What can biofabrication do for space and what can space do for biofabrication?

Biofabrication in space is one of the novel promising and prospective research directions in the rapidly emerging field of space STEM. There are several advantages of biofabrication in space. Under microgravity, it is possible to engineer constructs using more fluidic channels and thus more biocompatible bioinks. Microgravity enables biofabrication of tissue and organ constructs of more complex geometries, thus facilitating novel scaffold-, label-, and nozzle-free technologies based on multi-levitation principles. However, when exposed to microgravity and cosmic radiation, biofabricated tissues could be used to study pathophysiological phenomena that will be useful on Earth and for deep space manned missions. Here, we provide leading concepts about the potential mutual benefits of the application of biofabrication technologies in space.

Setting the stage: biofabrication, organoids, and space

Biofabrication (see Glossary) technologies, and in particular bioprinting, hold the promise to create 3D in vitro models that exquisitely mimic the complexity of our tissues and organs [1]. These models can be used to study the physiology of tissues and organs exposed to a variety of environmental conditions, such as microgravity ($\mu g$) and radiation, as encountered in space. Knowledge acquired from these models is crucial to understand the biological effects of the space environment for long-term manned missions, such as outlined in the ‘Moon village’ and ‘Mission to Mars’ programs (http://www.esa.int/About_Us/Ministerial_Council_2016/Moon_Village; http://exploration.esa.int/mars/; http://www.esa.int/Our_Activities/Human_Spaceflight/A_new_European_vision_for_space_exploration). Practical examples are not only well-known effects, such as osteoporosis for bone [2], but also muscle loss [3,4], cardiovascular [5,6] and lung capacity deficiencies due to microgravity [7], and the effect of radiation on sensitive organs such as thyroid, gonads, and intestine [8–11]. In addition to the more immediate effects, like acute radiation sickness, exposure to radiation also increases astronauts’ and cosmonauts’ risk to develop several cancers, genetic mutations, nervous system damage, and even cataracts [12,13].

Successful attempts to bioprint vascularized skeletal tissues, skin, heart patches, neuromuscular junctions, and rudimental organ structures on Earth have been reported, among others [1]. Despite these attempts, further steps need to be taken to move from proof-of-concept studies to real functional tissues and organs; in particular, for bioprinted soft tissues, such as blood vessels, which have proven to be more difficult to engineer [14,15]. However, these studies show that the potential of the bioprinting technology is close to reach. Bioprinting in microgravity 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 stem or pluripotent cells) and adult tissue-derived proteins as its bioink to create viable tissues on board the International Space Station (ISS). 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 [16]. So far, these systems are the only ones that have been successfully tested in space. Besides the developments of NASA and ROSKOSMOS, the European Space Agency (ESA) is also planning for the implementation of a 3D bioprinting and cell culture module on the ISS.

Typically, bioprinting comprises the automated deposition of cells or cell spheroids either alone or laden into a hydrogel carrier (a bioink) following a computer-aided designed model of the tissue construct that is needed. Most of the time, cell lines, primary cells, or adult stem cells have been used for bioprinting applications [17]. Recently, the advent of instructive hydrogels has also allowed the successful bioprinting of pluripotent stem cells, opening to broader applications where multiple tissues or organ patches could be conceived [18–20]. It is only very recently, however, that bioprinting of organoids has been reported [21–23]. This advancement opens up new horizons, as bioprinted organ models may become closer and closer to reality, initially to provide 3D in vitro models for personalized medicine and with possible implications in the near future for translational regenerative medicine.

Organoids, literally small organs, are microtissues that self-develop from stem cells (Box 1) and exhibit the histological and functional characteristics of mature organs [24]. Organoids created from human stem cells open up new unique possibilities for creating in vitro models of human diseases, studying new drugs and their possible toxicity, and finally for regenerative medicine and tissue engineering purposes [25–27]. The study of organoids is becoming an extremely promising and rapidly developing area in biomedicine. The use of organoids in space research will solve many important issues of space biology and medicine, such as the effect of weightlessness on the functional and morphological characteristics of human tissues; the effect of cosmic radiation on human cells, tissues and organs; and the study of the regenerative potential of human tissues, encompassing the reproductive and cognitive ability in space. Utilizing the effects of microgravity, the study of organoids under space conditions is also very relevant for drug R&D, cancer research, and stem cell research, with benefit for Earth. Planned experiments and technological challenges associated with the implementation of research using organoids in space are discussed later. Finally, the integration of organoids with biofabrication technologies (Table 1) is further discussed in the context of space biology research and its implication back on Earth. Particular attention is paid to the technological support of experiments with human organoids to study the effect of a prolonged stay in space, long-distance interplanetary travel, and constant work on future extraterrestrial planetary settlements.

