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BIOSENSOR INTEGRATED TISSUE CHIPS AND THEIR APPLICATIONS ON EARTH AND IN SPACE

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Abstract

The development of space exploration technologies has positively impacted everyday life on Earth in terms of communication, environmental, social, and economic perspectives. The human body constantly fluctuates during spaceflight, even for a short-term mission. Unfortunately, technology is evolving faster than humans' ability to adapt, and many therapeutics entering clinical trials fail even after being subjected to vigorous *in vivo* testing due to toxicity and lack of efficacy. Therefore, tissue chips (also mentioned as organ-on-a-chip) with biosensors are being developed to compensate for the lack of relevant models to help improve the drug development process. There has been a push to monitor cell and tissue functions, based on their biological signals and utilize the integration of biosensors into tissue chips in space to monitor and assess cell microenvironment in real-time. With the collaboration between the Center for the Advancement of Science in Space (CASIS), the National Aeronautics and Space Administration (NASA) and other partners, they are providing the opportunities to study the effects of microgravity environment has on the human body. Institutions such as the National Institute of Health (NIH) and National Science Foundation (NSF) are partnering with CASIS and NASA to utilize tissue chips onboard the International Space Station (ISS). This article reviews the endless benefits of space technology, the development of integrated biosensors in tissue chips and their applications to better understand human biology, physiology, and diseases in space and on Earth, followed by future perspectives of tissue chip applications on Earth and in space.

Keywords

biosensors; tissue chips; organ-on-a-chip; tissue engineering; in-space applications

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9.0Conflict of Interest

Dr. Yupeng Chen is a co-founder of Eascra Biotech.

1.0 Introduction

Space technologies have grown exponentially since 1957. Novel tools and techniques are often developed for microgravity research and development. Various standard technologies used in everyday life on Earth are adapted from space technology, such as global positioning systems (GPS), cordless power tools, three-dimensional (3D) Printing, and the development of tissue chips including biosensors. Although space explorations are one of the factors leading to the growth in space technologies at a steady pace, human adaptability to space is severely lacking, especially for long-term missions in space. With a future mission to Mars in mind, scientists are pouring in efforts to study the effects of microgravity, especially in a long-term mission, on human bodies.

Human bodies often undergo changes when exposed to microgravity, resulting in degeneration of the musculoskeletal system, cardiovascular deconditioning, and suppression of the immune system (Yeung et al. 2020). Many studies have been conducted to investigate these changes; however, the sample studies have been limited to the analysis of easily accessible bio-samples such as blood and urines, limited sample size, diversity, and concerns for privacies of astronauts. Efforts were made to speed up the human adaptability to space to rival the speed of evolving technologies. Nonhuman models such as rodents, tissue chips and organoids have been utilized to assess the potential risks of space travel (Giulianotti and Low 2019). Unfortunately, long-term orbital space flights for experimentations and flight opportunities are scarce and the cost of development of the hardware is high, not to mention research and development in research areas like cell biology and tissue engineering are not always a priority in space flight activities (Ulbrich et al. 2014). Therefore, many scientists are now utilizing microfluidic technology to further advance the development of tissue engineering on Earth seen in Figure 1. Several national programs were established to aid in multiple tissue research projects utilizing tissue chips.

Microfluidic technology was developed to precisely manipulate and control fluid flow in a microscale in the semiconductor industry. The success of this technology further provided a new “outlet” for researchers in both biology and medical science community in translating cells cultured in dishes to a more physiologically relevant *in vitro* model. The organ-on-a-chip (OoC) are being utilized to overcome the limitations of conventional in vitro studies in predicting human responses and uses minimal functional units in microscale. They are more relevant than animal studies due to the difference in physiology and cellular functions (Kim and Takayama 2015).

Tissue chips comprises of different microchannels where cells were cultured and observed over time (Huh et al. 2013). The addition of various injectable scaffold (Zhou et al. 2020b; Zhou et al. 2020c; Zhou et al. 2021) and the introduction of dynamic conditions such as mechanical loading or shear stress from the media flow can be mimicked to model the microenvironment of the native tissues (Choi et al. 2007; Hwang et al. 2008) as seen in Figure 2. These parameters allow the entire microchip to simulate the behaviors of various tissues and, subsequently, the organs of living organisms. The parameter modification allows scientists to better understand the inner workings of individual organs working separately or together. One of the advantages of the microfluidic systems over current models is the

dynamic nature of a 3D environment for cells to flourish. The fluids that flow through the channels of a tissue chip can apply forces to the cells that can accurately mimic the stresses those cell types regularly experience in the native tissue (Wu et al. 2020). Multiple parameters can be easily controlled and modified depending on the type of experiments (Lee et al. 2021; Sun et al. 2020; Yau et al. 2021). This allowed scientists to create conditions specific to the tissue type and possibly aid in the understanding of an organism as a whole. They can utilize the tissue chip to model diseases (Sands et al. 2020; Zhang and Chen 2019) (conditions can be accelerated in space) or screen novel therapeutic drugs (Lee et al. 2021; Sun et al. 2019; Yau et al. 2019b). Furthermore, current advances in biosensors in tissue chips on Earth have further promoted the applications of microfluidic technology in space. Implantable biosensors in tissue chips have the potential to help diagnose diseases, monitor levels of analytes in tissues, and deliver therapeutics to target diseases (Gray et al. 2018). The use of biosensors on Earth can assess cells behavior and microenvironment in real-time and can be applied to tissue chips in space.

On Earth, tissue chips are used to recreate organ systems that mimic patients or organisms. More importantly, these tissue chips can be seeded with cells from patient-derived sources allowing for personalization and increasing the possibility of drug screening, therapeutic testing, and gene editing in the future (Low and Giulianotti 2019). The inclusion of biosensors in tissue chips are highly encouraged and many have started to do so. For example, Rennert *et al.* have seeded the liver cells in a liver tissue chip to study the oxygen consumption using oxygen sensor (Rennert et al. 2015) and Shin *et al.* (Shin et al. 2016) utilized aptamer-based electrochemical sensor to monitor cardiac damage in their heart-on-a-chip study which will be discussed further in the next sections. Studying tissue chips in microgravity, however, is an innovative concept initiated in October 2016 by the government agencies (CASIS and NCATS) to utilize cutting edge technology in recreating OoC to better understand the role of microgravity on human health and diseases. The applications of tissue chips in space are a challenging effort because of limited opportunities to send test subjects up to the ISS for research and therefore limiting the number of iterations allowed for each attempt in a cost-effective way. This paper reviews the advantages and the applications of tissue chips on Earth, the integration of biosensors into these microphysiological systems. The paper also reviews the challenges and opportunities to utilize tissue chips in space, as well as the future direction of tissue chips in space. This paper further reviews the advancements and success of tissue chips development on Earth and in space, primarily through the collaborations between CASIS, NASA, NIH, and NSF. Together, researchers have developed multiple tissue chip systems as well as biosensors for understanding human health and diseases on Earth and in space.

2.0 State-of-Art Tissue Chips

On Earth, tissue chips have been utilized to help sustain cell and tissue functions for a long-term study (Kimura et al. 2018). The 3D systems often make up for the lack of relevant physiological stresses upon cells often encountered in two-dimensional (2D) systems and organoids where the fluid in the tissue chips can flow through the microchannels and accurately mimic the stresses like those experienced in the native tissues (Wu et al. 2020). The parameters to fabricate an optimal tissue chip can be engineered in a way to fit each

study. Cells can be guided to initiate responses through the numerous parameters applied causing cell signaling in the tissues (Wu et al. 2020). Biosensors can be integrated to monitor specific tissue functions in real-time. For example, cells can release cytokines and proteins that will trigger additional responses in other cells within the microchip, alerting the biosensor and thus collecting signals and data needed to monitor the cascade happening in real-time. Other than that, concentration gradients in the system can be adjusted with biosensors where microchannels' flow dynamics and orientation can be modified to influence cell functions at any point during a study. Figure 3 shows the advantages of using microfluidic tissue chips on Earth and translation of ground-based research to in space research.

2.1 Lung-on-a-chip

In the development of lung-on-a-chip, chemical etching was used to create the vacuum chambers in the polydimethylsiloxane (PDMS) membrane, where an etchant is used to dissolve large sections of the membrane, creating hollow channels which air can be pushed through to mimic breathing in the chip (Huh et al. 2010). PDMS is a common material used for the fabrication of tissue chips because they provide transparency for imaging, have a long shelf life, have excellent biocompatibility as well as high gas permeability for cell culture (Ashammakhi et al. 2018b). The presence of vacuum channels on either side of the chip imitates the movements seen at the alveoli-capillary interface to mimic the act of "breathing" like a pair of human lungs (Huh et al. 2010). These mechanical forces constitute a significant part of how pulmonary edema is induced in this study. In their study, this device was tested to determine if it could also effectively model organ function by introducing pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), into the system and observing cell activities to observe inflammatory response.

