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The biomedical challenge associated with the Artemis space program

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ABSTRACT

Human space flights fostered dreams and technological advances of humankind in the last centuries, since Jules Verne's visionary anticipations. However, exploration of the outer space challenges human adaptive response to a hostile environment in which cosmic radiation, microgravity, physical confinement, vacuum, and altered magnetic field, overall sum up to threaten human health. The Artemis program is organizing international efforts aiming to return to the Moon, and enabling a future Mars mission. A unified international human space flight policy should clarify the rationale, the acceptable risk, and the required countermeasures to reach the proposed objectives. To address such a challenge, proper medical and technical countermeasures should be developed for mitigating health hazard secondary to prolonged exposure to both radiation and weightlessness. Overall, development of artificial gravity devices, proper radiation shielding strategy, and the integrated network of biosensors that could provide a timely detection of health markers, constitutes indispensable requirements in supporting the next generation of human space flights. Thereby, to achieve such endeavors we need to bring in multidisciplinary skills and technologies in an integrated way. The Artemis program offers the opportunity to advance the basic knowledge and medical technologies in fulfilling such requirements, while adopting the prudent strategy of reducing to a minimum short- and long-term risk that space travelers shall face.

1. Introduction

New biomedical challenges are emerging as human spaceflight programs enter a new phase of space exploration. During the last 60 years, we gathered a remarkable body of information about living systems in Low Earth Orbit (LEO) [1], as reported by the NASA Human Research Program (HRP). The HRP has been conceptualized as a program of both basic and applied research, specifically focused to develop risk mitigation strategies in support of space exploration, as forecasted by the Vision for Space Exploration and the NASA Strategic Plan. However, much less knowledge is available for missions extended beyond LEO. Sadly, observations carried out on people exposed to microgravity for prolonged times (≥6 months) showed that previous hypotheses that systems would have adapted to the space environment proved to be largely incorrect [2]. Consequently, medical and psychological aspects become an issue of major importance when next missions - conceived for prolonged permanence either on the Moon (Artemis program) or during long-duration spaceflight (Mars) missions – are considered.

1.1. The Artemis program

In December 2017, President Donald Trump signed the Space Policy Directive 1, a document that marked a significant change in the US Space vision, establishing a joint public/private program for the human return to the Moon, followed by missions to Mars [3]. In 2019, that program was called Artemis, the sister of Apollo, the name under which the first lunar program started many years ago, calls for the NASA to lead a sustainable program of human space exploration across the solar system, "perhaps someday, to many worlds beyond" [4]. Workforces form different countries – namely US and European states [5] - are committed to reach those objectives by developing specific industrial infrastructures to support complex missions that would reinforce the Western leadership in space technologies [6].

The Artemis program includes a sequence of different steps - the Space Launch System (SLS) missions - currently scheduled from Artemis I through Artemis IV. According to Artemis I scheduled plan, the mission will place Orion spacecraft into a distant retrograde lunar orbit before returning it to Earth. The SLS will adopt the Interim Cryogenic Propulsion Stage (ICPS) second stage to send Orion to the Moon's South Pole.

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Orion will decelerate into a retrograde distant lunar orbit where it will stay for a week before coming back to Earth [7]. The SLS has unprecedented power capabilities: it is specifically aimed to ensure safe human travels and to support complex missions directed to the outer space.

Artemis II will be the first crewed flight, by including four astronauts that will perform several experiments in Earth orbit. The, Orion will perform a free-return trajectory around the moon, before returning to Earth. Artemis III – initially scheduled for 2025 and projected to use the SpaceX Starship – foresees to enable the crew in landing on the lunar surface. The mission aims to lay Human Landing System (HLS) in a near-rectilinear halo orbit (NRHO) of the moon. Once reached the HLS through the Orion spacecraft, the astronauts will transfer to the lunar surface. The astronauts are supposed to perform some extra-vehicular activities (EVAs) on the Moon before coming back to Orion on board of the HLS [8]. Finally, the Artemis IV is conceived as a crewed mission to the Lunar Gateway station in NRHO. A special role is expected to be played by the Gateway, a true "space station" positioned in lunar orbit, which will operate as multidisciplinary science lab, solar-powered communication hub, and as a location for future deep space missions [9]. Further assignments – from Artemis V through Artemis VIII– are going to be planned to land astronauts on the moon surface, with the objective of setting an outpost that would be instrumental in planning future space travels (Fig. 1).

The Gateway – fueled through solar electric propulsion - is a critical structure as it focuses on the minimum systems required to support a human lunar landing during the second phase. The Power and Propulsion Element (PPE) and the Habitation and Logistics Outpost (HALO) are the main components of the Gateway. The HALO is the pressurized living quarters where astronauts will work. HALO will serve as docking hub while providing for the overall control and recognition of the mission. Moreover, HALO will include the basic infrastructures and tools that would be instrumental in establishing a proper communication network, a centralized power hub, a center enabling surface excursions and, finally, in supporting a number of critical operational systems aboard Orion.

The Gateway will find its place tens of thousands of miles from the lunar surface, in a near-rectilinear 'halo' orbit, and it will serve as a transit station - a rendezvous point - for astronauts traveling aboard Orion prior to transit to reach the Moon surface. Moreover, the Gateway provides a command center and creates resilience and robustness in the lunar architecture. An outstanding feature is the proposed role of the Gateway for roundtrip voyages to and from the surface. Using the Gateway to facilitate astronauts landing on the Moon is an innovative strategy to test preliminary components, which could be incorporated into (reusable) lunar landers. The Gateway will enable access to the entire lunar surface: astronauts can be brought to the surface by first accessing a low-lunar orbit through a specific spacecraft recognized as the "transfer element". A different spacecraft (the "descent element") will bring crewmembers down to the Moon's surface. According to NASA's plans, the human presence on the Moon is scheduled by 2028.

Finally, it is worth mentioning that the strategic importance of Artemis will increase in the coming years, as the future of the International Space Station (ISS) was called into question by Russia's intention to abandon the project [10]. Nonetheless, the ISS still enable us in performing key experiments for assessing the influence of space-related hazards on living organisms, as well as testing critical technologies to allow prolonged human permanence in microgravity.

1.2. *Dissecting the problem: how to survive a hostile environment*

The space environment heavily challenges performance and health of space travelers, and we are just beginning to grasp the most relevant impact upon human physiology. Keeping crewmembers safe in space is an undisputable priority of the Artemis missions, and this goal will require an even more profound knowledge of those changes triggered by a still unexplored permanence in a different satellite, far from the Earth. More than just "quantitative" changes, it is presumable to expect new, unwarranted health modifications in these conditions. Undoubtedly, the

Launch date	Launch vehicle	Crew	Duration
2028 -2032 Proposed missions	SLS Block 1-2B Crew	TBA	30 – 180 days
Artemis V 2027	SLS Block 1B Crew	TBA	30 days
Artemis IV 2026	SLS Block 1B Crew	TBA	30 days
Artemis III 2025	SLS Block 1 Crew	TBA	30 days
Artemis II 2024	SLS Block 1 Crew	TBA	10 days
Artemis I November 2022	SLS Block 1 Crew	Uncrewed mission	25 days

List of Gateway logistics missions	
Launch date	Payload
2026 - 2028	Refuel for transfer and ascent module
2025 - 2027	Expendable lander ascent module – Reusable lander ascent module
2024	Expendable lander ascent module
November 2024	Power and Propulsion Element(PPE) - Habitation and Logistics Outpost (HALO)
-	

Fig. 1. List of Artemis missions. Missions in the program are aimed at exploration of the Moon, including crewed and robotic exploration of the lunar surface. Three flights of the Orion Multi-Purpose Crew Vehicle are currently planned for launch in the Artemis program in the early 2020s, beginning with Artemis I.

space station has offered priceless research opportunities to acquire several data. However, we are far from reaching the principal issues tied to the protracted human presence into a "hostile" environment, as such health threats represented by flying far from LEO.

We can argue that patients on Earth are people who live in a normal environment but can experience an "abnormal" physiology. On the contrary, humans flying in the space are individuals displaying "normal" physiology who live into an "abnormal" environment [11]. Space is perhaps the most hostile environment that humans have encountered: extreme variations in temperature, absence of atmosphere, microgravity, and cosmic radiation. All these factors represent redoubtable threats for preserving human life. The astronauts involved in long duration spaceflights must confront a set of dangerous and unforeseen issues, many of which require still unexplored solutions to allow a prolonged permanence far from our native planet [12]. In addition, exposure to space "stressors" can alter many physiological functions in the body in ways that could make it harder for crewmembers to perform critical mission tasks immediately after landing on a planetary surface, given that their adaptive response could be likely impaired.

