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NANOBIOSENSORS: TOWARDS REAL-TIME HUMAN MONITORING IN AEROSPACE MEDICINE AND OTHER EXTREME CONDITIONS

MSc Thesis

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1. Acknowledgements

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2. Abbreviations

AI	Artificial Intelligence
ANNs	Artificial Neural Networks
ASI	Italian Space Agency
CL	Chemiluminescence
CMOS	Complementary metal–oxide–semiconductor
CNTFET	Carbon nanotube field-effect transistor
CNTs	Carbon nanotubes
CQDs	Carbon quantum dots
DHEA-S	Dehydroepiandrosterone 3-sulfate
DLR	German Aerospace Center
DMI	DNA Medicine Institute
EGFET	Extended gate field effect transistor
EKT	National Documentation Centre of Greece
EMG	Electromyography
ESA	European Space Agency
EVA	Extra-vehicular activity
FADGDH	Flavin adenine dinucleotide-dependent glucose dehydrogenase
FDA	Food and Drugs Administration
G-LOC	G-force loss of consciousness
ICU	Intensive Care Unit
IEEE	Institute of Electrical and Electronics Engineers
IoT	Internet of Things
ISF	Interstitial fluid
ISFET	Ion-sensitive field-effect transistor
ITDM	Insulin-treated diabetes mellitus
IUPAC	International Union of Pure and Applied Chemistry
LDH	Lactate dehydrogenase
LFIA	Lateral Flow Immunoassay
LOC	Lab-on-a-chip

LOCAD-PTS	Lab-on-a-chip application development – portable test system
LOD	Limit of detection
LSPR	Localized surface plasmon resonance
Mbps	Mega-bit per second
MEMS	Micro-electromechanical systems
MIT	Massachusetts Institute of Technology
ML	Machine learning
MOF	Metal-organic framework
MOSFET	Metal oxide semiconductor field-effect transistor
MWCNTs	Multiwalled carbon nanotubes
NASA	National Aeronautics and Space Administration
NEMS	Nano-electromechanical systems
NFC	Near-field communication
NIRS	Near-infrared spectroscopy
NKUA	National and Kapodistrian University of Athens
NNI	National Nanotechnology Initiative
PAN	Personal Area Network
PCRAM	Phase-change random access memory
PDMS	Polydimethylsiloxane
pH	Potential of Hydrogen
PLGA	Polylactic-co-glycolic acid
POC	Point of care
PQQ-GDH	Pyrroloquinoline quinone glucose dehydrogenase
PVA	Polyvinylalcohol
QDs	Quantum dots
QoS	Quality of Service
RBCs	Red blood cells
RFID	Radio-frequency identification
r-HEALTH	Reusable Handheld Electrolyte and Lab Technology for Humans
RT-qPCR	Reverse-transcription quantitative polymerase chain reaction
SANS	Spaceflight associated neuro-ocular syndrome
SEE	Single-event effect
SET	Single-event transient
SOI	Silicon on insulator
SWCNTs	Single-walled carbon nanotubes
TFT	Thin film transistor
TID	Total ionizing dose
TNT	Trinitrotoluene
TRL	Technology readiness level
USB	Universal Serial Bus
WAIBNs	Wearable AI-biosensor Networks
WBAN	Wireless Body Area Network
WPAN	Wireless Personal Area Network

3. Abstract

Biosensors are promising tools for achieving point-of-care, real-time, human health, and performance monitoring. Nanotechnology can catalyze the process of biosensors miniaturization or can be used for inventing whole-new types of biosensors. The development of nanobiosensors, along with the maturation of artificial intelligence and Internet-of-Things applications, can inaugurate a new era for in-situ predictive diagnostics, telemedical practice, and general scientific understanding. This potential is of particular interest for medical fields responsible to ensure human health, safety, and performance in extreme environments, with utmost example: manned spaceflight and planets habitation. This review focuses on biosensing approaches in space, but extends further to biosensing applications in aviation, military, and sports, as other situations of extreme environmental conditions for the human body. Lastly, some miscellaneous types of nanobiosensors are mentioned, in order to provide an insight of the potential that future biosensing systems hold. Hopefully, this work will encourage nanobiosensor developers to work closely with the end-users, so that quality-by-design can be achieved, and thus the full potential of this next-generation technology can be harvested.

3.1. Keywords

Nanomedicine, Nanotechnology, Biosensors, Space medicine, Aviation medicine, Military medicine, Sports medicine, Extreme conditions, Artificial Intelligence, Internet of Things, Predictive diagnostics, Real-time monitoring, Physiology

4. Introduction

Information acquiring and communication of information (i.e. speech) are considered the comparative advantage of human beings over the rest of the intelligent biological species on Earth. Information is also the essential part for correct disease diagnosis and treatment in healthcare. Since the birth of medicine, scientists strive to create and advance methods for acquiring information about the human body. Today, there are fascinating pieces of technology for diagnosis in clinical laboratories. However, there is still a gap of information-acquiring capability when it comes to cases of dynamic parameters inherently related to the time continuum. These cases are especially present in situations where humans do not dictate their surrounding conditions – as in a stable and safe clinic –, but their behavior is rather dictated by the environment. In such cases, humans can regain control through decisions and countermeasures defined by the real-time information that they can acquire both for the environment and for their body.

It is known that the natural method of acquiring information in a human being are its senses (i.e. taste, sight, touch, smell, and sound). However, humans have created artificial systems that can "sense" a wider range of input information and can thus, enhance the overall perception capability. As technology advances, these "sensors" have growing practicality. They can be small enough to be carried wherever a human goes, they can acquire information continuously and therefore, be like sensory organs additional and comparable to the natural ones. This is among others what novel technologies (like nanotechnology) are aiming for, as it will be described further in the text. In this context, there will be an introduction to basic principles of biosensors, nanotechnology, and nanomaterials.

After being acquired, the information needs to be processed and communicated, in order to fulfill its worthiness. Therefore, there is also need for additional "information factories" besides the human brain. Hence, a brief description of the ideas of artificial intelligence (AI) and wireless communication systems is included in the text, all of these being the basis of the upcoming era of the Internet of Things (IoT).

Having these concepts in mind, this work discusses their application in extreme conditions for the human body. The current article is mainly focused on the support of human health during spaceflight and describes a collection of biosensing tools designed for that purpose, following their evolutionary stages throughout the years of human activity in space. However, biosensing approaches in other medically-concerning extreme environments are also discussed, such as aviation, military, and sports, as well as in clinical settings – where there is always space for improvement too –.

The information-compilation that is summed in this review article is expected to bring together the needs for human health, safety, and performance in extreme environments with the tremendous potential solutions that novel technologies (e.g. nanobiosensors) can provide. This mutual needs-solutions feedback can be the fertile ground for guiding technological development at the right direction.

5. Methods

The current work is a narrative review article. Therefore, it relied on thorough, but not necessarily exhaustive, search on scientific websites throughout the worldwide web. These websites comprise of scientific databases, journals, libraries, etc.

Some examples of databases that were used for scientific literature search are Web of Science, PubMed, Scopus, Google Scholar, Science Direct and Microsoft Academic. There were plenty of search terms that were used in various combinations, so that the maximum quantity of data was obtained in relation to the thesis subject. Such terms included: nanobiosensors, biosensors, health monitoring, WBAN, nanotechnology, medicine, predictive diagnostics, remote, extreme conditions, space, aerospace, aviation, astronauts, pilots, sports, physiology, human, wearables, implantables, IoT, planetary exploration, long-term, microgravity, telemedicine, theragnostics, military, operations, LOC, microfluidics, ICU, environment, etc.

Apart from the databases, specific scientific journals were also of great value, such as the "Biosensors and Bioelectronics" journal, the "Nanomedicine" journal and the "Aerospace Medicine and Human Performance" journal, formerly named as "Aviation, Space, and Environmental Medicine".

Furthermore, the literature search was significantly enriched by the online websites of organizations, agencies and institutions such as NASA (National Aeronautics and Space Administration), ESA (European Space Agency), the German Aerospace Center (DLR), the National Documentation Centre of Greece (EKT) and the Center of Aviation Medicine of the Hellenic Air Force.

There were no strict selection criteria for the found articles to be included, except for their relation to the subject. However, there was strong preference for more recent publications, as the subject evolves rapidly with plethora of new proof-of-concepts and technological upgrades every year, which in many cases solve problems that may be critical for the prospect of a medical application as the promising new "gold standard".

The outcome of the above described literature search was roughly one thousand articles, aiming to be used as a pool of scientific knowledge for the writing part of the thesis. All the

corresponding full texts were downloaded and stored on a reference manager software (Mendeley).

Although the thesis is a narrative review, which required a great deal of personal critical thinking for the selection of the scientific information to be written, the author's personal view is constrained in most of the text, except for the discussion part.

6. Theory

6.1. Biosensors

6.1.1. Definition

A sensor is a tool which detects (senses) raw information from a specific sample or a whole system and transforms it into an analytically useful signal.

Biosensors are a subcategory of sensors and are defined by the International Union of Pure and Applied Chemistry (IUPAC) as "devices that use specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals". However, biosensors can be more generally defined as tools which analyze analytes of biological interest regarding its quantity, structure, bio-composition or function and generate corresponding signals which can be read by the human user.[1] The biological element of the biosensor may derive either from a specific integrated compound of the device or from the analyte itself. However, it may not necessarily be an organic bio-compound (i.e. protein, nucleotides, microorganisms etc.) but also inorganic elements that can be related to a biological system (i.e. blood minerals, body motion, food toxicants, radiation, etc.). The biosensors are useful in many fields, such as medicine, environmental monitoring, food industry, defense, and sports.

6.1.2. General components

1. Analyte: The target substance of interest to be analyzed. An example of analyte is potassium, when the biosensor is built aiming to measure the concentration of potassium in a sample.

2. Bioreceptor: The part of the biosensor which interacts with the analyte and recognizes its presence (bio-recognition). Enzymes and antibodies are some very common examples of bioreceptors used in biosensors. Upon bio-recognition a measurable change is spontaneously generated in various forms (i.e. alterations of pH, temperature, mass etc.) depending on the interaction type of each specific biosensor. This measurable change is then processed by the transducer.

3. Transducer: Is the compound of the biosensor responsible for the process known as signalization, which is the conversion of one form of energy into another, usually electrical or optical.

4. Electronics: In this part of the biosensor, a suitably designed electronic circuitry amplifies the signal and converts it from analogue to digital form (signal conditioning).

5. Display: Is the final form of the processed information which is rendered on an interface that can be read and understood by a human. Examples of such interfaces may be a computer display, a smartphone screen or printed paper. Regarding the information displayed, it may be in the form of numbers, graphs, tables, or images, always according to the optimal convenience of the user.

6.1.3. Historical background

The Table 1 provides a brief description of the history of biosensors.[2]

Year	Milestones of scientific and technological progress
1906	M. Cremer found that the concentration of an acid in a liquid is proportional to the resulting electric potential between opposite located parts of the liquid.[3]
1909	Søren Peder Lauritz Sørensen introduced the concept of pH.[4]
1922	W.S. Hughes developed an electrode for pH measurements.[5]
1956	Leland C. Clark, Jr., the “father of biosensors”, invented the “Clark electrode” for oxygen detection.[6]
1962	Leland C. Clark, Jr. presented an amperometric enzyme electrode for glucose measurements.[7]
1969	Guilbault and Montalvo, Jr. invented the first potentiometric biosensor.[8]
1970	Bergveld invented the ion-sensitive field-effect transistor (ISFET).[9]
1973	Mindt and Racine developed the first lactate biosensor.[10]
1975	Yellow Spring Instruments (YSI) produced the first commercial biosensor, which measured blood glucose.[11]
1975	Lubbers and Opitz invented a fiber-optic biosensor for detection of oxygen and carbon dioxide.[12]

1977	Suzuki et al. developed the first microorganism-based immunosensor.[13]
1982	Schultz made a fiber-optic biosensor for detection of glucose.[14]
1983	Liedberg et al. developed a Surface Plasmon Resonance (SPR) immunosensor.[15]
1984	Cass et al. developed the first amperometric ferrocene-mediated enzyme electrode for glucose measurements.[16]
1992	i-STAT developed a handheld blood biosensor.[12]

Table 1. Historical background of biosensors

6.1.4. Basic features

- Limit of detection (LOD): is a term used to describe the minimum amount of the analyte that can be detected in a sample by the biosensor.
- Analytical sensitivity: is a term used to describe how small changes can be detected in the amount of the analyte by the biosensor.
- Resolution: is the smallest unit of measurement that can be displayed by the sensor.
- Selectivity: is the ability of the biosensor to detect only the target substance in samples that also contain other substances, admixtures, or contaminants.
- Linearity: is the term used to describe if the variable of the analyte is proportionate (linear) to the output signal (the mathematical formulation of y=mc would be depicted as a straight line in a graph, where y is the output signal, c is the concentration of the analyte and m is the sensitivity of the biosensor). It is notable that linearity may be limited to a certain range of values of the variable of the analyte, which is called “linear range”.
- Reproducibility: is the ability of the biosensor to produce the same results in repeated identical experiments.
- Stability: is the term used to describe that a biosensor is able to produce the same results under different conditions, which may be extreme temperature, pH, radiation, etc. Durability over time is also a factor of stability. This feature is especially important for biosensors used for long-term continuous monitoring.

