

Micro-incubator Based on Lab-on-Glass Technology for Nanosatellite Missions



L. Iannascoli, F. Costantini, N. Lovecchio, A. Buzzin, D. Caputo,
G. De Cesare and A. Nascetti

Abstract The study and quantification of the effects of the space environment on human body is a primary task for future manned deep space missions. The risk models for radiation exposures incurred by astronauts beyond low-Earth orbit, have different limitations due to the difficulty to have terrestrial parallels on which to base risk estimates. Indeed, no terrestrial sources fully reproduce the deep space energy spectrum and the multi directional flux of the cosmic radiation. In situ analysis would therefore be fundamental in order to enable reliable studies about the effects of the radiation environment on living organisms as well as to evaluate customized radiological countermeasures for astronauts. A micro-incubator suitable for cubesat missions for studying in situ the effects of the space environment on cellular cultures is presented. The device is based on lab-on-chip technology with integrated thin-film sensors and actuators for the active control of the environmental conditions of the cell culture and for the monitoring of its metabolic status. In particular, the device includes an incubation chamber connected to a microfluidic network for the supply of nutrients and/or pharmaceuticals. A second network is used for the distribution of carbon dioxide through a thin gas-permeable membrane. On-chip on-demand production of carbon dioxide can be eventually achieved from the pyrolysis of sodium bicarbonate stored in a separate reservoir with a dedicated thin film heater. The same network can be used to supply a controlled atmosphere from a pressurized tank. The on-chip hydrogenated amorphous silicon photodiodes are used to measure the light emitted by genetically-modified cell cultures that express a bio-luminescent behavior when subjected to given stress conditions. Accurate temperature control is achieved by means of additional on-chip thin-film diodes and a transparent indium-tin-oxide heater located beneath the incubation chamber. From technological point of view, the system relies on the combination of different thin- and thick-film fabrication

L. Iannascoli · N. Lovecchio · A. Buzzin · D. Caputo · G. De Cesare
Department of Information Engineering, Electronics and Telecommunications, Sapienza
University of Rome, Via Eudossiana 18, 00184 Rome, Italy
e-mail: lorenzo.iannascoli@uniroma1.it

F. Costantini · A. Nascetti (✉)
School of Aerospace Engineering, Sapienza University of Rome, Via Salaria 851, 00138 Rome,
Italy
e-mail: augusto.nascetti@uniroma1.it

technologies jointly used with the aim to achieve a compact, automated and low-power device that represents a viable solution for biological experiments aboard cubesat satellites.

Keywords Micro-incubator · Lab-on-chip · Nano-satellite · Space missions

1 Introduction

The greatest long-term risk for astronauts exposed to space radiation environment, beyond Low Earth Orbit (LEO), is to develop degenerative pathologies like cancer, experience central nervous system (CNS) decrements, exhibit degenerative tissue effects or develop acute radiation syndrome [1]. The intensity of the ionizing radiation becomes extremely relevant for missions outside the Earth's magnetosphere, which are currently under consideration within the solar system manned exploration missions being planned by the main space agencies as well as by private companies. Its therefore of fundamental importance to study and quantify the effects of the space environment on human body as a primary task for future manned deep space missions. Ground-based studies often suffer the limitations of the available risk models for radiation exposures beyond low-Earth orbit [2] arising from the difficulty to fully reproduce in laboratory the deep space energy spectrum and the multi directional flux of the cosmic radiation. In situ analysis would therefore be fundamental in order to enable reliable studies about the effects of the radiation environment on living organisms as well as to evaluate customized radiological countermeasures for astronauts.

As part of this framework, we propose a scientific payload that allows experiments on cell cultures to be carried out on nano-satellites by satisfying requirements such as compact size, automated operation and low-power consumption. This goal is achieved through the use of lab-on-chip technology, which enables the fabrication of extremely compact devices that integrate several laboratory functions that may range from sample preparation to the analytical outcome. Lab-on-chip devices have been proven to be powerful instruments to accomplish complex chemical or bio-chemical analysis on a single chip with very limited consumption of samples and reagents [3]. Such devices are ideal for space mission where experiments need to be almost totally automated, and volume, weight and power must be kept as small as possible. However, many lab-on-chip devices presented in literature still require external instrumentation for their operation (such as microscopes, cameras or pumping systems) making them less appealing to nano-satellite based space missions. The lab-on-chip device proposed here, instead, consists of a fully integrated micro-system [4], which includes the incubation chamber with the surrounding microfluidic network and on-chip integrated thin-film electronic sensors and actuators that eliminate the need for external desktop instrumentation and to perform experiments in a standalone fashion.