Space exploration exposes astronauts to a number of different threats, including vacuum, lack of breathable atmosphere, temperature extremes, radiation, microgravity, just to mention a few. Moreover, Lunar exploration will involve a lot of extravehicular activity and thus changes in pressure, inhalation hazards (dust), and maintenance of equipment. The overall risks induced by such factors are still only partially understood and have been extensively reviewed [13]. However, herein we will focus on three main challenges, 1) changes in physical forces (modified gravity and electromagnetic fields), affecting every level of the organism (from molecules to the entire human body); 2) exposure to cosmic rays and radiation hazard; 3) psychosocial threats secondary to the physical isolation and the disruption of fundamental chrono rhythms (Fig. 2).

1.3. Health threats

1.3.1. Microgravity

Our life is tightly embedded into the gravitational field and even our neuro-physiological cognitive processes have evolved to cope with it. Conversely, adaptation to reduced gravity values promotes a rewiring of cognitive processing, involving the cortical and subcortical regions intertwined with the vestibular cortex, to "reshape" the internal model of gravity' [14]. Microgravity is defined as "the condition in which people or objects appear to be weightless" [15], or "the complete or near-complete absence of the sensation of weight [16].

For a while, microgravity – i.e. every condition in which the gravitational field is below the "normal" value of 1 g – has been considered "irrelevant" for biological processes, given that the very preliminary experiments performed on the seventies revealed no major changes induced by weightlessness [17]. However, such possibility becomes real when the development of space technologies has opened to humankind the opportunity to fly into space. Since then, how gravity either modifies human physiology or enact the emergence of true pathological conditions, became a relevant field of investigation to ensure sustainable human health in the course of human space missions. Namely, microgravity can influence a wide array of essential biological functions, by interacting at different levels of the organism structure [12,18,19], while dysregulation of the immune system can constitute a relevant threat for long duration spaceflights [20,21] (Fig. 3).

Furthermore, synergistic effects of microgravity in combination with other environmental hazards – including radiation confinement and

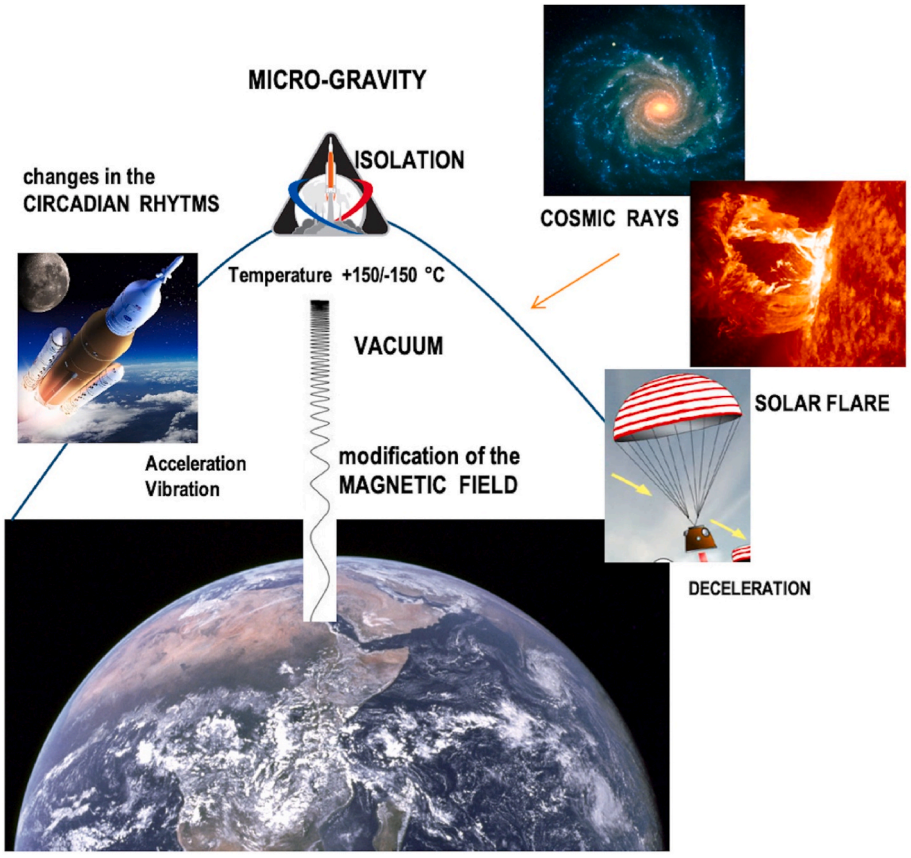

Fig. 2. The Space environment. Space is a hostile milieu, as some critical physical constraints tied to living processes are significantly modified. Those physical factors include the gravitational and magnetic field. Moreover, people flying in Space are exposed to extreme values of temperature, acceleration, and vibration, as well as to the deadly effects of GCR and solar flares.

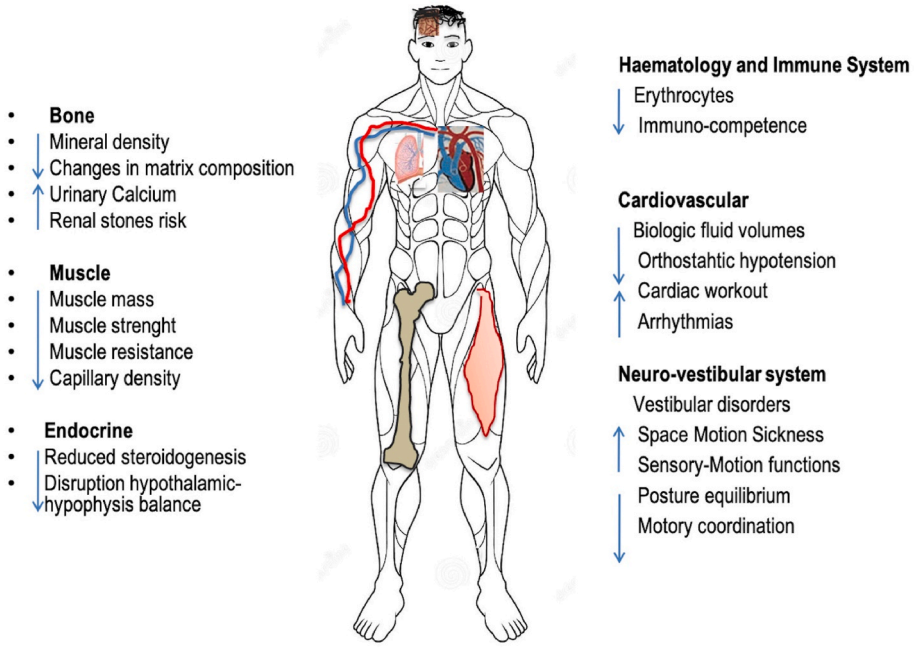

Fig. 3. Microgravity effects on Human Physiology. Microgravity can significantly influence several systems and organs. Specific risks have been identified regarding the malfunctioning of Bone, Muscle, Endocrine, Hematologic, Immune, cardiovascular and neuro-vestibular system.

disruption of circadian rhythms – can worsen medical problems and alter human performance in astronauts during long-duration missions [22]. Intriguingly, it has been observed that a common feature of very different cell types exposed to microgravity is the relevant increase of the oxidative stress associated with a slowdown of the proliferative rate. Noticeably, the use of antioxidants can efficiently inhibit the increase in reactive oxygen release. Overall, antioxidant-neutralizing drugs can preserve cell cytoskeletal architecture and restore cell proliferation rate and metabolism [23]. Special attention must be paid in investigating how weightlessness can impair bone and muscle metabolism, cardiac function, pressures of the human fluids, and cellular functions in almost all tissues.

1.3.2. Bone and muscle impairment

Medical data collected since the first human missions within [24], highlighted several adverse effects of weightlessness, including loss of bone density, decreased muscle strength and endurance, postural instability, redistribution of biological fluids and edema. Over time, these deconditioning effects can harm astronauts' capabilities and then increase the risk of accidental injury. In microgravity, astronauts put no weight on those muscles involved in assuming the erect position and, as a consequence, these muscles progressively weaken, with mass loss greater than 20% (in respect to pre-flight values) [25]. Generally, muscle mass is fully recovered after 1–2 months, once the astronauts return to Earth [26]. Moreover, astronauts may suffer a significant bone demineralization [27], developing osteoporosis-like features, as reported by case-studies and extensive bed-rest experimentations [28]. A significant loss of calcium from different regions of the skeleton and legs has been documented, and the increased calcium mobilization from bone repository may raise the risk of renal stone formation on long-duration missions [29,30]. Osteoporosis and bone remodeling are therefore a major concern, since impairment of bone architecture is likely to be progressive, and, once exceeding a threshold, may facilitate the occurrence of spontaneous fractures [31].

