CHALLENGE 4

SUSTAINING HUMAN LIFE IN SPACE

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1. INTRODUCTION AND GENERAL DESCRIPTION

On 20th July 1969, the Fresnedillas Control Station, near Madrid, received the first words of a human from the surface of the Moon. "That's one small step for [a] man, one giant leap for mankind", was the historical sentence recorded from Neil A. Armstrong, commander of the "Apollo XI" mission. Nowadays, fifty years after Armstrong's epic achievement, space exploration by humans is commonly recognized as a highly exciting and attractive challenge and a powerful booster for scientific and technological progress in order to improve the human life on Earth. This is true despite some criticisms (minority, but significant) that question the high costs that it entails (Rinaldi 2016). The establishment of permanent settlements in the Moon and Mars is becoming a realistic venture day by day. After a decade of successful rover explorations to the surface of Mars, both ESA and NASA, and more recently the agencies from growing economies in Asian countries, are working to promote a manned mission, first to the Moon, and then to Mars. The European Space Agency (ESA), of which Spain is an active member, adheres to these objectives and is strongly committed in supporting and participating in these programs.

The main aim of space life science is to understand how the space environment, and specifically altered gravity and radiation, affects the morphology, physiology and behaviour of living organisms, and to design countermeasures to enable terrestrial life, and particularly human life, to develop outside Earth. That is, how they perceive and respond to gravity and radiation and adapt to the space environment. There is a variety of disciplines, such as genetic, molecular, anatomical or physiological fields, which use a range of technologies to address these issues. In order to understand adaptations at the functional level it is necessary to identify and study adaptations at cellular and tissue levels. Also, basic research analysing biomolecules, cells and model organisms is necessary to progress towards exploration subsystems or bioregenerative life support systems. Figure 1 shows a scheme of the synergism between space biology and human research from NASA life sciences translational research (taken from Alwood et al., 2017). Thus, life science space research moves from biological systems to human health in order to support successful human exploration; through the horizontal integration of research between basic and applied researchers, along with vertically-integrated teams.

The main aim of the present chapter is to identify CSIC research teams that can contribute at any level on space life science research and how they can do this contribution. The goal is to engage researchers within the current open science program approaches in order to optimize resources and facilitate translational research (see Figure 2).

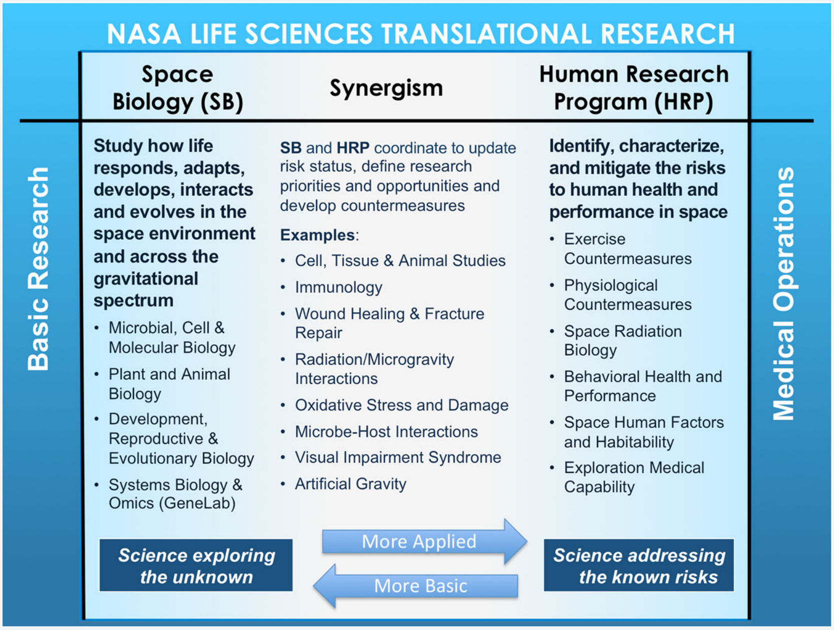
Although we do contemplate life science for space as a global research endeavour, the contents of this challenge can be gathered in three main categories:

Biomedical implications of space exploration: Human and animal space biology

The establishment of human colonies in space does not only depend on the appropriate technological developments, but also on the biological capacity of humans to live in a different environment. A return to the Moon, expeditions to Mars and beyond, and a rise in space tourism will lead to an increasing number of human spaceflights. Thus, it is crucial to consider the challenges to human health in space during long-term space missions and physiological changes that can take place during short-term altered gravity conditions. In addition, it is necessary to consider the possible influence of space radiation, available countermeasures and potential applications on Earth of knowledge gained when considering these issues.

For example, the establishment of the adult pattern of brain circuitry depends on various intrinsic and extrinsic factors, whose modification during development can lead to alterations in cortical organization and function. Since the

FIGURE 1–Space Biology and Human Research Bi-directional Synergism. Basic Space Biology research is done mainly with lower-level model organisms, cells, and tissues. The HRP conducts biomedical science mainly with humans. They synergistically and bi-directionally collaborate in maximizing opportunities for translating a subset of that knowledge to optimize the health and safety of the crew via applications supporting medical operations (taken from Alwood et al., 2017)



brain has evolved over millions of years in the presence of a constant terrestrial gravitational field, this environmental parameter may affect cortical development and the processing of information of the neurons and circuits exposed to space flights.

As well, the effect on human fertility will require careful consideration together with the necessity of ensuring human reproduction in long-haul missions of colonization. The capacity of humans to reproduce in space or in stations in other planets, under a variety of conditions of life with hypergravity or microgravity, and during or after exposure to radiation, represent new challenges which will determine the likelihood of success in these endeavours.