The present paper is arranged as follows. The system overview is reported in the next section with details of the system components reported in subsections. Here are also described the materials and methods employed for the fabrication of the microfluidic and electronic components. Finally, conclusions are drawn.

2 Micro-incubator for Cell Culture

With the purpose of studying the radiation effects on cell cultures, a microfluidic incubator has several advantages over conventional equipment, such as the ability to more closely mimic a cell’s natural environment by continuous perfusion culture or by creating chemical gradients [5], the conservation of samples and reagents and the minimization of disposables [6], besides low weight, volume and power consumption.

Figure 1 provides a schematic view of the proposed system for the maintenance and growth of cell cultures. The device is made up of two main elements: a polymeric microfluidic unit and a 50 × 50 mm² glass substrate (smart-glass) on which hydrogenated amorphous silicon (a-Si:H) sensors and thin-film resistive heaters are fabricated.

The smart-glass includes thin-film photodiodes that allow the implementation of luminescence-based analytical protocols commonly used in laboratory. Accurate temperature control is achieved by means of additional on-chip thin film diodes and a

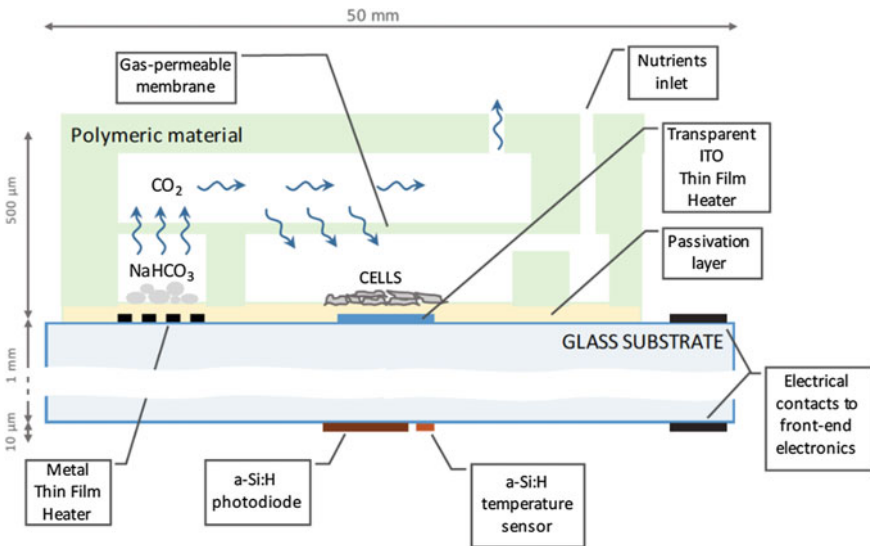


Fig. 1 Schematic view of the proposed micro-incubator for cell cultures based on lab-on-chip technologies

transparent indium-tin-oxide heater located beneath the culture chamber. Additional thin film resistive heaters are planned for the production in situ of carbon dioxide (CO_2) used to regulate the environmental conditions of the cell culture.

The polymeric microfluidic unit, which hosts the cell culture as well as the culture media and the supplies, is permanently bonded on the top face of the smart glass. The thin-film devices are fabricated on the opposite face to prevent any interaction between the ‘biological’ side and the ‘electronics’ side, thus making the system more robust and reducing the risk of contamination of the cell culture or of its supplies. The bottom side also hosts the electrical contacts needed to interface the device with the electronic control board.

2.1 Polymeric Incubation Chamber

One of the key benefits of the use of microfluidics for cell cultures studies is the ability to control parameters of the cell micro-environment at relevant length and time scales. In detail, an incubator system must be able to maintain the pH and osmolality of the culture within physiological levels and guarantee a regular refresh of cell nutrients [7].

According to these requirements, the building blocks of an incubator system are: an incubation chamber, a series of channels and reservoirs for culture media replacement, a thin gas-permeable membrane and a network for the distribution of suitable atmosphere. The device proposed here incorporates all of these elements.