[32]. Undoubtedly, studies on microgravity-related osteoporosis have provided priceless insights into bone physiology, and we are progressing in the development of some useful countermeasures. Working out regular exercise schedules in space, despite some preliminary disappointing results [33], showed that improving nutrition and resistance exercise during spaceflight can attenuate the expected bone mineral density (BMD) deficits previously observed after prolonged missions [34]. Namely, the newer advanced resistance exercise device (ARED), associated with bisphosphonate consumption, provided significant attenuation of bone loss compared with the older device although post-flight decreases in the femur neck and hip remained [35]. Definitely, although ISS crew can for now be considered sufficiently protected with a combination of diet/exercise/drugs, we still need new strategies for exploration-class missions outside LEO.

1.3.3. Cardiovascular system

The Cardiovascular System (CV) plays a critical function in modulating blood and fluids distribution according to different metabolic and environmental demands. In doing so, CV must principally accommodate with the gravity induced effects on fluid partitioning. Due to the gravitational vector, blood pressure is differently partitioned in the body according to a top-down gradient, i.e. with higher arterial pressure in the feet (200 mmHg) and lower pressure at the central nervous system (65–75 mmHg). In microgravity, this pressure gradient is no longer established due to acceleration, which reverses the gradient between gravitation and acceleration.

Redistribution of blood stimulates adaptive responses involving a number of systems, including the endocrine and the autonomous nervous systems [36]. Within few minutes after weightlessness exposure the increase in fluid in the upper body regions causes the "space motion sickness", a syndrome characterized by headache, vomiting and head edema (the so called "moon-face"), which usually resolves within few days. Fluid redistribution may not be the sole cause of the space motion sickness, given that sensory imbalance could also play a role. Indeed, muffled otolith signals upon entry into microgravity cause a "conflict" between real and anticipated hints from sense organs [37]. Redistribution of body fluids induces engulfment of the vascular tree in the superior regions, triggering mixed response from those mechanoreceptors that in turn activates a number of autonomic reflexes. Within a few hours, a compensatory vasodilatation occurs in the viscera, and concomitant increased urinary flux. In the same time, heart rates values decrease while arterial pressure increases [38], albeit other reports were

unable to confirm such findings [39]. Noticeably, physical exercise on board of the ISS can counteract arterial pressure changes [40]. However, evidence from the hindlimb-unloaded rat model showed that vascular smooth muscle cells could suffer from weightlessness and become atrophic as happen in skeletal muscle, with resulting hypotension [41]. Relevant differences have been observed in specific body districts: the lumen increases in the carotid and the basilar arteries, while was reduced in the femoral and anterior tibial arteries [42]. Overall, both vasoconstriction and vasodilatation responses to microgravity should be taken into consideration when evaluating spaceflight modifications on CV. Moreover, microgravity can influence CV also through modulation of a number of molecular factors that can influence CV performance, like nitric oxide (NO), which is significantly increased in several tissues [43]. In addition, it has been shown that mice have a decreased adrenergic response with a consequent impaired vasoconstrictive response [44]. Collectively, these findings suggest that the vasoconstrictive response altogether with the autonomic adaptive capability is impaired by microgravity and this may help explaining the deregulation of arterial pressure control that can cause orthostatic hypotension upon return of astronauts on Earth.

These effects may have also a significant impact on heart functions. Indeed, arrhythmias and abnormalities of the electrocardiogram - namely, prolongation of the QT interval – [45] have been frequently reported during spaceflights [46]. Cardiac arrhythmias increase the risk for myocardial infarction and this kind of risk cannot be underestimated [47]. Undoubtedly, microgravity exerts a dramatic stress upon the heart, which is constrained to adapt its functional capabilities to accommodate with the fluid imbalance experienced in weightlessness. We know less well what is going on at the cellular level. As for other types of cells, cardiomyocytes respond to microgravity by showing a wide array of appreciable changes, involving gene expression [48], ribosomes, mitochondria, and the endoplasmic reticulum, thus resulting in increased protein degradation, reduced turnover and, finally, atrophy [49].

1.3.4. Cells and molecular changes

An impressive number of alterations affecting cellular morphology and biology has been duly documented, involving essentially gene expression, mitochondrial function, cytoskeleton structure, epigenetic changes, and oxidative stress [50–52]. The search for “specific” molecular targets of microgravity turned out to be a futile exercise. Noticeably, human cell types cultured in microgravity undergo bewildering modifications in their morphology that lead to the appearance of two different phenotypes, denoted ‘adherent’ and ‘floating cell clumps’, simultaneously present in the same culture [53]. Noteworthy, this is a reversible process, which outlines how wide is the adaptive capability of living cells. Indeed, if a ‘clumps-organoid’ population is seeded again into 1 g, cells quickly recover the native phenotype. Conversely, if the same culture is replaced again in microgravity, the two above-mentioned phenotypes resurface once more. Similar results are obtained when the experiment is replicated starting with cells displaying an adherent phenotype. According to the self-organized theory (SOC) [54], the two morphological states behave as ‘sub-attractors’ of the shared cell-kind attractor in the multidimensional gene-expression space. The two sub-attractors can support the two observed phenotypes besides they are likely ruled by different gene-regulatory networks. Indeed, it has been observed that the gene-expression change occurs in a coordinate manner across the genome, showing only minor variations, while preserving the overall coherence, as defined by high autocorrelation values among genes along the different sub-state the system is traveling. Overall, these findings suggest that living into a modified gravitational field can have very relevant effects on very basic processes that drive both organism development and tissue repair. Acknowledging such a challenge constitutes a mandatory objective for understanding how life can reproduce and maintain its homeostatic equilibrium in space. Moreover, these results have fostered the development of specific technological devices for more sophisticate 3D-cultures, including the development of organoid techniques [55]. Noticeably, studies performed in microgravity conditions can serve as a novel paradigm for innovation, highlighting how architecture and physical interactions can efficiently shape the behavior of living structures.

1.3.5. Radiation hazard

Exposure to ionizing radiation is a major health risk to which space traveler are exposed. Any astronaut, while away from the protection provided by both the Earth atmosphere and the magnetic field that both shield our planet, are potentially subject to tissue and cell damage induced by Galactic Cosmic Rays (GCR), ionizing radiation and transient radiation from solar particle events (solar flares and coronal mass ejections) [56]. Astronauts in outer space are exposed to two forms of radiation: the first one is due to a chronic low-dose exposure to GCR, including electrons and positrons (2%), protons (85%), helium nuclei (12%), and heavier ions referred to as high-energy and high-charge particles (HZE; 1%) [57]. GCRs are highly energetic and penetrating particles that the spacecraft shield can hardly stop. The biologic impact of such charged particles cannot be overlooked, and secondary ionization processes that come from the primary particle track further amplify it. It has been calculated that during a prolonged spaceflight – like that planned to reach Mars – the total exposure would account for a total dose of about 466 mSv [58]. Based on the conventional risk assessment approach, the exposure limit for the astronauts’ career is of the order of 1 Sv for a one-way Mars mission, while the total exposure during a Mars expedition would account for ~662 mSv, regardless an additional variable contribution of SEP. In these conditions, the astronaut’s risk to develop cancer becomes unacceptable, according to the available scenarios (no more than 3% probability of cancer fatality estimated at the 95% confidence level) [59]. An additional risk comes from unexpected solar flares [60]. Furthermore, given that astronauts are generally exposed to only low dose of radiation when traveling LEO, specific studies should be undertaken to evaluate health effects caused by chronic and low-dose-rate exposure [61]. Principally, radiation hazard exerts the following, deadly consequences 1) carcinogenesis; 2) central nervous system damage; 3) tissue degeneration; and 4) acute/chronic radiation disease [62]. Despite the great achievements attained after 50 years of intense research, assessment of radiation risk and damage both remain an intricate subject, mostly because effects of radiation exposure depend on the complicated features of the general dynamics of the spacecraft [63], while the space radiation environment display stochastic features [64]. Radiation-related biological effects in space can enact well-known processes (protein and DNA damage, reactive species release) as well as other, less-understood mechanisms [65]. Definitely, space radiations produce biological damage that displays significant differences with that observed during experiments performed on Earth. This characteristic will lead to uncertainties in forecasting human risk, thus preventing an accurate assessment of the real effectiveness of possible countermeasures [66]. However, NASA’s Space Radiation Laboratory (NSRL) at Brookhaven National Lab acquired several years ago the capability of providing beam exposures using multiple ion species, better simulating GCR exposures [67]. Those studies have been conducted on animals, providing useful, new data [68,69].

