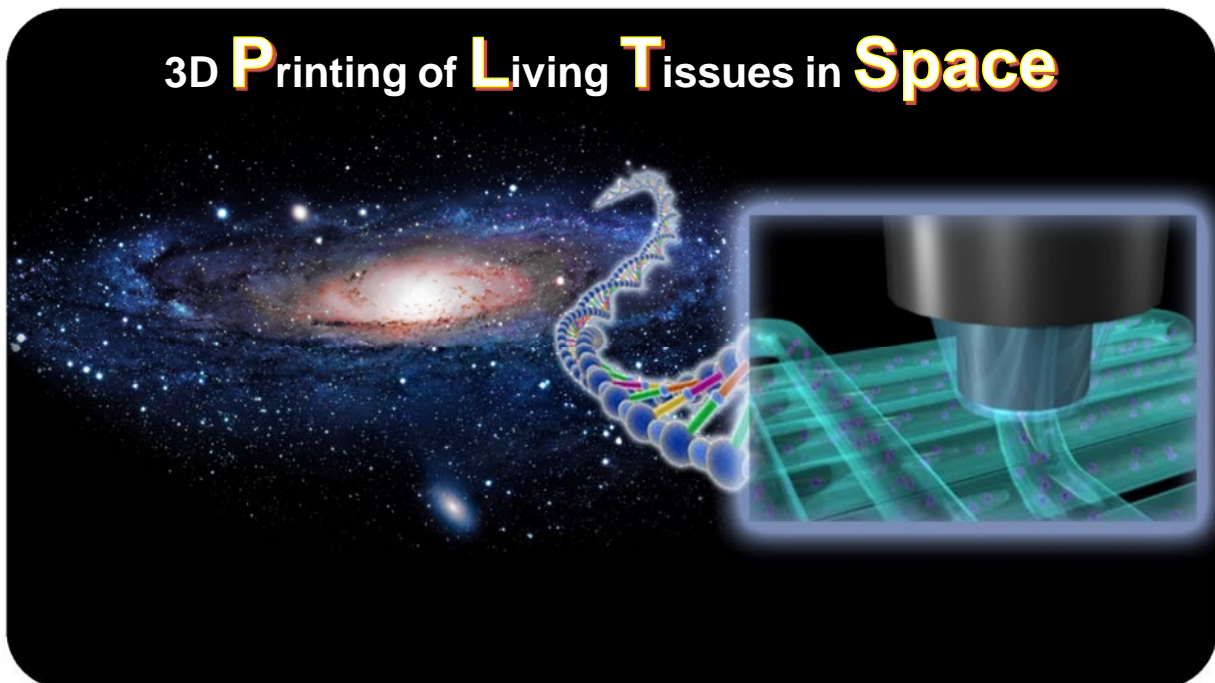


Executive Summary

PLT-Space

3D **P**rinting of **L**iving **T**issues in **S**pace



1 STUDY OVERVIEW AND BACKGROUND

Human exploratory missions to the Moon or Mars, are widely considered as the next logical steps in human space exploration and, lately, settlements. Such space exploration or settlement activities are involved with long exposure of humans in space and, in case of increasing distances to Earth, no abort possibilities. Crews on such missions have to be self-sustaining, not only concerning food and drink, wastes, but also with respect to medical treatment. Health issues are raised by the environmental conditions in space, such as the influence of altered gravity, radiation, isolation. In addition the health risks of illness or injury human beings are faced on Earth, are also present in space.



Figure 1-1: Challenges of Space Exploration

However, in case of long distance travels the medical infrastructure on Earth is not in reach. In order to protect human lives and health, such space exploration missions have to consider a medical infrastructure onboard, so that medical treatment of a wide range of health issues can be provided. A promising technology which can provide support in medical treatment and improves the autonomous functionality of the medical structure is 3D – Bioprinting. By printing skin, bones, organs or cartilage parts the medical treatment can be supported offering an important contribution to a medical infrastructure in space.