Effect of spaceflight on the human body

Spaceflight affects the human body in several ways, but we will focus here on the main health issues that astronauts will have to face in the context of a long-term space mission, namely, some effects of microgravity and radiation.

The effects of microgravity on humans

Since life began on Earth, the ‘only constant factor in evolution is the force of gravity’; all biological processes are accustomed to the ever-present force of gravity and ‘even small variations in this force can have significant impact on the health and function of the organisms’ (Figure 1).
human body is no exception to that principle and studies have already shown that microgravity has several effects on astronauts [28–30]. Furthermore, astronauts will be subjected to different gravity levels and going from 1 g to weightlessness to Moon gravity (0.17 g) or Mars gravity (0.376 g) (Figure 2).

The cardiovascular system is affected in weightlessness and the redistribution of blood and other fluids to the upper body may lead to dizziness, puffy face, and facial edema [31]. But the most

Box 1. Organoids
Definition of organoids and a brief history of the study of organoids
Organoids can be defined as 3D microtissues self-developing from stem cells and exhibiting the histological and functional characteristics of mature organs. It should be noted that not all scientists agree with this narrow definition of organoids. Some researchers prefer to use a broader definition, essentially equating organoids with mini-tissues from any cell types and formed in any way [79]. This definition does not embrace the achievements of recent years. More recently, pioneering works from the Clevers, Knoblich, and Lancaster labs primarily emphasized in their definitions the origin of organoids from stem cells and their self-development and self-differentiation [79,80].

Thus, these are at least three of the most important criteria for organoids: (i) organoids originate from their stem cells, (ii) organoids self-develop and self-differentiate, and (iii) organoids show authentic histotypic and organotypic histological structures and functions. Yet, the most interesting feature of organoids, and the reason they actually received such attention in recent years, is that they are made from human cells. An organoid is essentially a small ‘avatar’ or in vitro model of human organs [81].

Fundamental differences between organoids and tissue spheroids
Unfortunately, tissue spheroids are often mistakenly identified with organoids. In fact, significant and fundamental differences exist between these two types of microtissues. Tissue spheroids and organoids are often globular microtissues, composed of densely packed cells. However, this is where the apparent similarity between them essentially ends. Organoids are often irregular in shape, with jagged contours. Organoids are essentially a systematically cultivated teratoma, which often has an ugly shape and a chaotic structure. Organoids rarely consist of one type of cell, since they have an authentic histotype and an organ-specific structure and organization on histological sections. Organoids, unlike tissue spheroids, often have a lumen, which they cannot form without a natural ECM, such as Matrigel or its synthetic analogs. However, we would like to specifically draw attention to three fundamental differences between organoids and tissue spheroids: first, organoids are formed from stem cells and not from differentiated cells and tissues; second, organoids develop and are formed by self-organization and differentiation and not by assembling a cell suspension, as in the case of tissue spheroids; and third, in the case of organoids, as a rule, we are talking mainly about human cells.

Classification of organoids
Organoids are classified primarily by their origin: from embryonic stem cells, induced pluripotent stem (iPS) cells, or adult stem cells. The most popular classification of organoids is based on the type of organs they mimic or recapitulate in vitro. Examples are brain, lung, liver, kidney, exocrine and endocrine pancreas, intestinal, thyroid (or thyroid follicles) organoids, and organoids of the reproductive organs: the ovary and testis. Finally, in recent years, vascular organoids created from induced human pluripotent stem cells have received great attention [82,83].

The current state of the art of organoid bioprinting on Earth is listed in Table 1. In space, pioneer research work is currently ongoing or is in preparation in collaboration with ISS-certified manufacturing companies worldwide. However, only extrusion and magnetic levitation is currently available on the ISS.