6.1.5. Types

Biosensors can be classified to several different types according to the differentiating factor. A common classification of biosensors is technology-oriented and derives from the types of their components, meaning the bioreceptor, the transducer or the output signal. Some types of bioreceptors are antibodies (immunosensors), proteins (i.e. metabotropic, ionotropic biosensors), artificial proteins, enzymes, affinity binding receptors, nucleic acids (i.e. DNA-aptamer biosensors) and whole cells (i.e. microbial biosensors). Transducers may contain different types of transistors – such as MOSFETs (Metal Oxide Semiconductor Field Effect Transistors), ISFETs (Ion Sensitive Field Effect Transistors), EGFETs (Extended Gate Field Effect Transistors) and TFTs (Thin Film Transistors) - and the signal transduction may be electrochemical (i.e. amperometric, colorimetric, potentiometric, impedimetric biosensors), calorimetric, optical (i.e. fluorescence, bio-/chemiluminescence, Raman scattering, Surface Plasmon Resonance etc.), magnetic, or mass sensitive (i.e. surface acoustic wave biosensors, cantilever biosensors, etc.).[17]

Except for the purely technological features, biosensors can be divided to several other subcategories. Depending on the timescale of their function biosensors can be divided to two main types: biosensors for “long-term monitoring” and for “single-use”. Another division of biosensors could accrue from their different power sources. The two most common subdivisions are the “battery-powered” and the “self-powered”. The latter may use a variety of technological approaches, by which they can exploit energy that exists readily in their environment, such as the kinetic energy of human body movement when the biosensor is wearable. Furthermore, there are many different scientific fields that have realized the usefulness of biosensors and are already using them in several levels, from research to commercial products. The result is equal number of subcategories of biosensors depending on their purpose, such as medical biosensors (healthcare), environmental biosensors, food biosensors as well as biosensors used in the aerospace industry, the military sector and even in sports. Lastly, medical biosensors may be applied in different ways on the human body. Thus there might be biosensors that are handheld, wearable – meaning that they come to external contact with the skin much like clothes, watches, glasses, or embedded in a uniform – or

implantable – that is they need to be inserted in the human body via some surgical intervention –.

All the above variations are not exhaustive and can all be interconnected to a vast number of possible combinations depending on their use and optimization. However, an effort to describe the aforementioned classifications is depicted in Table 2.

Differentiating factor	Examples of variations
Bioreceptor	Antibodies, proteins, enzymes, artificial proteins, affinity binding receptors, nucleic acids, whole cells
Transducer	MOSFET, EGFET, ISFET, TFT
Signal transduction	Electrochemical, calorimetric, optical, magnetic, mass sensitive
Function timescale	Single-shot, long-term monitoring
Power source	Battery-powered, self-powered
Field of use	Healthcare, environment, food, aerospace, defense, sports
Human body location	Hand-held, wearable, implantable

Table 2. Classifications of biosensors

6.2. Nanotechnology

6.2.1. The vision

Nanotechnology has been holding many promises for the future of mankind since it was first introduced by Richard Feynman in his inspirational lecture "There is plenty of room in the bottom" in 1959. It has brought groundbreaking advances in most technological fields. All these unexplored special properties of the nanomaterials offered a giant pool of brand-new ideas ready to be tested for reconsideration of almost all technological products that were already in use. Could there be advanced products in means of effectiveness, production costs, or safety? Or even further, could there be totally new products that could be invented through the new solutions that nanotechnology offered? This excitement passed quickly from mere scientific curiosity to wide commercial and governmental funding in various projects around the world. And although many problems appeared and continue to appear in the way, such as nanomaterials' side-effects, environmental risks, or ethical and legal issues, there is remarkable effort for finding efficient solutions. During this scientific struggle, nanotechnology

is rapidly evolving and scientists from the National Nanotechnology Initiative (NNI) in 2007 made a plan of expected evolutionary progress as described below:

1. First generation (2000) - Passive nanostructures: These products are characterized by steady function and are already available in the market. Examples are coatings, nanoparticles, nanowires, and bulk nanostructured materials.
2. Second generation (2005) - Active nanostructures: The function of these products is evolving and many of them have already known commercial success. Some examples of this generation are transistors, amplifiers, sensors, targeted drugs, etc.
3. Third generation (2010) – Nanosystems: The main characteristic of these generation is that the aforementioned nano-products can be synthesized to create systems made of thousands of interactive gears that can work together. This generation is still under experimental development, but there is already progress in subfields such as bio-assembly, nanorobotics, nano-networks, and multiscale architectures.
4. Fourth generation (2015) – Molecular nanosystems: This generation describes the ideal technology of nanosystems, whereby the subunits of the system will be single molecules that will have special functionality according to their heterogeneity and special structure. Of course, it is apparent that more time is needed for technology to mature enough to this level of evolution.[18]

6.2.2. Basic definitions

The term “nanotechnology” describes the technology which is conducted within the nanoscale and more precisely within 1-100 nanometers. The various products of nanotechnology are called nanomaterials in general and nanoparticles are the basic components of a nanomaterial. Nanoparticles have special traits mostly due to their size, as the basic principles of macroscopic physics (i.e. Newton’s laws) begin to coexist with the laws of quantum physics. Thus, many materials with certain known properties in their bulk form may exhibit surprising new features when in nanoparticle form. Moreover, nanoparticles can behave in even more different ways depending on the final shape of the nanomaterial that they make up. There is a wide variety of shapes of nanomaterials developed so far, that can be classified according to the number of their dimensions which exceed the typical threshold of the 100 nanometers. Therefore, there

are nanoparticles with zero dimensions (quantum dots), one dimension (i.e. nanorods, nanotubes, etc.), two dimensions (i.e. nanosheets) and three dimensions (i.e. nanopores).[19, 20]

6.2.3. Nanotechnology in electronics and biosensing

6.2.3.1. Miniaturization - Nanofabrication

Along with the progress of electronics comes the progress of biosensors too. Electronic systems tend to become smaller by time – micro- to nano-electromechanical systems (MEMS to NEMS) – as an indirect result of the evolution of transistors, where the aim is to fit as many transistors in a chip as possible, in order to maximize processing power. This has led electronics and biosensors into the nanoscale. Thus, new manufacturing and fabrication techniques are needed. This is where nanotechnology plays its part by introducing tools and techniques both for top-down (e.g. electron beam lithography, focused ion beam nanolithography, nanoimprint lithography, etc.) and for bottom-up (e.g. single atom manipulation, self-assembly, etc.) manufacturing.[19,21]

6.2.3.2. Nanomaterials in nanobiosensors

Secondly, the addition of special nanomaterials as biosensor components is another intriguing contribution of nanotechnology because of their controllable attributes, the high surface to volume ratio, the advanced electric conductivity, etc. More specifically, some examples of nanomaterials and their advantages in biosensing are listed below:

- Carbon nanotubes (CNTs) are allotropes of carbon in the form of hollow cylinders made of rolled-up graphene sheets (carbon layers of width of a single carbon atom). They exhibit better capability in enzyme loading, aspect ratios, functionalization, and electrical communication.
- Quantum dots (QDs) are 0-dimensioned semiconductor nanocrystals which contain unique properties, such as exceptional fluorescence performance, quantum properties and tunability of band energy.
- Nanowires are 1-dimensioned nanostructures in the form of a wire with unconstrained length, but with width constrained to the nanoscale (0-100 nm) and can be made of various materials (metals, semiconducting materials, etc.). They are proven extremely

valuable regarding the properties of manufacturing versatility, electrical conductivity, as well as chemical and biological sensitivity.

- Nanorods are another type of 1-dimensioned nanoparticles and, similarly to nanowires, they can be made of a variety of materials too. One common difference between nanorods and nanowires is that the first usually have an aspect ratio (length/width) between 1 to 22. Nanorods perform well as plasmonic materials in sensing, are highly tunable in size and shape and can be effectively employed in MEMS/NEMS.[22]

6.2.3.3. Nanozymes

Another interesting addition of nanotechnology to biosensing are the nanozymes. The term describes nanostructured products with enzyme like functions, that can be otherwise called artificial enzymes. Since the origins of the term "enzyme" – coined by Wilhelm Kube in 1877 – the scientific community very quickly saw their extraordinary potential awarding the 1897 discovery of cell-free fermentation by Eduard Buchner with Nobel prize in 1907. And the leaps of science came by an ever-growing speed after the crystallization of urease by James Batcheller Sumner in 1926. In 1965 cyclodextrin compounds were first included in products aiming to mimic an enzyme and therefore, the term "artificial enzyme" made its appearance by Ronald Breslow in 1970. The first contribution of nanotechnology in artificial enzymes is recorded in 1993 with the DNA cleavage, induced by fullerene derivatives, which led to the coinage of the term "nanozymes" by Scrimin, Pasquato et.al in 2004. A more detailed perspective of the scientific timeline in the field of nanozymes is available online: http://weilab.nju.edu.cn/research/nanozymetimeline.html.

Nanozymes are gaining ground in biosensing, because they may offer solutions in existing problems in the use of natural enzymes. Specifically, they can be more stable, multi-functional, can be produced at lower cost and more massively. However, their most important advantage is the freedom of molecular design, deriving from the development of molecular imprinting techniques. Thus, instead of being constrained to the specific functionality of a natural enzyme, laboratories are able to produce artificial enzymes, optimized for the exact purpose of use (rational design). Several types of nanozymes have been successfully developed so far and a general perspective is given in Table 3. Nonetheless, it should be noted that the complexity of

such systems requires remarkable scientific research before a product can be really optimized and ready for use. [23]

Natural enzyme mimics	Material used
Peroxidase	Iron, vanadium, noble metals, carbon, metal-organic frameworks (MOF), copper, etc.
Oxidase	Gold, copper, molybdenum, platinum, etc.
Catalase	Lead, platinum, palladium, metal oxides, etc.
Superoxide dismutase	Carbon, cerium, melanin
Hydrolase	Carbon, gold, MOF

Table 3. Types of nanozymes. The first column depicts the natural enzyme that is imitated. The second column shows the main materials of which the nanozyme may consist.

6.3. Towards Internet of Things – A system monitoring approach

There is an ever-growing concept of creating connections between different, independent information sources (sensing devices), so that their data can be fused together and produce more possible outcomes through their correlation. Ideally, technology should be able to produce a single all-in-one device which could serve for all possible uses. However, this is rather utopic at the current moment. At the time being, most technological products (i.e. biosensors) are developed to undertake a single or a limited range of tasks by which they were inspired in the first place. And although technological advancements may come, which will enable the production of more sophisticated and multi-purposed devices, which will amass more and more utilities, most certainly they will still need to be connected to other devices as well. In terms of biosensing, it is evident that the more types of interconnected biosensors, the more complete perception of existing information will come as a result. Furthermore, the decision-making system which would be fed by the gathered information would become more accurate. Given all the above, there is strong reason in building networks that contain the various types of biosensors.

In the specific case of the continuous monitoring of health and safety parameters, a network should provide information for the following general aspects:

- The physiological status of the human of interest (Physiological sensors). In that, dynamic physiological parameters like vital signs (i.e. breath rate, heart rate, blood pressure, oxygen saturation, body temperature), blood glucose concentration, electrolytes concentrations, blood cortisol levels, immune status etc. could be measured continuously (or in short time intervals) and altogether produce packages of physiological status information: The physiological cluster.
- The human actions most prominently expressed through body movements (Biokinetic sensors). There is already much progress in the field of sports and fitness in general, where recording factors of body mobility throughout an event (i.e. football match) or a fitness workout program is of high importance. But except for sports, body movements could be interpreted in the actions that they serve, which could be actions of a pilot flying an aircraft, or a soldier marching in the battlefield, or just a worker in an industrial area where supervision would be a matter of safety. As a result, there would be a full map of a human's decisions and actions, which could be connected with the temporal dynamic changes of the physiological status as mentioned above.[24]
- The environment with which a human interacts (Ambient / environmental sensors), i.e. ambient temperature, humidity, incoming radiation, air/water composition etc. It is evident that in most cases a human does not have the choice of a stable and safe environment. On the contrary, the human will have to adapt his/her decisions and actions according to the uncontrollable environmental changes. However, a crucial part of this process is that the human apprehends these environmental changes in time. And for that part, the biological developed human senses may not be enough. More environmental parameters of interest can be monitored through sensors which can thus complement the human perception.[25]

In other words, there could be a human-centered "system monitoring" approach, where all that is needed is the human's passive status (physiological status), the active status (biokinetics) and the surrounding environment (ambience). Interestingly, this could be

achievable even with the lack of an "all-in-one" technology. A close to perfect solution could be given by the currently existing wireless technologies, which in combination with artificial intelligence would introduce each field (i.e. healthcare, defense, space travel, sports, etc.) to the Internet of Things – defined as a system of interconnected devices able to process and transmit data over a network without the need for human intervention –.[26]

6.3.1. Wireless communication systems

Technology has offered a wide variety of solutions of wireless connections to choose depending on the optimal usage of the interconnected nodes. From a spatial radius point of view, there are many categories of networks spanning from the nanoscale to global or even interplanetary networks. Concerning health and safety monitoring, the most relevant network protocols types are subclasses of the Personal Area Network (PAN) protocols, coded as IEEE 802.15, which entails connections between electronic devices oriented to serve an individual person's workspace. The Wireless Personal Area Network (WPAN) is normally functional in a radius of up to a few meters. Of course, WPANs can be interconnected with other WPANs and even further beyond WPANs to the internet, transitioning to wider networks along the process, such as Wireless Local Area Networks (WLANs) – IEEE 802.11. Applications of WPANs include telemedicine, biofeedback, ambient-assisted living, etc.[24,27,28]

6.3.1.1. Bluetooth – IEEE 802.15.1

Bluetooth is a technology for relatively short radius device communication with a maximum data rate of 3 Mbps (Mega-bits per second). Bluetooth Low Energy (BLE) is a more power-efficient variation of Bluetooth with decreased data rate (up to 1 Mbps), aiming for connecting smaller devices with lower power capacities. It is especially suitable for health monitoring devices.

6.3.1.2. ZigBee – IEEE 802.15.4

ZigBee is a type of network with even more power saving capability. However, the low data rate (up to 250 Kbps) is not enough for real-time health monitoring, but it would be rather suitable for distinct measurements.