Ground-based accelerators typically generate radiation of a fixed nature and energy, whereas cosmic rays display extensive energy spectrum and heterogeneous composition. Additionally, cosmic rays and microgravity can induce synergistic effects that cannot be simulated by our current technological tools. Therefore, it is imperative to perform a comprehensive research program to determine the biological risk arising from different types and energies of HZE particles and high-energy protons [70]. Spacecraft shields currently in use have adequately protected astronauts aboard the ISS, as well as crewmembers that in the past have visited the lunar surface. However, for longer flights conventional shields cannot limit radiation exposure below a threshold level without making space vehicles too heavy. Performing effective shielding is a

hard task in space, given that both the very high energy of GCR and the severe mass constraints of protective structures represent a serious hindrance in providing effective shielding. Massive shields are indeed impractical on spacecraft, although compact ‘‘storm shelters’’ are designed to protect from SEP events [71]. Alternative strategies focus on selecting mission periods with lower risk of SEP events, drug treatment and dietary supplements to mitigate radiation damage and enhance cell repair [72]. However, recently NASA has developed the "GCR Simulator" to generate a spectrum of ion beams that approximates the primary and secondary GCR fields experienced at human organ locations within a deep-space vehicle [73]. In this facility, the majority of the dose is delivered from protons (approximately 65%–75%), and helium ions (approximately 10%–20%) with heavier ions ($Z \geq 3$) contributing the residue. Noteworthy, to more closely simulate the rates found in space, sequential field exposures can be divided into daily fractions over 2–6 weeks, with individual beam fractions as low as 0.1–0.2 mGy. Preliminary reports suggest that the GCR simulator can provide sound results, enabling in addressing key issues for protection of humans during space travel [74].

Nonetheless, besides the advances carried out in the last decade, further efforts are needed in developing an effective shielding strategy for long duration missions [75,76]. To gain insight about the medical impact of extended human space missions, we are forced to extrapolate data provided by experiments performed onboard of the ISS or from short-duration flight missions, although none of the latter can fully reproduce the characteristics of a real flight when traveling in LEO. A recent study suggests a 600 mSv career effective dose limit based on a median estimate to reach 3% cancer fatality. However, these estimates do not consider the additive uncertainties of heavy ion radiobiology, and risks of cancer, as well as cognitive detriments and circulatory diseases. As a result, these “recommendations could have negative impacts on crew health and safety, and violate the three principles of radiation protection (to prevent clinically significant deterministic effects, limit stochastic effects, and practice ALARA), which would be a giant leap backwards for radiation protection” [77].

Furthermore, research accuracy in studying the potentially lethal consequences of radiation is severely hampered by the lack of reliable in vitro/in vivo models, the limited opportunity in obtaining reproducible results due to the impossibility to repeat the same experiment, not to mention the occurrence of unexpected, confounding factors. Overall, there is an urgent need to improve the understanding of the space radiation hazard, and develop appropriate countermeasures for prolonged permanence in the outer space. Noticeably, some attractive new solutions – based on a solenoid shaped, active magnetic shield design - have been proposed to reduce the radiation exposure on long duration, deep space missions. However, these attempts – albeit promising - pose significant technological challenges [78]. Thereby, we must recognize current limits to overcome them by specific, research programs [79].

Assessing the risk and evaluating possible countermeasures. The Apollo-era limits in properly assessing the health impact of spaceflights have surfaced only recently. More rigorous techniques of quantitative risk assessment (developed in response to the preliminary analytical procedures of the Apollo program), showed in hindsight that this program was “safe enough” in allowing people to fly in outer space. Calculations indicated crew survival chances higher than 98%, while mission success rates approximated 75% range for the first missions [80]. However, when adopting a similar approach in estimating risks of the Mars astronaut mission profiles, we obtain a worrisome scenario, as crew survival can hardly reach 50%. Furthermore, while early estimates of the cancer mortality risk after exposure to space cosmic rays were calculated from 400% to 1500%, accurate assessments indicate a four-fold increase [81]. In addition, space flight can expose astronauts to other significant health risks. From 1981 through 1998, 1777 single medical events occurred in the outer space: heart rhythm disturbances, anemia, kidney stone, space motion sickness and many others, including 141 events due to injury and 18 fatalities, during the Soyuz 1, Soyuz 11, and Challenger missions [82,83], it is surprising that such medical problems are only marginally addressed by future mission plans, which generally focus mostly upon technical and socio-economic implications of space explorations [84].

Undoubtedly, assessing how the risk increases with increased distance from Earth and mission duration is critical to mission success. LEO operations have evident advantages when compared to missions beyond LEO, given that space effects on living beings become more pronounced for longer exposure to outer space environment while increasing distance from Earth will pose technical hurdles that make even more complicated the management of medical emergencies. In detail: a) medical support is critically dependent on the possibility to provide telemedicine-based interventions in a timely fashion [85]. In absence of such a possibility, the crew should be trained to perform diagnostic and first aid measures in an autonomous manner. b) Medical assistance is critically tied to a re-supply chain of consumable resources - like drugs and food – that cannot easily be available according to our current model of space exploration [86], namely because drugs can deteriorate under the pressure of both radiation exposure and microgravity [87]. c) transfer to Earth of severely ill/injured crewmembers is achievable in an acceptable timeframe when astronauts are working in LEO. However, during longer missions – as such those foregone by the Artemis program - the evacuation of wounded astronauts will require unbearable times and a specific set of medical capabilities is hence needed to perform some kind of preliminary medical interventions to “stabilize” the patient. This simple conclusion implies we have to reframe a very different paradigm in respect to the current one – the Integrated Medical Model (IMM) Montecarlo simulation approach [88] - in order to design a suitable Crew Health and Performance (CHP) System that could afford such an issue [89]. Until now, only few estimates of medical risks for exploration spaceflight are available. The Design Reference Missions (DRMs) used by NASA to provide the basic guidelines to calculate the probabilistic risk assessment (PRA) has been developed for assessing risk during LEO operations and thus has important limitations when applied to missions beyond LEO. However, this PRA-based model - capable of integrating forecasts for extended missions - can still provide value in estimating a reasonable order of magnitude and bounding for risk. A recent study, which uses an integrated IMM model, shows that as the mission duration and distance from Earth increases, the overall risk grows it becomes uncertain, and cannot be grasped by current models [90]. Specifically, the simulation shows that both over-prediction and under-prediction likelihood can impair the overall estimate. Nonetheless, that model shows that as mission duration increases, missions beyond LEO will carry a level of medical risk that is equal to or greater than that experienced by astronauts for a prolonged period of time onboard of the ISS. Yet, differences between two scenarios – equipped with unlimited and limited “medical capabilities” respectively – are clearly recognizable and demonstrate that a proper implementation of medical resources can significantly dampen health risks, then increasing the likelihood of mission’s success. Overall, this kind of approaches highlights how critical is the prioritization of medical needs in the context of next coming missions [91,92]. A recent survey conducted by NASA [93] to identify medical research priorities, uses an evidence-based risk approach for ascertaining the likelihood of distinct risks, tailored for a well-designed mission. A specific risk rating – established to forecast the potential impact either on crew health performance or on long-term health outcomes – has been shown to be strictly related to the available level of medical support that can be delivered during the specific mission.

1.4. NASA and ESA programs

The human spaceflight program proposed by several US National Academy committees and further included in the NASA Strategic Plan, posits to address the most relevant issues and challenges linked to the realization of the Artemis project. That program aims at developing a

comprehensive understanding of specific threats and medical needs in order to establish those countermeasures that are required for ensuring the safest conditions for a long stay into an outer space environment [94]. Since human last set foot on the Moon – more than 40 years ago - human space exploration has been restricted to LEO. The longest lunar mission was carried out by Apollo 17, when three astronauts spent 12 days traveling to and from the moon. While in LEO the medical risk is well characterized and can be confidently managed, the risk for extended missions far from LEO – as such required to reach the Moon and eventually Mars – would likely increases exponentially. Previous projects – as the Mars One (MARSONE) – have indeed underestimated both biological and technological issues [95].

