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# **Bioprinting of Cardiac Tissue in Space: Where Are We?**

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Bioprinting in space is the next frontier in tissue engineering. In the absence of gravity, novel opportunities arise, as well as new challenges. The cardiovascular system needs particular attention in tissue engineering, not only to develop safe countermeasures for astronauts in future deep and long-term space missions, but also to bring solutions to organ transplantation shortage. In this perspective, the challenges encountered when using bioprinting techniques in space and current gaps that need to be overcome are discussed. The recent developments that have been made in the bioprinting of heart tissues in space and an outlook on potential future bioprinting opportunities in space are described.

#### 1. Introduction

Tissue engineering envisions the manufacturing of new tissues and organ systems for regenerative and personalized medicine. In a time of organ transplant shortage and high organ rejection rates,<sup>[1]</sup> the development of methods enabling patient-derived engineered tissue structures is therefore of high interest here on Earth, as well as in space. Amongst these different methods, 3D bioprinting holds the promise to create 3D in vitro models that

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#### DOI: 10.1002/adhm.202203338

exquisitely mimic the complexity of our tissues and organs<sup>[2]</sup> and is therefore also a promising strategy to mitigate organ shortage.<sup>[3]</sup> In space, the avenue of 3D bioprinting can result in more self-sufficient medical capabilities in case of human space missions beyond low earth orbit (LEO), which is crucial for a successful mission. In addition, microgravity (one component of the space environment), can further enhance the bioprinting process by enabling more complex geometries with voids, cavities, and tunnels, which would not be possible on Earth due to the gravitational force.<sup>[4]</sup>

Bioprinting under microgravity conditions has been so far mostly proven in

parabolic flights, showing feasibility to deposit cell-laden hydrogel constructs with softer gels, which is otherwise difficult on Earth due to the lack of self-standing capacity of such gel formulations. More recently, Techshot Inc., a US commercial developer and operator of spaceflight equipment, and nScrypt, a manufacturer of industrial 3D bioprinters and electronics printers, developed the 3D BioFabrication Facility, which uses adult human cells (such as (pluripotent) stem cells) and adult tissue-derived proteins as its bioink to create viable tissues on board the International Space Station (ISS). A bioink is commonly known as a solution of a biomaterial or a mixture of several biomaterials in a hydrogel form encapsulating the desired cell types. In addition, magnetic levitation bioprinting has been tested and validated on the ISS when, at the end of 2019, Russian scientists from 3D Bioprinting Solutions were able to bioprint bone tissue by growing fragments of bone structure in zero gravity conditions.

Although current advancements do not yet allow the manufacturing of organs for transplantation, 3D bioprinting of complex tissues in space facilitates the investigation of the impact of the space environment on biological processes that would otherwise be difficult to investigate in humans or animals. This is particular true for the cardiovascular system where invasive analysis is not possible on humans or animals. Exposure to the space environment causes accelerated cardiac aging and leads to pre-development of several heart conditions.<sup>[5]</sup> Radiation mainly causes inflammation, DNA damage, and senescence, while microgravity causes impairment of DNA damage repair and deconditioning of the heart that can lead ultimately to heart failure.<sup>[5,6]</sup>

Organoids are small 3D models that recapitulate the complex structure and function of an organ. They are generated from stem- or progenitor cells from an individual, thus an exceptional tool for personalized approaches.<sup>[4]</sup> As organoids can mimic human tissues closely, they allow for authentically studying human tissues without harming the individual.<sup>[7]</sup> Thus, cardiac organoids have been used to study cardiac diseases and drug response.<sup>[8]</sup> Thick, vascularized, and perfusable cardiac patches,<sup>[9]</sup> heart organoids<sup>[10]</sup> and vascular bed were already 3D bioprinted here on earth. Yet, in space, 3D bioprinted heart tissue is in its infancy, and knowledge acquired by projects that flew on the ISS using the 3D bioprinting facilities remain to be disseminated. Furthermore, current cardiac organoid models mainly represent the immature state of fetal hearts from a developmental perspective. Considering aging occurs in mature tissues, maturation of cardiac organoids is an important aspect. By integrating cardiac organoids with an organ-on-a-chip platform to create a heart-on-chip, it has been shown that cardiac maturation can be improved.<sup>[11]</sup> Organ-on-a-chips are microfluidic devices integrated with a biological system, allowing when needed for mechanical and electrical stimulation. Organoid vascularization can be achieved through addition of endothelial cells in the formation. More precisely, either by addition of endothelial cell-inducing growth factors during differentiation or through seeding of endothelial cells in the construct, leading to tubular formation or endothelialization of, for example, micro channels. However, by implementing organoids on an organ-on-a-chip platform, macro- and microvasculature can be integrated.<sup>[12]</sup> Considering that the vasculature is a main target for radiation and microgravity related aging processes, vascularization is an important addition when studying effects of the space environment.<sup>[13]</sup> However, multiple challenges remain before heart organoids can be bioprinted to produce cardiac bioreactors (e.g., automated heart-on-chips) in space, and therefore it is of extreme importance to shade further light on the current challenges.

In this perspective, we describe bioprinting techniques used on Earth and their applicability in space. More precisely, we discuss the current advancements in cardiac tissue bioprinting on Earth and in space with attention to bioink properties and challenges encountered in space.

# 2. Bioprinting Technologies on Earth and Their Applicability in Space

The most cutting-edge technique available now to researchers who are generating 3D cell-filled constructions for tissue engineering is 3D bioprinting. The ability of 3D bioprinting to perform in situ cell encapsulation, which makes it simpler for the created scaffolds to mature into functional tissue constructs, is one of the technology's many advantages. High precision and flexibility, in combination with multiple cell deposition, are additional significant benefits of 3D bioprinting.<sup>[3]</sup> Using this technique, it is possible to precisely place a variety of cell types and materials to create a hierarchical architecture that closely replicates the structure of the targeted biological tissue. Mainly used techniques for 3D bioprinting include inkjet, laser-assisted, and extrusion, although techniques such as magnetic levitation or derivative of previously mentioned technologies have also emerged.