In 2018, Yang *et al.* created a 3D environment with a nanofiber membrane scaffold in the lung-on-a-chip to mimic models found in *in vivo* studies (Yang et al. 2018). This group successfully tested the scaffold and cultured three cell types: lung cancer cells, fetal lung fibroblasts, and umbilical vein endothelial cells in the tissue chip, tracked with Cell Tracker technology. They developed a lung cancer disease model in the tissue chip with nanofiber and observed lung cancer cells replacing where the endothelial cells. They also successfully tested an anti-tumor drug into the diseased model. Further development of the lung tumor in tissue chips can further encourage the effectiveness of future novel anti-cancer drugs (Sands et al. 2020; Zhang and Chen 2019; Zhou et al. 2020a), with higher efficacy. On Earth, both studies have successfully modeled processes commonly seen within the lungs with tissue chip. In Huh *et al* (Huh et al. 2010), the mechanics of a chip is essential, where the movement of the channels dictates how the chip 'breathes.' With Yang *et al* (Yang et al. 2018)., their study showed that the tissue chip could accurately show the responses of both cell types in the lung as well as model the function of the entire organ (Li et al. 2022). It shows how this system can be used to test a wide range of lung diseases and conditions, simply by slightly modifying the structure of the device. It can also provide information about responses at both the cellular and organ level.

Since gravity greatly influences the function of the human lung, the absence of gravity may provide valuable insight into how microgravity affects the human lungs. Due to the limited sample size, not many long-duration microgravity studies (up to 6 months prior to 2014) (Prisk 2014) were performed. Although mimicking the lung tissue in tissue chips have been a success on Earth, the delicate structure of the lungs comprising of airspace and capillaries have been a challenge to simulate in space. Gravity plays a major role in the formation of the lung structure on Earth and therefore, could possibly affect the framework of the lungs when grown in space. The use of lung tissue chips has greatly benefited the understanding of human lungs on Earth. Worthen *et al.* (Worthen 2017) observed the lung host defense in microgravity utilizing their lung-on-a-chip system. Their project aim is to model the airway of the lung and bone marrow separately, then combine the models to mimic and understand the immune responses of the human respiratory system in microgravity (Worthen 2017). The lack of gravity has shown to influence the blood flow distribution and alveolar gas exchange in the lungs due to the structure of the lung and that the particles tend to suspend in the lungs rather than sediment like it would on Earth (Sciences 1998). Therefore, understanding how the lung works in microgravity, especially in a long-term mission, is important and can be further developed utilizing lung-on-a-chip. More in depths studies such as, pressure changes, ventilations, and gas exchange of lung tissues in tissue chips on Earth with the use of biosensors are encouraged to determine the feasibility of lung-on-a-chip in space. The incorporation of biosensors in these tissue chips and their studies in microgravity could benefit and accelerate the lung-on-a-chip progress on Earth and in space.

2.2 Intestine-on-a-chip

The functions of the intestines, specifically the digestion and metabolization of nutrients, are a crucial part of maintaining homeostasis. The villi are the major priority for all groups trying to develop a microchip because the tiny protrusions of the lumen in the intestines are used to increase surface area and therefore increase absorption of nutrients. In 2017, Shim *et al.* created a tissue chip that mimics villi found in intestines. The villi themselves were created from a collagen scaffold created from an alginate mold (Shim et al. 2017; Yau et al. 2019a). The group directly compared the effectiveness of 2D cultures in traditional Transwells to cells cultured on a 3D microfluidic chip to see how the two compares (Shim et al. 2017) and found that the 3D cell culture induced much better absorption of cells as well as better morphology (Shim et al. 2017). The addition of biosensors in this tissue chip would prove to be advantageous where the biosensors can either detect the nutrients absorbed or the wasted secreted from the tissue in real-time, and the experiments can be altered accordingly.

One of the major functions of the gut is to move food through the intestines to absorb nutrients that the body needs in the presence of various gut microbiomes. This is done through peristalsis, a process that pushes the food through the lumen of the intestines by contracting the area behind where the food is. The ability to manipulate the forces that act on a tissue chip is one of the main advantages of this type of system compared to *in vivo* model, especially during spaceflight. In the study by Kim *et. al.*, a microchip was created that included luminal flow and peristaltic movements. This was done with a vacuum channel on either side of the cell channel, through which air was removed to stretch the cell

channel outward. This allows researchers to change the conditions to induce environments that represent different diseases. The study was also able to study intestinal inflammation and injury by studying the height and structure of the villi as various types of bacteria were introduced into the chip (Kim et al. 2016). The amount and concentration of cytokines released by the epithelial cells were also measured and analyzed to fully understand the effect of the inflammatory bacteria (Kim et al. 2016). This information is vital because it allows researchers to understand the conditions in which cytokines are released then choose which cytokines should be inhibited to prevent an inflammatory response. However, a more faithful and accessible human *in vitro* systems are urgently needed to understand how specific diseases affect the gut. The lack of microorganisms and enzymes naturally found in the intestines incorporated into these systems (Shim et al. 2017) has been an obstacle for many researchers to model the physiological and pathophysiological processes in *in vitro* studies on Earth or in space.

2.3 Kidney-on-a-chip

The kidney is an important organ responsible for removing toxins from the bloodstream. The human kidney comprises of multiple cell types with different functional unit. The glomerulus is responsible for the filtration of the blood, while the proximal tubule accounts for much of the resorption that occurs within the nephron. Different parts of the kidney, such as the glomerulus and the proximal tubule, have been modeled on various kinds of chips to study different diseases that affect the kidney. In the study by Sakolish *et al.*, tissue chip with components that represented the glomerulus (filtration barrier) as well as the proximal tubule was created (Sakolish et al. 2019). They observed that the cells cultured in the tissue chips were healthier and more accurately responsive when shear stresses were applied to cells when modified the conditions to create the disease conditions (Sakolish et al. 2019) utilizing multi-chamber designs. The design has proven to be major advantage where the uptake and transport of drugs can be easily studied through the various chambers. In addition to that, the tissue chip developed for this study allows new cells to be reloaded and the tissue chips to be reused, making it a much more cost-effective and environmentally friendly option, especially during spaceflight.

Musah *et al.* showed that their tissue chip system has much potential to model other mechanisms seen within the kidney in both healthy and diseased states. Because the kidney is a complex organ to emulate, many are still developing more advanced kidney-on-a-chip to create an effective *in vitro* model (Musah et al. 2017). Other groups successfully modeled and perfected one aspect of the structure and function of the nephron (Mu et al. 2013) by creating two networks for the vascular and tubular parts of the nephron to mimic the passive diffusion that occurs during the resorption process through the basement membrane and interstitial space.

The ideal kidney-on-a-chip would comprise of different cell types to initiate cell-cell interaction to observe structural arrangements, cells signaling, fluid flow dynamic, cell function and many more (Ashammakhi et al. 2018b). While kidney-on-a-chip has been a successful endeavor for many researchers on Earth, further development to send kidney-on-a-chip to space may be required to achieve the same outcomes seen on Earth. Himmelfarb et

al. from the University of Washington, Seattle, were able to use kidney-on-a-chip to observe the effects of microgravity on the structure and function of proximal and distal tubule of kidneys in their microphysiological system. The success of Himmelfarb's kidney-on-a-chip in microgravity has laid the foundations for many future researchers in sending their tissue chips into space. Therefore, the next step in assembly of kidney-on-a-chip is to incorporate biosensors into tissue chips comprises of multiple compartments to observe the intracellular signaling and pressure gradient, metabolic activity, and endocrine functions in microgravity.

3.0 Integrated Bioelectronics for Tissue Chips

Integrating various bioelectronics and biosensors into OoC devices would allow the monitoring of tissue function such as metabolic activities and the collection of biological signals and changes in the microenvironment of cells and tissues. In general, biosensors can be divided into two types: chemical sensors (oxygen, metabolites, proteins, etc.) and physical sensors (current, mechanical activity, etc.). A recent review paper has detailed the developments of tissue chips integrated biosensors, including oxygen, metabolites (glucose and lactate), electrical resistance across cellular monolayers, electrical activities, mechanical activities, and electromechanical activities (Ferrari et al. 2020). In this section, we will focus on an emerging classes of tissue chips with integrated 3D bioelectronics and biosensors.

