseudomonas aeruginosa* (Worthen, 1UG3TR002198). On Earth, *P. aeruginosa* is a major cause of infection and can cause death in immunocompromised patients. Connecting the lung and bone marrow modules in a single linked tissue chip enables study of the innate immune system response to infection both on the ground and in the unique microgravity environment in low Earth orbit (Fig. 2a). Additionally, such research could shed light on how microgravity may affect the virulence of certain bacterial strains, which is important for not only people on Earth but also for astronauts in enclosed environments.

It is well documented that spaceflight has detrimental effects on the musculoskeletal systems of astronauts, and these effects are currently mitigated through specialized exercise regimes and diet (20). However, astronauts can and do occasionally sprain and strain their joints, which could lead to the clinical condition of post-traumatic osteoarthritis (PTOA). PTOA is a common condition in otherwise healthy (young to middle-aged) individuals affecting about 5.6 million people

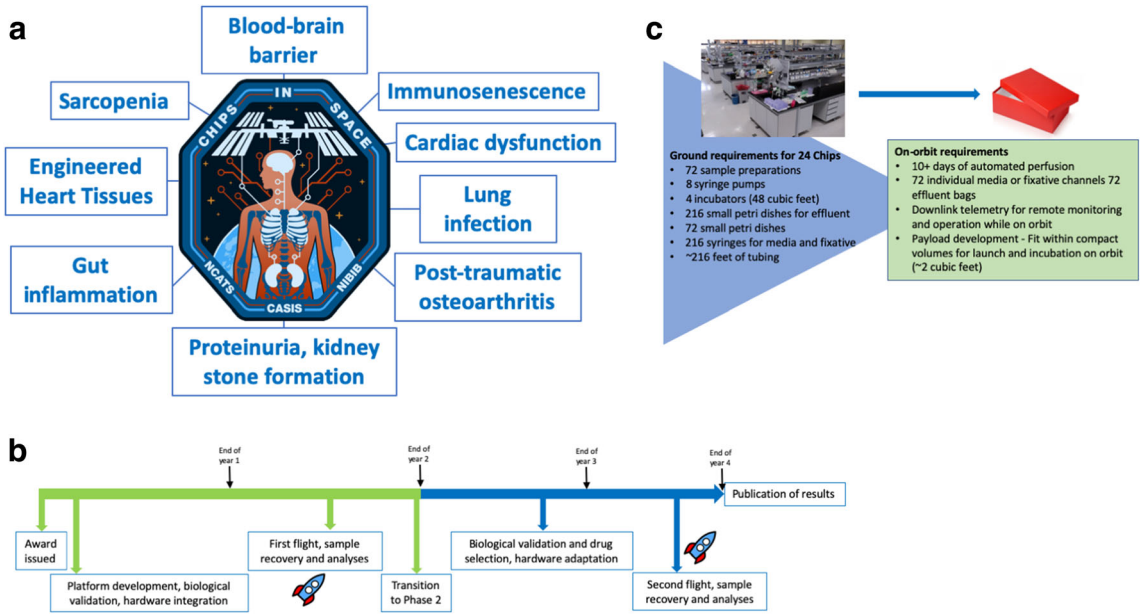

Fig. 1 Nine projects are supported under the Tissue Chips in Space program. (**a**) The 9 projects supported by the National Center for Advancing Translational Sciences, the ISS National Lab, and NASA, model a broad range of human organ tissues and disease states. (**b**) Project timelines. The biphasic projects allow for two flight opportunities for each team, with results from the first flight informing the science for the second. (**c**) Tissue chip design and adaptation for launch and integration onto the ISS involves extensive miniaturization and automation. The ground and flight requirements for the kidney chip are provided in this example and required reduction of volume from over 48 cubic feet to around 2 cubic feet for on-orbit operations – roughly the size of a shoebox.