6.3.1.3. Wireless Body Area Network (WBAN) – IEEE 802.15.6

WBAN is a versatile type of network aiming to satisfy the correspondingly versatile needs for human body measurements, both medical and non-medical ones. It is functionable in several different bandwidths for data transmission, in order to serve this versatility. WBAN standard also offers remarkable flexibility in terms of data rate (up to 10 Mbps), power consumption, range of function, number of nodes (256 per body network), nodes prioritization and security schemes. [28]

6.3.2. Artificial Intelligence (AI)

When so many sensors collect data continuously and transmit it over a network, the problem of data management comes forth. In fact, a more suitable term for this kind of data is "big data", which is quite fashionable at the moment, as the need for information of higher quality grows. Technology is progressing with leaps and bounds on the field of processors, as according to Moore's Law, the number of transistors on integrated circuits doubles every two years. Therefore, computers possess a stunningly increasing processing power capable of handling more and more data and in better time performance from generation to generation. Thus, as far as hardware is concerned, the problem of "big data" has already found its solution.

What about software? This is the Artificial Intelligence (AI) plays its part. First of all, what exactly is AI? It describes generally any kind of intelligence performed by an artificial product (i.e. machines), as opposed to humans and animals which exhibit "natural intelligence". The term AI was first mentioned by John McCarthy at a lecture in Dartmouth College in 1956 and has grown to a distinct scientific field along with the development of software engineering and informatics. AI has already facilitated many processes that previously necessitated the intervention of a human mind and has made its way in many sections of the human society, such as at work, in every-day routines, and even in state authorities. Concerning biosensor networks and especially continuous monitoring, the role of AI would be to handle the tremendous size of produced data, so that it can be useful and interpretable by the human user. For that purpose, the technology of "machine learning" has been developed.[29]

6.3.2.1. Machine learning

Machine learning (ML) can be defined as a wide spectrum of methods, techniques and evaluation of algorithms that aim at learning automatically through experience gained from data – called "training data" – to extract useful information or predictive models of a phenomenon or even make decisions without being specifically programmed to do so by a human.

In general, ML has two main aspects of roles:

- Decreasing data quantity (towards less need for power supply and memory storage of AI-biosensors)
- Increasing data quality (i.e. data consistency, monitoring accuracy, reliability)

There is a wide variety of methods to conduct machine learning, but they could be classified in two large categories:

- Supervised learning. In this type of machine learning, both the input and the output data of the training data set are predefined by a human (labeled data). Thus, after the computer has "understood" and "memorized" the desirable pattern, it can apply it in new raw data and provide information based on that pattern. Examples of methods of supervised learning are active learning, classification, and regression.
- Unsupervised learning. This is a more sophisticated type of machine learning, where the output of the training data set is not predefined by a human (unlabeled data). Instead, the computer is expected to create patterns of the training data on its own through processes like clustering, anomaly detection and dimensionality reduction. This is particularly useful in measurements of data that is too complex for a human to classify and computers take advantage of their processing power to work on more demanding algorithms (like Artificial Neural Networks – ANNs) and in multiple layers (deep learning). However, it is evident that these processes require much larger quantities of training data as well as more energy supply.[30]

7. Results

7.1. Biosensing in Aerospace medicine

Aerospace medicine is a unified term which includes both the fields of space medicine and aviation medicine. Each scientific field will be handled separately.

7.1.1. Space medicine

Space medicine is a field of medicine concerned with the health of humans during spaceflight, that is astronauts, or tomorrow's space tourists. Although it may seem like a subfield of medicine, it includes many more aspects in addition to medicine. Firstly, of course there is the clinical aspect, meaning disease prevention and treatment. Secondly, there is the human performance and functionality, that must be retained under any circumstances and variations of environmental parameters. Thirdly, space medicine contributes to systems engineering (e.g. spacesuits) aiming for human health and safety support.[31]

7.1.1.1. Environmental challenges of spaceflight

7.1.1.1.1. Microgravity

Beginning from the second and most busy aspect of space medicine, it is critical to underline the environmental challenges of spaceflight for the human body. In space, there are three main changes compared to the terrestrial environment that cannot be substituted by some form of technology yet. The first one is microgravity. This term is used to describe the almost zero gravitational forces that are applied on an object when the latter is far enough from celestial bodies (i.e. planets, suns, etc.). Microgravity exists also in the International Space Station (ISS), which is in low Earth orbit (LEO) and the distance from Earth is not far enough to eliminate Earth's gravitational force – in fact it is roughly 90% strong compared to Earth's surface. However, due to the cyclical motion of the ISS (as in orbit), the astronauts in ISS are in free-fall state and experience the weightlessness sensation and the same effects of microgravity. The effects of microgravity are most critical in fluidics, where hydrostatic pressure, buoyancy and sedimentation vanish, but diffusion, cohesion, adhesion, and surface tension gain significance instead.

7.1.1.1.2. Radiation

The second main difference of space environment is the increased exposure to radiation. Normally, the most dangerous part of incoming radiation on Earth is trapped by its magnetic field or filtered by its atmosphere. If an object is found outside of the protective features of Earth, it is vulnerable to solar radiation, i.e. solar energetic particles (SEP) consisting of protons, electrons, X-rays, alpha and gamma rays, etc., and to galactic cosmic radiation (GCR), which mainly refers to high-energy protons and heavy atomic nuclei. Moreover, Earth's magnetic field traps radiating energetic charged particles, thus creating the Van Allen radiating belts, which can pose an additional threat to objects or humans that pass through. Radiation can cause damage both to biological tissues and artificial structures, especially electronic systems. Although there is considerable effort for improving radiation shielding in spacecrafts, an astronaut on the ISS accumulate on average approximately 75mSv of radiation over a period of 6 months.

7.1.1.1.3. The spacecraft environment

A third challenge in spaceflight is living in a spacecraft, which is an artificial and confined environment. The ISS is about as large as two commercial airplanes, which is quite convenient for six people on board. However, a long-term stay in such a confined place can prove to be quite challenging, both for the musculoskeletal system – due to lack of physically demanding opportunities – and for the psychological status. Moreover, the fact that the crew is socially isolated and away from their beloved ones might promote behavioral changes at the expense of teamwork performance and even personal motivation.

7.1.1.1.4. Spaceflight environmental effects on the human body

All the above environmental changes are enough for provoking a series of effects in human homeostasis, physiology and nosology and most of them correspond to the thus coined term "space adaptation syndrome". Conversely, there are is also the "entry adaptation syndrome", which includes the effects taking place post-spaceflight due to Earth's gravity. Some of the mostly affected systems are, the cardiovascular, the musculoskeletal, the neurological and the immune system. The Table 4 provides a concise summary of these effects.[32,33,34,35]

System affected	Effect	Time frame
Cardiovascular system[36,37]	Altered blood distribution (Cephalad fluid shift)	First days in space until adaptation
	Puffy face – bird leg syndrome	First days in space until adaptation
	Decreased plasma volume (extravasation)	Long-term effect of spaceflight
	Dilation of cardiac chambers	As long as microgravity exists
	Cardiomyocytes atrophy	Long-term effect of spaceflight
	Vascular remodeling	Long-term effect of spaceflight
	Decreased production of red blood cells (RBCs)	Long-term effect of spaceflight
	Orthostatic intolerance	First 24 hours upon return to Earth (gravity)
Respiratory system[38]	Functional differences with no observed loss in respiratory efficiency	As long as microgravity exists
Musculoskeletal system[39,40]	Bone loss	Long-term effect of spaceflight
	Skeletal muscle atrophy	Long-term effect of spaceflight
Endocrine system[41]	Circadian rhythm desynchronization	Long-term effect of spaceflight
	Deteriorated sleep quality	Long-term effect of spaceflight
Mentality[42,43]	Social/Behavioral changes	Long-term effect of spaceflight

Neurological system[44,45,46,47,48]	Space motion sickness (vestibular disorientation)	First days in space until adaptation
	Diminished hearing, taste, and olfaction (due to puffy face)	First days in space until adaptation
	Diminished proprioception	First days in space until adaptation
	Increased intracranial pressure	Long-term effect of spaceflight
	Spaceflight associated neuro-ocular syndrome (SANS)	Long-term effect of spaceflight
Urinary system[49]	Increased tendency for kidney stones formation	Long-term effect of spaceflight
	Risk of urinary retention	First days in space until adaptation
Immune system[50,51]	Increased pro-inflammatory molecules	Long-term effect of spaceflight
	Opportunistic infections (e.g. latent viruses, pre-flight respiratory infections, etc.)	Long-term effect of spaceflight
	Altered microbiome	Long-term effect of spaceflight
	Increased carcinogenicity	Long-term effect of spaceflight
	Disturbed wound healing	Long-term effect of spaceflight

Table 4. Effects of spaceflight in the human body

7.1.1.1.5. Occasions of increased risk in spaceflight

Apart from the yet inevitable and constant exposure to the aforementioned parameters, space missions hold many more recorded risky situations and even more unrecorded that still lie in the unknown. Extra-vehicular activity (EVA) is a very demanding task for an astronaut with much preparation needed but is nonetheless a frequent practice. Throughout EVA an astronaut gets exposed to thermal stress and to risks such as micrometeoroids and space decompression sickness. The spacecraft itself and its systems may pose occupational hazards through possible failures, such as contaminated drinking water, toxic leaks (e.g. hydrazine), or other emergency situations such as fire or sudden cabin depressurization. Earth re-entry and landing are also a procedure with increased risks, although it is highly standardized for optimal safety. Danger is expected to be even higher when speaking of foreign planets exploration, as there are already lessons learned from the manned missions on the Moon, where lunar dust proved to be quite toxic upon exposure. Lastly, trauma risk is always present and should not be neglected.

7.1.1.2. The clinical challenges of spaceflight

With all these risks of space been noted, the usual medical risks of a human organism remain to be added to the total-risk equation. A common manned mission in the ISS lasts 6 months. Planned manned missions to Mars expect a spaceflight duration of approximately 30 months. These time frames are large enough for a healthy person to develop various type of disease that could temporarily reduce his/her performance, need medical support, or even threaten his/her life. In LEO missions, there may be some solutions facilitated by the proximity to Earth, which however cannot cover very acute incidents. Such a solution is telemedicine, which near Earth does not suffer from unacceptable communication latency. Additionally, drugs can be frequently renewed, so that they do not lose their efficacy due to chronic radiation exposure. And lastly, medical evacuation could be a solution, as there is readiness level of a few hours. Unfortunately, these solutions are not available for farther missions and thus the confrontation of such risks becomes even more challenging. A concise overview of the possible medical conditions that can be encountered during space travel is provided in Table 5.

Common / anticipated medical conditions	Occasional medical conditions	Possible medical conditions during long-term space travel/exploration	Unaddressed medical conditions
Space motion sickness	Renal stone formation	Radiation sickness	Cardiogenic shock
Nasal / sinus congestion	Acute urinary retention	Anaphylaxis	Malignancy
Constipation	Heart dysrhythmias	Osteoporosis	Acute glaucoma
Headache	Urinary tract infection	Seizure	Compartment syndrome
Upper respiratory tract infection	Near drowning (spacesuit failure)	Severe decompression sickness	Head injury
Minor abrasion	Serous otitis media	Limb amputation (lifesaving)	Hypovolemic shock
Minor Musculo-skeletal trauma	Minor decompression sickness (bends)	Chest trauma / pneumothorax	Shoulder / elbow dislocation
Corneal irritation	Contact dermatitis	Obstructed airway	Lumbar spine fracture
Insomnia	Gastroenteritis	Hemorrhage	
	Aspiration of foreign body	Burns (thermal / chemical)	
		Smoke inhalation	
		Diverticulitis	
		Appendicitis	
		Sepsis	
		Herpes reactivation	
		Cellulitis	
		Otitis	
		Dental problems	
		Eye penetration	
		Anxiety	
		Depression	
		Medication misuse	

Table 5. Spaceflight medical conditions list based on the work of P. D. Hodkinson et.al.[31]

7.1.1.3. Fostering countermeasures for crew health and safety

All the above being said, it is obvious why building countermeasures for health and safety during spaceflight is of paramount importance. They should also guarantee the high performance level of astronauts not only in LEO missions but also for long duration exploratory missions (e.g. manned mission to Mars). In that direction, since the beginning of space age, there has been remarkable progress in risks mitigation.[52] Currently, scientists strive to create valid tools able to pre-assess the risks of space missions[53] and accordingly pre-arrange the suitable medical resources and equipment needed for each mission[54] – everything loaded on a spacecraft must be chosen very wisely due to the limited available space –. There is also effort so that medical events management during spaceflight can be improved through mastering technical and non-technical skills by the crew members.[55]

7.1.1.3.1. Utilizing technological innovations as countermeasures

Furthermore, the novel technological advances in biosensors, wireless technologies, and miniaturized electronics have enabled the fostering of new perspectives in space medicine, such as the idea of point-of-care (POC) diagnostics and real-time monitoring. POC is the capability of performing a test directly where it is needed instead of a laboratory and thus, it is most valuable for healthcare in remote environments, especially in space travel. Real-time monitoring is expected to be a breakthrough in the whole medical way of thinking, as it has the potential to add a tremendous amount of knowledge and understanding of human physiology and even detect correlations to environmental triggers. This fact will bring new standards in preventive medicine – through predictive diagnostics –, as well as in timely disease diagnosis and treatment – through disease progress monitoring –. In space medicine, an additional aspect of great value of real-time monitoring is the continuous information about the space crew's performance status. Thus, there are increasing scientific attempts to adapt these technologies to the requirements of spaceflight equipment. [56, 57]