ESA began to develop a comprehensive program aimed at finding some countermeasures, namely against microgravity, by assessing the usefulness of artificial gravity tools based on a short-arm centrifuge. Nevertheless, an integrated roadmap for investigating those issues that are critical in establishing a convincing program of medical interventions is still lacking. In 2012, the European Union (EU) funded the project THESEUS (Towards Human Exploration of Space: A European Perspective). That program – in synergy with ESA - aims at developing a comprehensive life sciences research project to roadmap support for human space exploration. The success of such initiative relies on a well-coordinated series of intertwined programs, involving in a coordinated common work national agencies, stakeholders and universities. The specific focus is on establishing a human base on the moon and eventually laying the basis for future Mars exploration [96]. Within THESEUS, we can recognize three main objectives: (1) identify disciplinary research priorities, (2) putting emphasis on those fields that may potentially evolve to support technological transfer for Earth's benefit; and (3) build a European network to support the program. The principal, recognized fields include investigations on Systems Physiology, Human-Machine Systems interactions, Radiation, Health Care and Habitat Management [97]. Herein we will shortly describe a number of unavoidable tasks to empower the Artemis mission with those medical tools that could assure efficient health management.

1.5. Countermeasures and Research priority

1.5.1. What is microgravity and how it affects living organisms

A fundamental thrust of biomedical research should aim to explore the extent and nature of the effects of prolonged stay in weightlessness, to develop effective medical solutions, and to improve post-flight readaptation into a normal gravity field. To do that we have to reconsider what microgravity really is and how it interacts with fundamental living processes. In a thermodynamically open system, gravity acts as a constraint [98] - rather than a force – and a constraint implies some limits on independent behaviors. Consequently, a constraint on a dynamical process provides a reduction of its degrees of freedom defined by the physical conditions in whom biological/physical reactions occur. Degrees of freedom in a system are those provided by the dynamics of its internal variables, minus those that are "nullified" by constraints. Indeed, constraints usually act by restraining the system parameter ranges (i.e., limiting the range of quantitative forms its dynamics can take) albeit they can also have an "enabling" effect by allowing the system in accessing previously "unexplored" attractors [99]. Unfortunately, in biology constraints are usually treated as 'invariant' constants, like external factors in respect to the dynamics under study. This unfortunate situation is a consequence of the Lagrangian/Hamiltonian formalism adopted in modelling dynamics, which do not take into consideration constraints within equations based on a reversible form [100]. Yet, constraints, including those emerging from a gravitational field, actually reshape the Waddington's landscape (a schematic metaphor for the development of multicellular organisms) by changing the dynamical bifurcation tree – and thus can interfere with basic processes like differentiation, proliferation and tissue development [101]. Conclusively, changes in gravitational constraints may significantly modify the non-equilibrium thermodynamics of an open system in an unpredictable manner. In this condition no single organ, tissue or cell will be spared: microgravity would indeed influence the overall system. To cope with this complexity, we require reframing our approach, by including new theoretical assumptions by considering how multiple organ systems adapt to microgravity [102]. Namely, studies performed in Ground-Based Facilities (Random positioning Machine, 3D-Clinostats, and Rotating Wall Vessel) should shift from 2D-cultures to 3D-cultures of cells, which include both cells and their microenvironment (fibroblasts and extra-cellular matrix), to get a closest approximation of what really happen in vivo [103]. The use of multicellular spheroids and organoids is gaining momentum; yet, these models are still underestimated [104]. Indeed, associating 3D cultures and microfluidic technology is a strategic goal to develop a new generation of organ-on-chip. Combining these two techniques would enable us to design new platforms for combining both microbial and human cells into complex models, and thus more directly translating research to human applications [105]. New micro-physiological systems – identified by the National Institute of Health (NIH) Tissue Chips in Space initiative - belong to those new technological resources [106,107]. Another promising avenue is the development of organ-on-chip models. The use of these tools would provide new platforms for the construction of advanced models that can better mimic real processes and more directly translate research to human applications [108].

This physiological approach is mandatory in Space biomedicine studies to design affordable and beneficial countermeasures. Furthermore, the Artemis program would offer new opportunities to understand the true impact of different gravity conditions on human functions by allowing investigations to be performed on the lunar surface. It is well recognized that gravity acceleration on board of the ISS account of about 89% of that on the Earth, despite astronauts experiencing "weightlessness" because of the orbital motion of the space vehicle, i.e., a free-fall condition due to the centrifugal force exerted by the motion of the space vessel. Thereby, the effects of prolonged exposure to true Moon (0.16 g) or Mars (0.38 g) microgravity are unknown. Artemis missions will show us more accurately what happens in true long-term microgravity exposure versus the free-fall induced microgravity of the ISS environment [109]. Probably this opportunity will enable us to design proper, integrated countermeasures to prevent detrimental effects of weightlessness.

1.6. Artificial gravity

Technological devices are pivotal for counteracting the most relevant threats to which humans are exposed in microgravity [110]. Physical exercise provides appreciable effectiveness in limiting the most critical impairment of the musculoskeletal system. However, those measures proved to be of limited value in counteracting the full range of cognitive and sensory-motor changes occurring during space flight. Artificial gravity is essentially constituted by the "reconstruction" of a simulated gravitational field on a spacecraft, obtained by the linear acceleration or steady rotation involving some sections (or possibly the spacecraft in its entirety) of the vehicle [111,112].

The rationale behind the use of centrifugation posits that – due to the equivalence principle between gravitational and inertial mass - the gravity vector can be measured during rotation around an eccentric axis. This centrifugal force is an inertial force, which is function of the square of angular velocity (ω) and the radius (r) of rotation. The centrifugal force (Fc) experienced in such situation can be described as follows:

$$Fc = -mac, \text{with } ac = \omega^2 r$$

on an object of mass m at the distance r from the origin of a frame of reference rotating with angular velocity ω.

An astronaut at the edge of a habitat rotating at 4 revolutions per minute (rpm) about an axis located at 56 m would experience an

apparent gravity force as that perceived on Earth. Given that centrifugal force depends on rotation rate and radius, by either increasing or decreasing, the radius or the rate of rotation can change the value of the apparent gravity force. Yet, the radius of the structure will proportionally increase the cost and the complexity of the spacecraft, whereas, as the rotation rate increase, their impact on physiological and psychological responses will consequently increase too. Currently, it is widely recognized that artificial gravity could mitigate the effects of weightlessness in humans, given that short-duration sessions of artificial gravity have been demonstrated to be effective in mitigating a number of physiological responses, including orthostatic intolerance, neurovestibular as well as endocrine dysfunctions [113,114].

Under application of artificial gravity, muscle, bone, cardiovascular and vestibular apparatus are all stimulated as happen on the Earth [115]. However, the physiological response of other systems – including the respiratory, the endocrine, the vestibular system just to mention a few – to continuous/intermittent exposure to anything else other than Earth gravity and weightlessness is still unknown. We must plan detailed research to identify the minimum tier – from 0.16 to 0.38 G, accounting for the Moon and Mars gravity values, respectively – as well as the time intervals of artificial gravity exposure required to enact an effective physiological response. Furthermore, human adaptation as a function of rotation rate, gravity gradient, and Coriolis and cross-coupled accelerations should be ascertained. A preliminary, hypothetical solution would be a device that could provide a constant 1 G acceleration, given that even low G values can have relevant effects on human physiology. Besides being very preliminary, some studies carried out either in a full 14- day head-down tilt bed rest or during 14-day spaceflight demonstrated that cardiovascular deconditioning cannot be prevented by gravity values approaching those related to the lunar mass [116]. Nonetheless, those gravitational levels – as well as their duration and frequency - that can efficiently mitigate the deconditioning of physiological systems should be studied to provide “treatment” protocols to minimize weightlessness-related effects, at least. While some partial gravity simulators are already in use to simulate the effects of weightlessness on living systems, we are far from reaching an acceptable device that could “reproduce” an artificial gravity environment on board either of the spacecraft or on the lunar surface. Namely, we should investigate if artificial devices and protocols established by ground-based studies can be effective and safe in microgravity. A thorough validation in space is mandatory. Currently, small centrifuges that are available on the ISS for studying artificial gravity on biological processes include the ESA’s European Modular Cultivation System (EMCS), Kubik, Biolab, and JAXA’s Mouse Habitat Unit. EMCS focuses on experiments on plants exposed to fractional gravity levels (from 0.1 to 0.3 G) [117]. Kubik is an incubator for small living objects that can be exposed to increased gravity levels (from 0.2 to 2 G), while Biolab is a small centrifuge for a wide range of biological samples (including microorganisms, cells and tissues) that can generate artificial gravity levels from 0.01 to 2 G [118]. The Engineering Division at NASA Johnson Space Center has developed a 5-m radius centrifuge for human experimentation within the Deep Space Habitat (DSH), a device conceived for working into the Space Launch System [119]. Once that DSH model has been vindicated on ground, it should be studied in space.