Few studies have been done about aging/health changes in long-duration space flights, due to the low number of missions (8) that have lasted more than 300 days in the space (Garrett-Bakelman et al. 2019). In humans, exposure to microgravity caused aging-like changes, such as cognitive disturbance, bone density loss, mild hypothyroidism, increased stress hormones, decreased sex steroids, insulin resistance, impaired anabolic response to food intake, anorexia, altered mitochondrial function, and systemic inflammatory response (Garrett-Bakelman et al. 2019; Wang et al. 2009). Thus, space's impact on aging (by boosting the onset of diseases) is a growing research field, and alternative therapies and counter-measures are necessary to avoid or delay the onset of these aging-like changes described to date.

Besides, crews on space missions have to be self-sustained, which also implies medical treatment beyond aging mitigation. Diseases or injuries that humans face on Earth are a challenge in extra-terrestrial missions. Medical infrastructure must be reliable and it must cover as many clinical scenarios as possible and, preferentially, it has to be operated in a semi-automated manner for the lack of specialized personnel. Simplicity is another requirement as the space for medical equipment is limited. Additive manufacturing technologies can be used for manufacturing personalized tools including the manufacturing of surgical tools under demand.

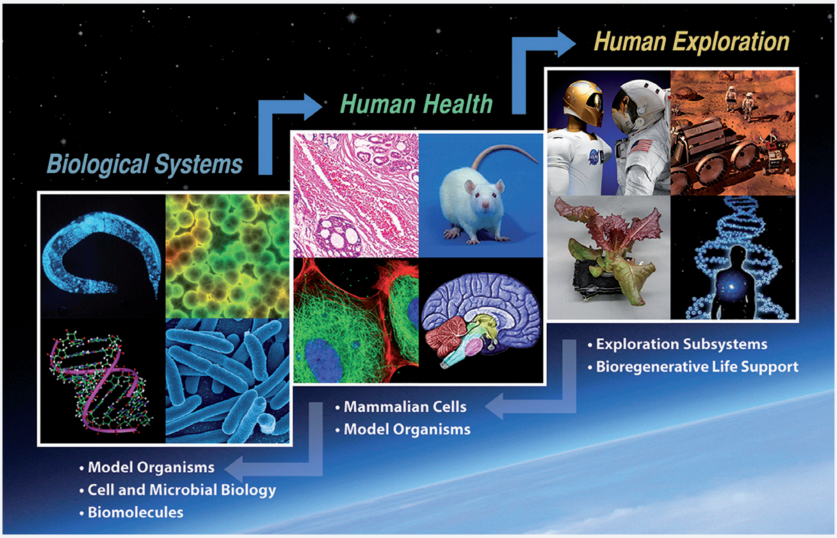
Bioregenerative Life Support Systems (BLSS): Plant space Biology

Plants are a necessary companion of Humans in Space Exploration.

https://www.esa.int/Science_Exploration/Human_and_Robotic_Exploration/Research/Plants

The objectives of deep space exploration by humans, including the Moon and Mars, require the implementation of a complex system of life support for space explorers, capable of supplying the elements necessary for sustaining their life (oxygen, food, moisture...) and of removing their waste products. The system needs to be bioregenerative, i.e. the components need to self-regenerate, without the addition of new elements brought from the Earth, and energetically efficient, only using the power sources available in space. Plants are a candidate to occupy a key position in these Bioregenerative Life Support Systems (BLSS). They indeed are being used in all the initiatives tested up to now, such as MELiSSA (Häder et al. 2018). There is no doubt that plants must accompany humans in space exploration ventures, since they offer the potential to provide food, replenish the air, filter water, and improve the mental health of the crew during long-duration missions in space. The achievement

FIGURE 2–The NASA Life Sciences Translational Path. It moves from basic research to human exploration applications with bi-directionality options. As knowledge is applied along this path (top arrows) questions may arise that can best be addressed by more basic research (bottom arrows) in order to support further progress toward successful human exploration (taken from Alwood et al., 2017).



of a true "space agriculture" is a fundamental objective of this global enterprise and it requires a full knowledge of the effects of the space environment on the plant biological mechanisms and the adaptive processes and countermeasures necessary to guarantee plant survival in these alien environments (Medina 2021; Figure 3).

In addition to the use of plants, human life support can greatly benefit from an adequate protein supply of animal origin. However, while seeds of different plant species are feasible to transport in space aircrafts to be seeded when necessary, transport of farm animals or embryos with the objective to raise a farm in the space, is not as easy task. A promising alternative to this problem would be the introduction of adequate animal species rich in protein content and having the ability for efficient waste processing. In addition to this, the animal farming model should be able to utilize inedible parts of plants to meet its nutritional requirements, everything integrated in a bioregenerative life support system.

A third approach to provide life support in space is by using food fermentation, the oldest way to preserve food in the Earth. It extends shelf-life, enhances palatability, increases nutritional value (e.g. vitamin production, increase nutrient bioavailability) and it may also deliver bioactive compounds and beneficial microorganisms (probiotics) (Marco et al., 2017). Fermented foods are essentially the result of microbial growth on a particular substrate and enzymatic conversions of food components. Lactic acid bacteria (LAB) are the main microorganisms involved in the fermentation of raw products. They are natural inhabitants of plants and the mammalian gut from where they have access to raw food and promote its fermentation. Currently, selected LAB are intentionally added to start fermentation and are generally known as "starter cultures" or simply "starters". Specific LAB strains are regarded as probiotics because consumption of viable cells exerts a positive effect on human health by improving human gut microbial homeostasis, including mental health through the interactions with the gut-liver-brain axis.