Vascularization of organoids
One of the key limitations in organoid development to generate functional tissues is vascularization. Indeed, development and growth of organoids occurs until a certain size, at which proliferation is stopped, followed by the appearance of a necrotic core. In humans, such necrotic core is evaded by proper vascularization of the tissue that ensures oxygen, nutrient, and metabolite exchange. Besides size limitation, the lack of vascularization also causes a lack of tissue maturation [84]. Despite the current technological advancements [85], achieving in vivo-like organ complexity and maturation in vitro remains a challenge [82]. Novel concepts and ideas are therefore awaited. Some of these ideas are the use of mesodermal progenitor cells [86], engineered human embryonic stem cells ectopically expressing human ETS variant 2 [87], modified lab-on-chip systems [88], or even using photosynthetic strategies [89]. Figure 1 depicts the overall process of mature organ generation from the building blocks (cell source, biomaterials, and scaffolds), through 3D bioprinting, vascularization, continuous monitoring, and, finally, organ maturation.
Serious effects of weightlessness are muscle atrophy and, more importantly, bone loss. During a spaceflight, humans in space lose an average of 0.5% to 2% of bone mass per month, or from 6% to 24% per year [32]. By comparison, postmenopausal osteoporosis causes a bone loss of 3% to 4% per year and senile osteoporosis causes a bone loss of approximately 1% per year.

Accelerated osteoporosis for astronauts (or spaceflight osteopenia) is a serious concern in the context of long-term space missions, because the more time crew members spend in space, the more they suffer from bone loss and the more there is a risk of injuries and fractures. Effects can be mitigated with specific exercises and maybe, in a far future, by using an artificial source of gravity, but today it is not enough to entirely counter the effects of microgravity on the bone [33].

For example, during a Mars mission, astronauts will have to face microgravity for several months and, after landing, they will be exposed to Mars (weaker) gravity during the time they spend there. As a consequence, they will land on Mars with certain bone fragility, increasing the risks of injury.

<table>
<thead>
<tr>
<th>Year</th>
<th>Tissue</th>
<th>Bioprinting technique</th>
<th>Refs</th>
</tr>
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<tbody>
<tr>
<td>2021</td>
<td>Kidney</td>
<td>Extrusion</td>
<td>[21]</td>
</tr>
<tr>
<td>2021</td>
<td>Lumens, branched vasculature and tubular intestinal epithelia</td>
<td>Extrusion</td>
<td>[22]</td>
</tr>
<tr>
<td>2019</td>
<td>Vascularized heart</td>
<td>Extrusion</td>
<td>[23]</td>
</tr>
<tr>
<td>2020</td>
<td>Liver and colon</td>
<td>Immersion</td>
<td>[71]</td>
</tr>
<tr>
<td>2020</td>
<td>Bladder</td>
<td>Microfluidic-based print</td>
<td>[72]</td>
</tr>
<tr>
<td>2020</td>
<td>Heart</td>
<td>Extrusion</td>
<td>[73]</td>
</tr>
<tr>
<td>2019</td>
<td>Vasculature</td>
<td>Extrusion</td>
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</tr>
<tr>
<td>2019</td>
<td>Heart</td>
<td>Extrusion</td>
<td>[75]</td>
</tr>
<tr>
<td>2018</td>
<td>Salivary gland</td>
<td>Magnetic</td>
<td>[76]</td>
</tr>
<tr>
<td>2018</td>
<td>Brain cancer (glioma)</td>
<td>Extrusion</td>
<td>[77]</td>
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IPS cells have been developed since then.

**Magneto-acoustic bioassembly**: a rapid formatative biotababrication of 3D tissues and organs with using physical fields as ‘scaffolds’ instead of more traditional solid scaffolds or scaffold-like hydrogels and bionics.

**Microgravity**: condition in which living materials or objects are in weightlessness.

**Noninvasive and nondestructive biomonitoring**: a method of estimation of viability and functionality of organoids without destruction and invasion.

**Organoids**: 3D microtissues self-developing from stem cells and having the histological and functional characteristics of mature organs from stem cells and their self-development and self-differentiation.

**Pluripotent cells**: cells capable of differentiating all germ layers (endoderm, mesoderm, and ectoderm) and the germline, but not extra embryonic tissues (e.g., inner cell mass, embryonic stem cells, embryonic germ cells, epiblast stem cells, iPS cells).

**Radiosensitive tissues**: usually highly proliferative tissues and organs, which die after exposure to radiation.

**Regenerative medicine**: Interface between engineering and life sciences, exploits the properties of living cells in combination with biomaterials, drugs, or genes to repair or replace tissues and organs.

**Scaffolds**: temporal and removable (usually biodegradable) supports in tissue engineering.

**Self-reporting genes**: usually labelled with GFP protein genes, which enable manifestation of cell viability and functionality using noninvasive and nondestructive fluorescent microscopy.