Artificial gravity systems cannot suffice and need to be implemented with physical exercise protocols and specific drugs/food recommendations. A promising approach is represented by associating exercise with artificial gravity within a centrifuge. According to this model, astronauts need to be fixed to a spot by wearing a yoke connected to a treadmill intended for performing a number of physical exercises. Various tools have been proposed to date, including the “Twin Bike’’ [120], the “Space Cycle’’ [121], and the human-powered centrifuge developed by NASA [122]. The basic assumption on which such models rely is that physical exercise would efficiently counteract microgravity related effects. However, conclusive results are still lacking, and more investigations are needed to ascertain the usefulness of such devices. Namely, few studies have been performed to take into consideration how the Coriolis force, the inertial force acting on objects in motion within a frame of reference rotating with respect to an inertial frame. Coriolis acceleration (Ca) and Coriolis force (Cf) are usually expressed by $Ca = 2(v \times \omega)$ and $Cf = -2\ m\ (v \times \omega)$, respectively, where v is the linear velocity of a moving object in m/s, ω is the angular velocity of a rotating system in rad/s, and m is the mass of an object in kg. Humans are well equipped with sensitive multi-axial acceleration sensors (semicircular canals and otoliths), suited to sense motions and fluctuations in a 1-G field. In a rotating structure, the movement of the head changes the orientation of these sensors according to the rotation vector. These are the so-called cross-coupled responses to angular motions in two planes, which can induce an unwanted signal related to the whole-body rotation. Cross-coupled Coriolis responses are disturbing to humans and can enact a wide range of troublesome symptoms, including neurovestibular instability, vertigo, nausea, emesis, and disorientation [123]. The Coriolis force is proportional to the linear velocity of the imparted motion, the mass of the moving object, and the rotation rate of the rotating environment. Noticeably, the magnitude of the Coriolis force does not depend on the radius of the rotating environment. However, once a specific g-level objective has been defined, the radius will impact on the Coriolis force. In these conditions, human movements will likely be further limited. Therefore, the adaptive performance of crewmembers in artificial gravity deserves to be reconsidered.

The gravitational cues and visual references play a role in supporting the neurological and neurovestibular systems. Absence of gravity dramatically influences static equilibrium and impact on the ability in performing those tasks that require motor function. As a result, 50–80% of astronauts experience impaired balance, altered locomotion, eye–head–hand coordination, and/or motion sickness during the first days of permanence in weightlessness [124]. Gravity changes disrupt the sensory input received from the vestibular system. This imbalance will generate a persistent conflict between expected and actual vestibular signals, especially when the organism is challenged by active motion [125]. Moreover, experiments carried out on the ISS have shown that microgravity causes structural and functional changes during the vestibular processing, namely by affecting hair and Purkinje cells of the cerebellum [126]. However, several ground-based as well as space flights experiments have unveiled the plasticity of both vestibular and neural networks in activating adaptive mechanisms [127]. It is likely that human brain uses two key strategies in facing gravity modifications: (1) by updating of a cerebellum-based internal model of the gravitational field; and (2) by re-processing of different kind of information to modulate the vestibular response to a very different milieu [128]. Overall, these findings support the hypothesis that active training can effectively promote a true adaptation to microgravity. Accordingly, astronauts should undergo special training to learn how to cope with this modified environmental context (i.e., limited movement in high angular rate short arm centrifuges), favoring a “rewiring” of the internal representation of gravity. Indeed, reframing the inner gravity model involves a re-processing of visual and non-visual signals. Moreover, it might reshape already existing connections between visual areas committed in the spatial-temporal analysis of visual stimuli and those operating in temporo-parietal-insular regions [129]. This means that – in some way – “guided” imagery and visual processing may facilitate adaptive behavior to different gravitational fields [130].

1.7. Sensors and telemedicine for advancing medical management

1.7.1. Telemedicine

Telemedicine is a key component of medical care for spaceflight missions and a significant expertise has been planned – since the establishment of the ISS - in providing medical support to crews staying in outer space [131]. The basic architecture of a telemedicine device includes tools for data acquisition, data processing and storage, as well as an organized network to support diagnostic procedures and the cross

talk between crew and medical staff. Monitoring of humans in space started with the first human flight, i.e. that performed by Yuri Gagarin in 1961. Basic health parameters of the Russian cosmonaut - heart rate, ECG, ventilatory frequency, electro-oculogram, galvanic skin response and thermography – were duly recorded [132]. Vital data in the early Russian and US space programs were typically transferred by one-way telemetry downlink, whereas today we rely upon a set of distinct modalities: real-time, store-and-forward, and just-in-time interactions. Real-time (synchronous) telemedicine does not have appreciable latency, whereas store-and-forward telemedicine allows collecting information that are supposed to be transferred in a second step. The "just-in-time" is a third modality - uniquely related to space medicine - and it means just what is said: data are received "timely" to permit patient's management. This is a quite arbitrary definition, as it is quite unclear what should be considered ""just in time", and if this time interval would be enough to plan an effective medical response.

However, the Artemis program requires space medicine to fulfill much wider-ranging objectives, and the project likely should address potentially severe conditions. Effective medical support through remote control requires satisfying three different aims: 1) establishing a proper telecommunication architecture, able to transmit high-quality images, with improved capability in signal processing and transduction; 2) enhancing the capability to harness critical clinical data; 3) widening the array of useful instrumental and biochemical parameters that can be recorded mostly through portable devices (ultrasonography) and chemical sensors.

Faithful transfer of information through the telemedicine network depends on a number of factors. Bandwidth, i.e., the data-carrying capacity of a communications system, is the most influential factor. For instance, increased potency and band capacity will be required to transmit medical images. A second factor is the distance the data must travel: temporal delays can grow to the extent that they preclude effective real-time interactions. Moreover, long-duration missions necessitate advancements in telemedicine-based technologies, given that during missions far from LEO, health monitoring shall no longer be real-time, as it will be subject to a significant time delay.

Astronauts, doctors, and personnel involved in spaceflight missions, should be trained to acquire the needed capabilities to cope with medical emergencies. A number of "guided medical procedures" can be performed according to previously planned "scenarios", as long as internet connectivity is available and could support imaging technology, even if crewmembers lack specific medical expertise. However, the effectiveness of telemedicine highly relies on two main requisites 1) the stability/fidelity of the connection, 2) the availability to operate in real time. This can be a serious problem, as Moon, and especially Mars missions would entail a delay of minutes (40 min when Mars is considered), which would render remote guidance impossible [133]. Furthermore, given time and distance constraints on medical care delivery required for Artemis and Mars programs, scientists are deeply committed to reshaping the basic requirements for ensuring a program of diagnostic assistance by means of reliable computer-assisted tools. This will involve the development of compact medical devices, an integrated informatics capability, and advanced artificial intelligence-based systems. Namely, deep learning in medical image analysis should further be improved to extract as much information as possible [134].

1.7.2. Biomedical sensors

To support the telemedicine program, development of a network of integrated sensors for monitoring a wide range of physiological functions represents an unavoidable, strategic issue.

Traditionally, heart rate and respiratory frequencies have been for a while the only medical parameters monitored during spaceflight. Advanced spacesuit designs incorporate self-contained life-support systems, and a number of other modular components [135]. Earlier attempts to monitor crew status have included direct measurements during Apollo missions to study heart rate, oxygen consumption and energy expenditure [136]. The EVA Physiology, Systems and Performance (EPSP) Project developed by NASA, has been conceived to recruit more data to monitor health performances. An integrated biomedical sensor system that can measure a number of biomarkers, providing data in real-time, has been already proposed [137]. However, the development of future diagnostic systems could be fulfilled only by achieving substantial progress in miniaturization, signal amplification, and data processing through innovative bioinformatic strategies. Since 2001, NASA and the National Cancer Institute signed a Memorandum of Understanding (MOU) to promote and support biomedical and technology research aimed at developing diagnostic sensors [138]. This endeavor provided interesting results by coupling different techniques that allow organizing newest sensors in multidimensional networks. We should implement the miniaturization process to make feasible the realization of sensory devices that could mimic those animal senses which have been demonstrated to perform excellent diagnostic tasks. It is worth noting that such devices can both test air and liquid samples with superimposable efficacy [139].