Precisely monitoring the physiological and biological properties of 3D tissues like organoids throughout their 3D and microporous interior, especially their electrophysiological behaviors, will significantly enhance our understanding of the development of organs such as the nervous system and the evolution and origins of neurological disease states (Di Lullo and Kriegstein 2017). Several recent studies represent initial works in the integration of flexible electronic sensors with 3D porous scaffolds for online monitoring and regulation of engineered cardiac, neuronal, and muscle tissue function (Cools et al. 2018; Dai et al. 2016; Feiner et al. 2016; Feiner et al. 2018; Kalmykov et al. 2019; Park et al. 2021; Tian et al. 2012; Wang et al. 2022), including using silicon nanowire-based nanoelectronic scaffolds (Tian et al. 2012) and cylindrical electrode arrays (Kalmykov et al. 2019).

Among those 3D platforms, an emerging 3D functional platform, which incorporates 3D bioelectronics and biosensors to interface with tissue chips, receives much attention. The 3D functional platform is assembled using a mechanically guided assembly technique (Figure 4a), which begins with the lamination of 2D patterns onto a pre-stretched elastomer as an assembly platform. Release of the pre-strain in the elastomer leads to compressive forces that induce geometrical transformations of the 2D patterns into 3D configurations in a process of controlled buckling. This technique is fully compatible with existing micro/nanofabrication techniques and is capable of fabricating 3D flexible structures of diverse geometries and material compositions, including polymers, metals, semiconductor silicon, microscale inorganic light emitting diodes (μ-ILEDs), and their heterogeneous combinations, at length scales down to micrometer (Guo et al. 2018; Kim et al. 2018a; Kim et al. 2018b; Liu et al. 2019; Shi et al. 2017; Wang et al. 2019; Won et al. 2019; Xu et al. 2015; Zhao et al. 2019) (Figure 4b). For example, Wang et al. developed a 3D electronic scaffold of precisely defined dimensions and microelectrode configurations and integrated it within engineered 3D cardiac tissues for monitoring and controlling tissue function and

for initiating on-demand, local release of drugs, each through well-defined volumetric spaces (Wang et al. 2020) (Figures 4c–e). Li et al. created cyborg organoids via the 3D assembly of soft, stretchable mesh nanoelectronics across the entire organoid (Figure 4f) (Li et al. 2019). More recently, Park et al. reported 3D assembled compliant multifunctional frameworks to enclose gently around the surface of brain spheroids to minimize the interference with the tissue development (Park et al. 2021). The 3D multifunctional device provides capabilities of electrical, optical, chemical, and thermal sensing and actuation for the monitoring and evaluation of spheroids (Figure 4g–k).

Vascularization is essential for controlled nutrition/gas delivery, waste removal, and efficient neural progenitor differentiation (Yin et al. 2016) to increase organoid lifespan, size, and structural and functional complexity (Garreta et al. 2020; Giandomenico and Lancaster 2017). Recent methods of engineering vascularization for in vitro microvasculature include self-assembly of endothelial cells into perfusable vascular networks within a microfluidic device (Campisi et al. 2018) and 3D bioprinting sacrificial vascular networks (for heart) (Skylar-Scott et al. 2019). One challenge is to achieve stable and mature microvascular networks within organoids that have proper composition and morphology of the native vasculature. Luan et al. recently engineered complex 3D microvascular networks via a robust, yet convenient route inspired by the mechanically guided 3D assembly approach (Figure 5) (Luan et al. 2021). Figure 5a–c shows a scalable 3D microvascular structure that adopts a stepwise change in the width of microchannels (100 μm, 30 μm, 10 μm), which geometrically mimics a basic vascular network. The fabrication begins with the formation of 2D precursors with embedded microfluidic channels from polydimethylsiloxane (PDMS) thin films using established procedures of molding and bonding in soft lithography (Duffy et al. 1998). Patterning these planar platforms into open geometries with a picosecond LPKF ProtoLaser prepares them for the 3D buckling process, which allows complex 3D geometries, open mesh architecture, and easy integration with functional components. In addition, electronics/optoelectronic components including μ-ILEDs, heaters and thermistors, and electrodes are integrated with the 3D microvascular networks for temperature sensing, thermal actuation, etc. (Figure 5e–h). Furthermore, by incorporating shape memory polymers and magnetic particles with PDMS, Wang et al. developed approaches to program 3D microfluidics (Figure 5i), which can be harnessed for applications like programmed drug delivery (Wang et al. 2022).

It should be noted that the existing tissue chip-integrated 3D bioelectronics mainly focuses on extracellular potential recordings, optical stimulations, and biophysical sensing, with limited demonstration of biochemical sensing, including metabolites (glucose and lactate) and protein biomarkers. For conventional 2D-based platforms, researchers have developed many kinds of glucose and lactate sensors integrated into OoC (Bavli et al. 2016; Lin 2014; Weltin et al. 2014), among which, enzymes-based electrochemical-based sensors are mainly used. However, for this kind of sensor, the frequent calibration and replacement of the sensor present a significant challenge. To that end, Misun *et al.* have developed a modular microfluidic and sensor structure that enables easy disassembly compared to conventional integrated OoCs, simplifying sensor replacement while obtaining high-precision measurement data (Misun et al. 2016). Monitoring glucose and lactate can also reflect the drug's toxicity and cell viability for drug screening. Weltin *et al.* analyzed the

relationship between the lactate produced and the amount of the drug Bosentan used. The entire process is monitored using enzyme-based electrochemical lactate sensors (Weltin et al. 2017). Due to the compatibility of assembling 3D platforms with existing methods to fabricate enzymes-based electrochemical-based lactate and glucose sensors, the inclusion of additional metabolites sensors would significantly expand the measurement capability of existing 3D bioelectronics to interface with tissue chips.

In addition to these small molecules of oxygen and metabolites (glucose and lactate), protein biomarkers, such as cytokines can regulate different cellular functions in the human body's immune process, such as the activation of antibodies. It also plays an essential role in cell signaling (Stenken 2015). Those protein biomarkers are typically detected by using immunosensors in which antibodies are used as biorecognition element. For example, Son et al. developed an integrated microsystem which consists of a microfluidic device for cell culture and a sensing chamber for the detection of secreted growth factors. The detection is based on the microbeads-based fluorescent immunosensor in which the fluorescent signal is generated from a sandwich complexes (antibody-coated capture beads and fluorescent detection beads). Different from the fluorescent method, Riahi et al. (2016) reported an electrochemical immunosensor for the detection of transferrin, a known liver biomarker, secreted from hepatocytes (Riahi et al. 2016; Son 2017). Nevertheless, antibodies suffer from limited stability and shelf-life, and lengthy and expensive production process. Different from antibodies, aptamers, single-stranded DNA or RNA molecules were found to be more stable than antibodies. Additionally, the conformational rearrangement of aptamers in the presence of cytokine enables the continuous and real-time monitoring, which is different from the single binding of using antibodies as sensor receptors. Shin *et al.* developed an aptamer-based electrochemical sensor to monitor creatine kinase (CK)-MB, an indicator for cardiac damage, in cardiac organoids cultured in a microfluidic platform (Shin et al. 2016). Therefore, integrating these aptamer-based biosensors into 3D platforms represents a future direction in order to spatially map protein biomarkers.

