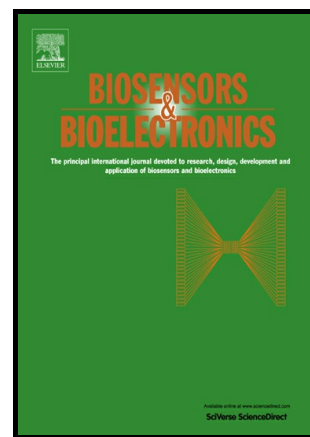


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Advanced biosensors for monitoring astronauts' health during long-duration space missions

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Abstract

Long-duration space missions pose important health concerns for astronauts, especially regarding the adverse effects of microgravity and exposure to high-energy cosmic rays. The long-term maintenance of crew health and performance mainly relies on prevention, early diagnoses, condition management, and medical interventions in situ. In-flight biosensor diagnostic devices and medical procedures must use few resources and operate in a microgravity environment, which complicates the collection and management of biological samples. Moreover, the biosensors must be certified for in-flight operation according to strict design and safety regulations.

Herein, we report on the state of the art and recent advances in biosensing diagnostic instrumentation for monitoring astronauts' health during long-duration space missions, including portable and wearable biosensors. We discuss perspectives on new-format biosensors in autonomous space clinics. We also describe our own work in developing biosensing devices for non-invasively diagnosing space-related diseases, and how they are used in long-duration missions. Finally, we discuss the benefits of space exploration for Earth-based medicine.

Keywords

Space medicine; Point-of-care testing; Biosensors; Spaceflight; International Space Station; Diagnostics.

1. Introduction

Space is an unnatural place for humans. From the first crewed spaceflights in the early 1960s to the current long-duration missions on board the International Space Station (ISS), a series of microgravity-related adverse effects have been reported. These include body fluid redistribution, muscle atrophy, and bone demineralization (Figure 1) (Nicogossian et al., 2016). In addition, spaceflight risks include exposure to high-energy cosmic rays (due to the lack of protection from the Earth's atmosphere and magnetic field), environmental contaminants or vacuum, and accidents caused by collisions with meteoroids or space debris. To mitigate these risks, crewmembers are selected according to stringent procedures, and careful prevention and protection strategies are implemented. Nonetheless, adverse health effects are still reported. Moreover, incidence is expected to rise with the advent of commercial crewed spaceflights, deep-space missions, and the colonization of extraterrestrial bodies. Therefore, to maintain their health in space in the long term, astronauts must be able to diagnose, manage, and perform medical interventions for spaceflight-related diseases and for common pathologies (e.g. acute appendicitis) in an effective, timely, and in situ manner.

For any medical procedure in space, astronauts must operate with a great deal of autonomy. This is because both medical equipment and specialized personnel are limited. Moreover, telemedicine approaches have restricted applicability due to the latency in communication, especially in deep-space missions (for example, the latency in Earth-Mars communication varies from 6 to 40 minutes depending on the relative position of the two planets). In addition, diagnostic devices for in-flight application must be designed to cope with various physical limitations (e.g. low weight, volume, and power consumption) and to operate in a microgravity environment, which affects many physical phenomena (e.g. fluid dynamics). Sample management is also critical, particularly for the non-invasive withdrawal, handling, and preanalytical processing of biological samples. In this context, crewed space stations in low Earth orbit, such as the ISS, are invaluable for investigating the effects of long-term exposure to microgravity, and for testing new technologies and devices for monitoring astronauts' health. Biosensor-based formats can potentially fulfil the above criteria to offer the necessary analytical performance for space medicine.

Herein, we critically discuss the state of the art and recent advances in developing biosensors to monitor astronauts' health during long-duration space missions. We focus on their fit-for-purpose development, with different biological recognition elements and physical transduction being used for space applications. We also briefly describe our own work in developing a biosensor, based on a lateral flow immunoassay format and chemiluminescent transduction, to measure salivary stress

biomarkers on the ISS during the ESA astronaut Paolo Nespoli's VITA mission (July-December 2017). Finally, we critically discuss the benefits of space exploration for Earth-based medicine in terms of future applications and perspectives of biosensors as point-of-care-testing (POCT) and point-of-need devices.

2. Requirements for biosensors for space

Portable diagnostic biosensors have already found application in areas such as emergency medicine (POCT) and resource-limited settings (Cummins et al., 2016). This is because they can perform clinical chemistry analyses with minimal pretreatment of samples to provide semi-quantitative or quantitative results in just a few minutes. As with terrestrial applications, procedures for bioanalysis in space should be as simple (or automated) as possible to reduce human error. The results must be easy to interpret. This is especially true for astronauts since they may perform the analyses several months after they received their training. Biosensor-based diagnostic devices for in-flight application must also meet specific criteria (Nelson, 2011; Karouia et al., 2017), which are often different to or more stringent than those required for terrestrial applications (Table 1).

One of the main requirements is to minimize resource consumption in terms of the instrumentation's weight, volume, storage conditions, and power consumption. Many of the miniaturized analytical devices proposed for Earth applications still need bulky external infrastructures to operate (e.g. pumps, application of magnetic or electrical fields, optics). However, the equipment for space must be very compact and fully integrated. Strategies to achieve this include a simplified biosensor design (e.g. using capillary forces to drive flows), miniaturization through on-chip integration of sensors and actuators and/or exploitation of shared resources (e.g. use of onboard PCs instead of dedicated processors), and a scaling-down of sample sizes.