**Space radiation**: the space radiation environment includes energetic solar particles emitted during solar flares and coronal mass ejections, as well as galactic cosmic rays, which are composed of electrons (2%), protons (95%), helium nuclei (12%), and heavier ions referred to as high-energy and high-charge particles (HZE; 1%).

**Spheroid**: a cluster of cells with a spherical shape, typically formed by allowing a cell suspension to settle into a droplet of media.

**Vascularization**: formation of new blood vessels, which involves vasculogenesis, angiogenesis, and arteriogenesis.
when facing Mars gravity, but they will also have some difficulties to totally recover their bone mass for the future [34].

Radiation
The Earth’s magnetic field and atmosphere protect us from approximately 99% of the radiation emitted by the sun and by distant stars. This is not the case when we stay in outer space. On
the Moon and even on the ISS, astronauts are exposed to ten times higher radiation than on Earth. In case of a deep space exploration mission, the radiation impact is worse because the astronauts stay outside the Earth’s magnetic field and the time of exposure will be longer [35]. Consequently, astronauts will be exposed to a range of effective doses [36] (Figure 2).

Radiation exposure damages the central nervous system and can alter cognitive function, but it also increases the risk of cancer. The risk is that because of the damages induced by cosmic radiation, the cells will not be able to completely repair and there will be an accumulation of mutations leading to diseases and cancer [37]. In extreme cases, it could cause an acute radiation sickness in the short-term [38].

Our atmosphere’s shielding can be seen as being equivalent to a 10 m high water column, while a spacecraft is only equivalent to a 20 cm water column and a spacesuit is equivalent to 1.5 cm water column [39]. As cosmic radiation is also different in nature, shields should be several meters thick (and composed of materials with different atomic mass) to completely shield astronauts, which is technically impossible today [40]. Hence, radiation is mitigated by partial protective shielding (with lower atomic mass materials such as hydrogen content materials), by shielding responses to dosimeters, alerts, and radiation sensors.

**Isolation constraints and difficulty in returning to Earth in a short time during a long Mars mission**

Another hazard that space travelers will encounter, in the context of a long-term mission, is obviously the duration of the mission and the distance from Earth. On the ISS, astronauts and cosmonauts can return home within hours in case of a medical emergency. Although this should be avoided, as the increased gravity forces experienced during Earth entry could aggravate the medical condition of the astronaut. Therefore, NASA Medical Operations have developed a comprehensive methodology to guide the crew members remotely. So far, no in-flight medical emergencies have occurred in the history of human spaceflight [41]. The ISS is also continually resupplied with resources and medical equipment. In addition, the ISS is equipped with an advanced life support pack, providing advanced cardiac life support and advanced trauma life support [42–44].

In contrast, in the case of a long-term expedition, it is months before a return to Earth is possible and there is no resupply. Therefore, it is vital that the spacecraft should be as self-sufficient as possible in terms of resources and even more in respect to medical capability. Facing a communication delay and the possibility of equipment failures or a medical emergency, humans in space must be capable of confronting several situations without support from Earth. Examples are emergency surgery, acute trauma, burns, and wounds. At the moment, critical aspects in surviving a trauma or a surgery are wound/suture behavior and healing in space. For these reasons, the spacecraft should host cutting-edge medical technology, like 3D bioprinting equipment, regenerative medicine capacities, surgical instruments, as well as (amongst other medical devices), microscopes and freezers for blood samples and tissues to be medically self-sufficient and to be able to face any kind of health issues.

Long-term travel, for instance, an extended stay on the Moon and, even more, a mission to Mars, imposes various technical constraints. Indeed, during such travel there are no possibilities to return in case of technical or medical issues. From a medical point of view, all eventualities should be considered and countermeasures have to be taken to avoid medical complications. In addition, the spacecraft and the crew members should have the ability to be medically self-sufficient, at least as far as possible, to face any kind of unexpected events. From this point of view,
translational regenerative medicine seems to offer promising prospects considering engineering of tissues like cartilage, bone, thyroid, liver, muscle, and heart, among others.

A combination of 3D bioprinters and an operational medical/surgical block containing samples of blood, bone marrow, and various types of tissues will help the mission to approach self-sufficiency and to be able to face critical situations requiring a medical intervention.