4.0 Tissue Chip Applications in Space

Tissue chips can be easily modified in a controlled, adaptable environment that is significantly easier and more reliable to create the disease state on a chip rather than trying to induce a diseased condition in an animal model with high variability. Therefore, tissue chips can be used in space for various experiments and testing, such as disease modeling. Cells in the human body experience a unique environment in microgravity and affect cell signaling and aggregation, resulting in changes in the overall 3D structural organization into tissues. Not only do the effects of microgravity exposure on genes, cells and, organisms result in changes similar to those seen on Earth (Capri et al. 2019; Honda et al. 2014), but it was shown that the onset and progression of diseases might be accelerated during spaceflight as well, providing new insights to access disease mechanism pathways otherwise takes a longer time to create (Low and Giulianotti 2019). Figure 6 shows the representation of researchers utilizing and integrating biosensors into tissue chip to OoC, tested on Earth and in space. The process of aging could be accelerated in microgravity (Biolo et al. 2003) and cause major dysregulation of the immune system. Schrepfer, et al. (2017) from UCSF have developed tissue chips aboard the ISS for up to a month is

to gain a better understanding of the influence of immune cells aging on the regenerative capacity of tissue-specific stem cells (Schrepfer 2017). To fully take advantage of the accelerated aging process in microgravity, a few researchers have developed osteoarthritis (OA) model in space (Rebecca Mae Black 2018). OA is a complicated model to obtain in the traditional *in vitro* model because several factors contribute to OA in the joint resulting in the degeneration of the joint (Occhetta et al. 2019). The researchers on Earth have included different triggers leading to OA to model the disease on Earth (Occhetta et al. 2019) while Rebecca Mae Black et al. 2018 from the Massachusetts Institute of Technology (MIT) have created a tissue chip to study interactions between cartilage-bone-tissues co-cultures of healthy joints as well as post-traumatic osteoarthritis (PTOA) in microgravity. They also tested for therapeutics that can prevent bone resorption and inflammatory responses to understand metabolic pathways involving PTOA pathology (Rebecca Mae Black 2018).

Spaceflight often has detrimental effects on the musculoskeletal systems of astronauts. For example, an absence of biomechanical loading due to microgravity can result in degradation of articular cartilage (Bader et al. 2011; Hinterwimmer et al. 2004). Because natural cartilage has limited self-repair ability (Huey et al. 2012; Lories 2011), it has been a challenge to regenerate authentic cartilage tissue after it degenerates. In space, the abnormal biomechanical loading caused by microgravity most likely also damages chondrocyte function and cartilage homeostasis. Therefore, Chen, et al. (2020) from University of Connecticut utilized DNA-inspired Janus base nanomaterials to develop a tissue engineered cartilage with a sustainable supply of mechano-responsive microRNA to restore cartilage homeostasis in long-term (Chen 2020; Griger et al. 2022; Lee et al. 2021; Sun et al. 2020; Yau et al. 2021; Zhou et al. 2020b; Zhou et al. 2020c; Zhou et al. 2021). Studies in microgravity using human tissues modeling muscle wasting, clinically known as sarcopenia, can significantly aid in understanding the disease. Skeletal muscle cells can be used in these microfluidic systems because the 3D structure of the chips creates a biocompatible environment for cells to survive. Malany et al. (2018) from the University of Florida studied the effects of microgravity on the electrical activity of human myocytes and tissues in almost real-time with the use of electrical stimulation to the tissues in a skeletal muscle tissue chip (Malany 2018). Before spaceflight, they established culture conditions from human myocytes, isolated from young, healthy, and older, sedentary volunteers, and obtained the biological data based on the cells from the donor tissue. Then, they fabricated a flight-ready chip with multiple chambers fully equipped with automation such as a fluid handling system.

In many circumstances, human-induced pluripotent stem cells (hiPSCs) have been utilized in tissue chip settings because it allows stem cells to differentiate into the intended cell type within the microstructure, eliciting a more accurate cellular response (Kimura et al. 2018) found in an in vivo study. Wu, et al. (2018) from Stanford University used hiPSC-derived cardiomyocytes cultured in tissue chips to form Engineered Heart Tissues (EHTs) and investigated the disease patterns observed in ischemic (Wu 2018). In this study, they can understand cellular mechanisms and differences that affect cardiac function under microgravity and Earth’s gravity. They also observed how drug prevent or respond to the changes in microgravity. These tissue chips were able to maintain a tissue-specific microenvironment and model cardiomyopathies, which has yielded deeper insights into

several rare and common causes of heart failure. At the same time, Kim, et al. (2018) from University of Washington researchers used iPSC-derived cardiomyocytes in a high throughput tissue chip platform with a scaffolding matrix containing electroconductive composite to further understand the progression of chronic heart diseases on Earth (Kim 2018). The data collected from the tissue chips aboard the ISS will provide a better understanding of how microgravity affects the human heart structurally and functionally. After the spaceflight, they will be able to understand the differences in cardiac function and physiological maturation between cells on Earth and in space.

5.0 Advantages and Limitations of Tissue Chips and Integrated Biosensors in Space

Traditional *in vitro* models often do not translate well *in vivo* and clinical studies. Therefore, sending traditional 2D *in vitro* models aboard the ISS is sometimes counterproductive. The evolution of the 2D *in vitro* models to 3D *in vitro* models such as organoids is more accurate and relevant. Organoids have been developing rapidly in the field of regenerative medicine but still often require polymeric matrices (like Matrigel) to suspend the cells within and form the shape as needed. The advantage of the tissue chips over traditional 2D models is the spatiotemporal orientation available for cells to expand and further differentiate. With the constant technological evolution, tissue chip is one of the best ways to emulate physiological conditions as well as disease modeling. It is widely understood that microgravity is one of the reasons for faster aging in astronauts, but limited bio-samples do not allow for an extensive study in humans. In that regard, tissue chips will be able to aid in further understanding of the mechanism of aging (up to 10 times) (Jayasuriya et al. 2016; Science 2013; Yang. 2012; Yu. 2012), especially when microgravity tends to accelerate many pathophysiological conditions resulting in the onset disease progression. The possibility of co-culturing multiple cell types neighboring one another is also an attractive advantage that tissue chips provide compared to the traditional model (Chen et al. 2016; Liu et al. 2018). This way, researchers could study the cell-cell interaction and cell-ECM interaction *in vitro* while observing them in real-time with biosensors, especially when they are subjected to microgravity.

The use of biosensor in tissue chips is beneficial, especially in the applications of tissue chips in space. On Earth, it is often difficult to monitor the biological signals on any biological experiments in real time. However, biosensors can amplify or convert these signals into electronic signals that are easy to measure. Compared with traditional biological experiments, biosensors combined with OoC are often very selective, resulting in a more accurate measurement of specific biological signals. In addition, unlike *in vivo* experiments, biosensors integrated with OoC require only a small amount of specific tissues or cells to operate, significantly reducing the time and cost of experiments. In the past decade, NASA has sent few biological samples into the ISS to determine the effects of microgravity has on different biological organisms. For example, in 2006, NASA developed a 12-well fluidic card with LED optical detection to study the gene expression of *Escherichia coli* (Ricco 2011). More recently in 2022, NASA sent 18 fluidic cards (comprises of 288 wells) to determine the deep space radiation effects on *Saccharomyces cerevisiae* using

Timepix-based linear energy transfer (LET) spectrometer (Kanapskyte et al. 2021; Ricco 2020). In addition to that, NASA helped developed Microfluidic Icy-World Chemistry Analyzer (MICA) to detect a panel of electrochemistry found on Europa's surface materials (Noell 2019). This MICA sensor technology can be converted to sense, process and oversee biological signals collected from the tissue chip, especially for a long-term space mission in the future.

The impact of space technology is often tremendous and improves lives on Earth. As such, having the opportunities to perform studies in microgravity often drives the development of innovative technology, which is beneficial to the people on Earth. When culturing cells on Earth, sedimentation tends to occur due to the gravitational pull of the Earth, especially in a 2D monolayer environment. A 3D environment would more closely resemble the tissue environment found in living organisms. However, in a scaffold-free 3D environment, cells would still be subjected to gravitational pull and settle on the bottom of the culture flask, negating the environment found in native tissue. The need to study materials in microgravity drives scientists and engineers to develop various instrumentations (Amselem 2019), biomaterial scaffolds (Chen et al. 2010; Chen and Webster 2009; L. Zhang 2008; Song et al. 2011), and cell culture methods (organoids) to develop 3D structures (Grimm et al. 2014). As a result, some researchers have created a temporary microgravity environment on Earth using free-fall conditions – where forces act against gravity (Amselem 2019) like the random positioning machine (RPM), rotating wall vessel (RWV) (Klaus 2001), drop towers and parabolic flights (Günter Ruyters 2006; V. A. Thomas 2000). RPM, a two-axis clinostat used for microgravity simulation, can reproduce effects observed in space, while RWV contains a chamber rotating around an axle, creating a continuous free-fall condition for cell culture, suitable for space applications on Earth.

While tissue chip studies on Earth are cost-efficient, it is a different story regarding to experimentation during spaceflight. First, a complete laboratory setup is often encouraged, if not, necessary, to begin research aboard the ISS. A major advantage of utilizing tissue chips on Earth is that the contents of the cell channels can be removed and collected for further analysis without disrupting the entire system. This allows researchers to get a better understanding of the cellular response because the cell secretions can be analyzed individually and at different time points throughout the experiment. However, there is a limitation on having the collection system and the microscope capability in the ISS. Therefore, the researchers must consider the technological limitation of equipment when observing tissue chips in real-time in space aboard the ISS. Because many steps that were done hands-on in person on Earth will have to be automated in Space, the hardware and software programming of how waste is collected and stored at defined time points and cell imaging must be adapted aboard the lab in the ISS.