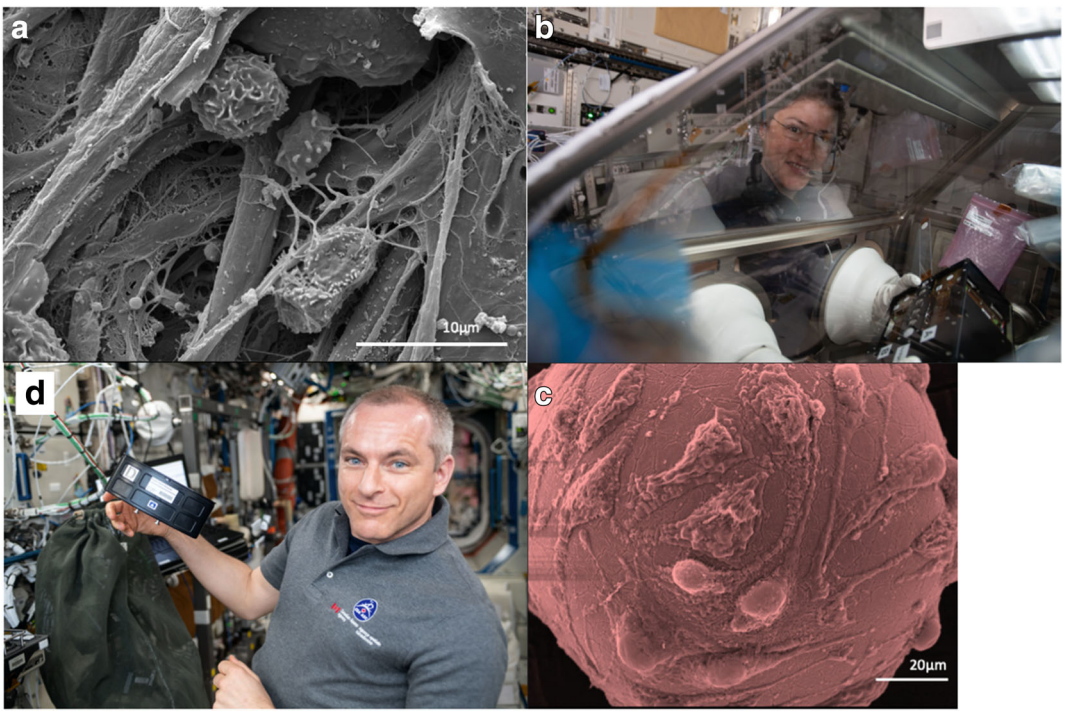

Fig. 2 Example images of cell culture from the projects and ISS crew undertaking the experiments. Clockwise from top left: (**a**) Scanning electron microscope (SEM) image of neutrophils in a vascularized bone marrow tissue chip. Credit: Andrei Georgescu, Biolines Laboratory at the University of Pennsylvania, & Children's Hospital of Philadelphia. (**b**) NASA astronaut Christina Koch works on the University of Washington kidney tissue chip investigation inside the Life Sciences Glovebox onboard the ISS. Image courtesy of NASA. (**c**) Electron microscopy image of a single smooth muscle cell attached to a microbead carrier (not pictured) after three days of simulated microgravity. Credit: UCSF Photo/Dr. Sonja Schrepfer and Dr. Dong Wang. (**d**) Canadian astronaut David St-Jacques checks on the MIT cartilage-bone-synovium platform onboard the ISS. Image courtesy of NASA.

in the United States and causing osteoarthritis of the hip, knee, and ankle. In a third project, researchers at the Massachusetts Institute of Technology have created a cartilage-bone-synovium MPS to study the interactions between these three tissues, which are critical for joint health and PTOA onset (Fig. 2d). The research team is testing therapeutics that prevent bone resorption (e.g., anti-sclerostin antibody) and inflammatory responses to understand some of the metabolic pathways involved in PTOA pathology (Grodzinsky, 1UG3TR002186).

Musculoskeletal Deconditioning

Loss of bone density and muscle mass in astronauts is well substantiated, with effects manifesting as early as within two weeks of microgravity exposure. While these losses are relatively well mitigated by specialized exercise equipment for astronaut use in orbit, the mechanism of bone density decrease is similar to that seen in osteoporosis, and age-related muscle wasting (sarcopenia) is a clinical problem in many elderly people. In a project from the University of Florida, a skeletal muscle tissue chip (Malany, 1UG3TR002598) will be launched to the ISS containing primary human myocytes from young, older, healthy and sedentary volunteers to study the effects of microgravity on the electrical activity of the cells and tissues in almost real time (via incorporation of microelectrodes and microscopes built into the platform). Electrical stimulation of the tissue from the microelectrodes could help prevent myocyte changes, and anti-atrophy compounds administered to the tissues during the second phase of this project could enable examination of how therapeutics could mitigate the sarcopenia-like phenotype of myocytes in microgravity.