The process of inkjet-based bioprinting involves applying either thermal, piezoelectric, or electromagnetic force in order to cause the release of small droplets of encapsulated cells onto a collection plate. This enables the deposition of thin layers and patterned constructs with high resolution. However, because of the dispensing mechanisms and non-contact nature, this technique is only compatible with low-viscosity bioinks, to avoid nozzle clogging, resulting in lower structural stability and cell density of printed constructs.<sup>[14]</sup> Yet, novel approaches such as the use of sacrificial material and optimization of printing parameters might alleviate this limitation.<sup>[15]</sup>

Laser-assisted bioprinting is a less commonly used bioprinting technique due to its low availability, high cost, and complexity.<sup>[16]</sup> Using two parallel slides, a laser-absorbing metal coated with bioink is sandwiched in between. Evaporation of the metal upon absorption of the laser pulses causes the bioink to fall on the lower slide resulting in controlled deposition. Among the many benefits of laser-assisted bioprinting are high cell viability, preservation of most cell function and morphologies, as well as the use of higher cell density. Furthermore, the clogging of nozzles by cells or materials, which is a significant drawback of nozzlebased approaches, is alleviated by this technique.<sup>[17]</sup> A derivative of laser-assisted bioprinting is stereolithography, which uses liquid polymers that crosslink when exposed to ultraviolet, infrared, or visible light.<sup>[18]</sup> The laser beam photocures the desired designs and unites them in a layer-by-layer method. Advantages are fast printing, high degree of precision, and resolution, which result in highly complex architectures. Cell viability is not impacted by the applied shear stress to cells in nozzle-based technologies, but suffers from the harmful effects of initiators and used laser beam wavelengths. In addition, the 3D bioprinter can employ absorption of one or two photons to obtain high-resolution printing down to the nanoscale.<sup>[19]</sup>

Extrusion-based bioprinting is the most common 3D bioprinting technique used in tissue engineering. It is based on the expulsion of viscoelastic bioinks through a nozzle in cylindrical filaments to produce 3D constructs in a layer-by-layer fashion. This technique allows the use of a broader range of bioinks with higher viscosity, resulting in higher mechanical stability of the generated biological substitutes.<sup>[20]</sup> Although different methods of extrusion are used (pneumatic or mechanical-based), the needed pressure for extrusion results in shear stress on the bioink, which can affect cell viability. On the other hand, promising results to generate myocardial constructs, heart valves, and blood vessels have been shown. For example, through the printing of prevascularized and functional multi-material constructs, Jang et al. used stem cell-laden decellularized extracellular matrix bioinks to generate cardiac patches to repair injured myocardium.<sup>[21]</sup> The used of novel bioink formulation further lead to the printing of electromechanically functional, chambered organoids, composed of contiguous cardiac muscle.<sup>[22]</sup> Using a combination of 3D printing and casting of poly-caprolactone and cellladen gelatin-methacrylate/poly-ethylene glycol diacrylate, Nachlas et al. fabricated a multilayered heart valve leaflet that mimicked the structure of natural leaflets.<sup>[23]</sup> Bioprinting of blood vessels, in particular of small diameter, remains a challenge. For instance, Zhou et al. used an advanced coaxial 3D-bioplotter platform to create novel, tunable, small-diameter blood vessels with biomimetic two distinct cell layers (endothelial cells and smooth muscle cells).<sup>[24]</sup>

In the pursue of soft tissue bioprinting, magnetic levitation emerged. The current approach in magnetic levitation is the use of ions such as gadolinium ( $Gd^{3+}$ ) and manganese ( $Mn^{2+}$ ) or radicals to paramagnetize suspending media that is positioned between two magnets, with same poles, facing each other.<sup>[25]</sup> Through the addition of cells encapsulated in hydrogels (called



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**Figure 1.** Principle of magnetic levitation. When cells become diamagnetic through the incorporation of certain ions (such as  $Gd^{3+}$ ) and are encapsulated in hydrogels, exposure to two magnetic fields will position the cells toward the minimum magnetic field strength region. As such aggregating the cells to form a tissue construct.

Table 1. Comparison of bioprinting technologies used on earth.

Evaluated parameters	Inkject bioprinting	Laser-assisted	Extrusion bioprinting	Magnetic levitation	Reference
		bioprinting		bioprinting	
Nozzle based	Yes (risk of clogging)	No	Yes (risk of clogging)	No	[29, 30]
Need of scaffold	Yes	Yes	Yes	No	
Dispensing mode	-Thermal -Piezoelectric	-Photothermal	-Pneumatic -Mechanical	None	[29, 30]
Printing speed	Up to 10 000 droplets per second	Up to 20 cm $s^{-1}$	Up to 5 cm $s^{-1}$	Not reported	[29, 30]
Cell density	Low (< $10^6$ cells mL <sup>-1</sup> )	High (< 10 <sup>8</sup> cells mL <sup>-1</sup> )	High (< 10 <sup>8</sup> cells mL <sup>-1</sup> )	Moderate (< 10 <sup>7</sup> per mL)	[29, 30]
Cell viability	High (> 85%)	High (> 95%)	Medium (70–80%)	High (> 90%)	[29, 30]
Resolution	Moderate (20–100 μm)	High ( $\approx$ 20 $\mu$ m)	Low (≈ 100 μm)	Not reported	[29]
Costs	Low	High	Moderate	Very high	[29]
Viscosity	Low (3.5–12 mPa.s)	Low to medium	Medium to high	Very low ( $\approx 1 \text{ mPa.s}$ )	[29, 30, 31]
Advantages of microgravity	-No need of scaffold	-No need of scaffold	-No need of scaffold -Lower viscosity of bioinks can be used resulting in higher cell viability	Reduced concentration of magnetic particles resulting in reduced cell toxicity	
Disadvantages of microgravity	Droplet deposition needs to be designed without the use of the gravitational force	Droplet deposition needs to be designed without the use of the gravitational force	Motor motion of platform need to be transferred to printhead	None	

building blocks) into the suspending media and upon exposure to the magnetic field, these hydrogels positioned themselves at the minimum magnetic field strength region.<sup>[26]</sup> Hence, by modifying the parameters of the building blocks (composition, stiffness, elastic modulus, porosity, or cell type), complex constructs with unique spatially heterogeneous material properties can be assembled in a scaffold-free, label-free, and nozzle-free manner.<sup>[25]</sup> Despite the U.S. government's approval of Gd<sup>3+</sup> chelates as contrast agents for magnetic resonance imaging (MRI), when used in high concentrations, Gd3+ chelates can cause cytotoxicity and osmotic pressure imbalance in cells. Mitigation strategies are the use of stronger magnets, the investigation of less toxic ions and reduced gravity environment, as found in space. Yet, recent advancement are promising in the field of controlled multicellular spheroid formation<sup>[27]</sup> and controlled construct alignment during tissue formation Figure 1.<sup>[28]</sup>

The aforementioned techniques were recently reviewed in the context of cardiac tissue bioprinting.<sup>[29]</sup> Nevertheless, we have

summarized the important aspects of each technique and indicated the advantages and disadvantages that microgravity would have if used in space (Table 1).