1.1 Mission Scenarios

The early phases of this study involved a thorough analysis of potential mission and medical scenarios, as well as expected health risks during these mission scenarios. Based on previous research on space exploration missions from space agencies, space exploration scenarios were evaluated with regards to the most probable medical situations. Those medical situations were then evaluated by their potential to be treated by 3D bioprinting technologies.

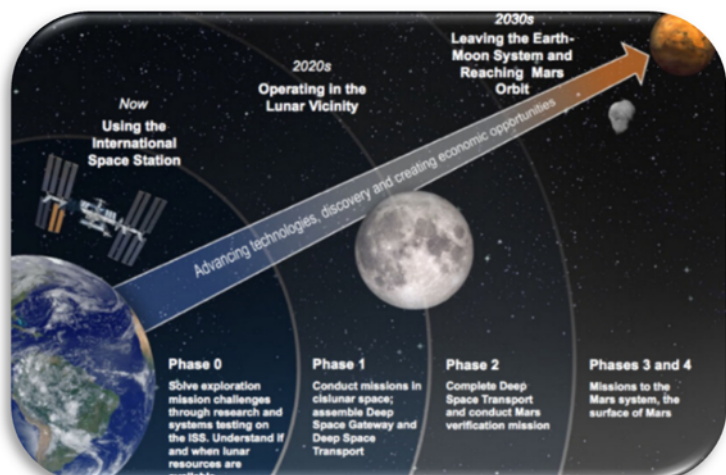


Figure 1-2: Space Exploration Visions
 (<https://www.nasa.gov>, 03.05.2019)

Figure 1-2 shows future visions of space exploration mission scenarios divided into close to Earth and distant to Earth missions. While journeys to the Moon or the establishment of a deep space platform in microgravity, such as LOPG, are considered to be doable in the near future, i.e. 2020s, missions to Mars are considered to be possible in the 2030s. Table 1-1 gives an overview of the characteristics of these mission scenarios.

Table 1-1: Characterisation of mission scenarios


	Close to Earth 🌐 0.16g - μ g-environment		Distant to Earth 🌐 0.37g - μ g-environment
Mission duration	Short < 6 months	Long > 6 months	Long > 6 months
Distance to Earth	<20*10 ⁵ km		>10 ⁶ km
Crew size	4 – 5 🧑	10 – 15 🧑	>15 🧑
Medical care	Medical first aid on site Serious injury: Stabilisation and return to Earth		Medical and surgical infrastructure
3D BP aspects	3D-printing for medical orthosis (no BP)		3D-BP relevant (Lab for operations and bioprinting)
Radiation Exposure (Al-shielded)	< 400 mSv	> 400 mSv	> 400 mSv
Gravity	0.16g - μ g-environment		0.37g - μ g-environment
Pot. Mission Start	~ 2020		~ 2040

The scenarios comprise generally the different phases of space exploration missions, i.e. travel time in microgravity and stay on a planetary body in reduced gravity. The mission scenarios are characterized by several categories which are important to evaluate the potential of the application of bioprinting technologies in these scenarios.

The **mission duration** and the **distance to Earth** are major key factors concerning the risk of health issues and the scope of available medical treatment. Generally, space missions with long mission durations, have a larger crew than short-term missions. Thus, the risk of health issues is increased by the crew size and by the time spent in space in isolation.

Close to Earth 🌐


A seriously injured person can be stabilized and returned to Earth. Applications of 3D printing would consequently be restricted to conventional methods for medical orthosis or non-surgical treatments, such as skin injuries (also by bioprinted skin).



www.esa.int

Distant to Earth 🌐

It is not possible to return to Earth in a reasonable amount of time (< 1 week). Thus a sophisticated medical infrastructure is required with one or two medical experts being part of the crew. For implementing bioprinting to a mission, then one of the crew needs to have specific training on bioprinting methods and their applications.



https://pics-about-space.com/mars-one-habitat?p=5

1.2 Health Risks and Injuries

During a space exploration mission, astronauts encounter health hazards, which may cause injuries, organ damage etc. and have to be treated.