Microorganisms in Space exploration

Microorganisms are major players ruling the maintenance and functioning of ecosystems on Earth and influence the development of plants and animals, including humans. Microorganisms rule the behaviour and well-being of plants and animals, including humans. Today, it is well known that the human microbiome rules human physiology and psychology. Similarly, microorganisms interact with plant growth and it is well known the role of plant-growth promoting microorganisms and their role in the rhizosphere. Plants and animals are not such independent living creatures; rather they live through a continuous interaction with their microbiomes, the microbial communities living on, or around, them. Consequently, space settlements will have to consider the presence of microorganisms which are required for humans, plants and animals' life. How these microbial communities are affected by different factors in space are gaps that need to be filled. Confinement, potential radiation, different gravity, nutrient and waste cycling, use of biofertilizers and probiotics, etc., are some factors to be considered on maintaining adequate microbiomes in plants and animals. There is even a potential to sustain human nutrition entirely out of microorganisms and current examples are represented by starting-up initiatives on the consumption of microalgae, yeasts, and numerous microbialy-generated nutritive complements. A support for the provision of vitamins and probiotics, as examples, which could be produced by microorganisms. The role of biotechnology of microorganisms in space would represent a major achievement for the adaptation of sustainable processes in human settlements beyond our planet.

The microorganisms also carry out some essential steps required for the complete cycling of elements such as denitrification, nitrification, sulphate reduction, methanogenesis, oxidation and reduction of metals, among others. These capabilities are highly valuable to generate sustainable human settlements. Thus, microorganisms are of relevance to be able to build sustainable support systems in space and other planets.

Microorganisms could be a tool for the transformation of planets or large-scale environments to be used either for human habitability or to carry out different processes. An example of this has occurred naturally on Earth. It is the great oxygenation event based on the massive growth of cyanobaceria on earth about 2500 billion years that resulted in an oxygenated atmosphere as we know it today. Environments appropriated for bioleaching processes to extract metals could also be generated by using microorganisms such as the case of Rio Tinto (Huelva) where metal transformations are naturally carried out by microorganisms (Garcia-Moyano et al. 2008) and, at present, these microbes and conditions are used industrially. Thus, the transformation of environments for different purposes is a future challenge for human development in space.

However, in space and on the surface of other planets of interest for the establishment of human colonies, such as the Moon or Mars, conditions are unfavourable for most microorganisms that could be used in life support systems. For instance, radiation exposure is much higher than on Earth, due to the absence of a protective atmosphere and magnetosphere. Besides that, on Mars the soils may contain toxic compounds such as perchlorate and toxic metals such as lead, cadmium or arsenic. Some microorganisms found on Earth can develop under aerobic (in the presence of oxygen) or anaerobic (in the absence of oxygen) conditions which result in the possibility to inhabit almost any imaginable habitat in our planet. Microorganisms, and especially extreme microorganisms (i.e., extremophiles), are a major natural resource to design long-term support systems which must be sustainable. So far, the extremophiles are the organisms that define the currently known limits of life. Thus, when bioprocesses need to be developed under unfavourable conditions (from the human perspective), the extremophiles (i.e., thermophiles, phychrophiles, acidophiles, halophiles, etc.) are the resource to look at for genes, proteins and enzymes (biocatalysts), mechanisms and potential solutions (Elleuche et al., 2014) to be used in long-term space support systems.

2. IMPACT IN BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

2.1. Biomedical implications of space exploration. Human and animal space research

Understanding the effects of space environment on the brain will reveal if long periods in space would induce significant structural changes. As the neocortex is the site of higher brain functions, the structural plasticity induced by microgravity may be of particular relevance for future prolonged human spaceflights. Also, generating models and simulations of the cortical circuits under situations of microgravity could particularly be relevant to model the effects of future prolonged human spaceflights that for technical reason cannot be performed experimentally. Providing a new understanding of brain plasticity will trigger interesting medical applications.

In a similar manner, the study of human reproduction in space and the associated technology that would be developed will found a direct application on Earth. The impact of food, or other environmental factors, on female and male fertility, and in offspring, will have to be implemented. There will also be a need to organize gamete, embryo and somatic tissue banks as an insurance against possible negative impacts of space conditions on fertility. Ethics and politics related to development and monitoring of reproductive issues will also be required.

The impact of aging-associated diseases is also specially relevant when thinking in future long duration space travel and other planets colonization.. Space-driven developmens to cure or delay aging-related diseases could have enormous social and economic impact. Besides an improvement in health and longevity in the elderly population, other social groups could be benefited. This is the case of patients suffering from premature aging diseases, cancer patients who are treated with agents that induce premature senescence of tumour cells, and old people suffering acute diseases (such as acute lung injury; ALI, and acute respiratory distress syndrome; ARDS).

Regarding space missions, the fact of being treated (vaccinated) against the onset of diseases that plague old age would reduce the consumption of medicines and health expenses, and would significantly improve the health and longevity of the older travellers. These aspects are especially important when thinking that these people will be confined in closed and reduced places for a long time or perhaps the rest of their life.

As well, developments in regenerative medicine based on tissue and organ bioprinting can find a direct application on Earth: i) The production of new generation of bioinks biologically active for capturing tissue-matrix properties, ii) clinical treatments for a variety of musculoskeletal defects caused by trauma or degenerative pathologies, iii) Personalised Medicine with Custom-made bioprinted Tissue Engineered Constructs (TEC) tailored specifically to the needs of each individual patient, iv) new platforms for testing radiation effects, drugs and cosmetics to accelerate the transition from animal to non-animal-based research and safety testing of new drugs for the treatment of numerous diseases. Space exploration offers a unique opportunity for making tissue engineered products a real choice for clinicians on earth. Added benefits include the possibility of providing remote heath assistance to low income or remote areas where the lack of specialized medical personnel is common.