7.1.2. Biosensing in Space medicine

7.1.2.1. Introduction to Nanotechnology in space science

7.1.2.1.1. Nanotechnology and the space science paradigm

Nanotechnology has the potential to play an integral and catalytic role for accomplishing biosensing during spaceflight. It incorporates the appropriate features for the space science paradigm "better-stronger-lighter-cheaper". Beginning from the "lighter" part, nanoelectronic devices especially – thanks to miniaturization – can promise lower mass and lower power consumption – which is directly translated to longer functional duration before needing recharge –. It is noteworthy that uplifting of 1kg to LEO costs about 25,000USD given the current space-transport technology. The promise of nanotechnology can also answer to the "better" part of the paradigm by offering devices of improved performance in terms of output accuracy, function stability, reliability, and all these in shorter time periods. The "stronger" part of the paradigm can be satisfied too. Nanotechnological products can be manufactured in a variety of methods, so that the exact demands of each purpose are met, e.g. material durability, flexibility, tensile strength, radiation resistance, etc.[58] Concerning the "cheaper" part of the paradigm, most nanotechnological products are yet in primary steps of development and thus, the costs per unit is not comparable to products that are already under large-scale production. However, it should be a matter of time until large nanotechnological production lines are developed.[59]

7.1.2.1.2. Nanotechnology and TRL scale

The capability of nanotechnological mass production has a direct relation to the so-called technology readiness level (TRL) scale that is used by space organizations, when it comes to efforts for developing and integrating new technologies in space missions. Of course, nanotechnology must first face the production challenges for the general market. But in the meantime, there is remarkable funding of nanotechnological projects for space applications. The common truth is that a product needs approximately 10 years of validity testing before being fully integrated to space missions or even more for replacing an older type of technology. Thus, it is not surprising that when a product is fully accepted in space missions, it may be 10 years behind the state-of-the-art technology. At the current moment, most nanotechnological

products, especially nanobiosensors, are still under early development stages and ranked in the levels 3-4 in the TRL scale (see Table 6). Of course, when it comes to more sophisticated and complex interconnecting systems – especially to already established space systems –, the optimization process may be extremely demanding.[59]

TRL	Definition
1	Basic principles observed and reported.
2	Technology concept and/or application formulated.
3	Analytical and experimental critical function and/or characteristic proof of concept.
4	Component and/or breadboard validation in laboratory environment.
5	Component and/or breadboard validation in relevant environment.
6	System/sub-system model or prototype demonstration in an operational environment.
7	System prototype demonstration in an operational environment.
8	Actual system completed and "flight qualified" through test and demonstration.
9	Actual system flight proven through successful mission operations.

Table 6. Technology Readiness Levels (TRLs) as defined by NASA (https://www.nasa.gov/directorates/heo/scan/engineering/technology/txt_accordion1.html)

7.1.2.1.3. Nanotechnology and space conditions

7.1.2.1.3.1. Nanotechnology and microgravity

Microgravity may provoke changes in the functionality and structure of nanomaterials and nanodevices. Micro- and nano-fluidic devices are expected to be influenced (e.g. bubbles formation) in the extend that fluid mixing proceeds without the phenomena of buoyancy, convection, and sedimentation. Diffusion, osmotic pressure, and electrostatic forces are rather prevalent in microgravity conditions. Thus, electrochemical interactions may also be affected, as the reagents may interact differently compared to Earth conditions. Accordingly, microgravity has already recorded effects in nanomaterial bottom-up manufacturing, especially for self-assembly processes. These alterations may seem problematic due to unpredictability of untested nanoproducts in space conditions, but there is also optimism that new manufacturing solutions and capabilities may be provided by the exact same differences

of a microgravity environment. Of course, thorough research is needed for understanding all aspects of nanomaterials behavior in space conditions.[60,61]

7.1.2.1.3.2. Nanotechnology and space radiation

Radiation can affect all types of materials and these effects are of varying severity depending on the quantity and quality of radiation exposure as well as the nature of the radiated material or device. Because this review focuses on nanobiosensors, it is worthy to examine in short, the effects of radiation in nanoelectronics. Electronic devices, especially memory and logic systems, are susceptible to damage from radiation exposure. There are three major types of radiation effects in electronics:

- Single-event effect (SEE) are sudden incidents of exposure to instant radiation which can inflict transient errors or failures of electronic devices, loss of memory data, or even total destruction of the exposed device. Although such radiation incidents happen also to devices on Earth's surface, space electronics must be protected against SEE. A damaged device on Earth can be fixed or replaced, but such an option is not always available in space. Moreover, an electronic failure at the wrong moment may cost a whole space mission's success.
- Total ionizing dose (TID) is the overall accumulated ionizing dose that a material receives. Thus, its effects are rather long-term, and the main problem is the decreased longevity of electronic devices in long-term space missions. However, mostly TID concerns unmanned missions, because the lethal dose for a human is much lower than the damaging dose for most common electronic devices.

The problem of radiation is becoming more worrying because many scientists fear that radiation susceptibility of electronic circuits will increase as they decrease in size. This worry is most intense since electronical circuits have shrunk to the smallest dimensions of the nanoscale (<100nm). Thus, there is a lot of effort on developing technological solutions for radiation protection, i.e. radiation hardening. Radiation hardening is divided into two major categories: physical, and logical hardening. The first category utilizes physical methods, such as adding radio-resistant materials to the circuit (e.g. Silicon on insulator – SOI technology).

The second category utilizes logical pathways as solutions, by integrating additional circuits that can have roles of error correcting, damage detecting, circuit saving, etc.

7.1.2.1.3.2.1. Potential solutions for improved radiation tolerance

SOI technologies would be an attractive solution for space missions, since SOI devices have been proven superior against SEEs compared to other technologies. In contrast, SOI devices are more vulnerable to TID, because their special structure tends to trap the ionizing radiation inside the device. However, the most worrying part is that SOI technologies have not been thoroughly tested on nanocircuits yet in relation to radiation tolerance.[62]

Conventional solid-state memory devices are proven to be susceptible to radiation damage. CNT field -effect transistors (CNTFETs) are a new generation of transistors based on carbon nanotechnology and their logic-gate designs have exhibited better resistance to single-event transients (SETs – are subspecies of SEEs) than silicon MOSFET- based designs.

Phase-change random access memory (PCRAM) is a type of memory, which does not involve charge transport and is thus immune to radiation damage. Conventionally, PCRAMs are made of chalcogenide materials and memory is stored in forms of material phases. An advanced technology of PCRAMs includes nanowires and has proven its superiority concerning the programming current and the input power needed.[63,64]

Bearing all the above in mind, it certainly is not in vain to test nanodevices in space missions. Of course, it will take time and extensive series of tests under several different conditions, until the right materials, structures and manufacturing processes are selected for the nanoproduct to exhibit the optimal resistance to radiation. However, this optimization is a step forward from what scientists face at the current moment. First, nanobiosensors must prove that they are worthy of integrating in space missions from a utilitarian point of view. What follows next is some examples of diagnostic devices for in-flight astronaut health monitoring, ranging from older technology but already tested products, to newer more sophisticated technological innovations that may yet need further development until they reach the required maturity for space equipment integration.[65]

7.1.2.2. i-STAT

I-STAT (see fig.1) is a handheld, portable blood analyzer developed by Abbot Labs as a commercial POC diagnostic tool. NASA began using i-STAT in the space shuttle missions and is still using it now in the ISS as a primary health monitoring tool for the crew. The device is capable of performing quantification analysis on more than 25 different blood analytes (e.g. electrolytes, glucose, creatinine, hematocrite, hemoglobin, blood gases, etc.) in very small blood samples (<100 μl) on single-use disposable assay cartridges in 120-200 sec per analysis. The sample of the cartridge is driven by capillary forces inside the device, where automatic enzymatic processing produces byproducts that are analyzed electrochemically by ion-sensitive and impedance-sensitive probes. The results are depicted on the integrated screen of the device. A drawback, however, is that there are different types of cartridges, each of which is able to analyze only certain types of analytes and thus, not all available choices of analytes can be analyzed simultaneously. Another disadvantage is the limited shelf time of the cartridges (4-6 months) which would be insufficient for long-duration space missions. Although i-STAT still constitutes a leading technology used by NASA, many scientific groups are trying to apply new technologies (nanotechnology, microfluidics, etc.) on upgrading features of the device, like making the cartridges reusable, or reducing the analysis duration, etc.[66,67]

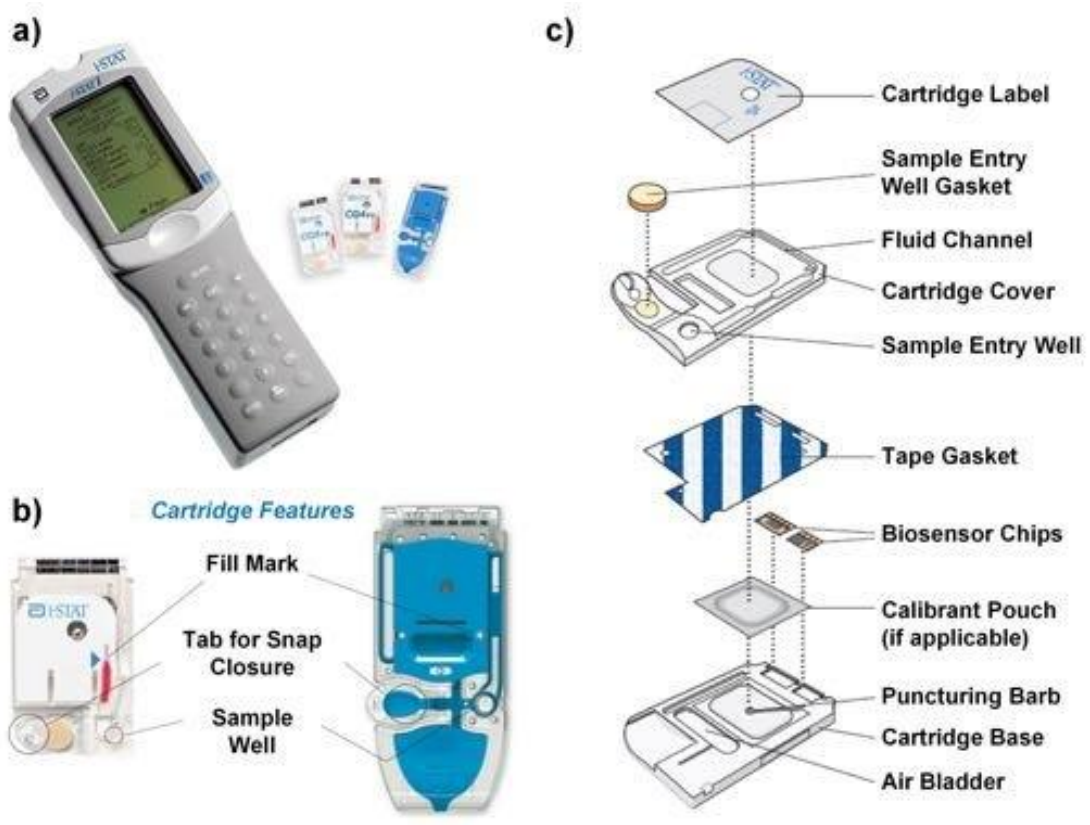

Figure 1. i-STAT system (image by Can Dincer, 2016)[68]

7.1.2.3. Reflotron IV

Reflotron IV (Boerhinger Mannheim) is a benchtop spectroscopic chemical analyzer which was used in "Mir" orbital station (1998) and subsequently in some of the earlier missions on the ISS. It analyzes biological fluid samples on disposable strips loaded with dry agents. This is an advantage of the i-STAT cartridges, because the "dry chemistry" approach of Reflotron IV ensures longer shelf time of its strips. Although Reflotron IV could also process a wide range of blood analytes, its use has been discontinued due to its bulky size, which is impractical for space missions.[69]

7.1.2.4. r-HEALTH sensor

The r-HEALTH (reusable Handheld Electrolyte and Lab Technology for Humans) sensor is a device developed by DNA Medicine Institute (DMI) in collaboration with NASA aiming to be used in long duration space missions. The device is a handheld POC system (size: 8x4x0.5 in^3), able to perform universal biomedical analysis in a single drop of fluid samples, utilizing nanostrip reagents, microfluidics, and high-sensitivity fluorescence optics. It has already been

successfully tested in microgravity conditions and it promises long multi-year reliability of function under spaceflight conditions (radiation, low humidity, lack of refrigeration). However, it is yet limited by the Food and Drugs Administration (FDA) regulation only for research and investigation purposes.[70]

7.1.2.5. LOCAD-PTS

The Lab-On-a-Chip Application Development – Portable Test System (LOCAD-PTS) (see fig.2) is a handheld sensor for environmental (e.g. surfaces, liquids, etc.) colorimetric detection and quantification of endotoxins or microorganism cell-wall components (bio-signatures of bacteria and fungi). It was applied on the ISS for the years 2006-2009 and operated with cartridges with dry reagents. The critical advantage of the system was the lack of need for culture growth and sending specimens back to Earth for analysis. The system also exhibited remarkable sensitivity. However, the inability to detect microorganisms directly was a reason for the LOCAD-PTS to be discontinued.[71]