A big step towards meeting low-resource requirements would be to switch to reusable components. Most POCT devices for terrestrial applications are single-use, in many cases based on disposable cartridges coupled with a reusable detector. However, the upload and space required for disposable devices and the bulky waste are prohibitive for space travelers on extended missions. The device reusability can be obtained by employing microparticles as a solid phase, which are then replenished with fresh ones for the subsequent analysis, although this approach does not fully accomplish the concept of zero-waste. Future development can be envisaged with the use of molecular switches as biospecific recognition elements, that exploit reversible binding with the target analyte (Xiong et al., 2017; Sheng et al., 2013). However, device reusability still poses critical aspects that must be carefully considered. These include the risk of sample carryover,

reduced analytical performance upon extended reuse, and the resources required to clean and reactivate the system.

A real breakthrough would be the development of reconfigurable modular analytical devices. While biosensors for Earth applications are designed for a specific type of sample and analytical procedure, those for use in space should be designed as a toolbox of modular components that can be plugged together in a customized fashion for a given analytical task. The assembled device should perform all the analytical tasks, including sample preanalytical treatment, and accept different types of sample (e.g. human blood, saliva, urine, but also food, animal, environmental samples). It should also enable a multiplex approach, where different types of assay may be performed together on the same sample (e.g. particle counting and characterization, quantitative detection of gases, electrolytes, organic molecules, proteins, and nucleic acids). This requires highly demanding technological development, which is far from being accomplished.

In contrast to terrestrial biosensors, the device for space must operate correctly in a microgravity environment. Chemical and biochemical reactions should be minimally affected by the absence of gravity. For example, there are controversial data about the effects of microgravity on the kinetics of enzymatic reactions. Some studies report that microgravity alters the catalytic efficiency of enzymes by affecting diffusion processes, thus facilitating the formation of the enzyme-substrate complex (Maccarrone et al., 2001). Subsequent studies found that microgravity did not alter any of the kinetic parameters or the equilibrium constant of the overall reaction (Ranaldi et al., 2003). Nonetheless, microgravity strongly affects many physical processes. This is especially true of fluid behavior, which is critical for biosensor development. In space, gravity and density effects (e.g. buoyancy) are negligible, so other forces such as surface tension and capillary and viscous forces become important. For example, there is no spontaneous separation between gases and liquids, which makes bubble management challenging. Bubbles could form within the device due to gases dissolved in solution or produced by chemical reactions, evaporation of liquids, or diffusion of air through seals or porous materials. This could clog fluidic channels or interfere with the detection process. Bubble management must therefore be considered when designing the device. In microgravity, capillary and wetting effects can dominate fluid flow. However, this is not necessarily a disadvantage, because of the possibility of driving flows or handling fluid microdroplets by exploiting capillarity or wetting of suitable hydrophilic/hydrophobic surfaces (Xu et al., 2017). Density-driven convection does not take place in microgravity, which means that it cannot be used to mix solutions. Mechanical stirring or carefully designed flow reaction chambers should be used instead. Furthermore, convective heat transport is inefficient. This influences heating and cooling processes, and can alter temperature gradients within solutions. There is still

limited data on these issues, which is why many of the current experiments on board the ISS are designed to further investigate the effect of microgravity on physical and chemical processes.

In anticipation of long-term space missions, another strict requirement is that devices and consumables should have a long shelf life and/or operating life (on a timescale of years for deep-space missions) without guaranteed access to refrigerated storage and in an environment characterized by radiation exposure. Some studies have reported the ability of recognition elements, either natural (e.g. antibodies) or synthetic (e.g. aptamers or molecularly imprinted polymers (MIPs)), to maintain their performance under space conditions. One study recently reported the stability of free and grafted antibodies in a radiation environment, which simulated the conditions of a mission to Mars (Baqu  et al., 2017). The authors suggested appropriate design features to further improve the long-term stability of antibody-based biochips (e.g. a surface density of antibodies much larger than the expected proton fluency across the chip). DNA aptamers (Baqu  et al., 2011) and MIPs (Izenberg et al., 2009), which present a higher stability, ease of production, and tailoring for assay design, were also shown to retain their properties when exposed to simulated space environments. AM Biotechnologies LLC (Houston) is collaborating with NASA to develop innovative second-generation aptamers (X-Aptamers) that incorporate natural and chemically modified DNA or RNA nucleotides. These are intended to overcome the common limitations of aptamers (e.g. lack of specificity, weak binding with targets) and antibodies (e.g. limited shelf life). These next-generation aptamers are easily synthesized and display a high affinity for specific biomarkers of interest to NASA (https://spinoff.nasa.gov/Spinoff2016/hm_3.html). This process is now commercially available, so anyone can make their own aptamers with a simple kit.

For collecting biological samples, the acquisition of capillary blood is preferred in order to reduce the invasiveness for the astronauts. Where possible, the sampling system should be integrated into the biosensor to avoid the need for sample transfer. The US Food and Drug Administration (FDA) recently approved a needle-free collection device for capillary blood. This appears promising for in-flight use (<http://www.tassoinc.com>). Other easily collectable biological samples (e.g. urine, saliva, sweat, feces, breath) could also be considered. These samples often require minimal preanalytical treatment, but they provide less information than blood. Moreover, in many cases, the analyte concentrations are much lower, even if they often do correlate with blood levels.

The safety of the spacecraft environment is another critical aspect in developing biosensors for space application. Adequate multi-level containment and safe disposal must be ensured to avoid the release of potentially hazardous substances and contaminated biosamples during operation or adverse events (e.g. depressurization). One of the best options seems to be biosensors that use disposable sealed cartridges with ready-to-use reagents and waste reservoirs. However, this option

does not meet the requirements of reusability and a low-waste approach. In addition, hardware design must follow a strict set of rules concerning for example sharp edges, touch temperatures and rotating equipment. In addition to hazards, limitations on available space crew time must be also considered.

