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APHRODITE: design and preliminary tests of an autonomous and reusable photo-sensing device for immunological test aboard the International Space Station

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Abstract: Preliminary results of the design and manufacturing of APHRODITE, a compact and versatile device for carrying out analyses of biological fluids during space missions that will be used as a technological demonstrator on board the International Space Station (ISS) for the quantitative determination of salivary biomarkers indicators of alterations of functionality of the immune system. The paper addresses the design of the main subsystems of the analytical device and the preliminary results obtained during the first implementations of the device subsystems and testing measurements. In particular, the system design and the experiment data output of the lab-on-chip photosensors and of the front-end readout electronics are reported in detail.
Keywords: lab-on-chip, chemiluminescence, hydrogenated amorphous silicon photosensors, biosensor, international space station

1. Introduction

APHRODITE is an analytical device jointly developed by the School of Aerospace Engineering (SIA), Sapienza University of Rome, the University of Bologna, and Kayser Italia. It is funded by ASI with the objective of producing a technological demonstrator to be launched to the International Space Station (ISS) for salivary biomarkers determination through an innovative biosensor. The core system is a lab-on-chip (LoC) with integrated thin-film sensors in a-Si:H able to host dual-analytes competitive chemiluminescence (CL) immunoassay.

One of the main challenges to be faced in future deep space missions is to protect the health and ensure maximum efficiency of the crew by preparing methods of prevention, *in situ* diagnosis, and/or through telemedicine and countermeasures aimed at contrasting the typical dysfunctions and pathologies of long-duration spaceflight. Indeed, the hostile environment causes important health problems, ranging from muscle atrophy, immunological and metabolic alterations due to microgravity to an increased risk of cancer caused by exposure to cosmic radiation [1]. With the exception of the few Apollo missions, all human activity in space has so far taken place in low orbit, so a return to Earth was always possible in case of need. In the case of biomedical research, samples collected on the ISS are generally stored at a controlled temperature until they are sent to Earth for laboratory analysis. This scenario will obviously not be viable for future manned missions beyond Low Earth Orbit (LEO). The development of diagnostic tools that can be used by astronauts themselves during a space mission is therefore a relatively recent priority in aerospace research.

A good design choice for such systems is the implementation of LoC technology, which permits miniaturization of the device by exploiting microfluidic approaches. Some of its most important advantages are the improvement in the efficiency of the analytical process, regarding the sample size, response time, cost, analytical performance, process control, integration, analytical productivity, and automation. Such devices have already been used on Earth in numerous biomedical applications [2], and this technology has recently been validated in orbit by AstroBio CubeSat [3].

In this paper, we report the system design and preliminary results of an innovative platform conceived to enhance space exploration by permitting the detection of numerous target analytes of interest in microgravity.

2. System overview

APHRODITE is a compact and versatile biosensor (**Fig. 1**) to be launched on the ISS for carrying out analyses of astronaut saliva for a quantitative determination of the levels of cortisol and dehydroepiandrosterone (DHEA), chosen as biomarkers for alterations of the immune system. The device format is properly designed to achieve ultrasensitive quantification of the target biomarkers present in sub-molar quantities in a non-invasive way. The biosensor is integrated with easy-to-use and portable instrumentation with highly miniaturized accessories and microfluidics that permit automatic operation and reconfigurability to perform different analyses.

The key technical solution of APHRODITE is based on the combination of a microfluidic chip with the use of functionalized microbeads (MBs). Analytical methods based on specific recognition elements, like immunoassays, are suitable for the development of LoC devices thanks to their high specificity and sensitivity which make it possible to detect analytes even at low concentrations in a small volume of complex matrices. Immunoassays are well-established bioanalytical techniques used extensively in clinical chemistry to determine a wide range of analytes. In APHRODITE, analyte-specific capture antibodies are immobilized on MBs [4], which are then loaded in the chip channel, kept in position using permanent magnets, and then eliminated by removing the magnetic field and washing, leaving the system clean and ready for the next analysis. Therefore, it is not necessary to functionalize the internal surface of the channels of the microfluidic chip like in most microanalytical devices, like for example in PLEIADES project [5]. Finally, optical detection based on measurements of CL photon emission by means of a-Si:H photodiodes has been chosen since it represents an ideal approach for LoC application because the required instrumentation is simple, and it has already been validated in microgravity [6].