In order to minimize the risk of health issues it is necessary to assess the most probable and most catastrophic ones (see Table 1-2).



Table 1-2: Potential health risks during space exploration scenarios

		Radiation	Reduced Gravity	General
Health Risk	Skin	Skin cancer	N/A	cryogenic burn, chemical and electrical burns, burns, cuts
	Organs	Tumor, organ damage	altered blood pressure due to gravity leads to organ disorders, heart problems	liver dysfunction, gall bladder disease, kidney stones, appendicitis
	Muscle & Bone	Bone cancer	Bone demineralization, decrease in exercise capacity	muscle rupture, fractures, amputation

The most common incidents in spaceflight so far involved skin (burns or cuts) or heart problems (arrhythmias) (*HUMEX, A Study of the Survivability and Adaption of Humans to Long-Duration Exploratory Missions, Lasseur C, (2003), ESA-SP-1264; Human Health and Performance Risks of Space Exploration Missions, (2009), NASA SP-2009-3405*). These medical events have been treated onboard. However, although being rare, the most catastrophic health incidents potentially may lead to mission abortion and/or death of one of the astronauts have to be taken into account.

Medical procedures can be distinguished into treatment without and with surgery. In order to assess the potential applications of 3D-bioprinting technologies it is useful to evaluate the corresponding medical events with regards to their conventional medical treatment procedures and how they can be modified or simplified for space exploration missions using 3D-bioprinting technologies.

In addition, also conventional 3D printing can be used to maintain the medical infrastructure by printing medical tools, splints for medical orthosis, etc. Of course, it has to be evaluated whether it is useful to pack a bag of substitutes on Earth and just bring it along the mission, or if it is easier and cheaper to have a 3D-printer which can print out the needed product on demand.

1.3 Medical Scenarios

As a result of the previous sections the following scenarios for on-site medical care procedures are considered with respect to 3D-(bio)printing:

- (1) Full medical treatment without surgery
- (2) First aid medical care and return to Earth for further treatment (e.g. surgery)
- (3) Full medical treatment including surgery

The first scenario considers medical events that can be treated by medical procedures without surgery, e.g. bandaging cuts, medication of organ or heart function disorders etc. The required equipment for conducting these procedures have to be either brought along and replaced by shuttle flights from Earth on a regular basis or, if limited or no regular flight connection to Earth is available, produced on site using 3D-printing technology. This includes conventional 3D-printing technologies for medical orthosis, such as splints.



The second scenario considers medical events that have to be treated by surgery, e.g. skin transplantation, organ transplantation, treating fractures, implementing stents or bypass etc. If the mission is close to Earth, the crew gives first aid and stabilizes the injured person so that a return to Earth is possible. Further treatment, such as surgery, is then provided by specialists on Earth. For distant to Earth mission scenarios this medical option is not available, since no fast return (< 1 week) is possible.

Consequently, in cases with no return to Earth option, full medical treatment, including surgery, has to be available in order to protect and secure the crew's health. As no flight connection and only a limited communication link to Earth are available a medical infrastructure has to be established, which can work autonomously. It is necessary to consider the most probable and most catastrophic medical events for such space exploration missions and use the support for medical treatment provided by 3D-bioprinters.



In the context of 3D - (bio)printing technologies long-term and distant to Earth missions are the ones with highest potential for their application as support technologies for medical treatment measures. However, since bioprinting methods generally have to be combined with surgical interventions, in particular 3D - bioprinting ones, it is important to not only test the 3D - (bio)printing methods in space but also install a surgical infrastructure as a testbed in space. In order to ensure the protection of human health, long-term missions to celestial bodies or deep space stations close to Earth offer a perfect laboratory for the implementation of a surgical infrastructure in space.