Long-term orbital space travel is often challenging due to the limited space flight opportunity as well as the high costs of hardware development to stow tissue chips in space. Currently, the footprint in maintaining tissue chips in a conventional lab is significant, as mentioned in a commentary by Yeung et al. (2020) (Weber et al. 2016; Yeung et al. 2020). A complete re-engineering of perfusion and environmental control system is often needed to meet spaceflight limitations due to stowage size constraints. While tissue chips are

just small devices containing human cells, the complexity of tissue chip sustenance needed to be miniaturized, simplified, and automated where various flexible tubings needed to be eliminated and only minimal hands-on involvement of crew members are required. NIH and CASIS have provided an opportunity for investigators to collaborate with implementation partners to overcome various challenges and aiding to translate the ground-based research to projects ready to be launched to the ISS.

The most critical shortcoming of the current biosensors on Earth is their sensitivity and accuracy. Existing biosensors on Earth are typically constituted of polymers and metal materials that reacts with biological tissues as the environment changes (temperature or humidity). However, their sensitivity decreases as the usage time increase, which may cause inevitable inaccuracy on results gathered. In addition to that, the rigors of launching from and the landing to Earth may contribute to biosensor insensitivity. Similarly, it is known that biological organisms would be affected by space radiation outside of the protective bubble of the Earth's atmosphere (Jamaji Nwanaji-Enwerem 2022; Li et al. 2018). Therefore, it is safe to presume that when the biosensors are subjected to different environmental changes such as being exposed to cosmic radiation, their accuracy would be impacted as well. Materials that fabricated the biosensors were only tested on Earth may not be suitable to be utilized in space which may require further advances in the field of material sciences. Although the sensor systems that can monitor multiple OoCs simultaneously have been developed on Earth, their stability and coordination are still insufficient compared with traditional biological experiments. In terms of wireless control, the stability of wireless signal transmission still needs to be improved. The materials used to construct biosensors is a key point to consider when assembling tissue chips with biosensor to avoid side effects caused by the environmental changes in space, vibrational effect during launching and landing. As technology continues to evolve, the ability of overcome the rigors of launching and touchdown should be applied to all future devices sent to space indefinitely.

In addition to withstanding the vibrational effect of launch and touchdown, multiple levels of containment of devices must be put in place, with a goal to protect the experiments in the container from various complications such as radiation, leakages, and contamination. More importantly, the layers of containments is used to protect the safety of the crewmembers on board (Low and Giulianotti 2019). In microgravity, aerosols do not sediment like it would on Earth. Therefore, the inhaled aerosols or particles will stay in the airway and transport to the alveolar regions of the lungs which is a risk to all the crewmembers in the ISS (G. Kim Prisk 2009). Any minute hazardous materials will pose a risk to the crewmembers onboard of the ISS due to the closed environment of the laboratory in the ISS (Yeung et al. 2020). There are many challenges that all parties may face in integrating platform and prepare the payload for flight where each step taken. Precise and intricate documentation to ensure good manufacturing practice and good laboratory practice are often challenging because the preparation for each project is unique which require different setup and systems, and therefore time-consuming. To counter that point, a universal and standard experimental setup system is encouraged, or the use of universal media may be necessary to relief some congestion encountered in payload preparation processes (Low and Giulianotti 2019). The capability for the research team to remotely monitor, detect, and change the settings is essential.

Multiple factors other than microgravity such as launching, installation, landing and cosmic radiation could play a major role in affecting the payloads in the ISS. When the OoC system is simplified and automated, it will be easier to control and monitor the system and harder disrupt cell culture systems in place (Low and Giulianotti 2019). While the tissue chips are elegant and simple, they go beyond just tissue chips when heading into space. A sturdy yet simple stowage platform may need to be developed for every type of tissue chip, depending on their need. An entire team of many engineers and scientists will have to work closely together to develop an entirely novel pumping, incubation, and imaging system customized to each type of tissue chip to adapt for flight readiness. The ongoing tissue chip studies have addressed these factors and supported through the collaborations with independent companies to the researchers in translating Earth-based lab research to flight readiness for launch (Yeung et al. 2020).

6.0 Future Technology That Can Contribute to Studies in Space and On Earth

As technology involves, so is the development of artificial intelligence. With enough data collected since the beginning of space exploration, a thorough computational simulations can be performed to screen how cells behave in a computational model prior to sending payloads to the ISS. Multiple models can be simulated for unexpected outcomes and missions can be designed to predict the success of a project. Multiple parameters can be put in place to ensure the highest chance of success in the computer simulations testing prior to loading projects into the ISS. Ideal nutrient and gas level can be monitored and controlled by artificial intelligence to provide the best environment for each tissue chip. Furthermore, the development of artificial intelligence can support crewmembers on board, relieving them of operating computer manually by having the ability to receive voice-controlled commands, make decisions to collect data, gathered a large volume of data and effectively processing them smartly.

The evolution of 3D technology in other fields has also influenced the field of tissue engineering, creating 3D *in vitro* model that mimics the physiology of tissues and organs, leading to the beginning of the 3D bioprinting field. The concept of 3D bioprinting allows for the precise placement of cells and biomaterials, in a pre-determined 3D position (Ramiah 2020). Bioprinting of organoids from human stem cells has recently been reported (Jonathan A. Brassard 2021; Lawlor et al. 2021; Skylar-Scott et al. 2019). It has opened new possibilities to create mini models of human organs, especially under the context of space biology research and its implication back on Earth (Moroni et al. 2022). Although these printing methods were proven successful, the printed constructs' static nature often does not accurately model the dynamic properties of native tissues. Therefore, four-dimensional (4D) bioprinting has emerged to produce constructs that are more physiologically relevant to native tissue, where these constructs are stimuli-responsive to pH, temperature, and magnetic attraction (Ashammakhi et al. 2018a). Soon, newly 4D bio-printed constructs coated with different materials such as polyethylene glycol (PEG) or poly(methyl methacrylate) (PMMA) (Bi et al. 2006) could be added or placed directly into tissue chips testing the effects of microgravity.

Since the use of scaffold is important to produce a native-like microenvironment for cell culture, some researchers have developed an injectable solid scaffold that can improve cell functions and differentiation (Zhou et al. 2020b; Zhou et al. 2021). In Zhou *et al.* 2020 (Zhou et al. 2020b), the researchers developed the self-assembled biomimetic Janus base Nano-Matrix (JBNm) for stem cell anchorage (Lee et al. 2021; Zhou et al. 2020c). In the subsequent paper in 2021 by the same group (Zhou et al. 2021), they successfully incorporated growth factors within the layers of JBNm through controlled self-assembly, forming a layer-by-layer (LbL) scaffold (Landolina et al. 2022). They showed that the JBNm achieved localized drug delivery and promoted stem cell anchorage for homeostatic tissue constructs. With advances in tissue chips together with solid scaffolds like the JBNm encapsulating drugs, integration of both technologies is possible. Further integration of biosensors into tissue chips containing injectable scaffold is expected to further study the tissue functions long-term. This will allow for development of new drugs and diagnostic once the mechanisms of diseases are fully understood. In this respect, using molecularly designed JBNm using self-assembly with biosensors into tissue chips in space is possible.

7.0 Conclusion and Future Perspectives

This review has shown that this technology can significantly improve the drug testing process. The efforts of researchers to develop and optimize organ-on-chip systems are successful. Multiple fields in regenerative medicine, such as tissue chips, bioprinting, biosensors, organoids, and biomaterials are moving forward simultaneously toward the same goal, the progress of human lives on Earth. Microgravity affects all levels of biological organisms differently than when on Earth. Research in microgravity can uncover a new understanding of living systems of novel directions of drug research and development as well as the acceleration of disease progression. Soon, tissue chips in space would contain cells with scaffolds in cell chambers connected through fluidic channels, just like how blood vessels connect organs in the body. The size, volume, and metabolic rate of the chambers can all be scaled to correlate to the organs in the body to more accurately model how they communicate (Sung et al. 2014). Biosensors can be implanted to assess and monitor the health of the organs in the chip in space, especially for long term missions. A novel drug can then be introduced into the system and based on the cell viability, electrical signals, and metabolic markers; the bodily response can be ascertained between a few organs as well as the larger organism as a whole (Sung et al. 2014). Tissue chips in space deliver a new solution for the future space program. However, the inclusion of more laboratory equipment in the ISS and a reduction in experiment setup size and more sensitive sensors may be required to conduct research on the ISS. As the development of space technologies and commercial space stations, the use of 3D tissue chips in microgravity may advance exponentially in the next decade. These microfluidic systems could be a great precursor to animal studies, saving drug developers time and money in determining which drugs can be successful. Therefore, OoC with biosensors have an integral role in the future of drug development and could very well permanently change this process for decades to come.