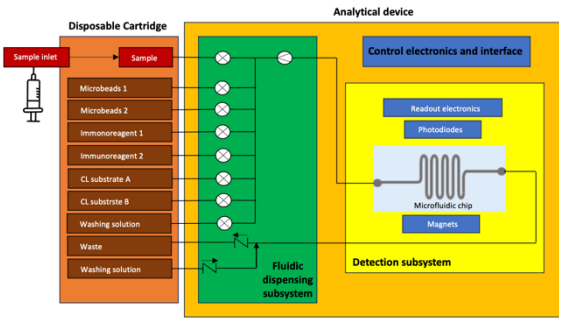

Figure 1 APHRODITE block diagram.

2.1. APHRODITE system design

From its design phase, APHRODITE is being developed to meet the requirements for space applications and to obtain certification for flight operations like compact dimensions, reduced weight, and energy consumption, adherence to ISS safety requirements, operation in microgravity and in the presence of relatively high levels of radiation. The design makes it possible to minimize the weight and volume of waste material, as well as the cold stowage (6 ± 4°C) space for storing the reagents until the time of analysis. APHRODITE's main subsystems consist in:

- Disposable cartridge. It is a removable container with a *series* of reservoirs to host the reagents connected to fluidic subsystems. The cartridge also includes empty tanks for the collection of waste solutions at the end of the analysis and for the saliva sample to be analyzed.
- Detection subsystem. It is the core of the system and it is mainly composed of a glass microfluidic chip, which incorporates 2 channels made of a combination of medical-grade adhesive, glass substrate and polymer (PMMA); each channel accommodates a volume of 15.31 µL and exhibits a surface area measuring 244.47 mm^2; an a-Si:H photosensor array composed of 30 thin-film photodiodes with a surface of 2x2 mm^2 arranged in a 6x5 matrix; a compact device for the generation of the magnetic field composed by a stepper motor that operates a cam able to control the position of 2 permanent magnets; a low-noise front-end electronic board (**Fig.2**) for the acquisition and analogic-digital conversion of the photocurrent signals coming from the photosensors; it was developed for PLEIADES project [5], permits the acquisition of all the sensors of the on-chip array simultaneously, has 32-channels and has a physical interface with the LoC Samtec MB1–150-S-02-SL card edge connector, a low-noise analog section with the circuit for current readout and the bias voltage supply circuit to operate the sensors and digitalize the signal, to time and control the analog part and a digital section for the USB interface. A custom Java GUI has been set up to control all the aspects of the system including the calibration of the sensor array, the movement of the magnetic mechanism, the visualization, management, and storage of the acquired signal, and automatic protocol execution.
- Fluidic dispensing subsystem. The fluidic dispensing subsystem consists of tubing, fluidic connectors, solenoid valves, pumps, and other elements necessary for the movement of fluids within the analytical device.
- Control electronics and interface. The control and interface electronics deal with the management of the detection and fluidic subsystems for the automatic implementation of the protocol and