**Why use organoids in space research?**

Why is it so important to use organoids in space research? First, human organoids are essentially small ‘avatars’ of a person, since they provide a unique opportunity to study authentic (histologically in structure and functionally similar in function to mature tissues and organs) human tissue. Secondly, this can be done without causing any inconvenience or damage to a person, since microtissues of a person are used, which have grown in vitro through self-organization. Thirdly, the genetic modification of the used stem cells provides unique possibilities for the creation of organoids that include self-reporting genes that are turned on when activated and expression of which can be easily visualized using fluorescent proteins and registered with built-in automatic biosensors.

**Organoids for studying deep space exploration**

Astronauts will encounter multiple space stressors during their missions, as previously described here. However, space radiation is the main biomedical problem associated with spaceflights into deep space [35]. In the last century, radiobiologists discovered that the most radiosensitive tissues are tissues and organs with a high level of proliferation. Thus, it would be logical to focus on research in space not only on the traditional study, as, for example, on the mechanisms of osteoporosis, but above all on the study of human organoids that recapitulate in vitro the development of organs with a high level of proliferation. These are mainly the intestines, bone marrow, and also reproductive organs such as ovary and testes. The effect of cosmic radiation on reproductive tissues can be studied initially on frozen samples of human organoids and then on living organoids using self-reproducing genes for apoptosis (programmed cell death) via fluorescent proteins [45]. Studying the possible effect of cosmic radiation on the differentiation of bone marrow cells into vascular endothelium in vascular organoids, as well as on angiogenesis and vasculogenesis, would be very important. Specially designed microfluidic perfusion chambers could assist in biomonitoring and visualize, in real time, angiogenesis as well as vasculogenesis from endothelial progenitor cells. Also, in vascular organoids, genetically modified stem cells could directly visualize in vivo the expression of tissue-specific cell markers, such as platelet endothelial cell adhesion molecule-1 (PECAM-1; CD31) or vascular endothelial growth factor (VEGF). The functionality of excitable and contractile tissues of organoids of the human brain and heart could be observed and visualized in real time using elegant and modern methods of optogenetics [46–48].

**Technical support for experiments with organoids in space**

Adequate technical support for the study of human organoids in space will require an additional solution to some still unresolved technological problems or challenges. First of all, the need for maximum automation of research is becoming increasingly obvious, especially in the case of using unmanned spacecraft and satellites for research. Minimizing and compacting devices is undoubtedly the second important technological imperative, especially in the case of space (satellite) research. The use of biosensors, built-in cameras, and other devices allows for noninvasive and nondestructive biomonitoring, in real time, of changes occurring in organoids in space. In the case of effective biomonitoring of experiments in space in real time from the Earth, the urgent need to return the organoids to Earth automatically disappears.
Alternatively, technologies for delivering organoids both to space and to Earth also require optimization, either by cryopreservation or by using specially designed or carefully selected stimulus-sensitive hydrogels. The study of the effect of space radiation can be carried out as simply as possible on frozen objects, provided that the appropriate cryo-equipment is available. Finally, the development of special bioassemblers and microfluidic perfusion devices is required for placement on board space stations or satellites. For ideal space experiments, it is also necessary to provide an adequate ground control, which means parallel experiments are conducted with similar human organoids on Earth under conditions of Earth’s gravity and normal Earth radiation levels, but applying the same time-line, temperature, and CO₂ profile as well as pH conditions.

**Ethics and feasibility**

The big question when talking about new technologies and ‘new medicine’ is: Where are we today? Is it feasible and worthwhile? Bioprinting already allows creation and transplantation of tissues, skin, bones, vascular graft, heart tissues, tracheal prostheses, and cartilage structure [49]. A next-decade goal is to create entire organs and nervous systems, which is essential to transplant muscles (Box 2).

Ethically, biofabrication in space brings new questions, such as whether new tissue or even entire organs could be simply printed off to replace injured/aged or dysfunctional body parts in space? In particular, the possibility to transplant tissues made from the astronaut’s own cells or replacing aged organs in astronauts during a mission with younger and healthier ones would be extremely beneficial. However, the prospect of being able to print or biofabricate human organs in space, like on Earth, is likely to have a profound ethical impact on long-term missions as it would have on society on Earth. Amongst others, it could potentially boost the average longevity and viability of astronauts. It can bring criticisms, however, that the ability to print organs and so extend life in space is not right, given the scarcity and shortage of organ transplants and potential biofabrication on Earth, which is already a great concern.