2 BIOPRINTING TECHNOLOGIES

2.1 Short overview of bioprinting technologies

3D bioprinting offers the possibility of fabricating tissue equivalents or even organ-like constructs, either autologous or allogeneic, by printing multiple cells, soluble factors and biomaterials simultaneously, following a precise predefined pattern.

A very common strategy used in bioprinting to form tissue constructs with embedded cells involves the deposition of layers of a bioink that after crosslinking will form a hydrogel matrix to provide structural support to the cells and other extracellular components embedded within. After an *in vitro* culture period, which depends on the tissue generated and is required for tissue differentiation, remodelling and maturation, the printed tissue or organ construct can potentially be applied to replace the function of damaged natural tissue.

3D bioprinting allows the inclusion of multiple cell types in the same layer or in different regions of the construct while maintaining a high spatial resolution. For that, cells are commonly mixed and embedded into their respective biomaterial prior to printing (bioinks). Therefore, biomaterials and cells are positioned together during the printing process enabling the fabrication of complex tissue constructs. As a side effect, very high seeding efficiency is guaranteed as the cells are immobilized in the scaffold material during manufacture. Depending on the nature of the deposition, two selected classes of techniques are presented:

- (1) Deposition of discrete spherical cell/biomaterial droplets or cell aggregates used as building blocks
- (2) Continuous extrusion of bioinks where cells are suspended and deposited in a strand-like fashion

The following schematic (Figure 2-1) shows the basic working principle of these two classes of bioprinting technologies: the left one belongs to the group in which cells/biomaterials are deposited in a discrete manner (1) whereas the method shown on the right side demonstrates continuous extrusion (2).

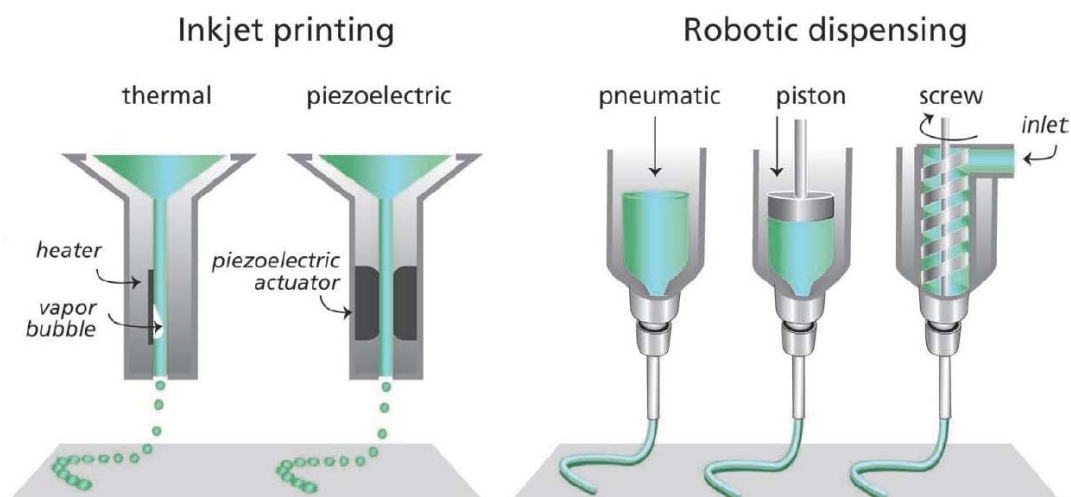


Figure 2-1: Scheme of the main technologies used in 3D bioprinting, (Malda J. et al., 25th anniversary article: engineering hydrogels for biofabrication Adv. Mater. 25 5011–28, (2013))

The main characteristic of the first group is that bioinks are released in small discrete droplets.

For the second group of technologies, where a continuous deposition of the bioink is aimed, different robotic dispensing methodologies have been traditionally used. The most common are normally known as “syringe-based extrusion” or “3D bioplotting” and the release of the

material can be achieved through diverse mechanical methods (Figure 2-1). Advantage of extrusion-based bioprinting is the commonly high speed of deposition and the compact architecture of the respective printers. As an example, Figure 2-2 shows the GeSIM BioScaffolder 3.1 as one of the typical devices used for extrusion printing and bioprinting.