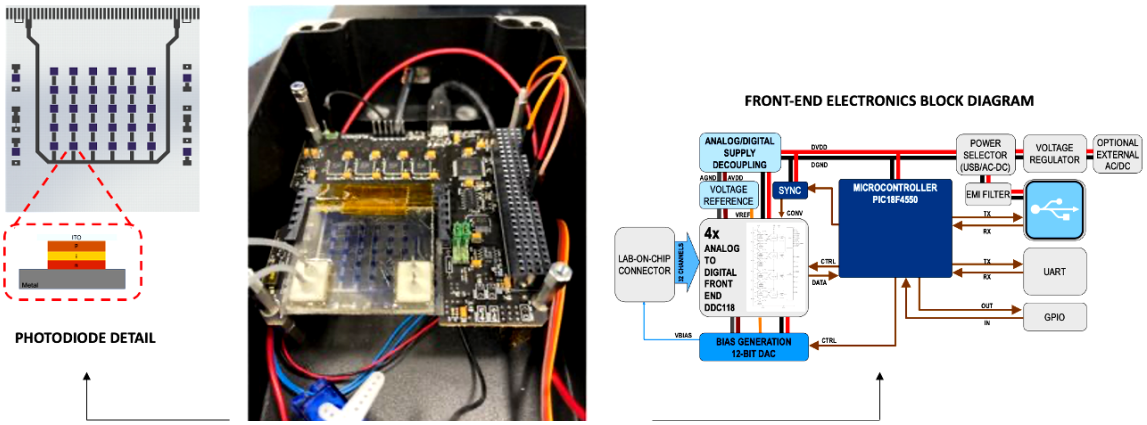

Figure 2 APHRODITE system setup and details. Left) photosensor array details. Center) Core subsystems setup. Right) Front-end electronic block diagram.

with the connection to the onboard computer for the storage and transmission of the acquired data.

- Mechanical housing. Its primary functions are to contain the device in an ergonomic way, to keep the detection subsystem in the dark, and to prevent the user from accidentally accessing the internal parts of the device; it includes the slot to insert the disposable cartridge and the USB interface.

2.2. APHRODITE protocol

The correct execution of an immunological analysis involves a series of operations that must take place in sequence at specific time intervals. The protocol for recognizing and detecting the target analytes is based on luminol/H_2O_2 reaction catalyzed by Horseradish Peroxidase (HRP), since it is a largely studied CL method and it can be easily implemented in miniaturized devices like LoC. The compactness of the final product volume and mass (1500 cm^3, 1.5 Kg) is a critical requirement to save payload launch costs and operations and to use the system in future missions. Furthermore, the choice of such a reaction enabled us to have long-lasting CL signals, which is of the utmost importance for correct dual-analyte detection.

The system is conceived to work on the ISS and, because of it, the space radiation effect will not interfere in a determinant way with the measurements and system functions since the typical radiation dose onboard oscillates between 0.0002 and 0.0005 Gy per day [7]. Nevertheless, APHRODITE is designed to be used potentially on a future Moon settlement, where the daily radiation dose is 2.6 times higher than on the ISS [8], or in a deep space trip to Mars. It is then clear the importance of including in the system design components that are radiation-hard and that have already been validated in orbit on board ABCS [3] in the Van Allen Belts, a complex and dangerous environment in the context of radiation.

Another key point of the experiment relies on the data production and analytical method. Indeed, during the analysis, the CL emission is detected by an array composed of a few dozen solid-state photodetectors optically coupled to the microfluidic chip. Using discrete sensors instead of imaging devices (CCD or CMOS) has a number of advantages. First, it is possible to achieve higher signal-to-noise ratio levels and thus improve the limit detection (LOD) of the system. It is also possible to sample the CL signal many times per second as well generating a limited amount of data, at least two orders of magnitude smaller than an imaging-based system that in any case would work at lower sampling rates. This is of the utmost importance for the effective use of such devices on the ISS and in future missions beyond LEO when data management will be even more complex. The high frequency of sampling allows one to accurately monitor the kinetics of the CL signal, providing additional and complementary information to that obtained from the signal intensity alone [9].

Regarding the protocol, schematized for a single measurement area section in **Fig. 3**, the disposable cartridge contains the following reagents, necessary for carrying out two different analyses on the same sample:

- Antibody-Functionalized MBs (Specific for cortisol);
- Antibody-functionalized MBs (specific for DHEA);
- Labeled immunoreagent (specific for cortisol);
- Labeled immunoreagent (specific for DHEA);
- Substrate CL (A);
- Substrate CL (B);
- Accessory solution for washings during the assay procedure;
- Accessory solution for the final washing.