Payloads used on board the ISS must be certified according to NASA regulations to ensure safety, compatibility with onboard equipment, and correct in-flight operation (NASA, International Space Station Program). For certification, NASA requires detailed information about the hardware (e.g. electrical schemes or chemicals used). This could be a problem for commercial systems, since sensitive data might have to be disclosed. Certification also implies extensive testing to evaluate criteria such as electromagnetic emissions and resistance to launch loads.

3. Biosensors to monitor astronauts' health

Space travel poses unique design challenges for portable POC diagnostics. Many biosensor formats have been proposed as in-flight diagnostic tools, although each one has disadvantages (Table 2).

3a. Clinical diagnostics biosensors. Despite some similarities in requirements (see above), it is not straightforward to take commercial POCT systems designed for clinical applications and successfully apply them in space. As a result, only a few devices have been used in space. The i-STAT analyzer (Abbot Labs) was first used on the space shuttle (Smith et al., 1997) and is now being used on the ISS (https://www.nasa.gov/mission_pages/station/research/experiments/373.html) to measure blood clinical parameters such as alkalinity (pH), partial pressure of carbon dioxide (ppCO₂), electrolytes, glucose, and hematocrit. This analyzer provides rapid results by analyzing a small amount of sample (less than 100 μ L) with disposable assay cartridges and electrochemical detection techniques. Its main disadvantage is the limited shelf life of its cartridges (4-6 months, even when stored at 2-8 °C). This is not an issue for the Reflotron IV biochemical analyzer (Boehringer Mannheim), owing to its “dry chemistry” approach, in which the biochemical reaction is initiated by applying the blood sample to a disposable strip preloaded with dry reagents. Blood enzymes and other clinically relevant analytes were first measured with this analyzer on the Russian “Mir” orbital station (Markin et al., 1998), then on the ISS (https://www.nasa.gov/mission_pages/station/research/experiments/533.html). Nevertheless, the benchtop unit for reflectance spectroscopy detection is bulky, limiting its applicability in space.

Chemical and biochemical reactions are well-established clinical methods for quantifying blood enzymes and relatively abundant species such as glucose. But detecting predictive diagnostic biomarkers at very low concentrations requires highly sensitive techniques. The best choice is

probably bioaffinity methods that exploit antibodies or synthetic recognition elements, such as aptamers or molecularly imprinted polymers (MIPs). This is because they can guarantee high sensitivity and selectivity. Proteins can be genetically engineered exploiting biotechnological advances to tailor their characteristics for the target application. For example, to enable continuous *in vivo* glucose monitoring, semisynthetic glucose recognition proteins were prepared that display improved thermal stability and apparent binding constant suitable for glucose measurement at physiological ranges (Smita et al., 2014). On the opposite side, a whole range of new binding elements can be obtained employing synthetic approaches. For example, DNA aptamer beacons were developed to evaluate bone loss during space missions by measuring a peptide deriving from human type I bone collagen (Bruno et al., 2011). Although there is rapidly growing interest in alternative specific recognition elements, antibody-based biosensors are still the most advanced and the most frequently used. The IMMUNOLAB is one example of a bioanalytical device based on immunological analyses (Kern and Eisenberg, 2015). It allows chemical-clinical analyses to be performed on blood, urine, or saliva samples on board the ISS. The integrated device performs both sample preparation (using quality-controlled commercial analysis kits) and target detection (via fluorescence microscopy).

Ames Research Center (ARC) is a major NASA research center at Moffett Federal Airfield in California's Silicon Valley. ARC is working with government, academic, and industrial partners to advance research in biosensors based on nanotechnology. An ARC-related group recently reported a label-free biosensor that uses carbon-nanofiber-based nanoelectrode arrays to detect cardiac troponin-I (a marker of myocardial infarction) with electrochemical impedance spectroscopy and cyclic voltammetry techniques (Periyakaruppan et al., 2013).

The fiber-optic fluorescence-based flow cytometry platform Microflow1 was designed as a portable and robust instrument. It was used on board the ISS for immunophenotyping and microbead-based multiplexed immunoassays. The Microflow1's performance was comparable to that of a commercial flow cytometer in a standard laboratory environment, demonstrating that its fiber-optic cytometer technology is inherently compatible with a space environment (Dubeau-Laramée et al., 2014). For further miniaturization, NASA supported the development of a portable microfabricated cytometer for leukocyte four-part differential count (i.e. lymphocyte, monocyte, neutrophil, eosinophil) from whole blood. This device exploited a sheathless microflow design and fluorescence detection (Shi et al., 2013).

A collaboration between NASA's Glenn Research Center and the DNA Medicine Institute (DMI) has produced a reusable microfluidic device that performs rapid low-cost cell counts and measurements of electrolytes, proteins, and other biomarkers. The reusable Handheld Electrolyte

and Lab Technology for Humans (rHEALTH) (bio)sensor is a compact portable device. It uses fluorescence detection, innovative microfluidics, and nanostrip reagents to perform a multiparametric assay from a single drop of blood or bodily fluid. It is designed to monitor astronaut health during long-term space flight (https://technology.grc.nasa.gov/documents/_6_Universalbiomedicalanalysissensor_SS-rHealth-2011.pdf).