Kidney Dysfunction

As bone mass is lost both due to osteoporosis and exposure to microgravity, an increased amount of calcium is leached into the blood and is filtered by the kidneys (21). Additionally, dehydration and high blood pressure can impair kidney function both on Earth and in space. Coupled with the fluid shifts around the body that astronauts experience in microgravity, these factors together can precipitate serious kidney issues and medical conditions including proteinuria and formation of kidney stones. In a project from the University of Washington, kidney proximal and distal tubule tissue chips are being launched to the ISS to investigate how microgravity affects cell polarization of proximal and distal tubule epithelial cells (which affects ion and solute filtration in the kidney) and to determine whether Vitamin D bioactivation and homeostasis within the kidney proximal tubule is compromised in response to extended exposure to microgravity (Himmelfarb, 1UG3TR002178). By creating disease state models of proximal tubule proteinuria (by elevating serum levels in cell culture medium) and growing oxalate microcrystals in distal tubule kidney chips to mimic kidney stone formation, the team aims to better understand how kidney function is affected by microgravity to give novel insights into disease mechanisms in patients on Earth (Fig. 2b).

Cardiac Tissues in Microgravity

Cardiovascular disease accounts for an estimated 31% of deaths worldwide each year, and heart attacks, heart failure, and arrhythmias represent a significant clinical burden globally. The cardiovascular system is uniquely stressed in microgravity conditions, and changes in heart size and beat function have been documented (22). These effects suggest that microgravity could be leveraged to investigate cardiovascular disease-relevant pathologies on tissue chips. Two research teams awarded through the Tissue Chips in Space initiative are developing cardiac tissues for spaceflight experiments. Stanford University researchers (Wu, 1UG3TR002588) are creating engineered heart tissues (EHTs) from diverse racial groups by generating human iPSC-derived cardiomyocytes (hiPSC-CMs) and investigating how alterations in cardiac function due to weakened heart muscles in the samples exposed to microgravity are similar or different to the molecular and electrophysiological disease patterns observed in ischemic cardiomyopathy. In the team's second flight experiment, high-throughput heart tissue arrays (HTAs) will be used for screening of drug candidates to prevent or reverse the phenotypic changes seen in response to microgravity, which could translate into new avenues for treatment of ischemic cardiomyopathy.

Another project from the University of Washington (Kim, 1UG3EB028094) will use human iPSC-derived cardiomyocytes in a high-throughput cardiac MPS on a platform with an extracellular scaffolding matrix containing electroconductive composites, which can help push the maturation of the cardiomyocytes. This is currently a challenge for all iPSC-derived cardiac cells, which generally display a fetal phenotype, limiting their utility for modeling aging-related pathologies. Real-time readouts from magnetometer-based motion sensor arrays will provide feedback on beat frequency and strength. In the team's second flight experiment, therapeutic and mechanical stimulation interventions will be compared to help improve understanding of the progression of chronic heart diseases on Earth.

Biological Barrier Disturbances in Microgravity

The separation of organ systems and maintenance of body function relies on many natural barriers—from the skin preventing pathogen infection to the blood-brain barrier preventing toxin access to the central nervous system. Tissue-specific

epithelial cells are critical for these barriers, and damage can contribute to a variety of disease states. For example, similar to the early stages of some vasculopathies and neurodegenerative diseases such as multiple sclerosis, changes in tight junction proteins and barrier integrity of epithelial layers have been seen in response to launch and simulated microgravity. To examine how microgravity conditions can affect the blood-brain barrier (BBB), the MPS company Emulate is developing a BBB chip (Hinojosa, 1UG3TR002188) containing neurons and vasculature on a commercially available tissue chip to study the integrity of the BBB under normal and inflamed conditions on Earth and in low Earth orbit. By exposing the tissue chip to spaceflight-relevant stressors such as hypoxia, BBB integrity can be monitored and assessed, providing critical insight into the burgeoning healthcare problem of neurodegenerative disease.