2.2. Bioregenerative Life Support Systems (BLSS) to sustain human life in space

The foreseen achievements of the study of plant biology in space are: i) to provide possible systems of food production in outer space, ii) to promote sustainable production of nutrients under extreme stress conditions, iii) to select crops and varieties adapted to environmental conditions out of the current ranges of our agricultural areas, iv) to increase resource use efficiency in plants for cultivation under minimal availability of resources, v) to achieve limited biosphere where humans can be integrated in a cycling system that allows a circular and sustainable food supply. These developments could allow the implementation of stable human colonies in the outer space or in extreme environmental conditions of the Earth. As a side effect, investigations to be performed with these objectives could be used in scenarios other than space application (poor soil conditions, extreme temperature, etc...), and put to good use to achieve the underarching objective of sustainable agriculture on Earth.

The same sustainability target can be achieved with the development of an adequate high-quality protein supply for astronauts and space settlers along big timeframes. It would provide with an easy-to-handle animal rearing technology integrated into bioregenerative life support systems capable to use plant wastes and human urine for animal feeding through highly efficient fermentative processes. Being able to reproduce the most appreciated organoleptic experiences of human culinary culture would have a positive impact in the social development of permanent, big lunar/Martian colonies.

Microorganisms can also contribute to the sustainability of bioregenerative life-support systems by the integration of an additional food supply. Overlapping with the ongoing research on the impact of gut microbiota and health, there is a window of opportunity to design food fermentation strategies as a dietary source of beneficial microorganisms to restore homeostasis of gut microbiota and mitigate associated risks to long-term human space settlements. The knowledge gained to modulate human gut microbiota to improve crew health could also be used in Earth-based applications.

2.3. Microorganisms in Space exploration

As commented above, long-term human settlements need to be designed based on sustainability. Microbial-based processes are an ideal solution for the recycling of wastes and the production of goods following green and circular strategies. Because of the major influence of microorganisms and microbial diversity on the maintenance of living beings, farming systems, and extra-terrestrial environments, the role of microbial diversity needs to be monitored and extensive utilized. Only in this way we will be able to avoid undesired biases of essential microbial systems and to achieve a successful, long-term habitability beyond Earth. The study of extremophiles will allow to improve the ability of life support systems to withstand the conditions of space and the surface of Mars and the Moon, to make possible the permanence of humans for long periods of time on space travel and planetary bases.

Soil bioremediation, treatment of health-hazardous residues, and other processes mediated by microorganisms that have to be developed for long-term human settlements in space will also undoubtedly find an application on Earth and help mitigating the conditions resulting from a possible climate change scenario, in which heat waves increase, producing higher UV radiation doses and droughts.

3. KEY CHALLENGING POINTS

3.1. Biomedical implications of space exploration. Human and animal space research

Effects of space environment on brain

In the brain, the main structure involved in the processing of cognitive information from a sensory perception is the cerebral cortex. The information from the outside world arrives into the cortex via thalamic afferent fibers and the information is processed to produce a response. The final product results from an interaction between three types of information: external, intrinsic and stored. The main neuronal type involved in this process is the pyramidal cell, whose dendritic spines play an integral role in the activity of spiny cells (reviewed in Yuste, 2010). Thus, our understanding of the synaptic organization of the neocortex largely depends on the knowledge available regarding synaptic inputs to pyramidal cells. In addition, dendritic spines are key elements in brain plasticity. Therefore, it is extraordinarily important in functional terms to know the distribution, size and proportion of cortical synapses.

Previous results in postnatal developing rats subject to microgravity conditions during a 16 days space flight (Neurolab mission) showed that microgravity leads to changes in the number and morphology of cortical synapses in a laminar-specific manner (DeFelipe et al., 2002). However, in this study only conventional electron microscopy and stereological techniques were used to estimate the density of synapses in a particular region of the cerebral cortex, the hindlimb cortex of rats. Methods have been developed to overcome these difficulties, for instance by means of dual-beam electron microscopy, where a focused ion beam (FIB) is used in combination with scanning electron microscopy (SEM). Moreover, other cellular and subcellular elements can be easily identified and traced through the series of images and thus all the components of the neuropil (axons, dendrites, synapses, glial processes, mitochondria, synaptic vesicles, etc.) can be studied at the same time as synapses.

To enable the next steps of human exploration such as cruises to reach destinations beyond low Earth orbit, it is critical to perform a more comprehensive study of possible microgravity-induced alterations of neuronal morphology and organization of synaptic circuits in various cortical areas, including the effects of longer duration space flights on brain neuronal circuits. Understanding the effects of space environment on the brain will expand previous results and reveal possible further changes in the design of the dendritic geometry of pyramidal neurons and synaptic connectivity, for a variety of sensory, motor, associational and memory-related regions. This will enable to find whether changes are selective for those cortical areas related to somatosensory and motor processing, whether there are certain critical periods of development in which they are more prominent, and to what extent synaptic plasticity occurs in the mature cerebral cortex in a microgravity environment. Furthermore, the modelling of neurons and circuits will enable to probe the distinct processing of information of the neurons and circuits exposed to space flights.