As previously mentioned, devices relying on capillary forces for fluid delivery (e.g. immunochromatography systems) are particularly suitable for microgravity conditions. A sensor platform can be integrated with a smartphone to monitor health and diagnose disease. This creates an innovative and cost-effective approach for space travel and low-gravity applications. Intelligent Optical Systems (IOS) (Torrence, US) is developing lateral flow assays for integration into a customized Holomic LLC (Los Angeles, US) smartphone reader to enable the quantitative measurement of early cardiac and liver function biomarkers in serum. Multiplexed liver panel (ALT, AST, ALP) and cardiac (troponin I) lateral flow assays have been developed and tested for sensitivity and cross reactivity with a prototype smartphone reader. Assays under development include metabolic blood chemistry panels (glucose and creatinine). Holomic is optimizing the reader to integrate a fully automated mechanical exchange mechanism for selected optical bandpass filters (Beshay et al., 2016).

Electrokinetics-based fluids delivery principles are also very suited for gravity independent applications. It is worth noting that NASA is at present working at the development of a fully automated device for planetary in situ chemical analysis in astrobiology missions, based on microchip electrophoresis, a miniaturized variant of capillary electrophoresis (Willis et al., 2015). It is expected that inflight biochemical analyses will greatly benefit from the technological advancements achieved in this field.

For nucleic acid biomarkers, another powerful biosensing approach is real-time quantitative polymerase chain reaction (RT-qPCR). Its potential in-flight applications range from measuring gene transcription (e.g. to study how microgravity affects biological processes) to rapidly diagnosing infectious diseases. In 2016, astronauts on board the ISS successfully deployed the Wetlab-2 research platform to quantitatively analyze gene expression via RT-qPCR. However, although dedicated equipment has been developed to facilitate the extraction and purification of nucleic acids, sample preparation in microgravity remains a problem, mainly due to the risk of sample carryover and contamination by the ISS environment (Oubre et al., 2013).

3b. Biosensors for water and environmental quality. Astronauts' health will be affected by the quality of their water, food, and environment. A spacecraft is a closed environment, in which air and water are continuously recycled. Their chemical and microbiological quality must therefore be monitored. In addition, future crewed deep-space missions will require the on-board production of food supplies. Biosensors will therefore also be needed to monitor food safety and quality.

Microbiological monitoring is crucial to prevent infectious diseases among the crew, food spoilage, and biofouling of the spacecraft's surfaces by so-called technophilic microorganisms, which can corrode alloys and polymers. Microbiological contamination is particularly hazardous in microgravity due to immune system dysregulation, an altered microbiome, and the long residence time of air-suspended microorganisms (Cervantes and Hong, 2016).

Monitoring air quality is another demanding task. It requires the parallel measurement of several targets, from the main air components (e.g. oxygen, nitrogen, carbon dioxide, water vapor) to trace gases (e.g. ammonia, carbon monoxide, volatile organic compounds) and particulate matter. Gas chromatography (GC) is the method of choice. To monitor air quality in real time, astronauts on the ISS have used the Volatile Organic Analyzer (VOA) and, subsequently, the Air Quality Monitor (AQM). The VOA is a benchtop GC-ion mobility spectrometer (GC-IMS) system. The AQM is a GC-differential mobility spectrometer (GC-DMS) system with improved analytical performance and reduced mass and size. Recently, the AQM was interfaced to a mass spectrometer to increase its ability to identify volatile compounds (Limero et al., 2015). New miniaturized ion sources have been developed to produce low-cost and simple analytical devices for the in-flight measurement of volatile contaminants (Bernier et al., 2016).

Currently, culture-based technologies are used on board the ISS to monitor air, water, and surfaces for microbiological contamination. However, these methods are time-consuming, detect only a limited number of microorganisms, and require that samples be returned to Earth for complete analysis. Therefore, a more expedient, low-cost, in-flight method is required to detect, identify, and enumerate microbes. The Lab-On-a-Chip Application Development-Portable Test System (LOCAD-PTS) was tested on the ISS between 2006 and 2009. With cartridges containing dried reagents, LOCAD-PTS used colorimetric reactions to detect and quantify Gram-negative and Gram-positive bacteria and fungi based on the presence of specific endotoxins or cell-wall components (Morris et al., 2012). However, this system cannot identify microorganisms. Molecular-based technologies are therefore being evaluated. Two systems (WETLAB-2 and RAZOR EX) have been deployed on the ISS to perform assays based on RT-qPCR. The RAZOR EX is being tested on board the ISS for water monitoring. With ready-to-use, freeze-dried reagents, it quantitatively detects targeted microorganisms or total bacteria in less than one hour

(https://www.nasa.gov/mission_pages/station/research/experiments/2109.html). Non-targeted approaches have also been proposed, such as the direct sequencing of DNA. These offer the advantage of very strong detection, since any microorganism can potentially be identified with the appropriate database. The MinION instrument uses a nanopore-based DNA-sequencing approach. It was successfully tested on the ISS in 2016 (https://www.nasa.gov/mission_pages/station/research/experiments/2181.html).

A portable electronic nose (E-Nose) was used in the Russian “Zvesda” module of the ISS to detect biocontamination in situ at the relevant locations and surfaces (Fleischmann and Lenic, 2013). The E-Nose was trained to determine a characteristic olfactory fingerprint for a dedicated sample (e.g. a spot with bacteria or microorganisms). The initial results on board the ISS demonstrated the feasibility of this label-free detection method and its suitability for long-term space missions.

3c. Biosensors for investigating the effects of and countermeasures to radiation exposure.