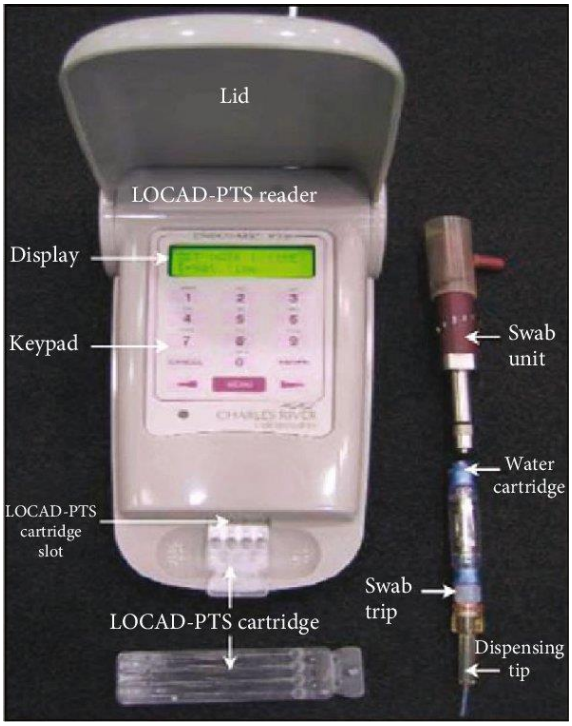

Figure 2. LOCAD-PTS (image by Yanwu Chen et.al, 2020)[72]

7.1.2.6. IMMUNOLAB

IMMUNOLAB (see fig.3) is a device by DLR, deployed on-board the ISS in 2015, aiming to provide reproducible in-flight immunological monitoring of the crew by analyzing bodily fluids (e.g. blood, saliva, urine, etc.). It comprises of a subunit for sample preparation (quality-controlled commercial analysis kits) and a subunit for detection and analysis, which utilizes labeled fluorescent microscopy techniques. The system is easy to handle and is expected to support even long duration space missions.[73]

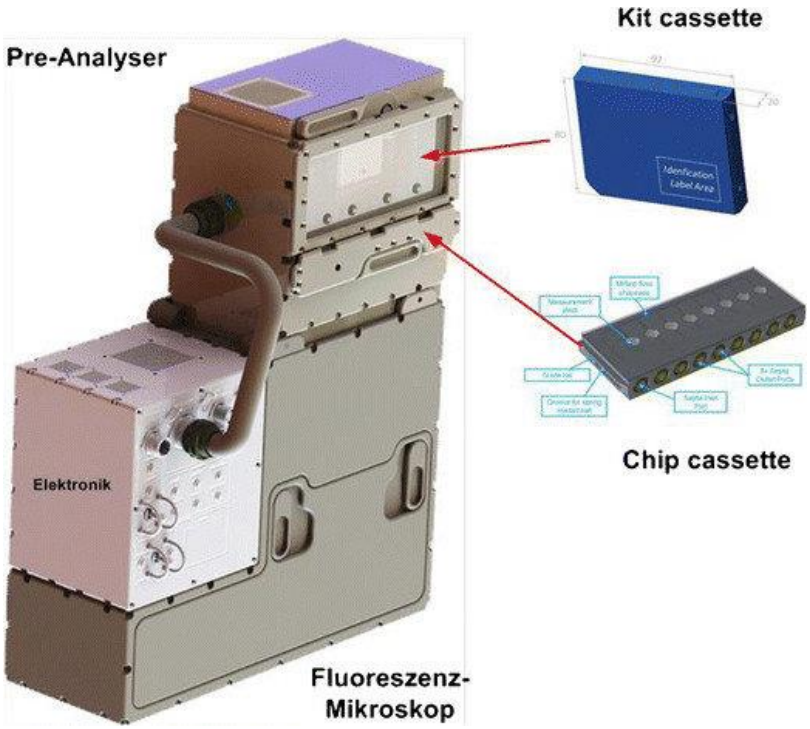

Figure 3. IMMUNOLAB (image by Christian Stenzel, 2016)[73]

7.1.2.7. Wetlab-2

The Wetlab-2 system (see fig.4) is a device based on the commercial product SmartCycler® (Cepheid) and has been deployed on the ISS for on-board quantitative gene expression analysis via real-time reverse-transcription quantitative polymerase chain reaction (RT-qPCR). This could offer an alternative solution for microorganisms detection. It fulfills several space mission requirements, such as simplicity (i.e. no need for warm-up, no routine maintenance, etc.), stable performance under microgravity, fast results (20-40 min), multiplexity (96 sites) and flexibility (the system can be expanded by only plugging an additional USB connection). However, it includes manual handling by the crew (e.g. separate module for sample

preparation, bubbles extraction, etc.) which might be time-consuming, and there is also risk of spacecraft contamination during sample preparation. Upgrading to automatic sample preparation will be critical for the system's further usage in space.[74]

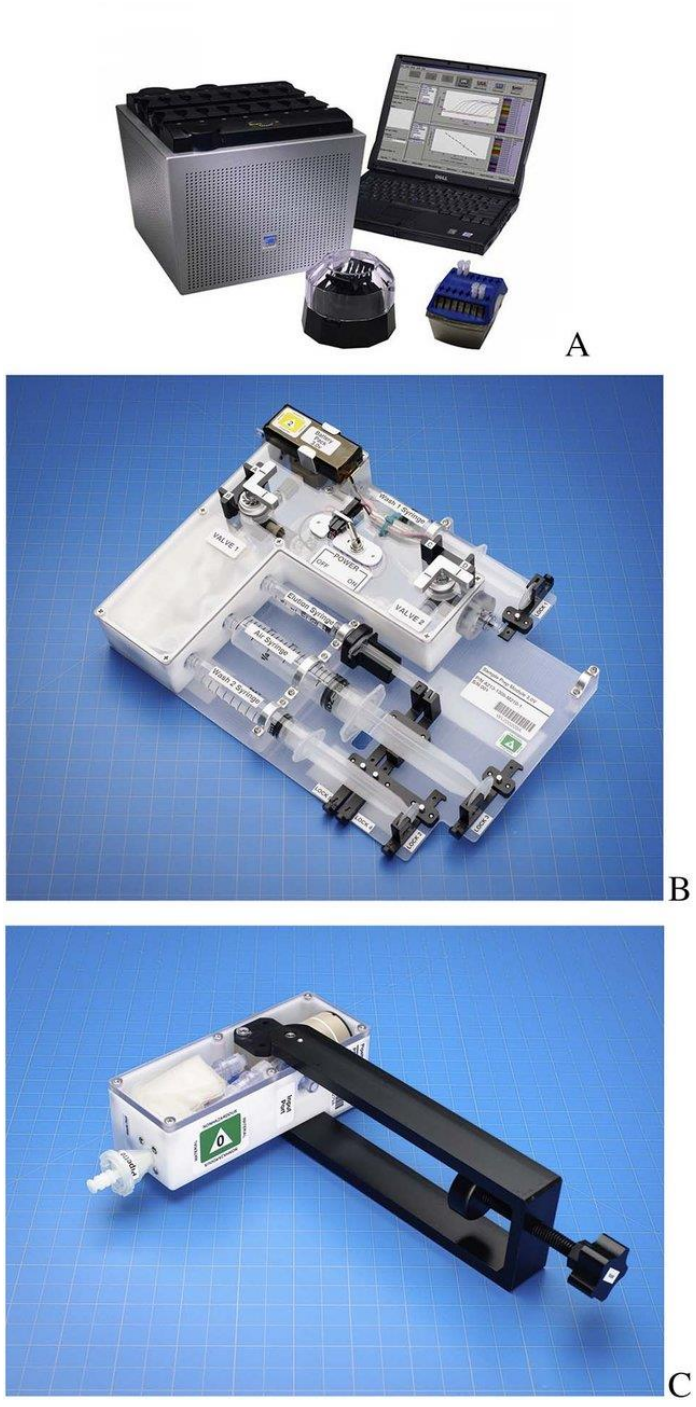

Figure 4. Wetlab-2. A: the SmartCycler® system. B: the sample preparation module. C: De-bubbler and pippete-loading device. (image by Fathi Karouia et.al)[74]

7.1.2.8. Razor EX

The RAZOR EX® (Biofire Defence, Inc.) system (see fig.5) is another platform for RT-qPCR, optimized for in-flight monitoring of microorganisms in microgravity in various sources (e.g. air, water, surfaces, food, etc.). It works on freeze-dried reagent pouches, which may be pre-determined to common pathogens, or customizable. Its mass is 11lbs, performs fast multiple analyses (up to 12 simultaneous analyses in 30 min) and has LOD 100 CFU/mL with <1% error rate. All necessary components are preloaded and lyophilized. The RAZOR EX® was deployed on the ISS in 2016 and is used as part of the Water Monitoring Suite, exhibiting results comparable to the ground-test controls.[74]

Figure 5. RAZOR EX® BioDetection System (image credit: Biofire Defence, Inc.)[74]

7.1.2.9. MinION

The MinION (Oxford Nanopore Technologies) sequencer (see fig.6) is a miniaturized (mass: 86 g, size: 9.5x3.2x1.6 cm) portable POC for non-targeted direct DNA sequencing and is based on nanopore technology. It was successfully tested aboard the ISS on contaminated mixtures for pathogen detection. Moreover, the non-targeted approach of the nanopore technology allows for even unknown biosignatures to be recognized, which could be appealing for research missions of extraterrestrial life as well. A serious disadvantage is the demanding library preparation (up to 4h), which forces DNA samples to be prepared on Earth before using them

to space. A promising solution for this might be the automated sample preparation module VolTRAX (Oxford Nanopore Technologies).[74,75]

Figure 6. Oxford Nanopore MinION sequencer (image credit: Oxford Nanopore)[74]

7.1.2.10. Microflow1

Microflow1 (see fig.7) is a compact (34x19x20 cm), battery-powered, fiber-optic flow cytometer, able to perform immunophenotyping and microbead-based multiplexed cytokine assays in space conditions. The device was successfully tested on the ISS in 2013. NASA has supported projects for further miniaturization and portability upgrades for the device. [76]

Figure 7. Microflow1 demonstrated on the ISS in 2013 (image by https://www.asc-csa.gc.ca/eng/sciences/microflow.asp)

7.1.2.11. Glucowizzard™

Glucowizzard™ (see fig.8) is an implantable miniaturized biosensor (size: 0.5x0.5x5 mm) for wireless continuous interstitial glucose monitoring. It is placed directly under the skin and is using light for power and communication. The biosensor utilizes the enzyme glucose oxidase for electrochemical (amperometric) analysis of the corresponding current. It also includes a coating of thermo-responsive hydrogel compound – polyvinylalcohol (PVA) and polylactic-co-glycolic acid (PLGA) – for controlled drug release (dexamethasone, etc.), aiming to mitigate the foreign-body response of the surrounding tissue. Thus, it can stay under the skin for more than 3 months. It has exhibited good biosensing characteristics: selectivity 99%, sensitivity 35nA/cm^2-mM, LOD 50 µM, no temperature dependence. Moreover, it can monitor the concentration of more analytes except for glucose, such as lactate, glutamate, oxygen, carbon dioxide, etc. This robust miniaturized device has gained NASA's attention and is undergoing tests under space conditions on the ISS since 2016.[65,77]

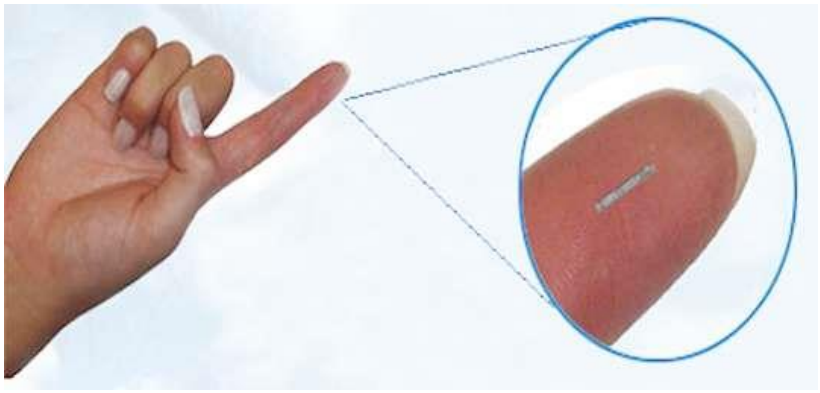

Figure 8. Glucowizzard™ (image by https://www.trendhunter.com/trends/biorasis-glucowizzard)

7.1.2.12. Intelligent Optical Systems (IOS) – Holomic LLC

In 2011 these two companies, in collaboration with NASA, undertook a project to build a portable biosensor based on lateral flow assays and fluorescence, integrated in a smartphone for readout (see fig.9). This is appealing for space missions due to the small size, compactness, portability, microgravity-compatible (since it relies on capillary forces), and cost-effective. The device can perform quantitative measurements of multiple blood biomarkers, such as

troponin, transaminases, glucose, and creatinine and continues testing for further optimization.[65]

Figure 9. Smartphone integrated biosensor by Holomic LLC and Intelligent Optical Systems (image by https://spinoff.nasa.gov/Spinoff2017/hm_3.html)

7.1.2.13. Volatile Organic Analyzer (VOA)

VOA (see fig.10) is a benchtop gas chromatography ion mobility spectrometer (GC-IMS) used for spacecraft air quality monitoring (i.e. oxygen, nitrogen, 23 different volatile organic compounds, etc.) with approximate time-to-results 3.5 hours. It was proven to function well on-board the "Mir" station (1997-1998) and the ISS (2001-2009), but it was a bulky device and its maintenance was demanding.[65]

Figure 10. VOA during in-flight maintenance on the ISS (image by https://spaceflight.nasa.gov/gallery/images/station/crew-12/html/iss012e10233.html)

7.1.2.14. Air Quality Monitor (AQM) - Microanalyzer

AQM Microanalyzer (Draper Laboratory) is a small-sized (25.4x15.2x13.2 cm, 3kg) gas chromatograph-differential mobility spectrometer (GC-DMS) for air quality monitoring on-board the ISS. It is designed to perform analyses on circa 20 volatile organic compounds twice per week and can be controlled by its integrated computer (see fig.11). It began testing on the ISS in 2013 and was validated for operational use in 2014. Soon after, a mass spectrometer was added on the device which enhanced its performance. Although AQM is a great advancement over its predecessor, VOA, scientific groups are trying to provide even further-miniaturized devices for air/water quality monitoring by using technologies like microplasma ionization.[78,79, 80]