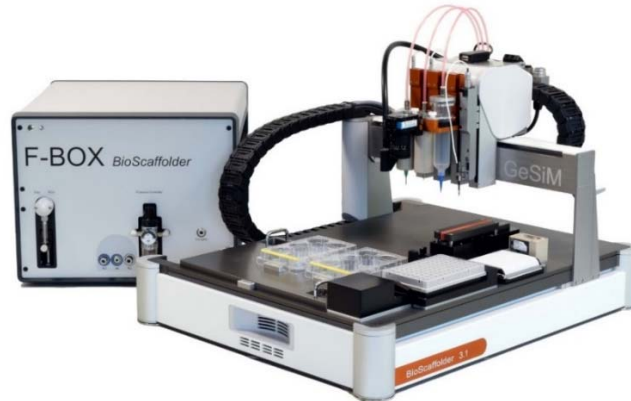


Figure 2-2: BioScaffolder 3.1, GeSIM (Germany) – a typical extrusion-based (bio)printer
(<https://gesim-bioinstruments-microfluidics.com>)

Extrusion-based bioprinting is a very versatile technology and it could be demonstrated that mixtures consisting of many different human cell types and a variety of synthetic and natural biomaterials can be utilized successfully.

Table 2-1: Classification of selected bioprinting technologies based on their deposition process

Process		Technology	Main features/ Characteristics	Advantages	Disadvantages
Physical support: bioink	Discrete	Inkjet	Deposition of single cells in low viscous inks.	High precision positioning single cells in two dimensions.	No volumetric constructs can be achieved because of the lack of mechanical stability of the low density bioinks employed.
	Continuous	Extrusion BP (syringe-based extrusion)	Cells are suspended in a viscous, polymeric solution (bioink) and extruded as strands.	Less cells are required.	Low resolution, defined by the nozzle diameter. As cells are diluted in the bioink formation of cell-cell contacts is hindered.

It is important to highlight that in bioprinting different steps can be found: pre-printing, bioprinting, and post-printing. It is essential to understand them as well as the requirements and limitations of each to translate this technology to locations outside of Earth:

- **Pre-printing:** In this phase, the main objective is to multiply the number of available cells, commonly obtained from a little biopsy.
- **Tissue printing:** The deposition of the cells and the biomaterials that will form the initial artificial extracellular matrix as a suitable space to grow and develop. More materials can be added at this step to improve the mechanical properties of the scaffold or the biological activity.
- **Post-printing and maturation:** Post-processing of the constructions prepares them for their normal function when implanted into the patient. Post-printing processes, like the crosslinking of the freshly printed construct, can be controlled by researchers while the maturation phase is mostly driven by cells and only some external parameters as culture conditions or mechanical stimulation can be modulated.

2.2 3D - bioprinting for medical treatments

The possibility of obtaining bioprinted living tissues to replace those that have been injured, or even complete organs in the future, can mean an important breakthrough for space colonization and mankind. As BP works on a semi-automatic manner, it would allow non-specialized crew to create different types of tissues, without the need of fully knowledge about it, with very few instructions and the help of the software.

Another advantage of having a bioprinter is that once this machine is available in space, it would be able to generate new different tissues as they are investigated on Earth, as the design can be modular, updated with new features and adjustable to the needs of the crew in each moment. This can be better understood, if the bioprinter is thought of as a kitchen robot, where different recipes can be pre-programmed and the final result will depend on the materials and tools previously introduced. Indeed, the same machine can be adjusted to work with thermoplastics (normal 3D Printing) or with cells and pastes, simplifying and reducing the necessary hardware – and therefore space and weight on board. As they can be installed in closed, sterile chambers, the risk of liquid leakage or contamination can be decreased.

In the following, the role of bioprinting in the medical treatment of skin injuries, bone fractures and organ issues is considered.