Exposure to ionizing radiation is one of the main problems for astronauts in long-duration space flight. NASA has set the maximum dose permitted for an astronaut’s overall career at 1-4 Sievert, depending on age and gender. Each crew member on board the ISS is provided with a personal passive dosimeter, and the ionizing radiation environment is continuously monitored by passive or active detectors placed throughout the habitable volume. The Tissue Equivalent Proportional Counter (TEPC) is a gas proportional radiation counter adapted to estimate the dose equivalent absorbed by astronauts [https://www.nasa.gov/mission_pages/station/research/experiments/TEPC.html]. The detector is surrounded by a plastic jacket, simulating the properties of human tissue, and a suitable internal gas (propane) provides an energy-deposition response similar to that of human cells. Next-generation TEPCs are currently in development, featuring improved energy resolution and sensitivity, lower weight, and the ability to be used in extravehicular activity. To reduce the risks of long-term space exploration in humans, it will be crucial to understand how exposure to space radiation affects living cells, and to evaluate the efficacy of countermeasures in a relevant environment (<https://www.nasa.gov/centers/ames/engineering/projects/biosentinel.html>). Various biosensors have been deployed on the ISS or on microsatellites for this purpose (Rabbow et al., 2009; Häder et al., 2009). The BioSentinel experiment will be launched in 2018. It will use nanosatellite technology to study DNA damage and repair in *Saccharomyces cerevisiae* cells exposed to deep-space radiation beyond low Earth orbit. Yeast cells were genetically engineered to grow and divide only upon radiation-provoked DNA-double-strand breaks and consequent activation of the yeast’s DNA-repair

mechanisms. The cells will be launched in a dry state and reactivated in space using microfluidic cards.

4. Next-generation biosensors to monitor health

In aerospace medicine, it is extremely important to monitor vital physiological parameters in real time. In this context, physical sensors are more commonly used than biosensors. In the space shuttle era, the Operational Bioinstrumentation System (OBS) was used to measure the electrocardiographic (ECG) signal of astronauts during the vehicle's ascent and re-entry (Cupples and Johnson, 2005). Nowadays, there is growing interest in using wearable wireless medical (bio)sensors to monitor health (Darwish and Hassanien, 2011). The NASA Ames Research Center and Stanford University have developed an improved wearable multiparameter-monitoring system (LifeGuard) (Montgomery et al., 2004; Mundt et al., 2005). The system comprises sensors to monitor physiological parameters (e.g. ECG, respiration frequency, blood oxygen, blood pressure, skin temperature), a small wearable computer with additional sensors (e.g. a 3-axis accelerometer to monitor activity), and an external display station. It can acquire and store data for several hours, or transmit it in real time using secure wireless technology. A further advance is the integration of sensors and signal processors on a textile platform. This approach would avoid the application of sensors on the skin, increasing comfort and dressing for astronauts. Canina et al. (2006) described a network of integrated fabric sensors embedded in the Bio-Suit, a space suit designed by Massachusetts Institute of Technology (MIT) in collaboration with the NASA Institute for Advanced Concepts (NIAC). This suit allows multiparametric monitoring, including ECG, pulse oximetry, and temperature. "Lab-on-skin" devices are also very promising. These electronic devices have physical properties (e.g. thickness, elastic modulus, water-vapor permeability) similar to those of the skin. Since these devices perfectly fit the epidermis, they allow long-term monitoring with minimal discomfort, even during complex activities (Liu et al., 2017). In most cases, skin-mounted sensors are used to monitor physical parameters, such as temperature, blood pressure, oxygen level, and electrophysiology. However, biosensing applications have also been reported to detect biomarkers in sweat. Although the technology behind the wearable sensors is very advanced, integration with real biosensors is still premature.

Implantable biosensors are one of the most promising approaches for the next generation of health-monitoring devices to continuously monitor specific biomarkers. In this context, researchers have developed an integrated glucose and lactate biotransducer and communications biochip to monitor physiological status during trauma-induced hemorrhage. This device can be implanted temporarily. It exploits microdisc electrode array, and encapsulates bioactive hydrogels to immobilize specific

enzymes (Kotanen and Guiseppi-Elie, 2010). The Glucowizzard™ implantable biosensor has also been proposed for monitoring glucose on board the ISS (https://www.nasa.gov/mission_pages/station/research/experiments/2750.html). It is extremely miniaturized for needle-based implantation, with drug-delivery coatings to completely suppress foreign body responses. It contains a robust sensing element for highly accurate glucose levels, and has a long lifetime (minimum of three months).

Despite these advances, there are still few wearable and implantable biosensors that can measure the concentration of specific analytes in real time. Further research is warranted here to develop reversible biosensors that offer continuous monitoring in real time.

5. Towards true space clinics

The deployment of biosensors for predictive diagnostics will be crucial for the early diagnosis and treatment of pathologies in deep-space missions.

Pharmacological treatment is usually the first-line therapy for a disease. However, preliminary investigations during spaceflight have revealed significant alterations in the pharmacodynamics and pharmacokinetics of drugs. Additional studies are needed to evaluate the efficacy and side-effects of medications used in space, and to define suitable therapeutic protocols (Kast et al., 2017).

As recently reported, the response to a given pharmacological therapy is highly subjective and each subject may require a specific therapeutic dose. In addition, physiological parameters are altered in space conditions. These parameters include gastrointestinal transit time and enzyme activity, organ perfusion, liver enzyme activities, and renal excretion. These alterations most probably alter the bioavailability of drugs, which will differ from one individual to another.