A second project from Emulate models another biological barrier, the epithelial mucosa of the gut and its interactions with sensory neurons and the microbiome (Hinojosa, 1UG3TR002595). Using Salmonella typhimurium infection of a mucosal epithelium innervated with enteric sensory neurons, the tissue chip will be used to investigate the immune response of incorporated immune cells in response to Salmonella in the absence and presence of probiotic bacteria. As foodborne infections cause 1.2 million illnesses each year, this project could provide valuable insight into the mechanistic degradation of the epithelial mucosa under stressed and infected conditions.

CHALLENGES WITH ADAPTATION OF GROUND-BASED SYSTEMS FOR FLIGHT-READINESS

MPS platforms have been developed to elegantly model human organ structure and function in ways that are not possible in other 2D culture systems or animal models. However, the footprint for these systems can be complex and substantial, which clearly presents challenges for launch to the ISS. Therefore, each project funded under the Tissue Chips in Space initiative has addressed the additional factor of adapting the necessary systems to make them smaller, more automated, and robust enough to withstand the rigors of launch and later, upon return to Earth, splashdown (Fig. 1C). Collaboration with ISS-NL implementation partners—independent companies providing commercial services to aid investigators in translating ground-based research into flight-ready projects for launch to the ISS—has been a critical part of the initiative. Implementation partners have developed miniaturized engineered systems including pumping, tubing, computational control boards, incubators, refrigerators, electrodes, and microscopes in sealed self-contained units that can support the appropriate number of biological tissues and replicates to gain meaningful results. To illustrate this challenge for the kidney project, lab equipment that could hold up to 1500 L of volume had to be reduced to a volume of approximately 50 L (23) and entirely novel pumping and incubation systems had to be engineered for some platforms. Implementation partners have also been instrumental in adapting systems that conform to NASA flight safety specifications, such as appropriate levels of containment for biological specimens and pathological bacteria such as *P. aeruginosa*, by integrating such containment into the system design and creating systems which can be safely used on orbit, for example in the Life Sciences Glovebox. While challenging, the process of overcoming these obstacles results in systems for use on Earth that are robust and more compact with increased automation and that are useful in a variety of situations, including laboratories and even extreme environments around the world.

CONCLUSION

In addition to the innovative biological research conducted through the projects detailed in this Perspective, the significant advances in automation and reduction in equipment size needed to conduct research on the ISS is simultaneously pushing the technical development of MPS on Earth. The integration of these complex biological systems modeling human disease into robust, reliable, automated hardware is predicted to accelerate the translation and accessibility of these systems from independent laboratories to a broader pool of end-users on Earth.

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REFERENCES

1. Waring MJ, Arrowsmith J, Leach AR, Leeson PD, Mandrell S, Owen RM, et al. An analysis of the attrition of drug candidates from four major pharmaceutical companies. Nat Rev Drug Discov. 2015;14:475.
2. Low LA, Tagle DA. Tissue chips - innovative tools for drug development and disease modeling. Lab Chip. 2017;17(18):3026–36.
3. Biolo G, Heer M, Narici M, Strollo F. Microgravity as a model of ageing. Curr Opin Clin Nutr. 2003;6(1):31–40.
4. Garrett-Bakelman FE, Darshi M, Green SJ, Gur RC, Lin L, Macias BR, et al. The NASA Twins Study: A multidimensional analysis of a year-long human spaceflight. Science. 2019;364(6436):eaau8650.