Skin



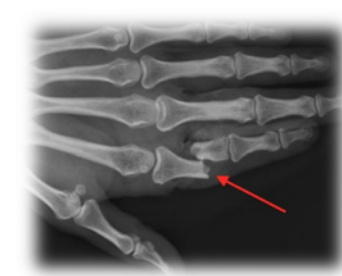
<https://www.ndr.de/ratgeber/gesundheit/Was-tun-bei-einer-Verbrennung,verbrennung110.html>

Conventional Medical Treatment: Skin grafting in severe cases

Bioprinting: Generation of autologous skin

Needs: Bioprinter and sterile environment (locally)

Bone



<http://www.handerkrankungen.de/frakturen/Fragen.htm>

Conventional Medical Treatment: Immobilization with cast, removal of injured bone segment and replacement (metal, ceramic, etc.)

Bioprinting: Generation of autologous bone segment for replacement

Needs: Bioprinter, operating room, surgical instruments

Organ



<https://www.t-online.de/gesundheit/krankheiten-symptome> (Staras/Getty Images)

Conventional Medical Treatment: Drugs and medication, partial replacement, full transplantation

Bioprinting: Generation of autologous replacement, creation of fully functional organs (future aim)

Needs: Bioprinter, operating room, surgical instruments

3 SPACE ADAPTATION

During a workshop in September 2018 leading scientific, medical and industrial experts gathered at ESTEC to bring together all areas of expertise to evaluate the potential and challenges for implementation of bioprinting in human space exploration missions.



Figure 3-1: Participants of the Workshop (from left to right, with core team highlighted in bold): Hugo de Oliveira (Biotis), Adrian Dragu (TU Dresden), Marcy Zenoby-Wong (ETH Zürich), Brigitte Godard (Medes), **Michael Gelinsky (TU Dresden)**, **Nieves Cubo (TU Dresden)**, **Daniela Knickmann (OH B)**, Vladimir Mironov (Bioprinting), **Sandra Podhajsky (OH B)**, Jos Malda (Uni Utrecht), Aleksandr Ovsianikov (TU Wien), Paul Gatenholm (Chalmers), Tommaso Ghidini (ESA), Alessandro Parolari (University of Milano), missing: **Klaus Slenzka (BH Luxembourg)**

Major goal during this workshop was to identify recommendations for Research and Technology aspects to enable future bioprinting in exploration missions. Leading experts from the fields of bioprinting, regenerative medicine, space medicine and space exploration participated providing a wide range of knowledge required to assess the potential application of bioprinting to support medical treatment in space. During this workshop the participants discussed the current status of 3D bioprinting, and the work still to be done to advance the practice from lab research to the point where it can help both terrestrial and astronaut patients – which include medical and technological, as well as regulatory challenges.

In Figure 3-2 the visions of space exploration is presented along a timeline. Short distance exploration in the lunar vicinity are foreseen to be available in the 2020s while long distance travel to the Mars, for example, will be possible after 2030. Medical treatment has to be adapted according to the mission scenarios, i.e. whether it is possible to return injured persons to Earth for further treatment. In case of long distance missions in situ treatment is essential. Based on these requirements, the potential application of 3D Bioprinting is analysed for the different mission scenarios, which is visualized in the upper half of the graphic.