To ensure optimal drug efficacy and safety in space, it will therefore be crucial to use real-time biosensors that can measure both the drug concentration in blood or other biological fluids and the biomarker indicators of therapeutic outcomes. This will provide prompt and accurate information about the drug's effect and the prognosis of a given disease. It may potentially reduce the need for surgical approaches to emergency situations.

For medical procedures that are not compatible with the crew members' medical skills, surgeons on Earth could operate on astronauts using telerobotic platforms. NASA has conducted telesurgery experiments on human phantoms in extreme conditions, including a zero-gravity environment (Lum et al., 2007). Nevertheless, telesurgery procedures outside Earth's orbit must cope with signal latency, which causes a time lapse between the surgeon's command and the robot's response (Korte et al., 2014). In the future, an approach based on data mining, specialized artificial intelligence, and augmented reality might be used to provide the surgeon with a simulated real-time

visual feedback (i.e. visual input is predicted before it is available from the remote sensors) (Haidegger et al., 2011; Thonier and Stephanides, 2001). Wearable or tissue-implanted biosensors will play a crucial role in the correct surgery approach when performed by crewmembers without surgical expertise.

Virtual reality technologies could also be used to support the in-flight diagnosis and treatment of certain pathologies. To date, the reported examples of virtual-reality-based diagnostics are mostly limited to endoscopic methods or ultrasound imaging (Willaert et al., 2012; Forest et al., 2007). However, collaboration between clinicians, neuropsychologists, researchers, and computer scientists could lead to further developments in this field (Tsirlin et al., 2009).

6. Our experience of biosensors for space medicine

MARS 500 was a ground-based simulation experiment conducted by the Russian Federal Space Agency (Roskosmos) in collaboration with the European Space Agency (ESA). It was designed to study the medical and psychological aspects of a crewed flight to Mars. Six crewmembers lived and worked for 520 days in a confined environment, which reproduced the main features of a round-trip flight to Mars, including limited resources and communication latency (Ushakov et al., 2014). A panel of non-invasive tests was carried out in breath to investigate how the gastrointestinal tract was affected by stress and related disease due to the long confinement in the module. In addition, we proposed using fecal calprotectin as a marker of intestinal inflammation. Fecal calprotectin was monitored with a reflectometric lateral flow immunoassay in a simple biosensor format. This revealed an increase in intestinal inflammation, with various degrees of intensity and persistence, in all the crewmembers (Roda et al., 2013).

“IN SITU Bioanalysis” was a more recent project, financed by the Italian Space Agency (ASI) in collaboration with NASA. Its goal was to develop and test a compact biosensor, which astronauts could use in flight to measure clinical biomarkers in saliva or other biological fluids in order to monitor their health. This biosensor is based on the chemiluminescence lateral flow immunoassay (CL-FIA) technique (Figure 2), an immunoanalytical approach based on the use of a nitrocellulose membrane on which the immunoreagents are immobilized in specific areas and relying on highly-sensitive chemiluminescence (CL) detection. The biosensor was designed to measure the salivary levels of cortisol, a chronic stress marker. It comprised a 3D-printed plastic cartridge containing a fluidic element with the LFIA strip, a port for sample loading, and pressure-activated reagent reservoirs and valves. The analysis required a simple manual procedure, and the flow of sample and reagents was obtained by pressing buttons on the cartridge or (across the LFIA strip) by exploiting capillary forces. Detection was performed using a separate CL reader based on an ultrasensitive,

thermoelectrically cooled charge-coupled device (CCD) camera in a “contact imaging” configuration (Mirasoli et al., 2012; Mirasoli et al., 2013). This approach had already been used successfully for several bioanalytical applications (Zangheri et al., 2015; Zangheri et al., 2016). In a telemedicine approach, the results were collected on an ISS laptop, then sent to ground personnel for processing and evaluation by medical experts. The biosensor has been designed according to most of the requirements listed in Section 2. Indeed, the analysis is carried out on a small volume of an easily collectable sample (saliva) that does not require any preanalytical treatment. The flow inside the cartridge is driven by capillary forces, thus operation of the device is gravity independent. In order to fulfill safety requirements, the fluidic element has been sealed and sample introduction takes place through an one-way valve to avoid any leakage of samples or reagents in the ISS environment. Moreover, the CL reader is an independent component of the device and it could be easily adapted to other analytical applications by developing cartridges for different analytes, also in multiplex formats. Finally, control of the CL reader by an ISS laptop PC eliminates the need of additional electronics. The biosensor was successfully used on board the ISS by the Italian astronaut Paolo Nespoli during the VITA mission (July-December 2017). As a technological proof of concept, the device demonstrated the feasibility of performing sensitive (nanomolar level) immunological clinical chemistry analyses directly on board the ISS. It could thus enable the monitoring of health, the early diagnosis of possible disturbances, and the timely activation of appropriate countermeasures (e.g. pharmacological therapy).

Future work will be aimed at further miniaturizing the device. This may be achieved by exploiting sensors based on complementary metal-oxide semiconductors (CMOS). CMOS sensors are cheap, small, and easily integrated in electronic circuit boards. Recently, a smartphone camera with CMOS sensors was exploited as a portable on-site detector (Roda et al., 2016). With a view to space applications, it was proposed as a fluorescence detector for paper-based analytical methods (e.g. LFI, FIAs) to quantify biomarkers in blood (Krihak and Tianna, 2014). Alternatively, thin film sensors can be easily integrated into lab-on-a-chip devices to provide good analytical performance (Caputo et al., 2013; Mirasoli et al., 2014).