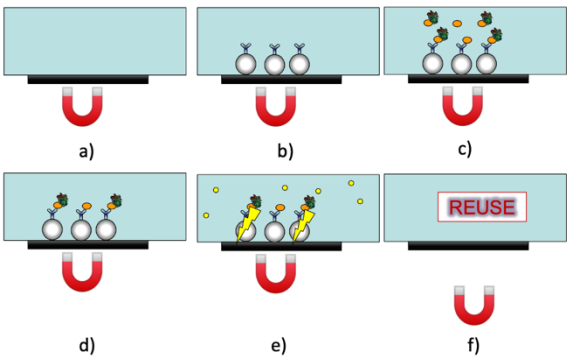

Figure 3 APHRODITE protocol representation. In a) the magnet is positioned in the channel, then the MBs are inserted in b); in c) the cortisol/DHEA HRP and the saliva

sample interact with functionalized MBs and then the channel is washed in d); the CL substrates are injected in the channel and the CL signal is detected in e); finally, the magnet is removed and the channel is washed for reuse in f).

3. Results and discussion

The data and results presented in this section of the paper have been acquired during an integration test session of APHRODITE main subsystems where analysis on DHEA was made without the saliva sample to check the performances of the system by the detection of the maximum CL signal. In the first paragraph, the method of the current and future measurements is presented.

3.1. Protocol run measurements

The first experiments made with the APHRODITE core subsystems were made to check the validity of the concept and its initial performance. Studies and measurements were carried out by the University of Bologna to select the most suitable buffer, a solution of 0.1 % bovine serum albumin (BSA) in phosphate-buffered saline (PBS), on MBs stability, functionalization protocol, and the cross-reactivity between DHEA and cortisol. The protocol has been tested and optimized firstly by direct injection of the reagents in the chip and then with a measurement with the integration of the basic subsystems including the analytical subsystem and a simplified version of the fluidic dispensing subsystem including the pump, valves, and microfluidic channels. In all the measurements, no saliva sample has been injected to evaluate the maximum output signal.

3.2. DHEA integrated protocol run

The presented results are based on an APHRODITE integration test measurement performed with a setup including the analytical subsystem, a fluidic dispensing subsystem with the channels, valves, flowmeter, bubble trap, and a first prototype of the disposable cartridge. The protocol was tested including 9 different runs of prepared solutions in the system; some extra runs were required to adapt to some contingencies during the measurement:

- 1st run: PBS 400 µL at 60 µL/min.
- 2nd run: 150 µL of MBs at 50 µL/min.
- 3rd run: 200 µL of PBS at 50 µL /min to wash the channel and leave only the suspended MBs by the magnet.
- 4th run: extra PBS run at 50 µL /min to check some unusual noise on the acquisition data; the problem was solved and related to the non-complete grounding of the acquisition board.
- 5th run: 150 µL of DHEA-HRP at 6.60 µL/min that interacted with MBs.
- 6th run: 2 cleaning runs with PBS, the first 100 µL at 16.7 µL/min in order to empty the channel favoring residual incubation, and the second of 500 µL at 50 µL/min in order to properly wash away every residual from the channel.
- 7th run: insertion of 300 µL of substrate A and 300 µL of substrate B at 50 µL/min mixed by closing and opening the respective valves.
- 8th run: after excluding the cartridge from the fluidic system to insert the reagent directly into the chip, an extra run of 400 µL of substrate A and B at 50 µL/min (200 µL A + 200µL B).
- 9th run: after system resetting with the insertion in the channel of 200 µL of MBs already reacted with the conjugate, 400 µL of extra substrate A and B (200 µL A + 200µL B) from the cartridge testing the mixing protocol.