#### 2.1. Bioink Properties for Cardiac Tissue Bioprinting on Earth

The creation of biomimetic functional cardiac tissues relies heavily on the appropriate microenvironment and cell density, as well as the structural and functional properties of the bioink. Specifically, the viscoelastic properties of the bioink, the inclusion of cellbinding motifs, and post-printing modifications of the bioink, that promote cell alignment, nutrient transport, and electromechanical synchronization all significantly contribute to tissue formation, maturation, and functionalization.<sup>[29]</sup>

The viscoelastic properties of bioinks are critical for achieving shape fidelity and printability of the printed construct. Higher the stiffness of the bioink, higher is the force that is required for dispensing it, which results in increased shear stress (in case of nozzle-based bioprinting systems). Excessive shear stress experienced by cells in the bioink can cause cellular damage, resulting in cell death. To mitigate this issue, bioinks with shear thinning and self-healing properties are necessary. After printing, the bioink should regain its initial viscoelastic properties to maintain printing fidelity.

The mechanical properties of bioprinted constructs are crucial for the maturation and functionalization of cardiac tissue. These properties vary depending on the developmental stage of the heart. The mechanical stiffness of embryonic cardiac tissue is low (<10 kPa), whereas neonatal and adult cardiac tissue have higher stiffness values (< 50 kPa).<sup>[32]</sup> The mechanical properties of bioprinted constructs should match those of native myocardium to support synchronous beating and optimal force generation (40–80 mN mm<sup>-2</sup>).<sup>[33]</sup> Dynamic flow conditions can be used to further stimulate the bioprinted constructs mechanically and promote extracellular matrix (ECM) remodeling, ultimately leading to the generation of mechanically mature cardiac tissue.<sup>[34]</sup>

The electrical properties come from mimicking the sinoatrial node of the heart, which initiates the electrical impulses, and purkinje fibers, which help to propagate them. In the embryonic and neonatal phases, cardiomyocytes can generate their own electrical impulses, and these electrically excitable cells can be printed using conducting bioinks. However, to obtain mature cardiomyocytes, an upstroke velocity of 150-350 V s<sup>-1</sup> and a resting membrane potential of 80–90 mV s<sup>-1</sup> need to be achieved, which was not yet obtained at the moment.<sup>[33]</sup> Conductive polymers like poly(3,4-ethylenedioxythiophene) – poly(styrenesulfonate) (PE-DOT:PSS) with photocurable hydrogels are excellent candidates for printing electrically active tissues.<sup>[35]</sup> The conductivity of polymers can also be further increased through the use and incorporation of nanomaterials.<sup>[36]</sup> Furthermore, electrical stimulation of the bioprinted construct is initially important, as bioprinted constructs made from decellularized ECM demonstrated higher force generation upon electrical stimulation compared to during spontaneous contractions.[37]

Functional properties are dependent on the inclusion of cellbinding motifs in the bioink formulation that promote cellcell interactions, communications, and hence cellular processes such as differentiation, migration, and proliferation. The development of these bioinks has led to an increased interest in both natural and synthetic biomaterials. Natural materials, such as polysaccharide-based (e.g., alginate, agarose, chitosan), proteinbased (collagen, fibrin), glycosaminoglycan-based (hyaluronic acid), and decellularized ECM have intrinsic properties similar to the native ECM and are biocompatible, but have limitations such as low mechanical properties, low immunogenicity, and low tunability. Synthetic biomaterials, on the other hand, have low immunogenicity and high tunability for physical, chemical, and mechanical properties, making them ideal for a wide range of modifications using pH, temperature, and crosslinking methods.<sup>[29]</sup> Hybrid bioinks are also being produced that combine natural and synthetic polymers to control the mechanical and biochemical properties while maintaining biocompatibility and degradability. One approach involves adding nanoparticles to biomaterials such as polycaprolactone to improve bioactivity, cell affinity, and tissue regeneration rates.<sup>[38]</sup> Conductive materials such as carbon nanotubes have been explored to recapitulate the conductivity of native myocardium in cardiac tissue engineering scaffolds.<sup>[38,39]</sup> Hybrid bioinks can be printed separately using microfluidic systems or multi-printer devices, and separately printed materials can be utilized as sacrificial materials to support special hollow structures.<sup>[40]</sup>

To date, no fully mature cardiac tissue has been generated. The search for the ideal bioink therefore remains and the following requirements should be used as guidance:<sup>[33]</sup>

- High elasticity and mechanical strength to withstand repetitive mechanical stress and non-linear elasticity during muscle contraction.
- Appropriate biodegradability that can last long enough for cell attachment and protect against proteolytic activity of diseased myocardium, without eliciting an immune response.
- Biocompatibility is non-toxic to cells and can support their survival.
- Facilitation of neovascularization and remodeling of the structure.
- Controlled electrical properties that do not interfere with the electrical conductance of the action potential.
- Outside-in signaling promotes optimal attachment, survival, growth, maturation, and function of cardiomyocytes in vitro, and supports functional cardiac contraction and integration into the host tissue after implantation in vivo.

#### 2.2. Advancements in Cardiac Tissue Bioprinting on Earth

#### 2.2.1. Conventional Bioprinting Approaches

The heart chambers are the main working units of the heart and further act as reservoirs for the blood that is pumped throughout the human body. Therefore, the heart chambers have been among the main interests to mimic in cardiac tissue engineering. Kupfer et al. developed a bioink containing GelMA, ColMA, and other ECM proteins capable of supporting high humaninduced-pluripotent cells (hiPSCs) viability and differentiation into cardiomyocytes.<sup>[22]</sup> The optimized bioink formulation successfully supported the differentiation of hiPSCs into cardiomyocytes after 32 days and was used for extrusion bioprinting of a two-chamber structure with input and output vessels inside a gelatin support bath. The bioprinted chambers showed high precision in the printed structure, high cell viability, and high population of cells, as well as a proper response to drug stimuli when calcium handling response was manipulated via drugs. Lee et al. utilized unmodified collagen as a bioink and adapted the gelatin particle synthesis to achieve microparticles with smaller size and narrower size distribution, uniform spherical shape, and adjustable mechanical properties of the bath.[41] This modified printing procedure was further used to bioprint left ventricle tissue with a sandwich structure composed of a collagen bioink outer layer for the shell and human embryonic stem cells-derived cardiomyocytes and cardiac fibroblasts in the core. The ventricles were cultured in vitro for 28 days and showed high cell viability, the formation of an interconnected dense layer with synchronized beating, and a spontaneous beat rate of 0.5 Hz, which could be paced by 1 and 2 Hz stimulation. Bioprinting of whole heart chambers with complex vasculature and mature functionalities remains a challenge.