Another aspect is the fact that, on Earth, tissues require some scaffold to hold all the structure in place, especially for cavities like the chambers of the heart. However, in a microgravity environment, much better biofabricated organs could potentially be produced. Faced with the exorbitant expense of printing organs, offering improved hearts or lungs created in space could be a method to attract people and a new market of rich people ready to pay for these improved space-grown organs and techniques.

At the moment, the focus is on using/biofabricating organs for life-saving surgery, also in space. However, there is a risk for enhanced organs to be used as a possibility for performance-improving in sport or other potential activities, since the technologies would be commercialized and thus available on the market for those able to afford them.

Finally, another ethical issue is: if many organs were changed in a person or an astronaut, arguably, what is then left would not be the human who was born, but some other creature. An important consideration when living in extreme and isolated space environments, lies on how to survive in a self-sustaining manner with a lack of immediate Earth-based medical assistance, equipment, and resources. Space travelers will need to cope with and to develop medical emergencies involving regenerative medicine within their own space habitats. On Earth, ethical issues of regenerative medicine are tied to medical decisions that might cause disagreement and conflict. In the same way, space regenerative medicine will be intimately related to bioethical standards that impinge on strictly medical decisions. However, a tunnel-vision-like perspective on medical
diagnosis and treatment in space, certainly from an ethical point of view, might prove very difficult and might force us to rethink in terms of developing further complementary perspectives on biomedical and ethical issues related to regenerative medicine. Furthermore, with the development of space tourism in the near future, that will pave the way for rather less-trained civilian space farers, additional medical but also ethical pressures on the future development of regenerative medicine for this particular population might be indirectly affected by a combination of higher risk of potential biomedical and health interventions and complications in space among the space tourists and a lowering of safety standards with regard to health requirements of space tourists prior to spaceflight.

**Concluding remarks and future trends**

Compared with the classical 2D cultures that allow cell organization only in monolayers, organoids are complex (3D) human tissues that are being developed with a closer recapitulation of the ‘authentic’ histological structure and organ function. When combined with biofabrication technologies, human organoids could hold the potential to further capture the complexity of different biological systems communicating with each other. If proven functional, such more complex 3D *in vitro* models could also hold the potential to advance over current animal models.

**Box 2. Ethics**

A central question is linked to ethical considerations related to regenerative medicine. Transplantation medicine and artificial organ advancements have drastically increased the potential of organs and tissues as parts and regenerative medicine is playing a role in furthering the ‘componentation’ (the organ is regarded as a simple commodity) of the human body and improving the image of human body parts. Furthermore, the progress in componentation of the human body, as well as the ‘enhancement’ of such ‘human body parts’, is one of the most prominent ethical dilemmas in regenerative medicine.

Indeed, the componentation of the human body is a first step toward what is called ‘commodification’ (the organ is regarded as a simple commodity) of the human body. The componentation and the commodification of the human body, regardless of the degree of regenerative medicine’s contribution, are seen as an ethical challenge to the traditional image of human bodies and the abstract concept of ‘human dignity’. However, in the future, a new ethical view of human bodies may arise, which is defined as ‘a perspective in which human bodies, organs, tissues, and even the bodies themselves are regarded as disposable tools like disposable cameras, syringes, or contact lenses’; therefore a new ethical view would be appropriate for a new reality.

Another aspect is that the majority of regenerative medicine advancements have many uncertainties and knowledge gaps in common. These characteristics make assessing the long-term effects of available options challenging. Safety and efficacy, patient consent, information, professional obligations, as well as equity and justice are among the main ethical concerns. The issues, as well as the underlying ideals, must be clarified, specified, argued, and prioritized. One issue is that values occasionally clash: some values can only be accomplished at the expense of others. When values clash, there are principles that can be used to assist decision-making.

The following are some additional ethical considerations. To begin with, well-informed decisions are based on two types of evidence: values and knowledge (or scientific evidence). The scientific evidence may include patient health, diagnosis, alternative therapy alternatives, costs, as well as regulatory knowledge, patient attitudes, and specific information regarding, for example, the origin of the cells used, cell properties, impact of prior studies, record of clinical evidence on efficacy and side effects, and the number of treated patients. A strong scientific foundation, as well as values that are debated, explained, stated, and ordered in priority, are required to reach ethically acceptable results in the long-term interests of all stakeholders concerned.