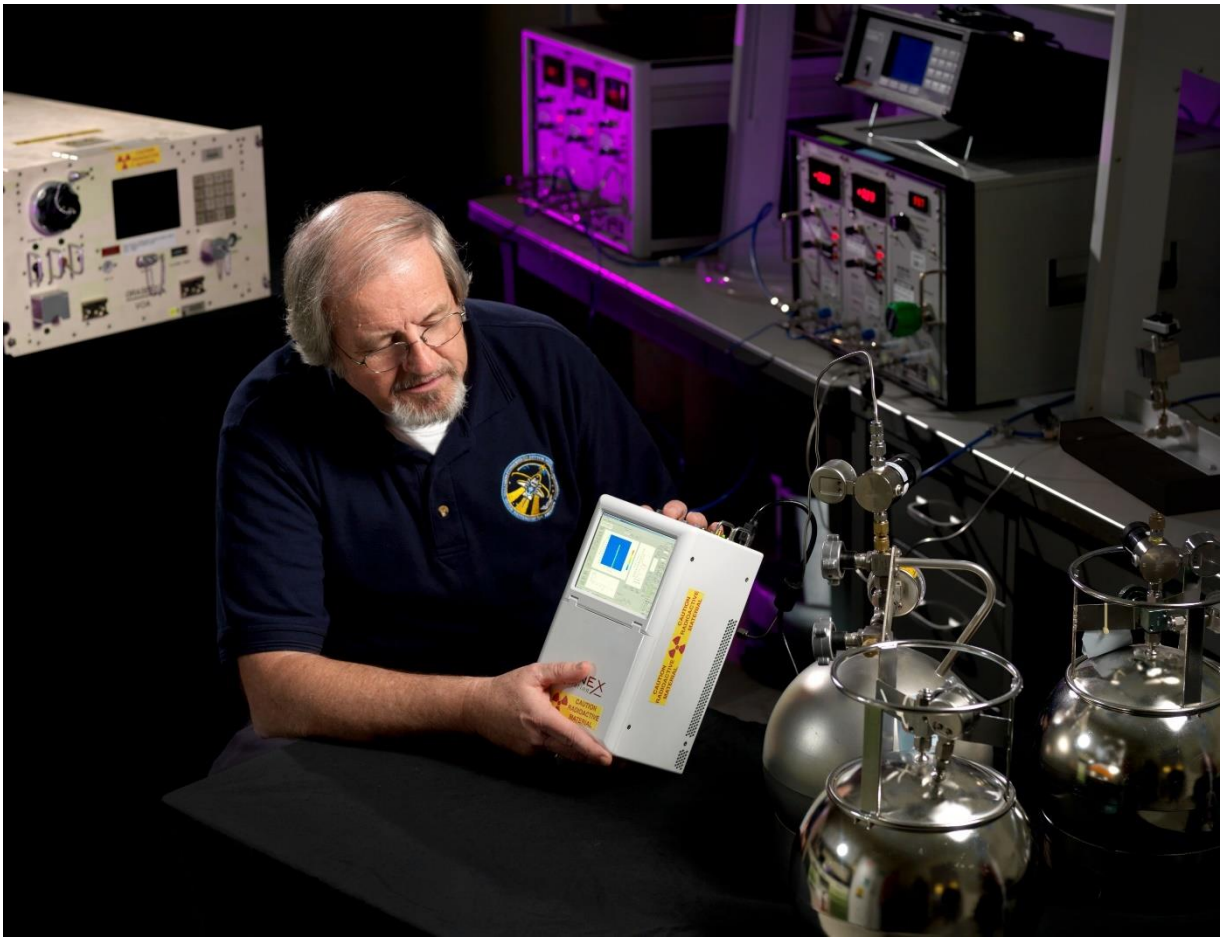

Figure 11. The AQM at the hands of the manager of the Toxicology Office of NASA (image by https://www.nasa.gov/feature/toxicology-and-environmental-chemistry)

7.1.2.15. JPL-ENose and E-Nose

JPL-ENose (Jet Propulsion Laboratory Electronic Nose), is a small-sized (volume 3.6 lt, mass 3.4 kg), low-power consumption, array-based sensing system, able to detect and quantify 11 chemical air substances and was designed for air quality monitoring on the ISS in 2008. The device can produce and transmit date continuously and in real-time. Its polymer-carbon composite sensors have a lifetime expectancy of 18 months.(see fig.12)[81]

E-Nose (see fig.13) is a portable device manufactured by Airsense Analytics GmbH, as a project of DLR, for experimental, in-situ, label-free detection of microbial volatile organic compounds on the Zvezda section of the ISS and was launched in 2012. It includes a sensor array of ten different types of metal oxides and was trained to identify the odor-patterns of the most

common microorganisms inside the ISS. However, more research is needed until the device becomes mature enough for routine utilization.[82]

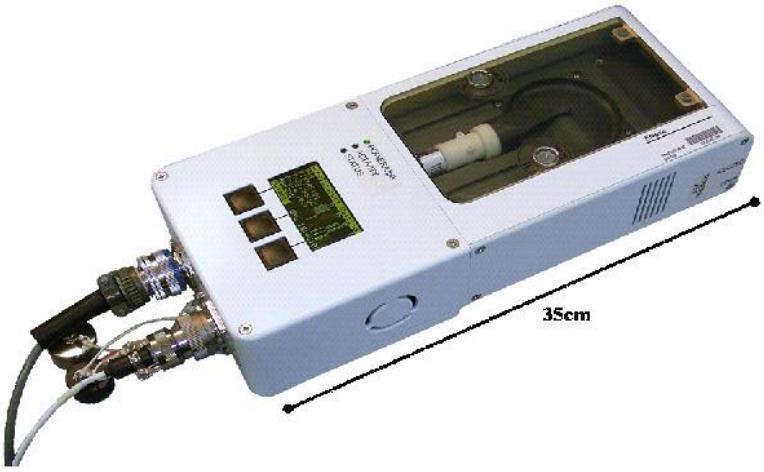

Figure 12. JPL-ENose (image by https://electrochem.jpl.nasa.gov/?page=research-sensors)

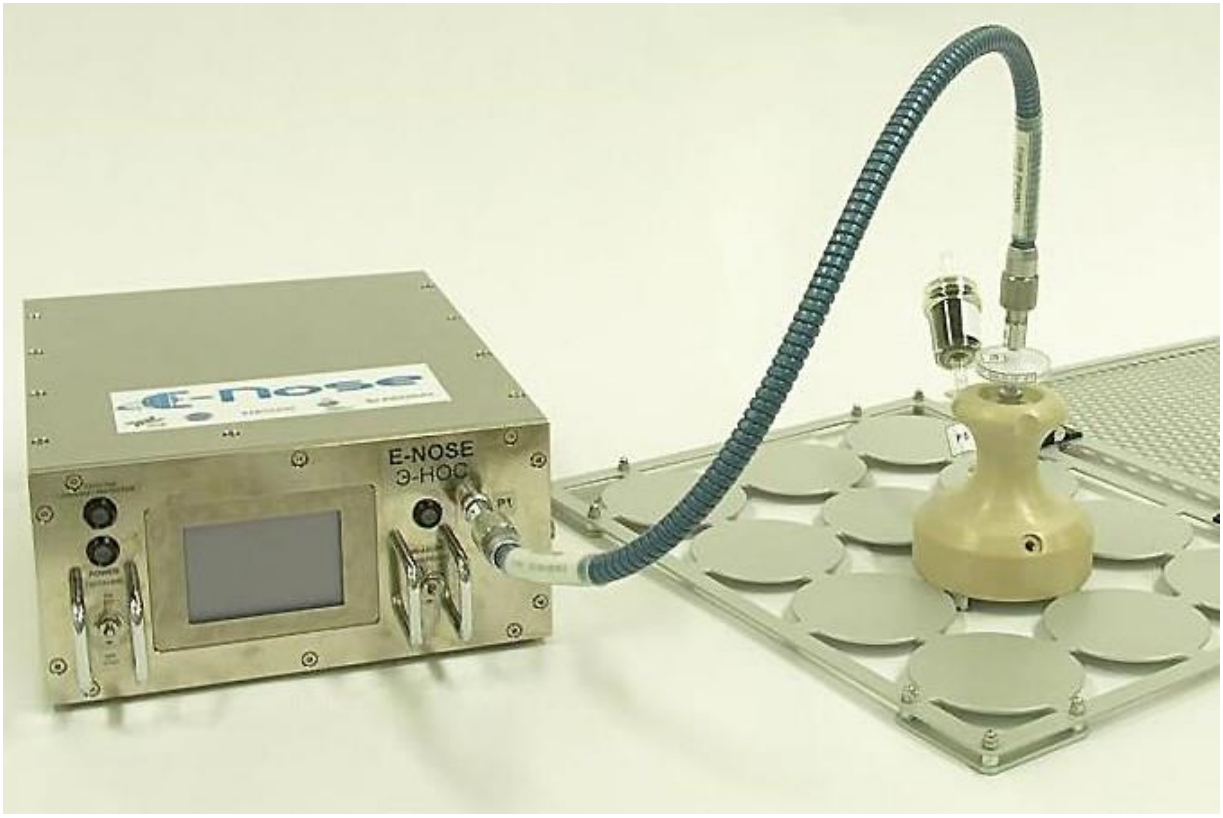

Figure 13. E-Nose (image by http://www.luftfahrt24.de/dlr-auf-auf-der-maks-2017/)

7.1.2.16. Tissue equivalent proportionate counter (TEPC)

TEPCs are dosimeters that can measure both the tissue-absorbed radiation dosage and the quality of radiation. Therefore, they are essential tools for long-term spaceflight health and safety. The TEPC utilizes a gas ionization chamber as a detector made by tissue-like materials. Although older versions of TEPC were bulky and impractical, new generations of micro-dosimeters are developed for low-energy consumption, continuous radiation monitoring that transmit data wirelessly. (see fig.14)[83,84]

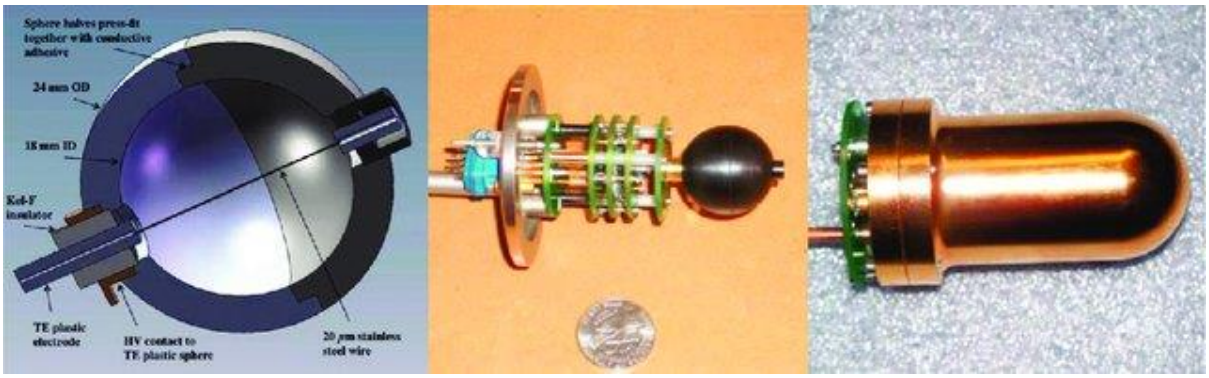

Figure 14. A next-generation TEPC (image by Straume et.al, 2015)[84]

7.1.2.17. Operational Bioinstrumentation System (OBS)

OBS was a real-time astronaut monitoring system, which could perform electrocardiogram (ECG) on the astronauts during body-stressful conditions, such as missile launch and re-entry. The ECGs were either sent to ground-stations in real-time, or they were stored in the systems memory. It has been used by NASA since the space shuttle era.[85]

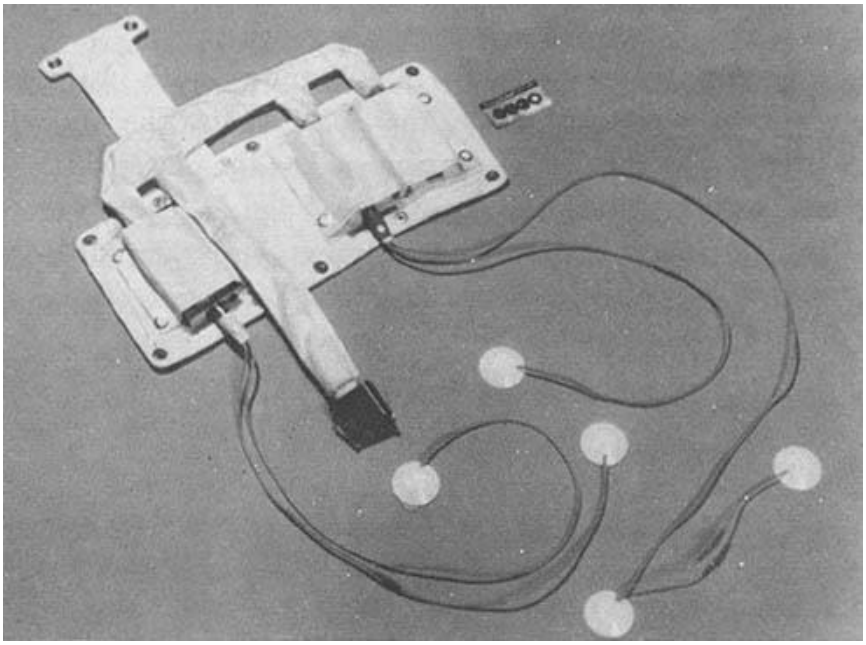

Figure 15. Bioinstrumentation system in the biobelt (image by https://history.nasa.gov/SP-368/s6ch3.htm)