Heart valves are essential in regulating blood flow in the body and any malfunction can result in severe health problems. Synthetic polymers alone have limited use due to their lack of cellbinding motifs. Hence, rapid crosslinking methods like photocrosslinking and ionic or temperature-based gelation have been used for printing complex structures. Duan et al. successfully bioprinted tri-leaflet valves with human valve interstitial cells (VICs) incorporated into methacrylated hydrogels using UV light.<sup>[42]</sup> They also printed anatomically similar valves with different cells incorporated into alginate-gelatin hydrogels. Hockaday et al. used photocrosslinking of alginate-supplemented with polyethylene glycol-diacrylate to fabricate aortic tri-leaflet valves with different inner diameters (12-22 mm).<sup>[42]</sup> Maxson et al. bioprinted a simple disk shape from collagen/mesenchymal stem cell bioink as a heart valve scaffold and observed resorption, ECM synthesis, stabilization, and remodeling stages.<sup>[43]</sup> Van der Valk at al. created a 3D-printed model of calcified aortic valve disease using hydrogels made of methacrylated gelatin (GelMA) and methacrylated hyaluronic acid (HAMA) that contained human VICs.<sup>[44]</sup> This approach successfully replicated the ECM of native tissue and maintained VICs in a state of inactivity under normal conditions. These studies demonstrate the use of 3D bioprinting to create heart valves with intricate shapes, varying levels of stiffness, and optimal cellular growth. However, bioprinted valve models have not been transplanted into patients yet due to several issues as degradation, mechanical mismatching, and poor functionality.

Cardiac patches have been used to augment cardiac functions and reduce scar size in the myocardial infarction region. Injecting cells at the site of myocardial infarction does not completely restore the heart's function due to inadequate oxygen and nutrient supply as well as the absence of a substrate for the cells to anchor to, leading to the eventual death of the injected cells.<sup>[45]</sup> Cellembedded injectable hydrogels are a promising therapeutic approach due to their ability to provide structural and cellular support. Yet, they have several limitations including poor mechanical properties, rapid degradation, and a lack of a vascularized network. To mitigate some of the limitations, Asulin et al. developed a cardiac patch that uses soft electronic components to stimulate cells and record heartbeat after implantation.<sup>[46]</sup> They used three different bioinks, including an ECM-based ink for encapsulating neonatal ventricular cardiomyocytes, a PDMS ink with graphite flakes used as an electrode, and another ink containing liquid PDMS used to passivate the electrode. The patch contained evenly distributed cardiac cells and successfully recorded tissue contraction and provided electrical stimulation. They also performed a cell viability assay after 12 days of culture, showing high cytocompatibility of the bioprinted constructs. Erdem et al. investigated the use of oxygenated bioinks to create a cardiac patch.<sup>[47]</sup> They used GelMA calcium peroxide (CPO) bioink, which they believed could release oxygen through hydrogen peroxide  $(H_2O_2)$ production and decomposition. They added various concentrations of CPO to GelMA and bioprinted cell-laden hydrogels at 4°C. They found that CPO had a significant effect on the extrudability, shape fidelity, and printing accuracy of the structures. The addition of CPO also increased the viability of seeded CMs and fibroblasts under hypoxic conditions. However, they also found that high concentrations of CPO (>1%) could be toxic to cells, reducing cell viability by nearly 60% after 4 and 7 days of printing. Despite this, their findings suggest that advanced bioinks Proper function and viability of cells depend on receiving the required oxygen and nutrition through the blood. However, the diffusivity of oxygen and nutrition is limited to small distances ( $\approx 200 \ \mu$ m), which makes the vascular network an essential component in tissue engineering. Skylar–Scott et al. used a bioprinting approach named "sacrificial writing into functional tissue (SWIFT)" to create perfusable vascularized cardiac tissue.<sup>[10]</sup> Maiullari et al. used microfluidic-head extrusion bioprinting to create vascularized cardiac tissues.<sup>[49]</sup> Mao et al. used coaxial electrohydrodynamic bioprinting (EHD) to create thick prevascularized cell-laden constructs.<sup>[50]</sup> All these studies demonstrated the potential of bioprinting in vascular tissue engineering and the formation of complex vascular networks in 3D structures. Thriving toward more controlled complexity, heart-on-chip systems also appeared.

#### 2.2.2. Approaches to Integrate Bioprinting with Lab-on-Chip Systems

The most commonly used printing techniques when integrating bioprinting with organ-on-chips are pressure assisted-, ink-jet-, light assisted- (part of the larger group previously introduced, laser-assisted), and microfluidic bioprinting.<sup>[51]</sup> Typically, the integration can be achieved using three main approaches, (1) printing the construct separately and placing it in a fluidic enclosure post-printing, (2) printing the construct directly inside the enclosure, or (3) fully bioprinting the entire chip and tissue construct in one-step (Figure 2). In Table 2, we summarize the current studies integrating bioprinting and organ-on-chips. In general, the studies included in the first category (1) create vascularized tissue constructs, but only a few studies have used this method so far. Where they generally make larger constructs and integrate them onto organ-on-chips. The second category (2) is more diverse with several different printing techniques, architectures, and target organs. Studies using this approach range from simple cell-laden microchannels to complex organ-onchips with vascular networks and integrated sensors. In the third category (3), more simple organ-on-chips have been fabricated so far. Mostly vascular constructs or perfusable cell-laden hydrogels have been fabricated using this approach. However, more complex bioprinted organ-on-chips with multiscale perfusable networks and high fidelity are being realized using light-assisted bioprinting techniques.[19]

One of the simpler ways to integrate a bioprinted construct with an organ-on-chip system is by first printing the construct and later placing it in a separately constructed enclosure (category 1). This avoids having to consider any compatibility issues between the printing substrate (in this case the enclosure) and the bioprinter, such as attachment to the print surface and accessibility of the printhead to the build volume. Several bioprinted organ-on-chip systems have used this approach for blood vessels<sup>[52]</sup> and the heart.<sup>[53]</sup> While being a simple and modular approach, perfusion of the tissue is a potential concern. Most enclosures are made to be larger than the tissue, resulting in a considerably lower flow rate through the compact tissue compared to





Figure 2. Overview of printing approaches for bioprinting organ-on-chips. 1) Printing the construct separately and placing it in a fluidic enclosure post-printing. 2) Printing the construct directly inside the enclosure. 3) Fully bioprinting the entire chip and tissue construct in one step. Created with Biorender.