The overall purpose of ethics related to this matter is also to improve the quality of the evidence base. To achieve this, improving publication practices, encouraging research integrity, publishing also the results of failed trials, and avoiding publication bias and selective reporting of the results of regenerative medicine studies are important steps to further develop. More and better access to scientific evidence is needed for patients, clinicians, and regulators. Reducing uncertainties and, if possible, filling knowledge gaps are both scientific and ethical obligations. Furthermore, debates are taking place about the potential costs, the use of stem cells, the risks of misuse, and experiments on the human body.

**Outstanding questions**

Can space constraints and limits provide new, ‘out-of-the-box’ solutions to further advance biofabrication technologies?

Is it possible to develop a magneto-acoustic bioassembler for rapid biofabrication of human tissue and organs from organoids in space with minimal nontoxic concentration of paramagnetic solutions?

Is it possible to fabricate human organoids working as radiation-sensitive ‘sentinels’ from genetically modified cells with self-reporting radio-sensitive apoptotic genes?

Is it possible to develop automated microdevices for noninvasive and nondestructive biomonitoring of viability and functionality of human organoids?

Is it possible to design and develop human vascularized and perfused tissue- and organo-specific microtissues (organ-on-a-chip) connected with organoids mimicking bone marrow to study the relative role of angiogenesis and vasculogenesis in pathogenesis of radiation-induced apoptosis associated with tissue and organ atrophy in space?

Is it possible to develop laser magneto-acoustic bioassemblers thanks to the use of space for rapid biofabrication on Earth of functional, vascularized, and perfusable complex human tissues and organs with low, nontoxic concentration of paramagnetic media?

Can the combination of human organoids and biofabrication technologies reach that level of organism complexity that is not possible with single organoids and not completed represented by animal models?

Do we need hydrogels at all or could we bioprint tissues, and in the future organs, with organoids only?

How can biofabricated constructs be preserved for months or years in a spacecraft?
which approximate only what could occur at the organism level in humans, are expensive both in space and on Earth, and are associated with ethical concerns on the well-being and sacrifice of animals. We expect, therefore, that human organoids will have stronger and stronger implications for space research as they are already providing on Earth, thus possibly replacing 2D cultures and reducing, if not replacing, research in animal models.

The technologies developed for the space environment and the results obtained from studies in space will have implications back on Earth. In fact, they are contributing to advance our understanding of relevant organ diseases and to develop treatments for these conditions, where space is used as an accelerator of ageing on Earth. In the case of the heart, for example, intrinsic processes that promote cellular ageing, such as inflammation and oxidative stress, exacerbate telomere shortening, chromatin remodeling, and epigenetic drift [50]. At a cellular level, ageing entails decreased replicative capacity and dysregulation of cellular processes. Consequently, a number of physiological functions are impaired, leading to arrhythmias, the reduction of aerobic capacity, and cardiac atrophy, ultimately resulting in two major age-associated cardiac pathologies: heart failure and atrial fibrillation [51]. The same technologies, in a more distanced future, could also be used to fabricate regenerative medicine solutions to promptly treat patients on Earth as well as space travelers that develop relevant conditions during long-term space missions.

Advances in piezoelectric inkjet bioprinting have greatly facilitated drop-on-demand delivery and provide full control of each individual nozzle and drop [52,53]. Using off-the-shelf microelectromechanical print heads, this technology has been applied to the printing of (human) tissues. Several investigators are using this approach to print single cells [54] and cell aggregates [55], demonstrating that printing of complex tissues [56] and eventually of whole organs [57,58] is technologically within our reach. Despite several commercially available additive manufacturing technologies capable of fabricating biological constructs [1,59], these systems still move in Cartesian coordinates, which limits the capacity to mimic the complexity of tissue organization. More recently, volumetric bioprinting with photosensitive hydrogels has been introduced, achieving more complex tissues and geometries [56,60]. However, this technology is still limited by the availability of a broad gamma of biocompatible photosensitive hydrogels.