7. Conclusions

Modern space medicine requires an interdisciplinary approach: physicians, biologists, chemists, and engineers must collaborate to develop devices for monitoring and diagnosis that satisfy the strict criteria of in-flight applications.

Several sensors are available for the in-flight measurement of physical parameters, such as body temperature, blood pressure, cardiac rhythms, and respiratory rhythms. However, a different

approach is required for biosensors designed to measure, in real time, a set of molecules in biological samples with high specificity and high detectability.

Portable diagnostic devices and biosensors developed for use on Earth (e.g. in emergency medicine or as POCT devices) can be adapted for in-flight application. This approach offers relatively simple, cheap, and rapid deployment in space. However, it does not guarantee the same performance as the ground-based counterpart. A real breakthrough can be obtained only through a paradigm shift. Even when based on mature technologies such as immunoassays or PCR, the successful development of biosensors for space applications will require substantial reengineering and a fit-for-purpose redesign of the instruments. We envisage that new-generation biosensor technologies will exploit nanomaterials to improve the mechanical, electrochemical, optical, and magnetic properties of biosensors. These can then be developed for single-molecule detection and high-throughput arrays. Nanoscale materials could be used to build more sensitive, specific, and adaptable sensors, overcoming the technical barriers that limit the application of conventional sensors in space environments (<https://www.nasa.gov/ames-partnerships/technology/cnt-biosensors>). However, despite interesting publications and proofs of concept, these biosensors are still at a lower level of technological readiness. Their numbers and use are expected to grow rapidly and exponentially, as researchers fully exploit the breakthrough technological advances, such as nanomaterials, molecular machines, and microelectronics.

A further critical aspect is sample collection and processing. Researchers are actively investigating the use of alternatives to blood (e.g. urine, saliva, sweat, faeces, breath) to enable the non-invasive withdrawal of samples and to reduce preanalytical sample treatment. Another area of investigation is procedures based on innovative needle-free technologies, such as that proposed by Tasso Inc to minimize discomfort during blood collection. Other emerging technologies that could find application during space missions are those that combine the real-time monitoring of physical parameters (e.g. cardiac rhythm, pressure, temperature) with the analysis of easily accessible bodily fluids (e.g. sweat) to quantify specific biomarkers, such as lactate or cortisol.

Flipping the perspective, it should be much easier to take diagnostic devices designed for use in space and transfer them back to Earth. Many technologies developed for space have produced concrete benefits in our everyday lives. These technologies include satellites for telecommunication, environmental monitoring, and weather forecasting; advanced materials originally designed for spacecraft components (e.g. fire-resistant or thermal-insulation fabrics); and even water purification systems now used in developing countries. There have even been important medical benefits, although these are not so widely recognized. However, medical diagnostics and therapies are nowadays undergoing a revolution in miniaturization and remote capabilities.

Biosensor devices designed for in-flight applications fit perfectly into this trend, with features such as reduced weight, reduced energy consumption, increased robustness, and consumables with a long shelf life. Moreover, extremely miniaturized biosensors based on nanotechnology will minimize the waste produced by disposable biosensors. In this context, the final goal is to eliminate waste entirely by replacing disposable biosensors with reusable ones. This would be advantageous for long-term space missions, but also for reducing the environmental impact.

We therefore expect that, in the near future, the development of (bio)sensing technologies for space will facilitate human exploration of space and substantially improve our lives on Earth, providing great benefits for medical and health services, environmental protection, and food safety.

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Figures captions

Fig 1. Main physiological effects of long term exposure to microgravity and diagnostic techniques and/or biomarkers suitable for their monitoring.

Fig 2. Biosensor employed in the “IN SITU Bioanalysis” project for the measurement of salivary cortisol levels on board the ISS: **a** sealed fluidic element with the LFIA strip and the reagents (inset: enlarged view of the LFIA strip in the fluidic element showing the area imaged by the CL reader), **b** 3D-printed plastic cartridge enclosing the fluidic element, and **c** CL reader. Scale checkerboards are 2×2 cm. **d** Detail showing the insertion of the cartridge in the CL reader. **e** The Italian astronaut Paolo Nespoli performing the experiment on board the ISS (image courtesy of NASA).

TABLES

Table 1. Main requirements for biosensors for space application, and comparison with POCT devices designed for use on Earth

Requirement	Strategies to fulfill the requirements	Open issues	Already implemented in POCT devices on Earth?
Low resource consumption	• Scale down sample volumes • Simplify (e.g. with passive processes such as capillarity) and/or miniaturize (e.g. with microelectronics to integrate sensors and actuators) devices • Scale down the disposable portion of the device • Develop reusable devices	• Small sample volumes may negatively affect detection limits • Reusable devices can lead to sample cross-contamination, surface fouling, loss of performance of biological components • Resource consumed for system washing and regeneration	• Reducing the device size and sample volume is a key features for POCT, although many miniaturized devices still rely on bulky external infrastructures to operate • Reusability is not often pursued; disposable devices are generally preferred to avoid cross-contamination

	• Use shared resources		
Flexibility	• Exploit massive multiplexing approach, also combining different assay types • Design reconfigurable devices following a mix-and-match modular approach (e.g. adaptable to different analyses and/or different types of samples)	• Technological readiness level is not sufficient	• Devices are most often limited in the breadth of measurements and sample types • Combination of different assay principles in one device is usually not pursued