the total flow rate through the chamber. Hence, nutrient and oxygen transport to the tissue core may not be enough unless dedicated vasculature is included in the tissue. Zhang et.al., therefore, showed through oxygen gradient simulations that using their bioprinted microporous multilayered scaffold, sufficient oxygen concentrations could reach the core of the construct. The estimated oxygen concentration is furthermore enough for highly oxygen-demanding cells, such as cardiomyocytes and endothelial cells. Additionally, they showed that the integration of the bioprinted construct with their organ-on-chip platform resulted in a lower presence of dead cardiomyocytes and endothelial cells due to perfusion.<sup>[53]</sup>

Another common approach is to directly bioprint the construct inside the organ-on-chip device (category 2). This allows for direct incorporation of the bioprinted construct with the premanufactured chip and includes both direct and sacrificial printing. One of the main advantages is the ease of fabricating or incorporating perfusable vasculature. A commonly applied approach to fabricate bioprinted tubes-on-chip (such as blood vessels) is through sacrificial bioprinting of cell-laden bioinks combined with either casting<sup>[54]</sup> or printing inside a bath<sup>[55]</sup> to create the outer housing. A similar approach, avoiding the use of sacrificial bioinks, uses either coaxial nozzles or microfluidic printheads,<sup>[56]</sup> thus generating a filament where the core is empty and the shell(s) contain cell-laden bioinks. By printing the coaxial filament, crosslinking it, and then casting an outer casing a perfusable structure is formed.<sup>[56]</sup> Another approach to printing inside the chip that does not involve casting or the outer casing is to directly 3D bioprint the entire structure inside the chip. This can be achieved using pressure assisted-, ink-jet- or laserassisted- bioprinting systems. These systems have in turn been used to fabricate on-chip models of vasculature<sup>[57]</sup> and heart<sup>[58]</sup> tissues.

One of the more complicated means to integrate bioprinting with organ-on-chip systems is to completely bioprint the entire chip, including the enclosure (category 3). This allows for direct integration of the tissue construct with the chip and easy integration with the fluidic system. Perfusable vessels have been bioprinted on a rotating rod and connected to a fluidic system, essentially creating a simple vessel-on-chip.<sup>[59]</sup> The filament of these vessels was printed using a coaxial nozzle to generate perfusable microchannels. Hence, these tubes incorporated multi-level fluidic channels, one of which is the main macro channel and the others are the micro channels around the wall of the tube structure. This allowed for mechanical stimulation through the macro channel and nutrient transport using the micro channels.

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Year	Manufacturing method	Region of interest	Significant advance	Reference
2014	Extrusion	Vasculature	Developed a functional, perfusable vascular channel using 3D-bioprinting	[52]
2015	Microfluidic	Heart	Developed a method to generate vascularized heterogeneous scaffolds using microfluidic bioprinting	[53]
2016	Extrusion	Heart	Generated novel endothelialized bioprinted cardiac tissues	[53]
2019	Stereolithography	Vasculature, Alveoli, Liver, Osteogenic	Developed a method to print complex, biocompatible, functional vascular networks	[60]
Bioprintir	ig inside of organ-on-chips			
Year	Manufacturing method	Region of interest	Significant advance	Reference
2016	Extrusion & casting	Vasculature	Developed a 3d-bioprinted thrombosis-on-chip model	[54]
2017	Extrusion	Heart, liver, lung	Developed an integrated heart-, liver- and lung-on-chip using bioprinting	[53]
2020	Digital light processing (DLP)	Heart	Developed a method to print suspended cardiac tissues with specified alignment on chip using DLP	[58]
2020	Extrusion	Vasculature	Developed a layered vessel-on-chip	[57]
2021	Pin type printing	Heart	Developed a method to bioprint cardiac tissues on a sensor-covered substrate with high spatial accuracy	[58]
Full biopr	inting of organ-on-chips			
Year	Manufacturing method	Region of interest	Significant advance	Reference
2011	Inkjet bioprinting	Vasculature	First study enabling macroscale 3D-bioprinting with a microscale resolution using bioink droplets	[61]
2017	Extrusion	Heart	Established a technique to fully 3D print a microphysiological device to use for the fabrication of a heart-on-chip	[62]
2017	Extrusion	Vasculature	Fabricated a multilevel blood vessel-on-chip through crosslinking of 3d-bioprinted hollow fibers	[59]
2018	Microfluidic	Vasculature	Developed a system for multichannel coaxial microfluidic bioprinting of tubular constructs	[56]
2020	Two-photon polymerization	Vasculature	First study to fully bioprint an organ-on-chip with micrometer resolution and live encapsulated cells using 2PP	[19]

More conventional on-chip tissue constructs of the vasculature have further been fabricated using advanced light-assisted printing techniques,<sup>[19,60]</sup> one of which uses two-photon polymerization (2PP). Using 2PP, Dobos et. al developed a method to generate microvascular and capillary-like networks on chip with a feature size down to a few microns.<sup>[19]</sup> They further show that their printing allows for direct encapsulation of cells and that they are therefore able to directly pattern vascular networks.

#### 2.3. Bioprinting Techniques Used in Space

When compared to terrestrial operations, 3D bioprinting in microgravity presents a variety of novel obstacles, but also opportunities that necessitate tailored engineering adaptations of current terrestrial technology. Microgravity provides a unique solution to printing complex organ structures, as minimal gravity removes the need for scaffolding structures to support complex tissue shapes.

In space, two bioprinting techniques have been used so far. In 2018, "Organ.Aut" was launched from Roscosmos, which bioprinting technique is based on magnetic levitation.<sup>[30]</sup> Their experiment involved a series of sequential phases (Figure 3). Hermetically sealed cuvettes with admixed thermoreversible hydrogel protected human chondrocyte tissue spheroids (further referred to as "chondrospheres") from undergoing undesirable preliminary fusion and attachment to the walls of cuvette during delivery to the ISS on earth. Onboard the ISS, first the paramagnetic medium was injected into the hydrogel containing the chondrospheres. The mixture was then cooled to 17°C in a temperature-controlled chamber for 90 min, resulting in the "gel-sol" phase transition of the thermoreversible hydrogel. Because of this process, chondrospheres were unbound and allowed to roam freely. After 1 h in the magnetic field, six cuvettes containing the chondrospheres were fused together into one tissue construct and kept in a temperature-controlled environment (+37°C) for 2 days. Finally, the acquired 3D tissue structures were fixed in 4% formalin and kept at room temperature for 2 weeks until they were brought back to earth for further analysis. Analysis of the 3D tissue construct indicated that the chondrocytes were able to maintain their viability and SCIENCE NEWS \_\_\_\_\_

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**Figure 3.** Overview of Organ.Aut printing process in space. A) Cuvettes containing fixative solution, growth media containing paramagnetic gadobutrol, and chondrospheres in thermoreversible nonadhesive hydrogel. B) The main steps of the experiment carried out on the ISS were the activation of cuvettes by cooling them down to 15°C, magnetic construction of 3D tissue constructs at 37°C, and fixing. C) Return of cuvettes to earth transportation. Reproduced with permission under the terms of the CC-BY license.<sup>[30]</sup> Copyright 2020, the Authors. Published by the American Association for the Advancement of Science.