The CL signal was correctly detected with a maximum of 7.47 pA. **Fig. 4** shows the moving median of average acquired data. A point worth mentioning that was already verified by the preliminary measurements done by Bologna personnel and it appeared also in this session is that the MBs tend to deposit at the bottom of the reservoir due to gravity. Because of it, some experiments may be affected by the fact that all the MBs may not be correctly injected in the channel causing a weak output signal. An easy and quick fix is shaking the recipient before the analysis. The problem should not occur in microgravity, but still, it needs to be addressed and solved for the GM and future measurements.

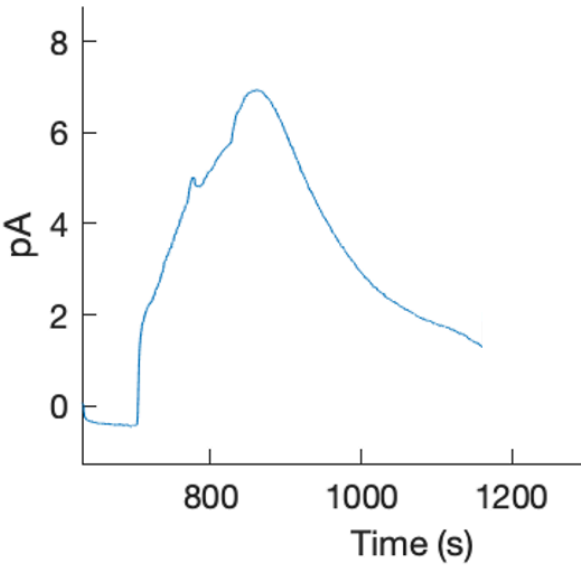

Figure 4 DHEA run output signal.

Finally, in **Fig.5** a detail of the presented data is provided with a focus on the washing part; the exponential decay of the signal (pA) during washing has been highlighted.

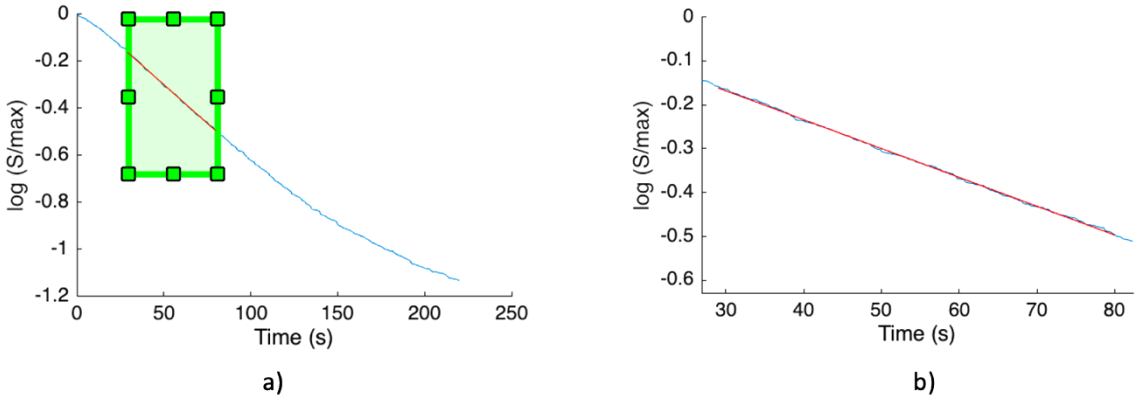

Figure 5 a) Detail of the output signal during washing. b) exponential decay of the output signal during washing.

4. Conclusions

APHRODITE design has been validated through some preliminary measurements and integration of the core subsystems. The next steps will be to integrate a final ground model to make new measurements with saliva with known concentrations of cortisol and DHEA and to start the test campaign to qualify the device to fly to the ISS.

Once validated in flight, APHRODITE could immediately be used in many studies conducted on the ISS to investigate the effect of the space environment on the human organism. It could also be adapted to the determination of other biomarkers of interest, simply by supplying new Disposable Cartridges with the necessary reagents.

The first integration and tests of the subsystems show that the design concept works correctly with good performance levels.

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