7.1.2.18. LifeGuard

LifeGuard (see fig.16) is a wearable device, developed by NASA Ames Research Center and Stanford University, for real-time monitoring of physical and basic health parameters of astronauts. It consists of a wearable computing system, internal sensors (3-dimensional accelerometers, skin thermometer), external physiological sensors (ECG and respiration electrodes, pulse oximeter, auscultative blood pressure monitor), and a small screen for display. All data are measured and can be transmitted in real time wirelessly (e.g. via BlueTooth) for a maximum battery duration of 8 hours. An upgraded version is under development, aiming to increase performance features and to expand input connections (such as environmental monitoring).[86]

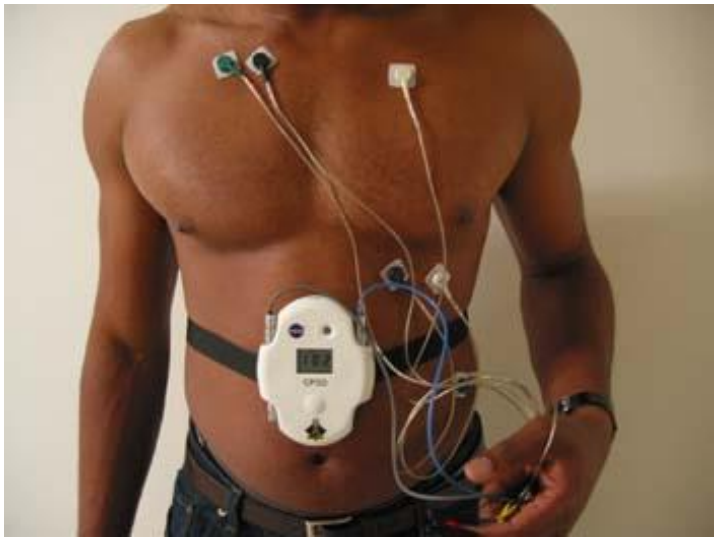

Figure 16. LifeGuard (image by https://www.nasa.gov/centers/ames/news/releases/2004/lifeguard/lifeguard.html)

7.1.2.19. Bio-Suit

The BioSuit (see fig.17 and fig.18) is a concept for the next-generation spacesuit, developed by Massachusetts Institute of Technology (MIT). It is based on the revolutionized idea of mechanically countering the lack of ambient pressure in space by the elastic forces of advanced textile materials applied directly on the skin. Furthermore, the BioSuit is designed to integrate textile sensors for real-time multiparametric monitoring (e.g. vital signs, ECG, physical movement, etc.). An even further upgrade of the BioSuit will be to add actuators that could enhance mobility.[65,87]

Figure 17. MIT student works on BioSuit knee joint (image by https://appel.nasa.gov/2012/01/11/building-the-future-spacesuit/)

Figure 18. Professor Dava models the BioSuit (image by https://appel.nasa.gov/2012/01/11/building-the-future-spacesuit/)

7.1.2.20. IN-SITU Bioanalysis / VITA mission

VITA mission (2017) was a part of "IN-SITU Bioanalysis" project, supported by the Italian Space Agency (ASI), aiming to test the feasibility of on-board the ISS application of a biosensor (see fig.19) for analysis of salivary levels of cortisol. The biosensor is based on Lateral Flow Immunoassay (LFIA) and enzymatic (horseradish peroxidase – HRP) chemiluminescence (CL)

detection and consists of a microfluidic element, the LFIA strip and the CL reader, that is a charged-coupled device (CCD) camera. It has salivary cortisol LOD 4 ng/mL with time-to-results about 15 min and can be connected to an external computer via USB (Universal Serial Bus). It uses 3D-printed disposable cartridges. The device functioned well in space conditions, avoiding microgravity problems (e.g. bubbles formation), tolerating mechanical stress (e.g. vibrations, depressurization, etc.), and taking all the precautions for contaminant leakage by design. Its buffers have an approximate expected longevity of 1 year. Since the biosensor's feasibility was proven, it can be further miniaturized and upgraded for detection of more bioanalytes, the replacement of disposable components with reusable ones, and the expansion of their shelf-lifetime.[88]

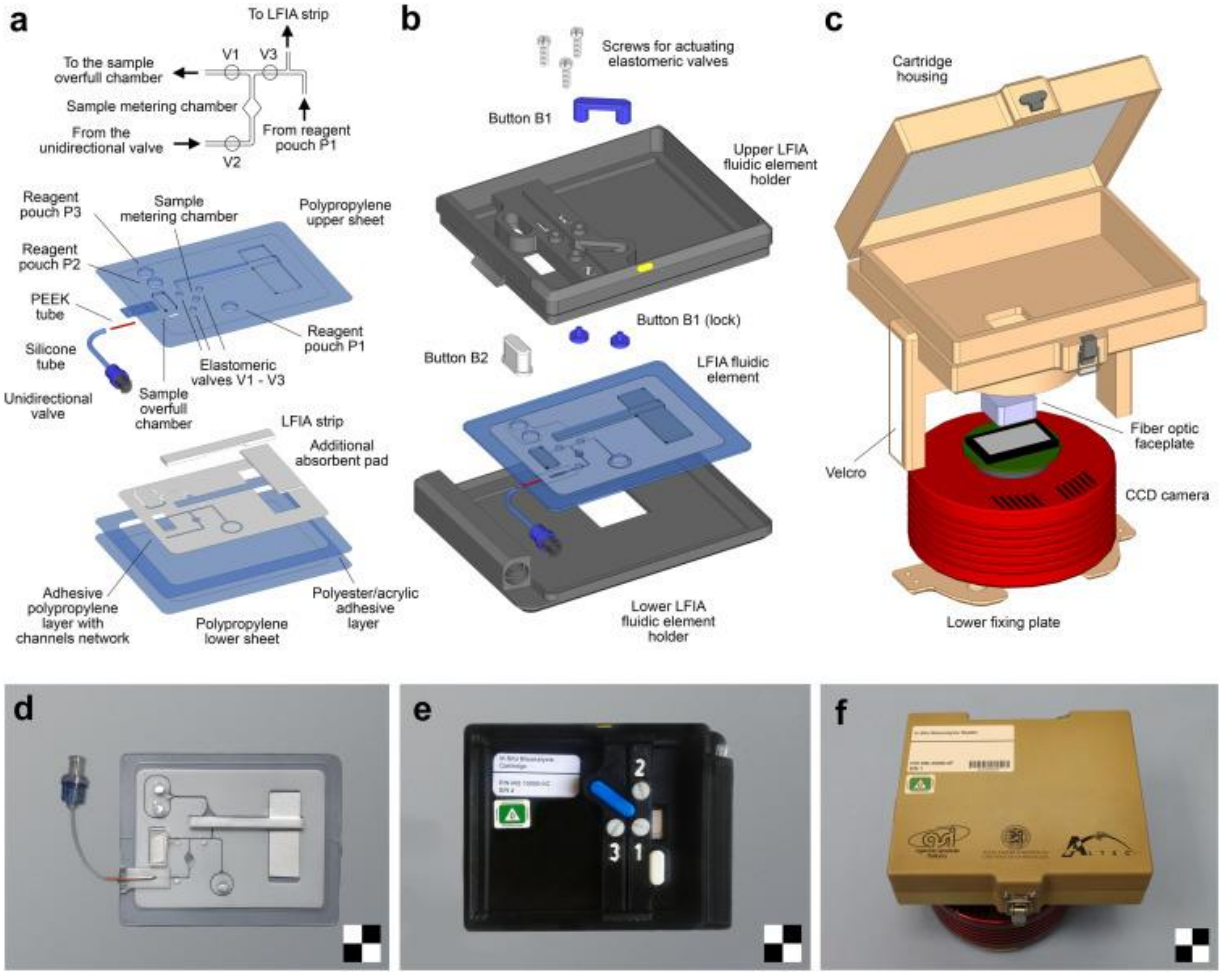

Figure 19. The biosensor of VITA-mission. a,d) fluidic element, b,e) LFIA cartridge, c,f) CL reader. (image by M. Zangheri et.al, 2018)[88]

7.1.2.21. Superhydrophilic–superhydrophobic microchips

A new proof-of-concept study demonstrated superwettable microchips as a platform for microdroplets biosensing aspiring to be used in space conditions. The capillary forces of the superhydrophilic nanodentrici coating of the microwells, along with the superhydrophobic substrates promise to conduct highly sensitive biomarker analyses (e.g. calcium, glucose, etc.) in microgravity conditions eliminating problems, such as bubbles formation. The small size of the device, as well as the designed ease of use (direct naked-eye colorimetric monitoring) could make it an attractive biosensor for development for future space missions.[89]

7.1.2.22. A promising field-effect nanobiosensor for real-time pathogen detection

A preliminary study (Mishra et.al, 2013) developed a radiation tolerant field-effect based nanobiosensor for label-free microorganism detection. The biosensor includes a combination of highly doped polysilicon nanowires and silicon nano-channels and was successful at detecting E. coli genome via impedance measurements almost in real time. The initial results of total-radiation-dose testing were satisfying as well. These facts, along with its small size make it promising for space missions. However, the biosensor still needs more work to reach maturity for in-space utilization.[90]

7.1.2.23. NINscan - Wearable brain imaging with multimodal physiological monitoring

NINscan is a name given to a series of wearable biosensor prototypes (see fig.20) for real-time synchronized multimodal physiological and performance monitoring, based on near-infrared spectroscopy (NIRS). NINscan can provide hemodynamic deep-tissue imaging (diffuse optical tomography – DOT), electroencephalography (EEG), ECG, electromyography (EMG), electrooculography, accelerometry, gyroscopy, blood pressure, respiration rate, body temperature, etc. NINscan has been developed to small dimensions (<500 cm^3, <0.5 kg), can operate continuously for about 24 hours (battery-powered) and has already been tested in microgravity conditions (e.g. parabolic flight). The NINscan prototypes have already achieved remarkable unobtrusiveness, robustness, ease-of-use, and low cost. Thus, they show great promise for multiparametric real-time monitoring of astronauts, as well as in other earth-conditions where close and spherical monitoring could be critically beneficial (e.g. during high-

intensity sports). However, further upgrades are feasible, in terms of higher sensitivity, miniaturization, and advanced computational algorithms.[91]

Figure 20. NINscan application during parabolic flight (image by https://flightopportunities.nasa.gov/technologies/90/)

7.1.2.24. A LED-based sensor for hazardous gases monitoring

A scientific group (Terraciano et.al, 2017) gained proof-of-concept (on a NASA's high-altitude balloon) for a sensor based on absorption spectroscopy and light-emitting diode (LED) technology. The tested prototype was designed to analyze only one substance (carbon dioxide), but after its success, the scientists proposed a multiplex sensor that can be designed to detect more gaseous substances in the same platform. This concept can lead to real-time air quality monitoring, as part of a fire alarm system of a spacecraft, or even embedded on spacesuits, always in small-sized and robust sensors with minimal energy-consumption. However, further research and development are needed.[92]

7.1.2.25. A biosensor for DNA radiation damage

Heger et.al (2016) developed a 3D printed stratospheric probe as a prototype for monitoring DNA radiation-induced damage. The probe utilizes the fluorescent properties of carbon

quantum dots (CQDs), which when in complex with DNA, can offer measurable data for the changes of the DNA. The prototype was tested successfully – it detected the radiation-induced DNA damage of Staphylococcus aureus – under the high ultraviolet (UV) radiation conditions of the stratosphere, managing to retain its functionality intact despite the harsh external conditions (e.g. extreme ambient temperature). This concept would be interesting for long duration space missions, where space radiation can pose a long-term threat to DNA integrity.[93]

7.1.3. Aviation medicine

Aviation medicine is A branch of aerospace medicine which copes with the health and performance of aircraft pilots, referring to either civil passenger flights, or to high-performance flights (e.g. military, aerobatic, etc.). In aviation medicine, the problem of a human living in an isolated and inaccessible environment is not so prominent as in space medicine, because the duration of each flight is only some hours and can be aborted at any time in case of emergency. However, aviation medicine poses extremely high safety standards, aiming to minimize probability of accident to zero rate. Thus, medical concerns for flight safety may derive from chronic medical conditions of the pilots (such as diabetes mellitus), or from drowsiness during a long duration flight. As far as the high-performance flights is concerned, the challenge there is maximizing performance without crossing the human limits. In that case, there are more problems to handle, such as loss of consciousness due to hypoxic events, or due to g-forces (G-LOC), or generally fatigue-deriving performance decrease, that could compromise flight safety.[94] Some concepts for human health and performance monitoring are described below.