Figure 4. Overview of BFF printing process in space. a) The Techshot BioFabrication Facility's interior print volume. A bioreactor cassette is attached to retain the printed construct, and two syringe holders are put on the face of the leftmost SmartPumps. b) Bioprinting on the ISS using a low viscosity material c) and higher viscosity material. d) Filling of BFF bioreactor post bioprinting. e) Side view of bioprinted construct inside sealed and filled bioreactor. Reproduced with permission.<sup>[63]</sup> Copyright 2022, Wiley-VCH GmbH.

physiological activity similar to 3D cultures on earth.<sup>[30]</sup> In addition, mouse thyroid gland, meat, and bones constructs were tested (https://www.space.com/cosmonaut-cartilage-engineering-on-space-station.html), despite details of the successful biofabrication have yet to be reported.

In 2019, the BioFabrication Facility (BFF) was launched from the ISS National Lab, which bioprinting technique is based on extrusion<sup>[63]</sup> (**Figure 4**). In order to allow the printed tissues to cohesively form on a cellular level, BFF is utilized in conjunction with the Techshot's bioreactor cassettes. These cassettes store the

produced tissues for several weeks after initial printing. For bioprinting, a gantry platform with precise linear motors and 3-axis motion applied to the print head rather than the printing platform has been designed to lessen acceleration stresses on the construct during printing. To ensure printing detail at a medically meaningful size, the BFF's linear drives offer a resolution of 100 nm, repeatability of 500 nm, and accuracy of 1 µm. The highvelocity motors in this motion control system provide a sizable print area. With a single motor, the Z-axis can move at speeds of > 400 mm s<sup>-1</sup> and has a travel of 50 mm. Although each drive is capable of greater values, they are restricted to 0.5 G acceleration in order to reduce imparted vibration and motion artifacts. Within the print volume, filtered and humidified air is circulated to ensure a stable condition for hydrogel printing. So far, BFF is outfitted with four independently controlled dispensing systems for hydrogels. To further advance the bioprinted construct, the bioreactor cassettes include thermal, chemical, mechanical, and electrical impulses similar to what stem and progenitor cells experience during in vivo tissue development in the womb. Through these signals, a cohesive solid as simple as a tissue-analog monoculture or as complex as a neo-organ can be achieved.

The first experiment intended to generate a cardiac tissue-like structure composed of cells and extracellular matrix specific to cardiac, nerve, and vascular components. In total, 100 layers of bioink were dispensed resulting in a construct volume of  $\approx \! 19.3 \ \mathrm{cm}^3.^{[74]}$ 

#### 3. Current Challenges in 3D Bioprinting in Space

On earth, challenges in 3D bioprinting focus on the printing resolution, selection of scaffolds, generation of controlled vascularization, controlled heterogeneous cell-type composition and their related spatial distribution, as well as the biomaterials used in bioinks. These challenges remain in space, although the need for a scaffold for structural support is reduced due to the microgravity environment. However, in space, additional challenges are emerging.

First, logistics in packaging and transportation need to ensure that the biological material safely arrives on the ISS. To ensure the safety of the astronauts, multiple containment levels are needed to avoid any leakage of fluid into the ISS. This means that any connectors or components of the bioprinter where liquid exchange occurs also need to be leakage proof. In addition, because of microgravity, lower viscosity bioinks can be used, which avoid shear stress on the cells, increasing cell viability as well as decreasing clogging. However, as fluid motion differs in space, the bioinks could wet the print tips, forming large masses of fluid that would impact the printing process. Hence, to solve this issue for the BFF, a cleaning process was developed in which a 4% hydrogen peroxide solution went through the print tips and was collected in a waste compartment.<sup>[63]</sup> Indeed, microgravity has a significant impact on fluid dynamics and surface tension. On earth, the cohesive force of surface tension competes with gravity, which causes liquids to distort and pull molecules apart. In space, microgravity reduces the effects of hydrodynamic shear and hydrostatic pressure, making surface tension the dominant force.<sup>[64]</sup> This results in molecules staying in tight spheres and films, maximizing intermolecular attraction. Additionally, the absence of gravity causes droplets to form almost perfect spheres,

and the movement of fluid spheres and films is slower in space compared to movement on Earth.

Second, to ensure that the biological samples remain alive. different storage temperatures are needed. For the Organ.Aut, chondrospheres were included in a thermoreversible hydrogel stored at 4°C until printing.<sup>[30]</sup> In the case of the BFF, cardiac cells were stored at  $-95^{\circ}$ C while the hydrogels were packed separately and stored at 2°C.<sup>[63]</sup> Yet, when cells are separated from the bioink, mixing systems such as the cell-laden alginate-based bioinks described by Kostenko et al., are needed. The cell-laden alginate also has the advantage to have viscosity tunable bioinks that can be stored for a longer period of time (up to 7 days at 15°C).<sup>[65]</sup> To note, during return from the ISS, biological materials are exposed to different levels of gravity (3 to 6g), which could impact the 3D printed constructs.<sup>[66]</sup> For the return, freeform cell-laden cryobioprinting as demonstrated by Ravanbakhsh et al. could be a solution.<sup>[67]</sup> However, maturation of the bioprinting construct would then not be possible. Therefore, innovative solutions are needed where current protocols used for cryopreservation of cell aggregates are adapted to 3D bioprinted constructs or eventually investigating through the implementation of hibernation protocols.<sup>[68]</sup>

During the maturation process, the 3D bioprinted construct needs to be incubated at 37°C and supplied with adequate nutrients, oxygen levels, as well as physical (mechanical, electrical, optical) and biochemical (e.g., growth factors) cues. This is of particular importance in bioink formulation for cardiac tissues, where sufficient electrical conductivity, adaptive stiffness, mechanical loading and unloading, controlled release of biomolecules and vascularization should be ensured to enable tissue maturation. Hence, further research on responsive biomaterials is needed<sup>[69]</sup> while considering the impact of microgravity. The decrease in gravity-induced fluid motion could be a critical issue. While diffusion is not gravity-dependent, convection rate is and plays a crucial role in moving metabolic products, especially larger molecules, through mass transport. The reduced fluid convection rates and slower solute diffusion in microgravity could also lead to impaired heat and biomolecule exchange, potentially favoring the transport of smaller solutes.<sup>[70]</sup> Consequently, the integration of 3D bioprinted constructs into a fluidic system that drives convective flow would help to obtain the physiologically relevant environment that is needed for tissue maturation and survival.