Human reproduction in space

Many genetic and environmental factors affect human reproduction with impacts on fertility and offspring health. Extended travel in deep space presents potential hazards to the reproductive function of female and male astronauts, including exposure to cosmic radiation, microgravity, hypergravity, psychological stress, physical stress and circadian rhythm disruptions. Only a few studies have examined the effects of microgravity on female reproduction (Mishra and Luderer 2019). They have found disrupted oestrous cycling and follicle development, which are a cause for concern. Exposure to microgravity during space flight and to simulated microgravity on Earth disrupts spermatogenesis and testicular testosterone synthesis in rodents. Studies performed on Earth in rodents exposed to experimentally generated high charge and energy (HZE) particles have shown a high sensitivity of ovarian follicles and spermatogenic cells to these particles (reviewed by Mishra and Luderer 2019).

In mice exposed to microgravity or artificial gravity at the International Space Station (ISS) and returned to Earth, only a decrease in accessory gland weight was detected in relation to control mice caged on Earth. There were no overt microscopic defects or changes in gene expression in the reproductive organs as determined by RNA-seq. Spermatozoa from mice kept at the ISS could fertilize oocytes in vitro at levels comparable to control males. Development of these fertilized eggs to birth and postnatal growth or fecundity of offspring showed no significant difference in relation to controls. Thus, short-term stays in outer space do not seem to cause apparent alterations in male reproductive function and offspring viability.

There has been little work carried out on human semen yet. Frozen sperm samples subjected to space-like microgravity conditions appeared to be as viable as those that remained on Earth. Mobility, vitality and DNA fragmentation was not altered when compared to its properties in conditions of gravity on Earth. A more detailed study on human (and bull) sperm function (NASA Micro-11 mission) allowed for live sperm assessments in spaceflight using several fertility biomarkers used clinically on Earth. Cryopreserved sperm were thawed at the ISS and several functional tests were carried out to assess motility and the ability of sperm to develop fertilizing capacity. Overall, results suggest that sperm functions related to fertility are altered in spaceflight. Studies on fresh human sperm are lacking. So far, research has apparently been carried out comparing semen samples collected before and after space flight, but no studies are available yet with semen samples collected at the ISS because astronauts seem to refuse to supply the samples. Future results should further explore sperm function in space with a view towards automated sperm analysis systems and the establishment of sperm banks outside Earth and also examine impacts on sperm DNA integrity.

Sex in space would be difficult and dangerous for a series of reasons. There are mechanical challenges and the threat to fertilization and the developing embryo posed by cosmic radiation. The development of appropriate space suits that could allow for conception in low gravity and aid pregnancy will be required. Altogether, many gaps require attention, including the effects of microgravity, hypergravity and radiation on the male and female reproductive tracts, hypothalamic-pituitary regulation of reproduction and prenatal development of the reproductive system, as well as the combined effects of the multiple reproductive hazards encountered in space.

Human aging and cellular senescence

In recent years it has been shown that the cause of the main diseases associated with aging is the accumulation of senescent cells in different tissues. These cells are metabolically hyperactive, and with harmful effects when they accumulate in the body in a chronic way. Importantly, it has been described that simulated microgravity promotes cellular senescence in neural rat cells and human intervertebral disc cells (Wang et al. 2009). Thus if the microgravity is a trigger of cellular senescence (as radiation does as well), and due that these cells play a causal role of several aging-associated diseases, it could explain some physiological aging-related changes described in astronauts.

An emerging and exciting approach is the clearance of senescent cells. It was recently demonstrated that elimination of senescent cells delays the onset of aging-related disease, thus establishing for the first time a direct role for cellular senescence in the onset of disease. No side effects were observed in mice during progressive elimination of senescent cells, demonstrating that age-dependent accumulation (continuous presence) in the organism is not essential. However, this strategy is based on genetic interventions, which cannot be used in humans. Pharmacological approaches, by using drugs that selectively kill or "silence" senescent cells (senolytics and senomorphics, respectively), are another strategy, although based on a chronic treatment scheme, which in some cases has led to negative side effects. A possible approach to solve this problem would be the targeted and progressive clearance of senescent cell accumulation by stimulating the adaptive immune response (vaccine development or targeted immunotherapy). Immunotherapy is defined as the stimulation of the immune system to recognize and kill disease-associated cells and recent data demonstrated that vaccination (T-cell response) in spaceflight was not affected, unlike other processes (Garrett-Bakelman et al. 2019). Such a vaccine could prove successful for the natural aging process, by improving health and increasing longevity, and important for microgravity-related environments. On the basis of the accumulated experience in this field, senescent cells are ideal targets for directed immune therapies. Importantly, there are no published data about the effect of immunotherapy on senescent cells, thus highlighting the novelty of this approach.

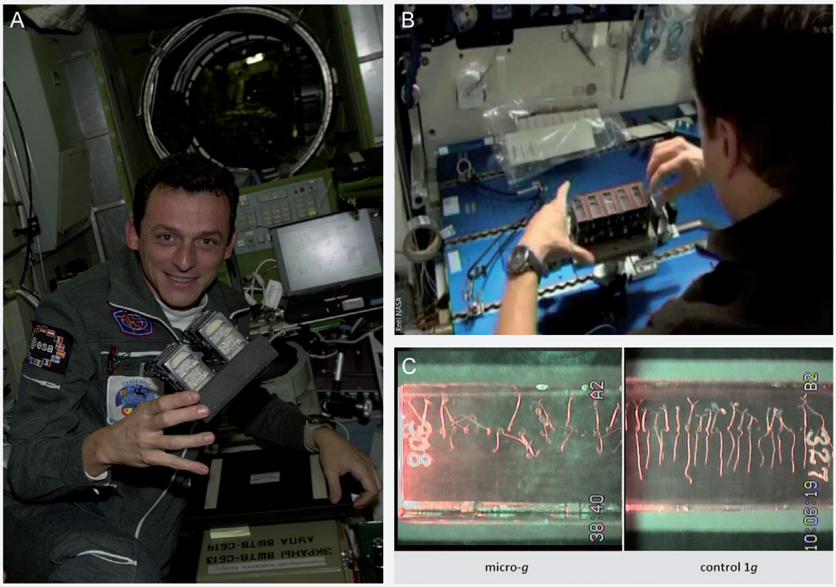
Countermeasures for regeneration of tissue and organ damage in space
The future challenge to improve the medical autonomy of the crew is the use of bioprinting, an additive manufacturing technology that will allow to grow human tissues in space, to treat various pathologies such as severe burns or complex bone fractures.