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Abbreviations:

CASIS	Center for the Advancement of Science in Space
NASA	National Aeronautics and Space Administration
NIH	National Institute of Health
NSF	National Science Foundation
ISS	International Space Station
NCATS	National Center for Advancing Translational Sciences
2D	two-dimensional
3D	three-dimensional
OoC	organ-on-a-chip
PDMS	polydimethylsiloxane
EHTs	Engineered Heart Tissues
CNT	carbon nanotube
JBNt	Janus base nanotubes
JBNm	Janus base Nanomatrix

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Highlights

- Multidisciplinary review on biosensors, microfluidic devices, and tissue engineering
- Applications of biosensor integrated tissue chips on Earth and in space
- In-depth discussion of the future development of tissue chip systems in space
- Biosensor integrated tissue chips to better translate into clinical studies

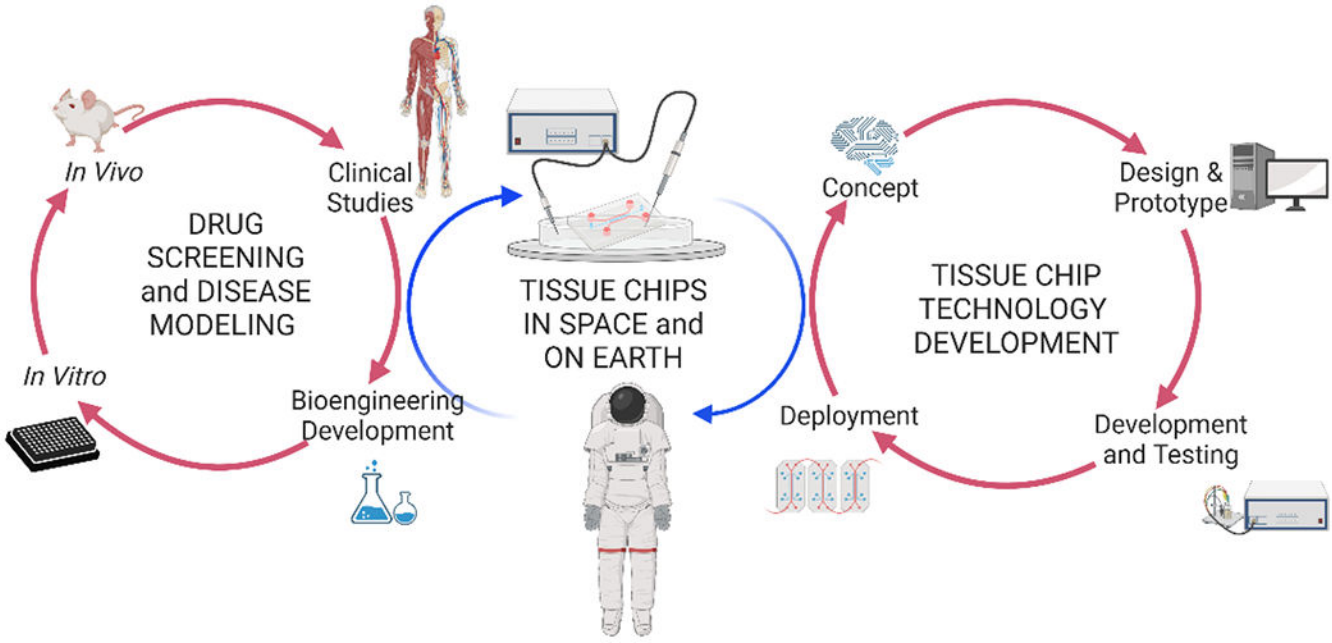


Figure 1.

The evolution of biological and technological research advances the progression of bioengineering studies in space. Several national programs have helped multiple research projects to utilize the tissue engineering and microfabrication advances to create tissue and organ-on-chip with and without biosensors platforms to mimic human physiology. Through this, scientists can better understand the role of microgravity on human health and diseases.

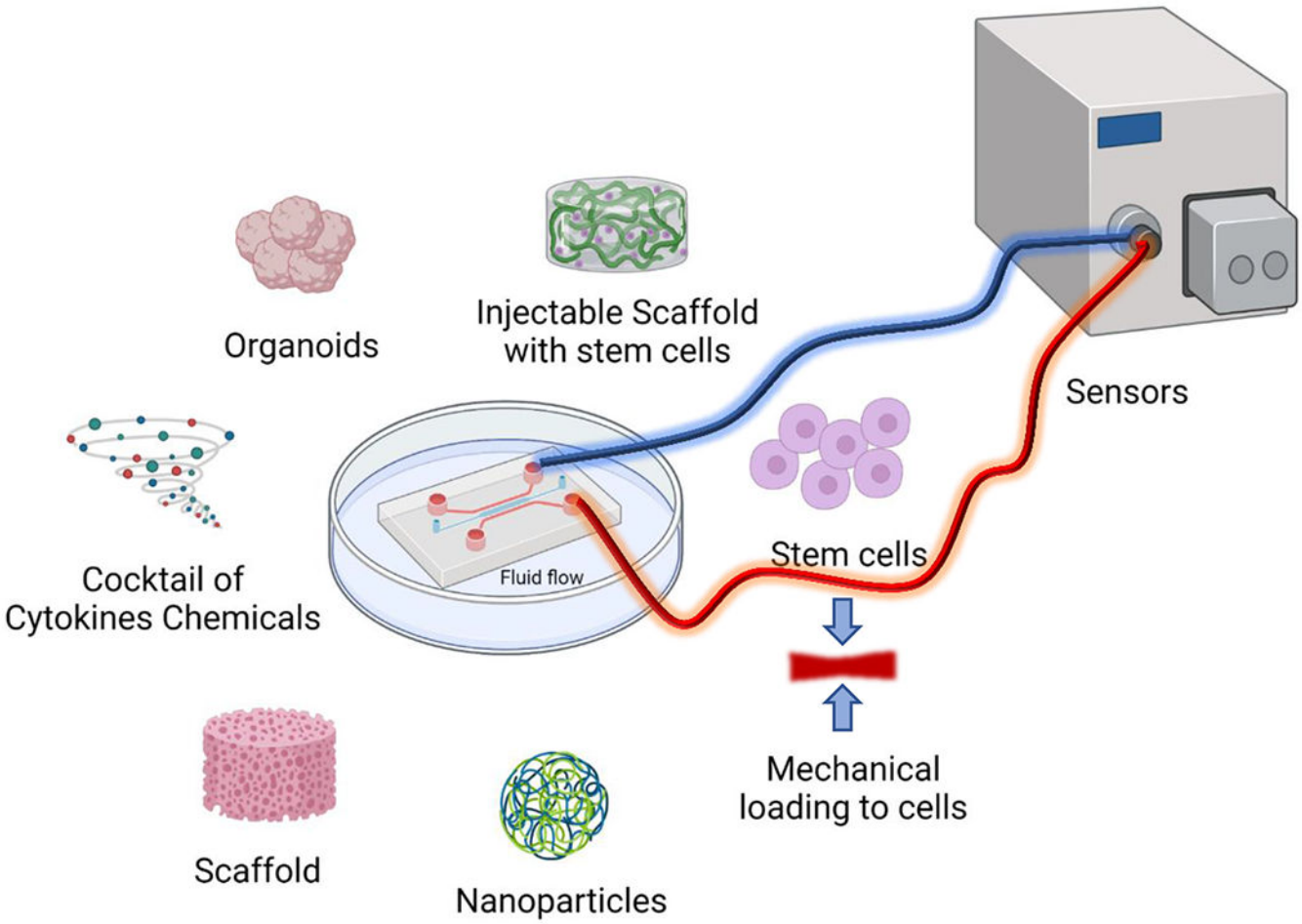


Figure 2.