Biosensors should be able (1) to ascertain the earliest molecular signatures of disease, (2) to generate a detectable signal in real time, (3) to provide suitable data that in turn could (4) support a diagnostic-making process according to a validating clinical algorithm. It is imperative to address a number of issues linked to sample management, including safe acquisition, handling, and pre-analytical managing of biological samples. Indeed, biochemical sensors need to be non-invasive, i.e. they should be preferentially focused on managing breath air and/or salivary specimens. The FDA has authorized a needle-free device for collecting capillary blood; blood-based devices are however invasive and the sampling system should be included into the sensor device, thus avoiding sample transfer procedures [140]. On the contrary, breath, saliva and urine samples require less pre-analytical processing. Noticeably, in some instances, some analytes under consideration are present in higher, valuable concentrations in salivary or urinary specimens than those recorded in blood [141].

Biosensors in space should reduce to minimum resource consumption, including instrumentation's weight, volume, and storage requirements. Moreover, the sensor's equipment for space must be "compacted" and miniaturized through on-chip integration of actuators, able to exploit capillary forces to drive flows and to economize resources (namely by looking at re-useable components). Furthermore, sensor devices for space must be designed to operate correctly in a microgravity environment. Some reports have highlighted that microgravity may affect enzymatic kinetics [142], while others did not confirm such preliminary reports [143]. Undoubtedly, microgravity strongly affects several biological processes, mostly ruled by physics principles, like sedimentation and fluid behavior [144]. Whereas some effects (like buoyancy) can be overlooked, others – including surface tension, capillary and viscous forces - cannot be ignored, as they have proven to play appreciable effects on living organisms in weightlessness conditions. Spontaneous partitioning between gases and liquids can become a challenging task, namely making bubble management problematic. Yet, the possibility of driving flows or handling microdroplets by capillarity or well-designed hydrophilic/hydrophobic surfaces can help in overcoming such hurdles [145]. Several other issues – density-driven convection, temperature-dependent effects on solution gradients - just to mention a few – still wait for rigorous investigations on the ISS. Finally, both sensors and consumables should have a long operating life in a shielded environment, due to the hazard posed by radiation exposure. Preliminary data are promising, showing significant stability of free and grafted antibodies – specifically DNA aptamers [146] and molecularly imprinted polymers (MIPs) in a simulated Mars environment [147] (Fig. 4).

Consequently, available commercial sensors used for clinical purposes on Earth are not immediately suitable for space applications. Some instruments have been used in space to measure some blood parameters

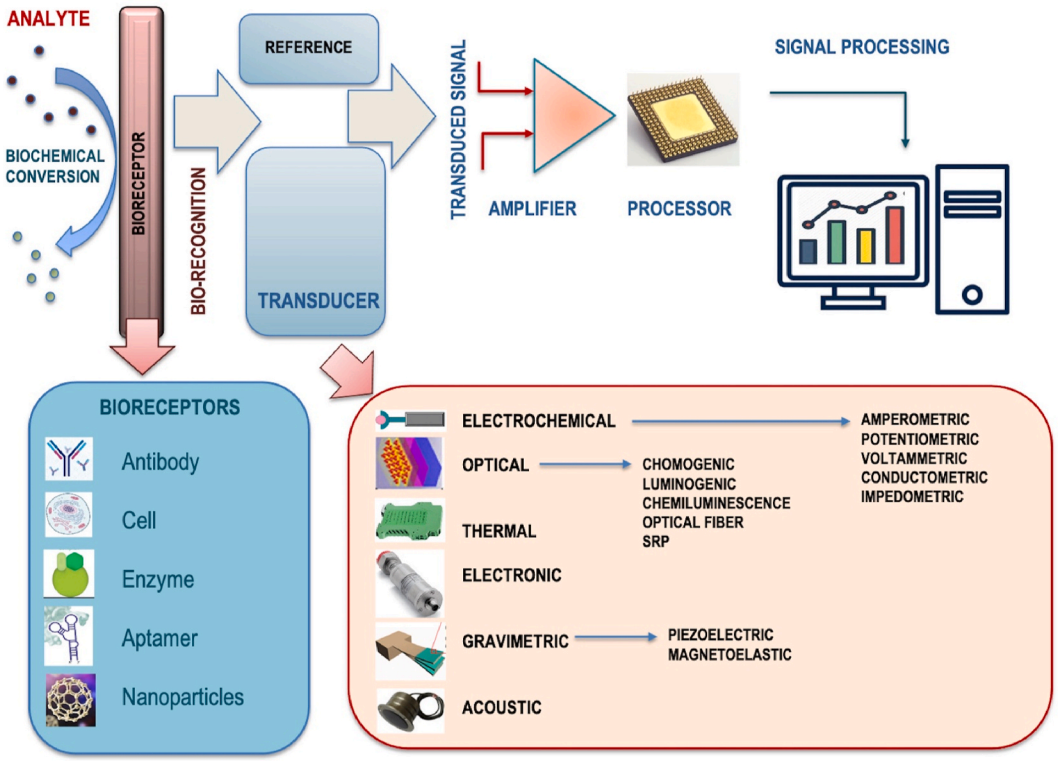

Fig. 4. General structure of biosensor for biomarker detection.

(pH, CO2, electrolytes, glucose and hematocrit) [148]. This kind of sensors requires less than 100 μL of blood, and it relies on small electrochemical detection techniques. However, a main drawback is the limited autonomy of its cartridges (4–6 months, even when stored at 2–8 °C). The Reflotron IV biochemical analyzer, in which the biochemical reaction begins when biological sample is applied to a strip preloaded with dry reagents has overcome this problem. However, the bench-top unit for reflectance spectroscopy detection is huge. The instrument related hindrance will likely limit its use in spacecraft habitats [149].

Interesting results have been recently provided by "IN SITU Bioanalysis" project, supported by ASI, aimed at developing a biosensor for monitoring health markers in salivary samples. The sensor is designed according to an innovative design, based on the chemiluminescence lateral flow immunoassay (CL-FIA) technique, in which immunoreagents are immobilized in specific areas [150]. This sensor was tested to measure salivary levels of cortisol, detected through an ultrasensitive charge-coupled device camera designed according a "contact imaging" configuration [151]. The analysis is carried out on a small volume of salivary sample, without requiring any pre-analytical processing and, noticeably, capillary forces drive the flow inside the cartridge, thus operation of the device is gravity independent. Moreover, sample introduction is performed through a one-way valve to avoid any leakage in the station milieu. The biosensor has been successfully utilized on board the ISS during the VITA mission and it is still in use [152]. This device proved to detect nanomolar concentrations of the analyte. Overall, a next generation of sensors requires developing multifunctional technologies to develop an interface, which should include nanostructured new devices, based on novel materials/composites that support linked recognition and signal detection. This approach is likely to help in facilitating the shift from recognition to guided, medical intervention.

1.7.3. Identifying new biomarkers through a systems-biology approach

Basic research is still needed to identify those markers that are specifically modulated under microgravity exposure. As evidenced by some preliminary reports, in weightlessness human beings showed an altered pattern of biochemical markers, involving glucose and bone metabolism, stress response, adaptive endocrine signaling [153,154], just to mention a few. Extensive studies to identify specific patterns of protein/microRNA/metabolites perturbations occurring during spaceflights should be performed according to a metabolomic approach [155]. These kinds of studies imply we first recognize through a high-throughput approach – conducted with Liquid Chromatography/-Mass Spectrometry – that would allow to integrate the overall parameters into a diagnostic algorithm [156]. Once recognized, these specific targets could be "incorporated" into specific, "spatialized" biosensors. These advancements will pave the way for the making of a "space personalized medicine" given that the approach integrating genomics, transcriptomics, proteomics, metabolomics, and bioinformatics can help in recognizing personal biological profiles. Studies are under way to develop an aptamer-based technology for assessing the proteome and metabolome changes occurring during extended missions, toward the Moon and Mars [156]. Overall, such considerations stress the need to pursue a systems biology approach to develop personalized medicine programs for astronauts. This statement stems from two convergent concepts. First, molecular networks interact dynamically in influencing the individual susceptibility to any specific environment. Second, specific space stressors – microgravity, radiation hazard, disruption of circadian rhythms, psychological factors – affect significantly and in a non-predictable manner upon the individual susceptibility. By no doubt, the development of such a comprehensive Omics-based platform could help in assessing diagnosis and needed countermeasures.