Bioprinting involves the use of bioinks, materials that combine biological and hosting materials. Hydrogels are normally used as the hosting material for bioinks. They are mostly appropriate for soft tissues but they do not present the required mechanical properties for hard tissues such as bone or cartilage, where other more complex approaches are needed.

Current technologies in development for bioprinting on Earth are:

- *Extrusion*: viscous materials are pushed through a nozzle and deposited on strands following the designed pattern. Physical and chemical processes are required to shift the viscoelastic nature of the material to stabilize the structure. The shift can be triggered by the addition of ions, variations in temperature or pH, solvent evaporation or photocrosslinking.
- *Inkjet:* droplets of low viscous liquids are directly deposited onto a substrate. The work with solid materials in powder forms requires the use of a resin that acts as binder.
- *Selective laser sintering (SLS):* a laser beam is directed across a powder bed to raise temperature and melt "a path". Ceramic, metallic or polymer substrates can be used. Laser-induced forward transfer (LIFT) requires ultrashort laser pulses to induce vaporization on a metallic layer acting as a mask. The mask protects cells from the high power beam and results in droplet formation that is collected on a substrate.

FIGURE 3–Images of Spanish biological experiments in the International Space Station (ISS), originated in CIB Margarita Salas – CSIC research center. A: The Spanish ESA astronaut Pedro Duque during the Cervantes Mission (2003) showing an experimental container of one of the Spanish experiments. Credit: ESA/Pedro Duque. B: Processing of samples in space corresponding to one of the runs of the NASA-ESA Seedling Growth Project (2013-2018) on plant biology. Credit: NASA. C: An experimental image of the Seedling Growth Project. Seedlings of the plant model species Arabidopsis thaliana germinated and grown for 6 days under microgravity, in space (micro-g), compared with control seedlings grown in the same conditions, but under terrestrial gravity (control 1g). Credit: F.J. Medina (CIB-CSIC).



- *Stereolithography:* similar to SLS. Here the laser is used to obtain local polymerization on organic substrates. 2-photon stereolithography can write 3D structures inside a monomer solution within a submicron range.

For space applications, gravity is an important factor determining not only the material and technology requirements, but also the three-dimensional growing of tissues and organs. Reduced gravity has shown to cause dramatic physiological changes. Cardiovascular, musculoskeletal, nervous, gastro-intestinal and immune systems respond to microgravity in a similar way to changes associated with ageing. Recent microgravity experiments found that human thyroid cancer cells and human endothelial cells form multicellular spheroids rather than the flat single-cell layers typical on Earth (Bradock, 2019). In microgravity, spheroids can develop into tubular structures resembling the intima of rudimentary blood vessels, even without scaffolds. However, for more complex tissues this self-assembly methodology is not enough, and another technique (like 3D bioprinting) is required to mimic the tissue environment.

In the ISS, Russian researchers have just started to use a magnetic levitating technology to create spheroids of mouse thyrocytes, to imitate a thyroid gland, and chondrocytes to produce cartilage. Both sort of construct showed living cells with a normal morphology. Also, a Biofabrication Facility (BFF) with a bioprinter has been recently installed by the US at the ISS to test different biomaterials for tissue engineering. These are likely to be the first steps in using microgravity studies to create tissues for transplantation or drug development. It also provides new research lines that enable the testing of bioinks created on Earth, opening new opportunities for bioprinting researchers and institutions.

3.2. Bioregenerative Life Support Systems (BLSS) to sustain human life in space. Food supply

Plant growth in space

Growing plants successfully in space requires a full understanding of the biological mechanisms of response and adaptation to the conditions of the spaceflight environment. It provides conditions that are inaccessible on Earth, such as growth in microgravity and exposure to cosmic radiation, providing a unique opportunity to dissect responses under conditions that plant biology has not encountered during its evolutionary history (Figure 3). Additional challenges of spaceflight experiments come from limited access and available space on orbiting platforms.

The culture of plants on planets and satellites other than the Earth, such as the Moon or Mars, necessarily requires the creation of a "greenhouse" in which the plant is provided with the necessary environmental elements to enable its development. These elements include light, water, temperature, oxygen, CO_2, aeration, and nutrients. Furthermore, microorganisms are required to achieve a fully functional and sustainable environment for plants. In addition, plants need a substrate capable of anchoring the root and sustaining root development. There are published experiments that demonstrate that terrestrial plants can grow in an analogue of lunar soil or Martian soil, provided that this substrate is supplemented with additional elements and substances that provide the plant with water, mineral salts and nutrients that it needs for its survival and development. In this regard, the example of potato cultivation on Mars described in the movie "The Martian" (2015) is very illustrative. It is certainly science fiction, but it raises with rigor and solid scientific and technological arguments the problems and their possible solutions. Regarding gravity, centrifugal culture at 1*g* does not seem technically feasible and, with respect to radiation, available shields do not seem to achieve 100% shielding, as evidenced from the data obtained in the ISS (Maalouf et al. 2011; Medina et al. 2015).