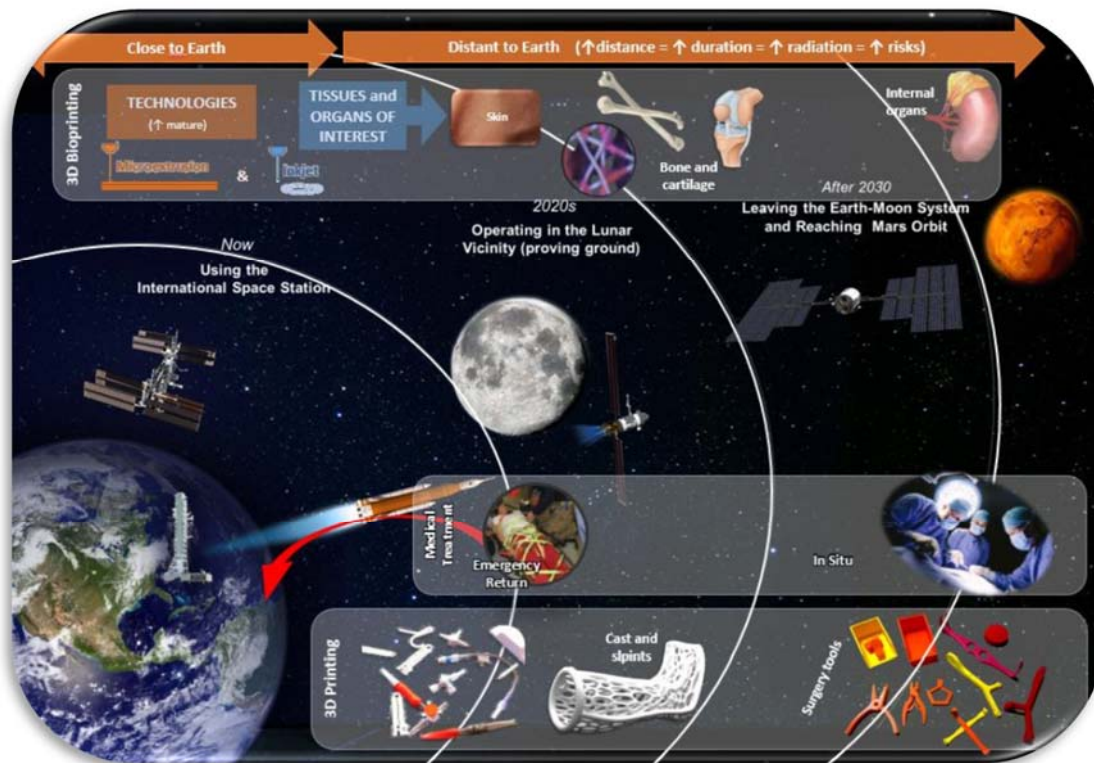


Figure 3-2: Bioprinting feasibility for different mission scenarios
(presented @ Biofabrication 2018, Würzburg)

In a follow up trade analysis the most suitable matches were identified and then the most reasonable research and technology needs identified. Amongst them are a bioprinting multitool for all mission types, and for longer mission durations a bio-printing laboratory for pre- and post-printing aspects as well as an operation room for surgeries.

In the field of bioprinting there are several major issues that have to be tackled to print more complex structures, and also wound healing with bioprinted tissue under space conditions will need further research, prior to application in an exploration mission.

SPACE ADAPTATION OF BIOPRINTER TOOL

- Development of **Bioprinter Multitool**: Printer, Incubator, Bioreactor, Sterile Bench
- **Bioprinting Technology**: Research on Bioinks, cell behaviour, wound healing in altered gravity
- Implementation of **Bioprinting Laboratory**: Pre-printing, Tissue printing, post-printing process
- Implementation of **operational theatre**: Surgery in space

3.1 Near Term Missions of all types

For mission scenarios which are feasible within the near future, i.e. within the next 10 years, some basic aspects of research and development of the bioprinting technologies for space applications are considered.

First the bioprinter multitool is the key element for future medical applications of bioprinting technologies in space and therefore serves as the basis for future research and development tasks. The components of the bioprinter multitool incorporate:

- a bioprinter,
- a bioreactor for cell culturing,
- an incubator for maturation,
- a sterile bench
- all required support systems, such as waste handling or electronics, as well as a data management and control unit.



The combination of all these components into one tool saves space and simplifies the handling of the bioprinting process. The overall system shall provide an all-in-one application, i.e. starting from cell and bioink preparation, then printing the desired tissue, up to cleaning, removing and maturation of the tissue until it is ready to be applied in a surgical intervention in space.