5. Hughes-Fulford M, Chang TT, Martinez EM, Li C-F. Spaceflight alters expression of microRNA during T-cell activation. FASEB J. 2015;29(12):4893–900.
6. Xu H, Wu F, Zhang H, Yang C, Li K, Wang H, et al. Actin cytoskeleton mediates BMP2-Smad signaling via calponin 1 in pre-osteoblast under simulated microgravity. Biochimie. 2017;138: 184–93.
7. Camberos V, Baio J, Bailey L, Hasaniya N, Lopez LV, Kearns-Jonker M. Effects of spaceflight and simulated microgravity on YAP1 expression in cardiovascular progenitors: implications for cell-based repair. Int J Mol Sci. 2019;20(11):2742.
8. Cazzaniga A, Locatelli L, Castiglioni S, Maier JAM. The dynamic adaptation of primary human endothelial cells to simulated microgravity. FASEB J. 2019;33(5):5957–66.
9. Zhang X, Li L, Bai Y, Shi R, Wei H, Zhang S. Mouse undifferentiated spermatogonial stem cells cultured as aggregates under simulated microgravity. Andrologia. 2014;46(9):1013–21.
10. Masiello MG, Cucina A, Proietti S, Palombo A, Coluccia P, D'Anselmi F, et al. Phenotypic switch induced by simulated microgravity on MDA-MB-231 breast cancer cells. Biomed Res Int. 2014;2014:652434–4.
11. Crabbé A, Nielsen-Preiss SM, Woolley CM, Barrila J, Buchanan K, McCracken J, et al. Spaceflight enhances cell aggregation and random budding in Candida albicans. PLoS One. 2013;8(12): e80677–7.
12. Jha R, Wu Q, Singh M, Preininger MK, Han P, Ding G, C, *et al.* Simulated microgravity and 3D culture enhance induction, viability, proliferation and differentiation of cardiac progenitors from human pluripotent stem cells. Scientific Reports. 2016;6:30956–30956.
13. Shi W, Xie Y, He J, Zhou J, Gao Y, Wei W, et al. Microgravity induces inhibition of osteoblastic differentiation and mineralization through abrogating primary cilia. Sci Rep. 2017;7(1):1866–6.
14. Xue L, Li Y, Chen J. Duration of simulated microgravity affects the differentiation of mesenchymal stem cells. Mol Med Rep. 2017;15(5):3011–8.
15. Fuentes TI, Appleby N, Raya M, Bailey L, Hasaniya N, Stodieck L, et al. Simulated microgravity exerts an age-dependent effect on the differentiation of cardiovascular progenitors isolated from the human heart. PLoS One. 2015;10(7):e0132378–8.
16. Grigoryan EN, Radugina EA. Behavior of stem-like cells, precursors for tissue regeneration in Urodela, under conditions of microgravity. Stem Cells Dev. 2019;28(7):423–37.
17. Capri M, Morsiani C, Santoro A, Moriggi M, Conte M, Martucci M, et al. Recovery from 6-month spaceflight at the international Space Station: muscle-related stress into a proinflammatory setting. FASEB J. 2019;33(4):5168–80.
18. Honda Y, Honda S, Narici M, Szewczyk NJ. Spaceflight and ageing: reflecting on *Caenorhabditis elegans* in space. Gerontology. 2014;60(2):138–42.
19. Crucian BE, Choukèr A, Simpson RJ, Mehta S, Marshall G, Smith SM, et al. Immune system Dysregulation during spaceflight: potential countermeasures for deep space exploration missions. Front Immunol. 2018;9:1437–7.
20. Sibonga J, Matsumoto T, Jones J, Shapiro J, Lang T, Shackelford L, S *et al.* Resistive exercise in astronauts on prolonged spaceflights provides partial protection against spaceflight-induced bone loss Bone 2019;128:112037.
21. Smith SM, Heer M, Shackelford LC, Sibonga JD, Spatz J, Pietrzyk RA, et al. Bone metabolism and renal stone risk during international Space Station missions. Bone. 2015;81:712–20.
22. Khine HW, Steding-Ehrenborg K, Hastings JL, Kowal J, Daniels JD, Page RL, et al. Effects of prolonged spaceflight on atrial size, atrial electrophysiology, and risk of atrial fibrillation. Circ Arrhythm Electrophysiol. 2018;11(5):e005959.
23. Yeung CK, Koenig P, Countryman S, Thummel KE, Himmelfarb J, Kelly EJ. Tissue chips in space—challenges and opportunities. Clinical and Translational Science. 2019;0(0).