The first space experiments, more than 50 years ago, showed that plants could survive and grow in space, although alterations were soon reported. Major improvements in culture facilities, mostly after the assembly and operation of the International Space Station (ISS), have allowed concluding that microgravity itself does not prevent plant growth and reproduction. This occurs despite the alteration of fundamental cellular and molecular processes that have been reported to take place at the early developmental stages and/or as an early response to the microgravity environment. They include alteration of cellular processes, changes in gene expression and epigenetic modifications, which should produce significant effects on the plant growth and development (Herranz and Medina 2014). The experimental evidence of the achievement of a full seed-to-seed life cycle in space means that plants should trigger immediate adaptive responses to overcome the stress conditions of spaceflight throughout the successive developmental stages (Zupanska et al., 2019). However, the physiological mechanisms by which plants overcome and counteract the adverse environmental factors of space, specifically gravity alteration and increased radiation are still unknown. The investigation on these adaptive processes and mechanisms is one of the most important and decisive challenges of space plant biology in the coming years. In particular, the research efforts focused on the effects of spaceflight on the plant genome will be considered under a further separate heading in this document. Additionally, how the microgravity condition changes gene and protein expression by using the novel repertoire of -omics methodologies will be also explained here.

An alternative approach in space plant research has consisted of the *in situ* direct production of vegetable crops in ISS, which may serve as fresh food to supplement the packaged diet of astronauts. This has been (and still it is) a specific objective of NASA with the Veggie (Vegetable Production System) and APH (Advanced Plant Habitat) facilities. For instance, to study the effects of space conditions, red romaine lettuce was successfully grown in three tests in the Veggie incubator with two different harvest methods, and yields were comparable to growth on Earth, as well as different physiological markers analyzed and the microbial communities associated to plants.

Selection of crops for stable agricultural systems can be carried out primarily by choosing species adapted to wide ranges of conditions in the Earth, assuming that they are more adaptable to new situations than the crops that cannot prosper in extreme environments of the Earth; and, second, with crops able to grow with minimum requirements of soil, nutrients and other inputs. Indeed, some crops are able to produce edible organs without soil and with limited availability of resources, such as lettuce (*Lactuca sativa L.*) or tomato (*Solanum lycopersicum L.*). In addition, this crop selection should ideally cover most of the Human nutritional needs.

The role of microbial communities for plant growth is also a factor to be investigated to enhance plant productivity and to solve some of the stresses of plant growth under space conditions.

The global challenge thus consists in releasing crop varieties adapted to the target conditions – ranging from outer space to human settlements on other planets – for a sustainable production of food.

Human food supply: animal protein

Some animal candidates for space protein source have already been discussed in the scientific literature, namely aquatic animals (fish species, snail, and amphipods) poultries, mammals and insects such as silkworms. However, none of them has met the necessary requirements to be used for this purpose.

Yellow mealworm (*Tenebrio molitor L.*) is a kind of coleopterous belonging to *Tenebrionidae* family. During its larval and pupal stages is rich in protein and it is easy to rear. In China it has become a popular dish for human consumption, whereas in Europe its main use has been limited to provide a protein source for animal feeding (Gasco et al., 2019, Motte et al., 2019).

Previous research has been already carried out concerning analysis of the amino acid composition of *Tenebrio molitor*. According to this, this insect contains all the essential amino acids needed for human nutrition and, in most cases, those contents are above the requirements proposed by FAO/WHO/UNU (FAO/WHO/UNU, 1985). It has been reported that the essential amino acid content of yellow mealworm is higher than that of pork, lamb and bean, and close to that of beef and fish (Li et al., 2013). However, some studies are still needed to characterize, beyond its amino acid composition, the identity and nature of yellow mealworm proteome. We need to go forward the simple compositional analysis by implementing high throughput Omic approaches allowing performing a detailed proteomic, lipidomic and metabolomic profiling of yellow mealworm.

Most common feeding sources in yellow mealworm farms are wheat bran together with various kinds of vegetables and fruits. In bioregenerative life support systems it is common to produce a certain amount of wheat bran and around 3 to 5 times the amount of rice and/or wheat straw. On the other hand, it is known that anaerobic fermentation contributes to increase the digestibility and protein content of straw. Consequently, production of fermented straw could become, together with other alternatives, an interesting feeding source for yellow mealworm in the space, contributing to the improvement of life cycle closure. Previous studies have shown that the growth rate of larval *T. molitor* is lower when plant wastes (fermented straw) was included into the feeding regime as compared to feeding with a conventional diet containing wheat bran and cabbage leaves. The explanation could be the low nitrogen content in the fermented straw (Li et al., 2013). This model would be improved considering the potential use that astronauts could give to excrements of yellow mealworm larvae as plant fertilizer.

Human food supply: fermentation processes

Certain microorganisms are currently applied in bioregenerative life-support system (BLSS) for in situ regeneration of resources to support long-term space explorations. An example of such BLSS is the Micro-Ecological Life Support System Alternative (MELiSSA) pilot plant located in Barcelona and developed by the MELiSSA consortium where bacteria are used for organic waste and water recycling: http://www.esa.int/Enabling_Support/Space_Engineering_Technology/ ELiSSA_life_support_project_an_innovation_network_in_support_to_space_exploration

In parallel, several research groups are studying the so-called "spaceflight syndrome" using model bacteria to understand bacterial physiology under spaceflight conditions (Morrison et al., 2019). The majority of these experiments have been carried out with either model bacteria or pathogens that represent a threat to human health. Nonetheless, due to the growing interest on the potential role of probiotic bacteria on astronaut's health, preliminary ground-based space simulation experiments have already started with some LAB. Therefore, one can anticipate that the body of knowledge will develop in the near future to provide further evidence for the intended use of these bacteria to sustain human life in the space.