Some challenges for reach in regards to applying bioprinters in space exploration:

- **Microgravity:** The reduction in gravitational forces results in decreased buoyancy-driven flows, rates of sedimentation and hydrostatic pressure. In general, fluid dynamics are also altered, and there is a near absence of convection. In this sense, both 3D printing and bioprinting can be deeply influenced. Microgravity would also affect manipulation of liquids like cell culture medium in general, needed to keep the constructs alive until implantation.



https://de.123rf.com/photo_50432665_brave-astronaut-im-au%C3%9Fenbordeinsatz-auf-dem-mars-.html/

- **Viscosity of bioinks for extrusion and inkjet bioprinting:** Viscosity can be a function of temperature and may be altered by shear thinning effects and therefore needs to be adjusted for different environmental conditions. Thus, to obtain vital tissue constructs in reduced gravity conditions, it is mandatory to investigate the dependence of the printed tissue and its quality on the viscosity of the bioinks.



<https://tu-dresden.de/>

- **Radiation:** During space exploration missions, the spaceship, the crew and all hardware will be exposed to higher radiation levels and different radiation composition than on Earth. Thus, radiation will influence electronics, (bio)materials and cells and might as well be quite harmful to bioprinted tissues *in vitro*.



- **Characterization of the bioprinted tissues:** Due to the effect that the radiation and altered gravity levels have on the behaviour of cells, and therefore on the bioprinted tissues too, the tissue quality and maturation, such as mechanical properties, cell density etc., should be performed along the process chain to check for the correct development of the construct. Such online analytical tools are yet not available as the dimensions of bioprinted constructs (at least of those, relevant for clinical applications) are not accessible for common microscopic techniques.



Thus, further research and development for new hardware, such as syringes, sensors, e.g. maturation and tissue quality sensor as well as scientific research for the space adaptation of biological aspects, such as properties of bioinks in altered gravity, is needed until the bioprinter multitool is ready for applications in space.

Selected Aspects to consider for Research and Development

- **Space adaptation of bioprinter**

Development of sensor to monitor maturation process and tissue quality

Evaluation of waste and recycling options



- **Research on biological aspects in altered gravity**

Analysis of properties of bioinks and medium spreading

Analysis of cell behavior and vascularization

Study of wound healing process with bioprinted skin



- **Research on cell damage in radiation environment**

Study effects of heavy ions on tissue and different shielding possibilities

Ground based experiments in particle accelerators



3.2 Long Term Missions of all types

For long term travels to planetary bodies far away from Earth, such as Mars, not only the technology to apply the bioprinter in space has to be available to print the tissue, but also the medical infrastructure to perform surgery and medical interventions supported by bioprinting methods needs to be established. Therefore, a 3D Bioprinting Laboratory as well as an operational theatre have to be established on site for medical treatment of injured astronauts.

3D bioprinting lab facility

The 3D - (bio)printing lab shall provide room for the 3D printer(s), cell culture bioreactor, resources for bioinks etc., so that all required pre- and post- printing processes can be conducted in this room. This implies the processing of the cell sample of the astronaut giving the input for the bioprinter, the preparation of the bioink before it can be mixed with the cells. Then the tissue is produced and printed, which ideally works automatically using monitoring devices and allowing an automatic control of the printing process. After finishing the printing the tissue has to be matured and its functions have to be trained before the tissue can be transplanted on a human body. Moreover, the facility have to be cleaned and sterilised. All these tasks shall be performed within the laboratory, before the tissue is used for surgical operations.



Operational theatre and surgery in space



The operational theatre shall be a room where operations with open wounds can be conducted. It needs a sterilisation tool for medical tools and disinfection of hands and arms of surgeons. Package waste from sterile medical tools, waste from operations etc. has to be reduced and instead recycling options and 3D printing of medical tools have to be taken into account.

The integration of an operational theatre is preferred to be in a

station on a planetary body, i.e. moon or Mars, as it simplifies the development and logistics compared to an integration on a spaceship.