In this respect, space could offer new opportunities to develop biofabrication technologies further. The lack of gravity might provide an environment where magneto-acoustic forces could be more easily modulated without the need for high magnetic or acoustic fields [61,62]. These special conditions would result in an easier control of the floating building elements in bioprinting. At the same time, microgravity conditions would provide a biophysical environment where more dynamic hydrogels could be developed without the limitations of the force of gravity. A microgravity environment would also allow us to answer to an everlasting question in the field: do we need hydrogels at all or could we bioprint tissues, and in the future organs, with organoids only? This is not an easy question to answer, because hydrogels have been initially used as bioinks with the purpose to provide a physical support to cells to move from 2D to 3D bioprinting. However, in recent years bioinks have advanced more and more, to include instructive biological cues able to better recapitulate the cell–extracellular matrix (ECM) microenvironment [17], thus moving from shaping to biological function support. In this respect, the use of molecularly designed hydrogels by using self-assembly and supramolecular principles have been proposed as bioinks and shown to better recapitulate the multiscale hierarchical organization and dynamics of the ECM [63,64]. Yet, these hydrogels are often more fluid than conventional bioinks and more difficult to process in gravity conditions on Earth. In space, microgravity conditions could facilitate the integration of such dynamic and molecularly designed bioinks.
within bioprinting approaches, thus resulting in biofabrication with higher precision and molecular diversity. In addition, the volumetric deposition in space microgravity allows control of how different organoids can come together in space, thus providing more fundamental tools to better understand organoid–organoid fusion and tissue morphogenesis. Ultimately, the space environment allows biofabrication of 3D *in vitro* models in a biophysical environment where ageing is accelerated, therefore providing also a functional research environment for ageing back on Earth or for drug development and testing with relevance for us earthlings.

Moreover, biofabrication delivers new solutions for future space programs. In the most immediate, we could foresee a biofabrication station available for plans of a Moon colony or a manned mission to Mars, as an emergency solution, to biomanufacture tissue patches to treat injuries. Organoids with self-reporting genes providing a fluorescent signal about cell death, for example, which is detectable and readable by special devices, are ideal and sophisticated biosensors (or ‘centinels’) for real-time biomonitoring of space radiation. Whereas tissues can certainly be engineered until 2030, organs remain still a dream, but one that can be realized (at least in form of organ patches) in the next two decades, especially if we consider the positive impact that space could have on biofabrication development, as discussed earlier. Moving beyond tissues and organs, biofabrication could also provide a framework to standardize biological constructs for other purposes. We could envision, for example, the bioprinting of engineered food [65]. It is possible to fabricate *ad hoc* nutrients and currently efforts to engineer meat have gained a lot of momentum [66,67]. Beyond food, the use of vegetal forms of life with specific properties, such as production of energy and oxygen, has also been shown to be feasible [68,69]. Ultimately, we could also foresee the use of biofabrication technologies to make 3D *in vitro* models to study bacteria and virus infections in space, on one side to understand the risks of bringing Earth species on other planets and, on the other, serving as a possible testbed to be better prepared in the distant future for testing new viruses found on new planets (Figure 3).

These technologies give great hope in the context of a long-term space exploration mission as it would be a serious and flexible response to some hazards astronauts will face in deep space. Several diseases caused by space stresses might be treated by the potential ability to replace damaged tissues or organs. Weakened bones and muscles should also be treated and, if necessary, replaced using 3D bioprinted grafts.

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Figure 3. A vision for biofabrication in space. Drawings from Erik Wallert (www.erikwallert.nl).
Biofabrication technologies could provide one of the key solutions to ensure that the mission is self-sufficient and that the astronauts are able to encounter some complex challenges while traveling in deep space without the possibility of returning to Earth for months. Ambitious projects such as a Mars mission need several new technologies and medical capacities, as well as studies on the use of regenerative medicine in space. In the context of space exploration, the feasibility of using regenerative medicine technologies also depends on the miniaturization ability, as equipment and 3D bioprinters should fit in the spacecraft. Questions on how to preserve the biofabricated constructs for months or years in the spacecraft, if needed, will have to be answered (see Outstanding questions). For instance, the current state of knowledge does not allow preserving blood samples for such a long period.

Historically, research in space placed certain constrains and limits (e.g., space, volume and size limits, need for automation, work in unusual and often harsh conditions, need for certification and validation to operate, safety concerns, distal monitoring), which is actually very good to enhance and even induce creativity by forcing researchers and engineers to think untraditionally, ‘out of the box’, thus eventually improving biofabrication technologies also on Earth. The feasibility and added value of implementing 3D printing technologies into future exploration missions still have to be evaluated, especially regarding current knowledge, but there is some space determinism leading us to believe that these technologies would help not only in successfully managing all kinds of difficulties encountered during a mission to Mars, but also to find new medicines and treatments on Earth.

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Declaration of interests
No interests are declared.

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