1.8. Take up the challenge

What we do not know about human physiological limitations hinders our ability to plan a human exploration campaign beyond LEO, as that forecasted in the Artemis program. In any event, the most limiting factor that makes human space exploration a risky endeavor is strictly

dependent on safety issues. Therefore, the principal barriers to human exploration far from LEO are those given by the limits of medicine. Namely, the question is how to assess an acceptable level of risk that can be reasonably tolerated? Let us remember that space exploration remains a challenging task, and that the uncertainties cannot be restricted to the technological challenges, even if the crew would be "limited" to two astronauts, as planned in simplified scenarios [157].

Human space exploration must proceed across prudent, progressively increasing steps, aimed at reducing immediate and long-term risks to an acceptable level. Furthermore, potential hazards must be addressed within the context of a comprehensive program finalized to implement our basic knowledge of biology in space.

In 2004, NASA released a "Vision for Space Exploration'', stressing the future objective of Mars colonization [158]. Current focus on the Artemis program reinforces such an objective by identifying Moon's settlement as a preliminary step. To address such issues the Human Space flight Architecture Team was established in 2012 to inform NASA's Human Explorations and Operations Mission Directorate regarding possible missions beyond Low Earth Orbit (LEO) [159], with the goal to set up the risk standards necessary to provide preliminary guidance to Moon and Mars mission planners and hardware designers. Hitherto, NASA's Human Research Program identified 32 principal health risks that should be adequately investigated for developing either preventative measures or efficient mitigation treatments [160]. A similar survey has been made by ESA [161] and other national space agencies [162].

According to almost all the surveys done until now, there is a shared belief that our current knowledge is inadequate to ensure astronaut health safety, as long duration and exploration spaceflights would likely expose the crewmembers to levels of known risks beyond those forecasted by currently accepted biomedical standards. Humans flying into outer space would also be exposed to a wide range of risks that are still poorly characterized [163]. Conclusively, the only way to reduce health hazard below the acceptable risk, is by improving our fundamental and applied knowledge on Space Biomedicine. Specifically, three main goals should be attained: a) providing a reliable shield protection from radiation exposure; b) developing a device for achieving artificial gravity conditions; c) performing an integrated network for remote diagnosis, which should include telemedicine advanced programs and a new generation of sensor devices for capturing a wide array of biomarkers. It must be stressed that to counteract microgravity-induced effects on human physiology by employing artificial gravity devices is an absolute requirement. Unfortunately, the proper level of the artificial gravity and the desirable daily duration to artificial gravity in preventing major weightlessness effects are both unidentified. Similarly, recognizing to what extent artificial gravity may induce relevant side effects is an absolute requirement if we are to minimize long-term medical risks in lunar/Mars-bound exploration missions.

The development of Artemis program will expose astronauts further to a number of "new" challenges, mostly related to the transition from microgravity to different hypogravity regimens, as those present on the Moon (0.16 g) and Mars (0.38 g) surface. This is not a trivial aspect given that exposure to microgravity even for very limited times can trigger cortical reorganization of the sensory-motor control and severely hinder human movement, producing significant damage [164,165]. Currently, we consider that a number of factors, other than Moon hypogravity – including terrain features, Extra-Vehicular Activity (EVA) suit as well as challenges in controlling the Center of Body Mass (COM) with respect to the base of support (Center of Pressure) – can contribute in determining the instability recorded on lunar surface [166]. Moreover, vestibular adaptations and dysregulation of postural control and locomotion can further complicated this situation finally increasing the risk of fatality/injury on the Moon terrain [167]. Investigations carried out with different gravity regimens showed that vertical and forward work as well as total internal, external and mechanical work changed considerably when compared to 1 g, thus indicating unexpected, large effects linked to the gravity field [168]. Regarding the biomechanical parameter recovery (ability of the human body to safe energy by behaving like a pendulum-like system), a number of different consequences related to the exposure to hypogravity environment have been recorded, ranging from small to large modifications depending on locomotion features. Similarly, in a Moon gravity environment, a significant reduction for hip and knee range of motion using the tilted and vertical body weight support systems has been observed. Overall, most of the spatio temporal parameters showed extremely large effects across the different microgravity/hypogravity conditions investigated.

Elucidating how transition from different gravity regimens influence sensory-motor capability is critical to inform development of pre-flight, in-flight, and post-flight programs to foster the more appropriate countermeasures, including the design of the lunar habitat and EVA suit [169]. This topic – i.e., the human locomotion in low-gravity environments – becomes a central issue in the last 30 years, and a relevant body of information has been released since then, dealing principally on the biomechanics, energetics and general viability of our gait repertoire. As current exercise-based countermeasures appear to attenuate but not prevent 'space deconditioning', new strategies have been investigated. A first candidate is offered by plyometric exercises (hopping and whole body vibration), which showed to exert beneficial effects, especially in those settings wherein resources will be limited compared to the possibilities available on the ISS [170]. This approach is based on the evidence that individuals experiencing low gravity regimens – even for a limited time of exposure as such provided by parabolic flights – prefer "to run" instead of walking, as this would provide a much higher Froude numbers than predicted by the inverted pendulum model and the dynamic similarity principle [171,172].[1]

Additionally, other ground-based methodologies have been developed to simulate hypogravity and related countermeasures by using gravity "compensation" or "offloading" systems such as vertical body weight support (BWS) [173], supine suspension [174], or lower body positive pressure (LBPP) [175]. Noticeably, the use of BWS tools to reduce mechanical loading for mimicking exposure to a reduced gravitational loading, is a priceless device for modeling those adaptations that astronauts could experience in future space missions. Additionally, results from this kind of studies can also aid in developing new therapeutic interventions in patients suffering from neuromuscular/orthopedic/neurologic disorders that may benefit from advanced rehabilitation approaches [176].

In the perspective of the Artemis program, it would be required to know those minimum thresholds of gravity values that are "sufficient" to enable locomotion and maintain the functionality of physiological systems [177]. Indeed, a strong correlation ($R > 0.88$) has been observed between cardiovascular function [178], oxygen consumption, metabolic rate [179] and gravity values ranging from 1 g to μg. These results are promising, as they suggest that consequences to the exposure to Mars and Moon gravity field may be less challenging when compared to the effects experienced in true microgravity. However, limits of our models become relevant when facing data obtained from locomotion on the Moon, wherein the environment characteristics – including rough surface, pervasive dust, reduced atmosphere, large fluctuations in temperature, weighty spacesuits – contribute in hindering human locomotion [180]. Recent experiments performed on different fields (soft/complex landscape) can presumably provide useful insights into this intricate matter [181].

Since 2010, space agencies have promoted a series of studies aimed at identifying the biological hazard of a prolonged stay in space, well conscious that "an adequately safe system is not necessarily one that

[1] The principle of dynamic similarity [172] states that dynamically similar bodies have the same gait when the horizontal speed of COM v is normalized as the dimensionless Froude number: Froude = v^2/gL, where L is the leg length and g is the acceleration of gravity.

completely precludes all conditions that can lead to undesirable consequences" [182]. Moreover, for copying with challenges associated with the Artemis program, health systems would be required to provide medical care, environmental monitoring, and improvement of crew performance. The biomedical approach is expected to evolve toward increasingly higher levels of self-sufficiency. In addition, crewmembers should be taught to perform autonomously basic diagnostic procedures, eventually with the aid of more sophisticated telemedicine and biosensors devices. Nonetheless, on-site medical care – including surgical capabilities - would be needed to establish proper medical response to major and minor illnesses. These arguments are gaining momentum in the perspective of future travels, given that as the Moon becomes accessible to a "wide" public, private commercial missions will support space tourism at an increasing pace, as well as diversified scientific activities [183].

Europe should seriously consider such issues if a decision has to be made about the opportunity to build an autonomous spacecraft for future crewed space explorations, as suggested by previous NASA reports [184]. In Europe, these issues have been underestimated during the last two decades, as documented by the limited resources allocate to funding space biomedicine programs. It is time to review in depth the scientific policy of ESA and national space agencies too, in order to meet the basic requirements outlined above. Space Biomedicine should become an integrated component of university teaching, and space biology research should enter into the main institutional research programs as an autonomous, specific area of study. This would inspire enthusiasm to a new generation of young students and researchers within a proper academic frame.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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