Tissue chips can be customized to fit each healthy and/or diseased organ to screen novel therapeutic drugs. Parameters include changing the fluid flow, adding mechanical loading can be adjusted together with the addition of stem cells, scaffolds, and organoids to the tissue chip to mimic the physiological conditions of native organs. Cocktails of cytokine chemicals can be added to model certain diseases and detected with biosensors. These conditions can be accelerated when tested in space.

Advantages and Applications of Tissue Chips on Earth and in Space

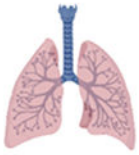
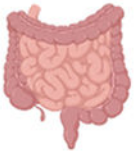
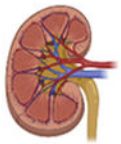
	Lung on a Chip	Intestine on a Chip	Kidney on a Chip
			
Advantages on Earth	• Understanding the mechanism of lung with vacuum channels to mimic "breathing" • Model lung diseases	• Creation of villi to absorb nutrients • Ability to manipulate peristalsis with tissue chip	• Formation of different tissue chips for different part of kidney • Drug screening
Applications in Space	• Accelerated aging and diseased models • Understanding immune response of human respiratory system in microgravity	• Studied bacterial infection in human gut subjected to microgravity • Ability to manipulate forces to mimic perstalsis remotely	• Understanding the mechanisms of healthy and diseased states with different functional units
Further improvements to be added	• Scaffolding needed for formation of lung structure • Inclusion of biosensors to detect biochemical releases and gas exchange	• Addition of different gut microbiomes • Detection of cytokines with biosensors	• Inclusion of biosensors to detect • Automation to detect and dispose waste and ability to collect nutrients as needed

Figure 3.

Advantages and applications of tissue chips on Earth and in space. Tissue chips on Earth have provided major advantages, such as providing a 3D microenvironment for cells to develop and differentiate to tissues and organs. Different parameters can be manipulated, experimental progress can be monitored real time. Many have started translating these ground-based research to in space applications with the support of the government funding. However, further improvement is encouraged to expedite the success of the next generation of vitro model.

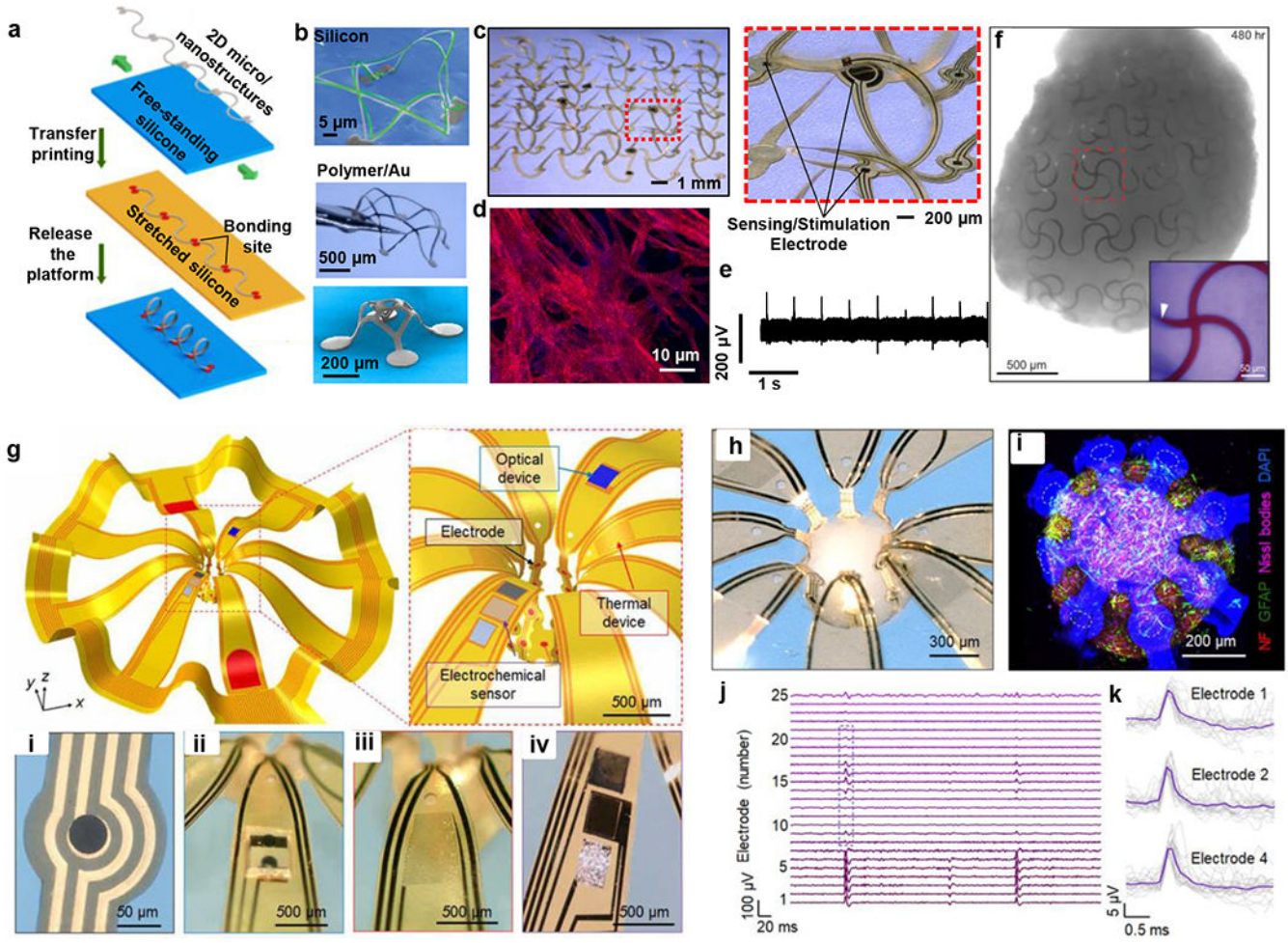


Figure 4. 3D electronic sensors interfacing with tissues.

(a) Schematic illustration of the 3D assembly process. **(b)** Images of 3D microstructures. Reference for Figure 2 **(b)** is from (Wang et al. 2019; Xu et al. 2015) **(c)** Optical image of a 3D electronic scaffold. **(d)** Confocal microscope image showing the assembled cardiac tissue within the electronic scaffold. **(e)** Extracellular potential recordings from an electrode embedded within the cardiac tissue. Reference is from (Wang et al. 2020). **(f)** Phase color images showing a cardiac organoid with embedded mesh electronics. Reference from (Li et al. 2019). **(g)** Simulation results of a compliant, multifunctional framework, with a magnified view and optical micrographs to highlight the functional components including 25 microelectrodes (Pt black, diameter of 50 μm, image i), μ-LED, thermal actuator and sensor (Au trace in serpentine geometry), and electrochemical oxygen sensors (Pt black, Au, and Ag/AgCl as working, counter, and reference electrodes, respectively). **(h)** Optical image of a cortical spheroid enclosed in a 3D multifunctional device designed for electrophysiological recording. **(i)** Confocal microscope image of the spheroid in a similar 3D mesostructure, formed in a transparent polymer without microelectrodes. **(j)** Representative field potentials recorded from all 25 microelectrodes in the system. **(k)** Overlaid plots of 30 spikes from microelectrodes. Reference for Figure 4 (g–k) is from (Park et al. 2021)