Stability	• Employ (or develop) biospecific reagents stable in space conditions, possibly at room temperature	• The long-term stability of many reagents in a space environment is not fully assessed	• Devices are often designed for limited storage times (weeks or months). • The need for controlled-temperature storage is not always considered a drawback.
Gravitational independence	• Carefully design the fluid handling • Use gravity-independent processes (e.g. centrifugal forces, electrokinetic fluid handling, capillarity, wetting)	• Some processes create problems in microgravity (e.g. bubble formation, convective heat transfer) • The behavior of physical-chemical processes in microgravity is not fully understood	
Safety	• Design devices following the regulations from NASA and other	• Flight certification may require disclosure of sensitive	• Safety requirements for POCT devices on Earth are less stringent

	Space Agencies	information about the device • Fulfillment of safety requirements complicates the design of the device	
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Table 2. Biosensor formats proposed for space application with their main advantages and disadvantages

Format	Advantages	Disadvantages	References
Lateral flow assays Intelligent Optical Systems (IOS) (Torrence, US)	- Stability of reagents and of recognition elements, either natural (e.g. antibodies) or synthetic (e.g. aptamers or molecularly imprinted polymers) - Capillary flow efficiency in microgravity - High simplicity and rapidity - Small size of the disposable equipment - Possibilities for integration with smartphone readers and for the development of multiplex formats	Not reusable	Beshay et al., 2016 Roda et al., 2013 IN SITU Bioanalysis project (https://www.asi.it/en/node/50989)

Dry chemistry Reflotron IV biochemical analyzer	• High stability of reagents • Small size of the disposable equipment	• Not reusable • Bulky detection instrumentation not suitable for space • Limited breadth of assay types	https://www.nasa.gov/mission_pages/station/research/experiments/431.html https://www.nasa.gov/mission_pages/station/research/experiments/533.html
Electrochemical devices i-STAT Abbot	• Fast, simple, integrated POCT • In some cases reusable • Possibility of exploiting nanotechnologies to improve performance	• Refrigerated storage for consumables • Fouling of electrodes caused can decrease performance	Periyakaruppan et al., 2013 Morris et al., 2012
Microfluidics rHEALTH LOCAD-PTS	• Multiparametric assay in a small volume of blood or bodily fluid • Reusability of the microfluidic network • Massive multiplexing can be attained	• Bubble formation can be an issue for microfluidics	https://technology.grc.nasa.gov/documents/_6_Universalbiomedicalanalysisenssor_SS-rHealth-2011.pdf Morris et al., 2012
Arrays IMMUNOLAB	• High multiplexing ability • Automated analysis • Easy and safe	• Expensive and sophisticated fluorescence microscopy instrument	Kern and Eisenberg, 2015

	sampling procedures	• High volumes of reagents required for operation • Cold stowage for reagent • Small operational flexibility	
Nucleotide technologies <i>Nucleic acid amplification</i> Wetlab-2 research platform; Razor EX Sequencing MinION	• Rapid diagnosis and wide identification of markers	• Sample preparation in microgravity • Possible contamination by the environment, especially for amplification techniques	https://www.nasa.gov/ames/research/space-biosciences/wetlab-2 https://www.nasa.gov/mission_pages/station/research/experiments/2109.html https://www.nasa.gov/mission_pages/station/research/experiments/2181.html
Flow cytometry Microflow1	• Portable, robust and miniaturized instrument • High multiplexing ability	• Time consuming • Complex instruments prone to problems with the microfluidics system • Lengthy and complex sample preparation • Warm-up, laser	Dubeau-Laramée et al., 2014 Shi et al., 2013

		calibration and cleaning for each use	
Implantable biosensors GlucoWizzard™	• Extreme miniaturization for implantation with needle-based device • Robust sensing element designed for accurate analyte quantification • Long life-time	• Invasiveness • Still at an early stage of development	Kotanen and Guiseppi-Elie, 2010

Highlights

- Advances in biosensors to monitor astronauts' health during long-duration space missions.
- Perspectives of new format biosensors in autonomous space clinics.
- A biosensor for non-invasive diagnosis of space-related diseases used aboard the ISS during VITA mission was presented.
- Benefits of space exploration for Earth-based medicine.

Body fluids shift, heart atrophy, hematological alterations

Electrocardiography
Ultrasound examination
Cardiorespiratory fitness evaluation
Blood pressure and hemodynamic measurements
Intraocular pressure measurement
Blood biomarkers of cardiovascular disease, impaired glucose metabolism and inflammation

Cellular and humoral immune response impairment, latent viruses reactivation

Blood immunophenotype analyses
T-cell function evaluation
Blood cytokine levels

Kidney stones and acute urinary retention

Ultrasonography examination
Urine biomarkers (e.g. calcium, uric acid, calcium oxalate)
Urine pH

Muscles atrophy

Electromyography
Muscle strength measurements
Bioimpedance
Muscle biopsy

Cephalic fluids shift, neurovestibular impairment, vision alterations, cognitive and behavioral alterations

Neuroimaging analyses
Measurement of intracranial pressure
Evaluation of ocular structure and function
Blood stress hormone levels
Cognitive performance tests

Exposure to ionizing radiations

Cytogenic assays (e.g. dicentric chromosome assay, chromosome translocation assay)
Blood microRNAs
Gene expression profiling

Gut microbiome alteration, reduced appetite and gastrointestinal motility

Gastrointestinal motility measurements by breath tests or ultrasonography
Blood level of gastrointestinal peptides (e.g. ghrelin, leptin)

Bone demineralization

Bone densitometry
Blood/urine calcium levels
Urine uric acid and sodium urate
Blood biomarkers of bone and calcium metabolism (e.g. bone-specific alkaline phosphatase, osteocalcin) and of collagen degradation (e.g. N-telopeptide deoxypyridinoline)