3.3. Microorganisms in space exploration

Extremophilic microorganisms have developed complex molecular mechanisms to adapt to the conditions of extreme environments on our planet, such as high radiation doses, high and low temperatures, high salinity and toxic compounds such as perchlorate and heavy metals, among others. Molecular inventions developed by extremophilic microorganisms could be transferred to other microorganisms and plants to expand their capability to adapt to the particular extreme conditions of space during manned missions, to the surface of the Moon, or of other planets such as Mars.

For instance, microorganisms used to produce fermented foods and to recycle waste, and plants could be modified to resist higher doses of UV and ionizing radiation. In this way, their level of cellular stress and the frequency of mutations in their genomes would be reduced, optimizing their performance in spatial conditions. These organisms would be modified with genes involved in the radiation resistance of bacteria, archaea or algae naturally exposed to high doses of UV (e.g. Andean highlands) or ionizing radiation (e.g. uranium mines and nuclear power plants), such as genes related to DNA damage repair, production of pigments or in protection against oxidative damage.

Another limitation for the human colonization of other planets is the composition of the soil, which for example on Mars contain toxic compounds such as perchlorate and heavy metals could be harmful to plant growth. Therefore, food plants, microorganisms that promote plant growth or those used in soil bioremediation could be modified to resist and/or degrade toxic compounds. Bacteria and archaea resistant to perchlorate and others that also reduce it to chloride have been isolated from various extreme saline environments, such as Big Soda Lake (USA) and hypersaline soils. On the other hand, microorganisms (including unicellular algae) resistant to high concentrations of toxic metals and metalloids have been identified in acid mine drainage environments, such as in the Rio Tinto (Spain) (García-Moyano et al., 2008). Genes involved in resistance to perchlorate, or to toxic metals and metalloids from microorganisms of those environments could be used to modify other organisms that facilitate human exploration and eventual settlement on Mars.

The molecular mechanisms of resistance to some of the extreme conditions that can limit the maintenance of life outside our planet, such as radiation, perchlorate, toxic metals and metalloids, have not yet been well characterized in the microorganisms most resistant to these conditions. In addition, there is an important bias in the mechanisms that are known, since they have been studied mainly in microorganisms that can be cultured in laboratory conditions.

To better understand microorganisms and their capabilities it is necessary to complement culture-independent and -dependent techniques. Cultures allow to grow and evaluate metabolic processes whereas -omics (genomics, transcriptomics and proteomics) and molecular biology permit the analysis of the genomes and their regulatory mechanisms. The retrieval of genetic information, preferentially from extremophiles (showing highly stable biomolecules), to be expressed in microbial model systems is a procedure to achieve large-scale expression of selected genetic information (genes and their regulation), use of microorganisms or their enzymes as biocatalysts and achieve biotechnological processes for sustainable human settlements beyond our planet.

The use of microorganisms and their enzymes in biotechnological processes in space will require accurate and rapid monitoring systems which, at present, are barely available. These systems could include, for example, fluorescence lifetime correlated techniques which can provide quick, sensitive, *in situ* evaluation of the status of microbial and enzymatic processes. Ideally, the combination of biological, genetic and physiological, tools complemented with engineering design will aim to final applications for sustainable strategies for the settlement of humans in space.

3.4. Space –omics. Use of spaceflight generated biological datasets

In the next couple of decades spaceflight life-support systems will require to generate and exploit all the knowledge from space biology experiments performed in microgravity and cosmic radiation conditions from human bones to plant biology effects. The objective to promote more efficient and safe environments to support our settlements out of Earth will be realistic thanks to the scientific activities that began on board the ISS twenty years ago. Scientists who have had the opportunity to develop biological experiments in the space environment have collected a significant amount of biological samples and scientific results but the limitations on Space Research on experimental design made quite complicated to compare the results and reach similar conclusions. In addition, not all the samples have been processed and the results published conveniently, as stated at scientific meetings by our European colleagues. They have compiled more materials than they can analyse and complementary biological information for spaceflight experiments is not usually made available to the community. In the case of the genome scale -omics techniques, while results are usually deposited in the databases by publisher's request, data does not use to be processed following a single scientific criterion.

NASA has paved the way in answering such needs by implementing the GeneLab database (Ray et al. 2019). The GeneLab initiative was launched in 2014 and has positioned the American scientific community working with NASA at a clear advantage, as their work is more readily accessible and usable than the work from scientists who depend on the ESA for storing information. GeneLab has been prioritizing the deposition of data obtained in spaceflights. However, samples and data which are spaceflight-relevant are also collected, covering a full range of adverse experimental conditions, either in space experiments or by simulation in terrestrial analogues. To list a few examples: experiments with various levels of gravity, with high radiation, with extreme temperatures and suboptimal composition of the atmosphere and culture media (nutrients, pH, humidity...). GeneLab is considering extending the database to other types of data, such as datasets obtained from other astrobiology analogues with similar suboptimal environments, or from a more diverse range of ionizing radiation. Finally, GeneLab has launched in early 2018 the Analysis Working Groups (AWG), divided in four topics: Plants, Microbes, Animals-Humans, and Multi-Omics. AWGs are now comprised of ~120 scientists across four different countries (including CSIC researchers), and members meet monthly to: establish and adopt standards for sample processing and data analysis for space omics; to discover new biology from mining this large array of data; and to publish jointly in peer-reviewed journals.

There exists a need to promote a unified criterion for the incorporation of all scientific data obtained at the European level in a single database to homogenize all the ESA projects, including highly valuable samples that have been exposed to spaceflight environments and preserved without proper analyses.

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