Third, if the printing process is controlled on Earth, loss of signal due to communication breaks between the ISS and ground control needs to be taken into consideration. In that regard, the development of automated systems with the implementation of artificial intelligence and machine learning is needed. Automation allows for greater control over bioprocesses, leading to more precise and efficient process operation, and hence, reducing risk in the supply chain through enhanced quality control and quality assurance.<sup>[71]</sup> Yet, it implies 1) the standardization of equipment, biological materials, and protocols; 2) continuous real-time nondestructive monitoring and feedback loop capabilities. Standardization ensures reproducibility and comparability for the complete process, but needs to be implemented at all levels from the material source and individual processes to data acquisition, validation, and reporting. Yet, these standards remain to be elaborated although efforts are currently being made here on Earth, in ADVANCED SCIENCE NEWS \_\_\_\_\_ www.advancedsciencenews.com

particular for human stem cell-derived tissue constructs.<sup>[72]</sup> Advances in in-line analysis of organ-on-chip systems with sensors (electrical, electrochemical, and optical)<sup>[73]</sup> and real-time monitoring with a feedback loop<sup>[74]</sup> further promote the development of automated systems that will aid the translation into pre-clinical and clinical use of 3D bioprinted constructs on Earth and in space. Therefore, consideration of the material type of organ-on-chip systems is important. Beside biocompatibility, selected materials need to be transparent, biocompatible, sterile, have low absorbance capabilities for biomolecules as well as compatibility with fluidic connections and electronic systems.

Finally, due to radiation levels in space, two aspects need to be considered. First, the electronic component needs to be radiation hardened. Initially developed for nuclear applications, radiation hardening by process, radiation hardening by shielding, and radiation hardening by design are the three main methods that have been extensively studied to minimize the damaging effects of radiation on electronic components.<sup>[75]</sup> Second, although protected by the Van Allen belt, the perceived level of ionizing radiation that is equivalent to 6 months on the ISS is 80 mSv, which corresponds to four whole-body CT scans.<sup>[76]</sup> However, the largest source of uncertainty in health risk assessment related to ionizing radiation is the inadequate understanding of biological mechanisms and effects. Particularly, the extent and nature of interactions between heavy ions and human tissue.<sup>[77]</sup>

# 4. Current Status of Cardiac Tissue Research in Space

In space, initial cardiac tissue research concentrated on human stem cells and their differentiation into cardiac progenitor cells or cardiomyocytes (Table 3). Baio et al. cultured neonatal and adult human cardiovascular progenitor cells (CPCs) on the ISS and investigated changes in the expression of microRNAs and genes related to mechanotransduction, cardiogenesis, cell cycling, DNA repair, and paracrine signaling.<sup>[78]</sup> Interestingly, only neonatal CPCs showed higher levels of expression of markers associated with early development (POU5F1, NANOG, SOX2, GATA4, KIT, PDGFRA, ISL1, KDR) and exhibited a greater ability to divide, replicate and migrate. Camberos et al. also cultured human CPCs and provided evidence that microgravity (simulated and real) inhibits the Hippo signaling, driving adult cardiac cells to express higher levels of active YAP1.<sup>[79]</sup> Looking more specifically at global gene expression, Camberos et al. showed stemness induction in both neonatal and adult human CPCs after 30 days on the ISS, but more pronounced in neonatal human CPCs.<sup>[80]</sup> These experiments indicated that CPCs, in particular nenonatal, are more sensitive to further acquire stemness properties in space. Using hiPSC-derived cardiomyocytes (hiPSC-CMs), Wnorowski et al. investigated the gene expression, structure, and function changes that occurred in space compared to ground control.<sup>[81]</sup> A decline in calcium recycling was observed in space, together with a global gene expression change. Specifically, motifs enriched in genes upregulated in space-flown hiPSC-CMs were associated with transcription factors known to regulate hypertrophic pathways. Cryopreservation of CO<sub>2</sub>-independent culture of 3D cardiac progenitors was successfully differentiated into cardiomyocytes in space.<sup>[82]</sup> A subsequent study from Rampoldi et al. differentiated cardiac progenitors into cardiomyocytes that showed improved Ca<sup>2+</sup> handling and increased expression of contractionassociated genes, after 3 weeks in space, while 3 days in space upregulated genes involved in cell proliferation, survival, cardiac differentiation, and contraction in cardiac progenitors.<sup>[83]</sup> Altogether, these experiments indicated that successful differentiation of human CPCs into cardiomyocytes might be more effective in space.

Although cardiac tissue was bioprinted using the BFF, no other studies have been published so far. Nevertheless, promising projects have been accepted in recent years that will further improve our understanding of heart tissue engineering in space (Table 3).

#### 5. Future outlook

In the rapidly developing field of bioprinting, one of the novel promising, and future research directions is the bioprinting of materials in space. The practice of bioprinting in space carries with it a number of potential benefits.<sup>[4]</sup> To begin, it is conceivable to bioprint constructions, because of microgravity, using more fluidic systems and hence more biocompatible bioinks. Second, the lack of gravity means that complicated geometries such as voids, cavities, tunnels are, as in contrast to on Earth, self-supported in microgravity environment. Third, initial experiments seem to indicate that stem cells perform better in microgravity.<sup>[79,83]</sup> Cardiac tissue is one of the most complicated organ to engineer where microgravity has the potential to bring groundbreaking advancements.

Despite the current state of the art, scientific, technological as well as experimentation, integration, and operations gaps remain. On one hand, scientific gaps include i) how microgravity affects nutrient and gas exchange in 3D bioprinted constructs; ii) the definition of assessment parameters to evaluate bioink printability under microgravity; iii) the establishment of tissue maturation protocols, and iv) how microgravity induced changes through mechanoreceptors influence the 3D bioprinted construct. On the other hand, technological gaps include i) the development of bioinks that should be compatible with microgravity as well as methods to safe mix different bioinks prior printing; ii) improving the reusability of bioinks to reduce the shipping of resources from Earth, and iii) the development of the use of multimaterials within one printing job. Finally, experimentation, integration, and operations gaps include i) the development of novel bioinks, which can be stored with live cells for long periods; ii) the identification of optimal storage conditions of cell culture material to ensure maximum reusability; iii) the optimization of experimental protocol to meet ISS safety requirements as well as protocols for experiment retrieval post-flight.