7.1.3.1. Biosensing in Aviation medicine

7.1.3.1.1. In-flight glucose monitoring

Pilots with insulin-treated diabetes mellitus (ITDM) could avoid possible hypoglycemic events during flight more efficiently if they had the chance to monitor blood glucose levels continuously. Subcutaneous continuous glucose monitoring (GCM) has been approved by FDA for glucose control. Commercial biosensors, such as Dexcom G5® Mobile (see fig.21) or FreeStyle Libre™ (see fig.22), are able to collect real-time data of interstitial glucose and transmit it wirelessly to external devices, such as smartphones. Because blood glucose takes 10-20 minutes to diffuse to the interstitial space, this latency is corrected via algorithms. Such

devices have already proven their value in clinical settings and at-home self-monitoring, but they should become aeromedically certified before they are put in aeromedical practice.[95]

Figure 21. Dexcom G5® (image by https://www.dexcom.com/en-AU/g5-mobile-australia)

Figure 22. FreeStyle Libre – Abbot Diabetes Care Inc. (image by https://jdrf.org.uk/news/abbott-libre-supply-warning/)

7.1.3.1.2. Preventing hypoxia-driven loss of consciousness

In-flight hypoxic events are quite rare, given that modern airplanes have pressurized cabins and oxygen supply systems. However, there is always the possibility of system's failure, which could result in hypoxia of the crew. Therefore, the pilots' training include training in hypoxia simulation, so that they can distinguish a possible hypoxic state through the memorized symptoms that they might feel. However, it is also possible that they start to lose consciousness before they notice anything. A study (Rice et.al, 2019) utilized dry-EEG real-time monitoring during flight simulations with provoked hypoxic events. The results where that 20-40% of the events, the EEG showed signs of hypoxia without the pilot noticing it. These results signify the potential benefits of developing unobtrusive biosensors for in-flight hypoxia monitoring.[96] In that direction, a scientific group developed a probe for buccal real-time monitoring of blood flow and tissue oxygenation, based on fiberoptic NIRS. The hypoxic events were detected successfully in the initial tests, but further optimization is needed in terms of algorithmical processing and probe fixation.[97]

7.1.3.1.3. Preventing G-LOC

High-performance aircrafts (such as fighter aircrafts and aerobatic aircrafts) are able to do flight maneuvers that produce acceleration forces that exceed the limits of the human body. Therefore, pilots are trained thoroughly in order to control the flight within their limits. However, situations like for example fighter missions often push pilots to their limits and there is increased risk for G-LOC. Since a growing number of physical and physiological biosensing systems becomes available, scientists have started using them in research for better understanding the in-flight g-forces and their body effects.[98] A study, in particular, utilized a commercial off the shelf wearable biosensor, called Zephyr BioPatch™ (see fig.23), which consists of a patch and a biomodule, altogether weighing 33g. The biomodule (with diameter 0.7 cm) is multifunctional (triaxial accelerometry, ECG, respiration rate measurement, thermometer), battery-powered and can transmit data wirelessly. The study showed that in-flight monitoring can alter our understanding of flight physiology, providing proof of pilot adaptation to g-forces.[99] Another scientific group used EMG sensors for in-flight real-time monitoring of the gastrocnemius muscle and developed algorithms warning for upcoming G-

LOC.[100] Biosensors have thus the potential to increase flight safety by enhancing the pilot's situation awareness, along with retaining maximum flight performance.

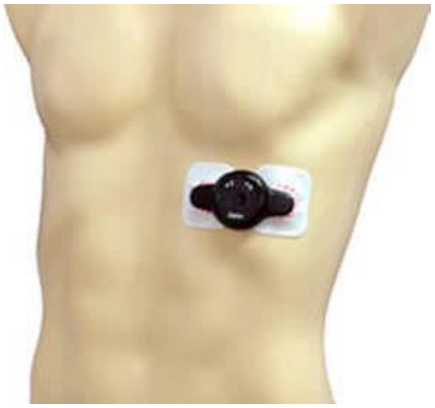

Figure 23. Zephyr BioPatch™ (image by https://healthmanagement.org/products/view/ecg-patient-monitor-wearable-wireless-biopatch-zephyr)

7.2. Other biosensing applications for human monitoring

7.2.1. Biosensing in Military medicine

Military operations are extremely demanding for the human body, both due to external risks (e.g. incoming fire, harsh environmental conditions, etc.) and individual performance limits (e.g. fatigue, psychological stress, etc.). Therefore, it is of paramount importance that the individual soldier is continuously fully aware of these risks (situational awareness). In addition, a military operation almost always relies on teamwork and thus, it is equally critical that all soldiers-members of the team remain operational throughout the mission, or else the mission will most likely fail. For these reasons, the development of technologies that will allow monitoring of real-time operational status of the team members is considered a primary goal for upgrading the combat ability of the armed forces. There are several theoretical monitoring applications that are under development: individual real-time physiological monitoring throughout each mission and detection for upcoming failure due to stress load, timely detection of external threats, casualties detection and management, optimization of individual fitness habits towards operational readiness, long-term damage from exposure to hazards, etc. Despite the existence of many commercially distributed physiological biosensors, most of them can neither provide reliable military-useful information nor are customizable to meet

mission-specific operational needs. Therefore, military biosensors need to be developed from scratch and are thus still in early stages of development.[101] Some examples from recent scientific literature are biosensors for human thermal-work strain optimization[102], compensatory reserve measurement for field care of severely injured soldiers[103], detection of explosive substances (such as trinitrotoluene – TNT)[104], and detection of chemical-biological warfare elements (such as sarin) in the blood.[105] Although there is still much research ahead before military biosensors are perfected, the augmentation of situational awareness that will derive from them will fundamentally change the way of war planning.

7.2.2. Biosensing in Sports medicine

Sports medicine is the field of medicine dealing with the health and body performance of athletes. Unlike aerospace and military medicine, where human performance is a common interest, sports medicine has the privilege of not to be bound by extremely strict and solid safety protocols. This fact along with the continuous race for performance maximization constitute sports medicine more susceptive to innovation. Nanobiosensors are no exception to this.

Towards performance and athlete training optimization, a self-powered (via conversion of mechanical to electric energy) nanobiosensor was developed for real-time motion monitoring and big data processing. The biosensor is composed of tetrapod-shaped ZnO nanowires and can monitor the athlete's kinetics along with environmental parameters, such as ambient temperature and humidity.[106]

Since sweat is almost present during exercise, there is a growing variety of biosensors monitoring physiological substances in the sweat. As such, a wearable (on the wrist) self-powered enzymatic (lactate oxidase) amperometric biosensor was developed based on a hydrophilic porous carbon film upon a flexible substrate (polydimethylsiloxane – PDMS) for monitoring lactate concentration in sweat and transmit sports big data in real time.[107] A similar effort produced wearable perspiration analyzing sites as biosensor platform, based on ZnO nanowire arrays (with lactate oxidase modification) and PDMS substrate, that extracts lactate in-sweat concentration via the hydrovoltaic effect from the electric double layer (EDL) that forms due to the interaction of the flowing sweat and the nanowires.[108] Another variation of

wearable in-sweat lactate biosensing is mounted on eyeglasses, is based on osmium-complex and suffers less from data noise (due to the relative stability of the forehead).[109] Because the so-far mentioned biosensors exhibit relatively weak sensitivity, a new non-enzymatic approach decreased in-sweat lactate LOD by three orders of magnitude. It is an amperometric biosensor based on porous nanostructured nickel oxide and electrocatalytic lactate detection.[110]

Lastly, biosensors could also be useful in the detection of doping substances. An example is an electrochemical antibody-based biosensor platform, that was designed to detect the substance dehydroepiandrosterone 3-sulfate (DHEA-S). Notably, the biosensing platform can be modified to detect other doping substances as well.[111]

7.2.3. Biosensing in clinical healthcare

Disease diagnosis and monitoring in clinical practice is conducted through clinical examination and laboratory confirmation. Most laboratory tests are made by strictly validated machines that are stable units inside the laboratory of each clinic. Although they may possess unmatched quality features in terms of sensitivity and specificity, there is lack of flexibility, which may be critical when a cross-sectional test is not enough to monitor the course of a disease. To fill this gap, biosensors take the role of point-of-care diagnostic tools. Some of them, like the pulse oximeter, are already well established in clinical practice. Other more sophisticated biosensors are still under development. A recent in-vitro study demonstrated a more sophisticated biosensor for post-treatment real-time monitoring of flow rates in a cerebral aneurysm model. It is a batteryless, implantable, stretchable, wireless biosensor, made of silver nanoparticles and polyimide.[112] Another biosensing platform, based on microfluidics and localized surface plasmon resonance (LSPR), was designed to monitor the inflammatory responses of infants after cardiopulmonary bypass surgery, by measuring multiple serum cytokines. It is a biosensor made of antibody-conjugated gold nanorods, with remarkably high sensitivity, low time-to-results (40 min) and requires very small quantities of sample.[113] Another research group developed a biosensor for detection of dopamine depletion, as part of an implantable intelligent theragnostic device for Parkinson's disease treatment, aiming to provide feedback for controlled release of the pharmaceutical agent.[114]

7.2.4. Other nanobiosensors

To continue with some other examples of upcoming lactate biosensors, a study demonstrated a minimally invasive, semi-implantable, microneedle-based biosensor for continuous monitoring of lactate levels in the interstitial fluid (ISF) of the skin. The microneedles are made of gold and are functionalized with the addition of multiwalled carbon nanotubes (MWCNTs) and the enzyme lactate oxidase and the biosensor exhibited satisfying performance (i.e. sensitivity, selectivity, stability, response time).[115] Another enzymatic electrochemical lactate biosensor was opted for flexibility, by using materials such as chitosan film cross-linked with glutaraldehyde and graphene nanowall.[116]

Glucose is another popular biomarker for real-time monitoring and several studies have approached biosensing techniques. A new-generation glucose biosensor was demonstrated, based on highly sensitive In_2O_3 nanoribbon FET transistors, integrated with a gold side gate, and functionalized electrodes (with glucose oxidase, chitosan and single-walled carbon nanotubes – SWCNTs), for detection of glucose in various body fluids (e.g. sweat, saliva).[117] Another study showed a wearable contact lens optical glucose biosensor, based on a glucose-selective hydrogel films that alters its optics when absorbing glucose (Bragg diffraction) and can be measured by a smartphone camera.[118]

Other research groups attempted to combine glucose and lactate biosensing in a single platform. As such, a minimally-invasive, enzymatic biosensor was developed based on gold surfaced microneedles and functionalized MWCNTs with lactate oxidase and flavin adenine dinucleotide-dependent glucose dehydrogenase (FADGDH), for simultaneous lactate and glucose monitoring in the ISF.[119] Similarly, an amperometric biosensing platform was proposed, based on MWCNTs functionalized with pyrroloquinoline quinone glucose dehydrogenase (PQQ-GDH) and lactate dehydrogenase (LDH), for dual glucose and lactate monitoring.[120]

Another research group created a biosensor for POC, IoT-compatible, free-calcium monitoring. The biosensor was 3D-printed, and its function was based on impedimetric microfluidics and golden multi-electrode interdigital sensors. With satisfying performance results and low-cost

and easy-to-manufacture methods, the researchers proposed this biosensing platform for several different biomedical applications.[121]

Biosensors can also be used for blood cytometry. A study demonstrated a portable, battery-powered, impedometric, microfluidic biosensor for blood cell counting, able to transmit the results to smart devices wirelessly via Bluetooth. Notably, the device's comparison test against a high-end bench-top impedance spectrometer showed no disadvantage in sensitivity.[122]

Furthermore, many studies provide biosensing approaches for pathogen detection. A recent study presented a biosensor for malaria detection (Plasmodium falciparum), by utilizing specific DNA aptamers specific for Plasmodium falciparum lactate dehydrogenase (PfLDH), coated on magnetic microbeads for magnetic-guided microfluidic capture and colorimetric detection. This biosensing platform could be opted for equipment-free POC testing in malaria-endemic regions.[123] Another innovative suggestion was a textile-printed biosensor for sensing the exposure to influenza A by utilizing influenza protein-specific antibodies. The biosensor consists of common textiles combined with nanoporous polyamide as a substrate for silver conductive electrodes and graphene oxide transduction film. The biosensor can potentially be connected to IoT platforms and thus, contribute critically in the management of epidemics.[124]

As already imposed, the lab-on-skin approaches are the final goal for biosensing development. Then, the way will be paved for more sophisticated and multifunctional skin-attachable electronics that will reach maximum flexibility independent of human actions.[125] An even more interesting approach was presented by a recent study, by which a skin-attachable biochemical-physiological monitoring biosensor can at the same time be a means of art and body beautification, like a normal tatoo.[126]

8. Discussion - Conclusions

Biosensors have revolutionized the means of human health, safety, and performance monitoring, due to their application flexibility, exempting the need of sending samples for tests in laboratories. Instead they provide diagnostic information in the point of care. Admittedly though, earlier versions suffered from various weaknesses, like low sensitivity in most wearables, inflammatory reactions in most implantable devices, low battery-life, etc. The introduction of nanotechnology in this point is the catalytic element not only for overcoming these weaknesses, but also for reaching unprecedented levels of miniaturization and diagnostic performance – creating thus labs-on-chips. While most possible applications of lab-on-a-chip concepts have not yet been fulfilled, there is even further potential towards lab-on-textile and even lab-on-skin technologies, which will have zero impact on human mobility while "wearing" them.

Space medicine is particularly interested in closely monitoring humans in space and nanobiosensors seem to be the ideal means to satisfy that interest. As described in this article, the numerous attempts for testing biosensors on-board the ISS share a common trend: early bulkier instruments are pushed to miniaturization and increased functionality. Although lot of effort is needed for each new product to be opted for the strict requirements of spaceflight, once validated, the new-generation nanotechnological systems may pave the way towards safe long-duration manned space missions and even planets habitation.

Aviation medicine is another field that could utilize the capability of real-time health and performance monitoring in extreme conditions. Adoption of such technologies can lead to safer flights and more accurate control whilst pushing the human body to its limits on extremely physically-demanding flights.

Other fields that can harvest the benefits of real-time performance and point-of-care monitoring in limited-resources environments are military operation planning, sports (e.g. for mountain-climbing, training optimization, etc.), and pre-hospital life support by first responders or even the patients themselves (e.g. with possible addition of telemedicine or virtual health assistance services).

Beside the tremendous practicality that nanobiosensors can imply for medical events management, it is expected that a vast amount of data will thus emerge for the human physiology. Given that this big data be properly processed by AI and machine learning algorithms, and synchronized with other environmental data via IoT technologies, it will be a huge leap-forward for the understanding of the human body and nature in general.

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