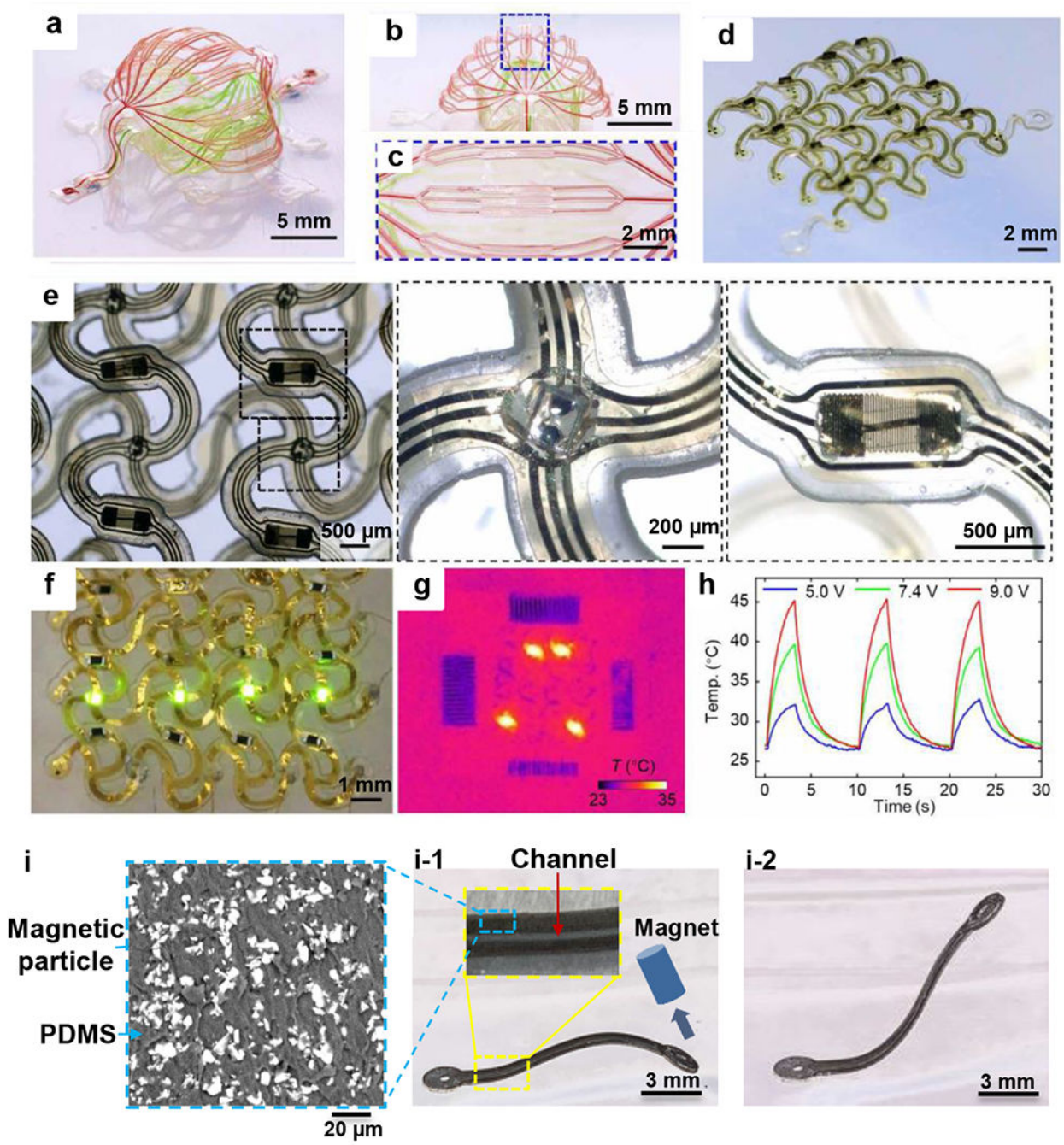


Figure 5. 3D hybrid microfluidic and electronics systems.

(a-c) Optical images of a 3D microfluidic network from a 3D view (a), side view (b) and magnified view (c). **(d)** Optical image of a 3D system with integrated electronic components (μ-ILEDs, heaters and thermistors, and electrodes). **(e)** Top view optical image of the system, with magnified views of a μ-ILED and a microfabricated serpentine trace, respectively. **(f)** Image of μ-ILEDs during operation. **(g)** Infrared image of local heating performance. **(h)** Plot of temperature versus time near a heater element during cyclic operation (10-s period and 3-s duty cycle). Reference for Figure 5 (a–h) is from (Luan

et al. 2021). **(i)** Shape programming of a ribbon structure made of a bilayer of shape memory polymers and magnetic PDMS composites *via* magnetic forces. The insets show the magnified views of the microfluidic channels and magnetic particle distribution in magnetic PDMS composites. Reference from (Wang et al. 2022).

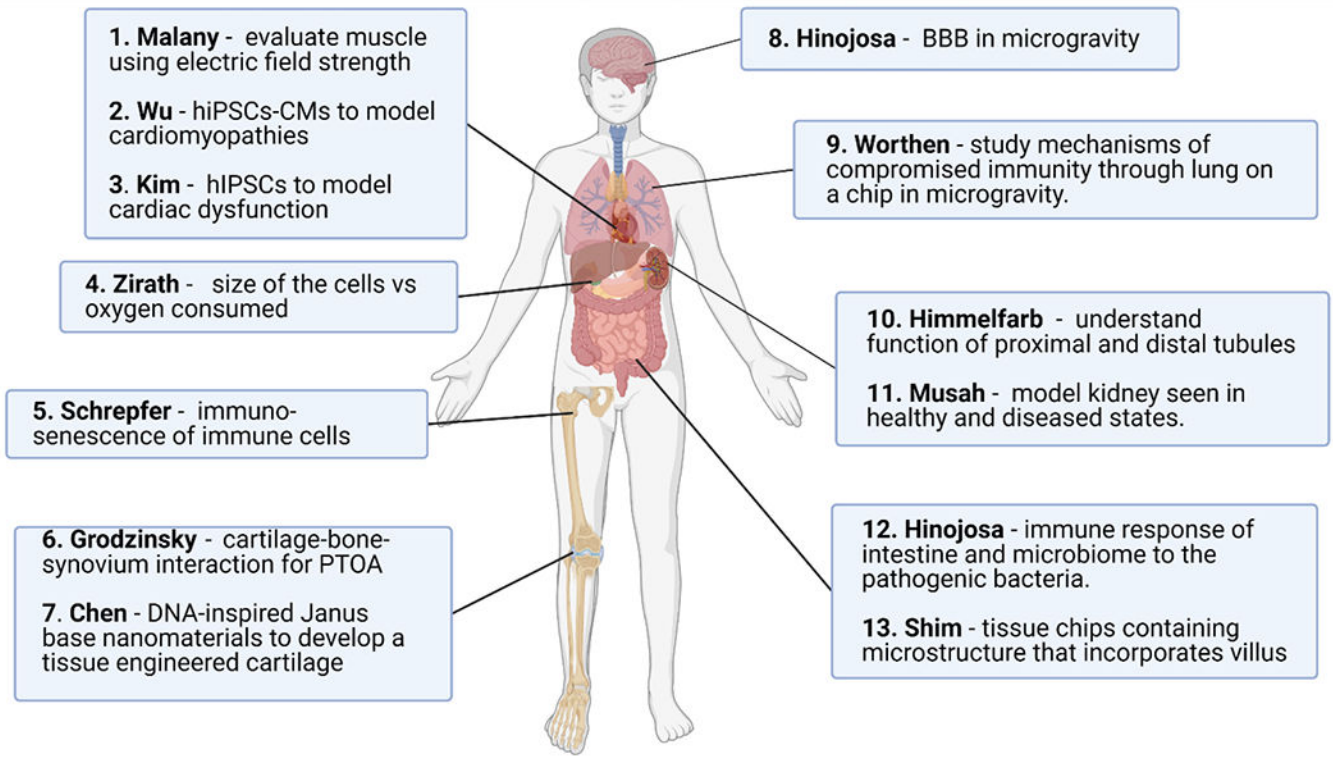


Figure 6.

Various groups integrating tissue engineering, tissue chips and biosensors to study human physiological condition and model diseased organs. (Malany 2018) took advantage of an automated tissue chip system to model muscle and evaluate muscle wasting in space by using electric field strength through electrodes. (Wu 2018) and (Kim 2018) utilized human-induced pluripotent stem cells (hiPSCs) to model cardiomyopathies as well as cardiac dysfunction and testing the effects of microgravity. (Zirath et al. 2018) studied the relationship between the size of the cells and the amount of oxygen consumed with tissue chips, while (Schrepfer 2017) studied the immuno-senescence of immune cells by observing the interaction between tissue and mesenchymal stem cell and endothelial progenitor cells. (Rebecca Mae Black 2018) working with Grodzinsky *et al.* studied the mechanisms of PTOA by looking into the interaction between cartilage-bone-synovium while (Chen 2020) successfully developed cartilage tissues by utilizing DNA-inspired Janus nanomaterials. (Hinojosa 2017, 2018) took advantage of microgravity to study its effect to the blood-brain-barrier (BBB)(Rice et al. 2022) and investigated the immune response of various cells and microbiome to the pathogenic bacteria using automated tissue chips while (Shim et al. 2017) used similar strategy to mimic microstructure of villus. (Worthen 2017) studied the mechanisms of compromised immunity in lung-on-a-chip in microgravity. Both (Himmelfarb 2017; Musah et al. 2017) studied the effects of microgravity in kidney and model the mechanisms of healthy and diseased states of kidney in tissue chips.