Furthermore, organs-on-chip are proving to be powerful tools for biomedical research here on Earth. Recently a landmark paper showed that the authors could improve the predictability of liver toxicity caused by certain drugs using a liver-on-chip compared to animal models.<sup>[84]</sup> Considering that the most commonly encountered drug toxicity during pharmaceutical development is cardiovascular toxicity.<sup>[85]</sup> there is incredible potential for using cardiac tissue engineering and heart-on-chips for pharmaceutical purposes. By integrating bioprinting with organ-on-chips, more complex and more accurately recapitulating models of

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#### Table 3. List of experiments conducted on the ISS using human stem cell-derived cardiac cells.

2D and	3D	in	vitro	assays

Year	Cells	Duration	Significant advance	Reference
2018	Human adult and neonatal cardiac progenitor cells	12 days	<ul> <li>Neonatal CPCs exhibited increased expression of early developmental markers, enhanced proliferative potential, and increased migratory capacity</li> <li>Adult CPCs experienced little change in the expression of genes indicative of their developmental state but exhibited changes to migratory capacity.</li> </ul>	[78]
2019	Human adult and neonatal cardiac progenitor cells	12 and 30 days	-Microgravity inhibits the Hippo pathway in a time-dependent manner	[79]
2019	hiPSC line from peripheral blood mononuclear cells	5.5 weeks	<ul> <li>-Microgravity exposure resulted in changes in hiPSC-CM calcium-handling properties</li> <li>-2635 genes were differentially expressed among flight, post-flight, and ground</li> <li>-Pathways related to mitochondrial function were enriched in space-flown hiPSC-CMs</li> </ul>	[81]
2021	SCVI-273 and IMR90 hiPSCs	3 weeks	-Cryopreserved cardiac progenitor spheres were successfully cultivated in a spaceflight culture module without CO <sub>2</sub>	[82]
2021	Human adult and neonatal cardiac progenitor cells	30 days	-Transcriptomic analysis indicated dedifferentiation and enhanced stemness	[80]
2022	SCVI-273 and IMR90 hiPSCs	3 and 22 days	<ul> <li>Cryopreserved 3D hiPSC-cardiac progenitors differentiated efficiently in space into cardiomyocytes</li> <li>Microgravity cultures had increased sphere sizes and cellular proliferation</li> <li>Beating cardiomyocytes in microgravity cultures had improved Ca<sup>2+</sup> handling</li> <li>Microgravity cultures had upregulated genes in cardiac contraction</li> </ul>	[83]

Outlook research of accepted projects					
PI	Funding	Title	Objectives		
Kim, Deok-Ho	NIH-NCATS	A human iPSC-based 3D microphysiological system for modeling cardiac dysfunction in microgravity	To assess differences in cardiac function and physiological maturation between cells maintained in normal gravity and microgravity environments		
Wu, Joseph c.	NIH-NCATS	Effect of microgravity on drug responses using engineered heart tissues	To reveal key functional and molecular differences that drive phenotypic changes in heart tissues under the influence of microgravity		
Kevin, Costa	NSF-CASIS	Microphysiologic model of human cardiovascular stiffness-related diseases in microgravity	To characterize a multi-tissue in vitro microfluidic human organoid model of the cardiovascular system; to test micro-cardiovascular chips on the ISS; and to identify novel disease biomarkers and pathways postflight		
Xhunhui, Xu	NSF-CASIS	Engineering stem cell-derived cardiac microtissues with metabolic regulators in space to promote cardiomyocyte maturation	To establish a multipronged approach combining microgravity, tissue engineering, and metabolic regulation to promote the maturation of cardiomyocytes derived from human-induced pluripotent stem cells		
Laurel, Kuxhaus	NSF-CASIS	Studying the effects of microgravity on 3D cardiac organoid cultures	To compare and contrast the morphology, viability, and altered energy metabolism in 3D bioprinted cardiac organoids under microgravity and Earth's gravity; to study the epigenetic changes in 3D bioprinted cardiac organoids under microgravity and assess how these changes may affect the development of cardiac atrophy when compared to Earth's gravity.		

human physiology can be achieved.<sup>[86]</sup> Furthermore, considering the favorable conditions for bioprinting in space, even more advanced and better recapitulating organ-on-chips could be developed. Hence, space could be utilized as an advanced platform to generate crucial models for pharmaceutical purposes on Earth. Likewise, it is incredibly expensive and practically difficult to perform animal studies in space. Additionally, the total amount of astronauts that are currently in space or have resided in space is still quite low. Therefore, conducting studies on the effects of the space environment to understand what challenges we may encounter on future and longer missions is limited. Even more difficult are pharmaceutical and clinical studies to develop or repurpose drugs for space applications, as they would require a large number of animals or humans according to today's standard methods. Therefore, the use of organs- and especially hearton-chips will be crucial to study the effects of the space environment and, in the future, develop space-specific drugs or treatments. By integrating bioprinting with organ-on-chips, in space, models that are even more accurate may be generated. By further complete bioprinting the organ-on-chip systems in space, transportation of solely raw materials to space would be needed, further increasing the availability.<sup>[4]</sup>



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The ideal bioprinters for space must be risk-free, fully automated, versatile, portable, and easy to operate. They might be based on several versions of bioprinting technologies, such as extrusion-based and magnetic-based bioprinting, (both of which have already been investigated in space). While we extend human presence in space, other needs might arise. Hence, the systematic examination of 3D bioprinting in space will progress bioprinting technology and these new fascinating research frontiers will advance tissue engineering not only for space exploration but also here on Earth.

### Acknowledgements

K.T. and E.R. contributed equally to the writing of the original draft. B.B. revised the interim drafts and the final version. L.M. and S.B. supervised the writing and revised the final version. This work was supported by the ESA PRODEX grant IMPULSE (PEA 4000109861). We are also grateful the EIC Pathfinder Open project PULSE (Grant Agreement number 101099346) for support.

### **Conflict of Interest**

The authors declare no conflict of interest.

### Keywords

bioprinting, bioinks, cardiac tissues, space

Received: December 21, 2022 Revised: April 18, 2023 Published online: June 14, 2023

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