As is shown in Fig. 2 the polymeric microfluidic structure is made up of different levels, each of which has a specific objective. The first layer at the bottom of the microfluidic structure, marked as 1, is the one which is bonded to the smart glass: this layer hosts the incubation chamber, inlet and outlet ports for fresh nutrients and waste respectively, and a reservoir for Sodium Bicarbonate (NaHCO_3) that can be used for the on-chip production of CO_2 by thermal decomposition of NaHCO_3 . Geometry and dimensions of the culture region are fundamental design aspects for a microfluidic system with cell cultures [7]. In the present design, a cylindrical incubation chamber

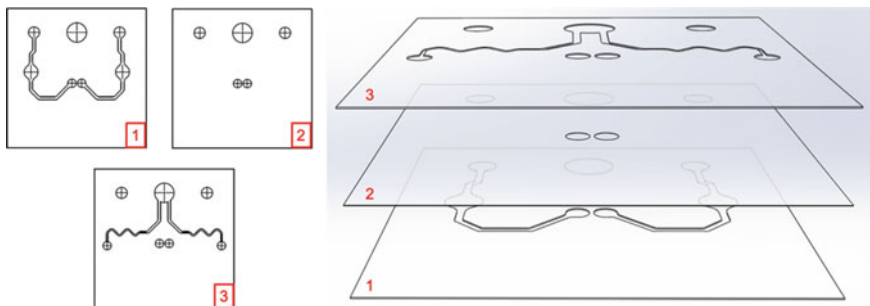


Fig. 2 Layout of the three layers that make up the microfluidic part

whit a volume of 100 μl has been chosen. The second layer of the structure consists of a 50 μm -thick gas-permeable membrane to ensure gas-exchange with the incubation chamber. The last layer hosts a network for the distribution of carbon dioxide across the chip. Access holes are also made in the top layers in order to allow chip loading. Eventually, a top sealing layer can be used during the experimental phase (not shown).

The polymeric microfluidic structure can be fabricated using different materials or combinations of materials. For this work, Pressure Sensitive Adhesives (PSA) have been chosen. The advantages of these materials rely on low cost, easiness to assemble multilayer structures and rapid prototyping processes; this is suitable especially in the preliminary stages of system implementation. Moreover, experiments with PSA technology were already performed in space environment, mainly on the ISS. Each layer is fabricated separately by patterning the PSA by means of a cutting plotter and then assembling the entire structure layer by layer. The position and geometry of the different microfluidic elements have been affected by the fabrication constraints of the smart-glass chip considering the expected temperature distribution during operation.

2.2 On-Chip Sensors and Actuators

In order to design, test and characterize the active components of the smart-glass a set of intermediate devices has been preliminarily fabricated. In particular, two glass substrates one with sensor-side only and one with heater-side only (Fig. 3) have been produced.

The heaters for the control of the temperature of the incubation chamber are made in Indium Tin Oxide (ITO), which is transparent, in order to allow the optical monitoring of the cell culture by means of the photodiodes that are fabricated on the opposite side of the substrate. The heaters are passivated by a 5 μm -thick SU-8 layer to ensure electrical insulation from the microfluidic part that is assembled on top of them.

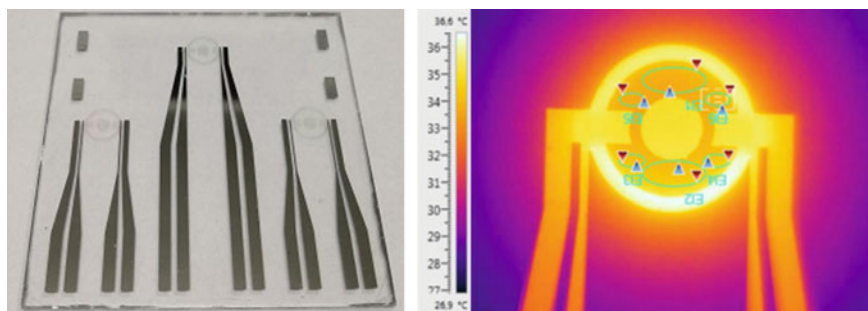


Fig. 3 Left: fabricated sample of the smart-glass heaters. Right: heater characterization by thermal imaging

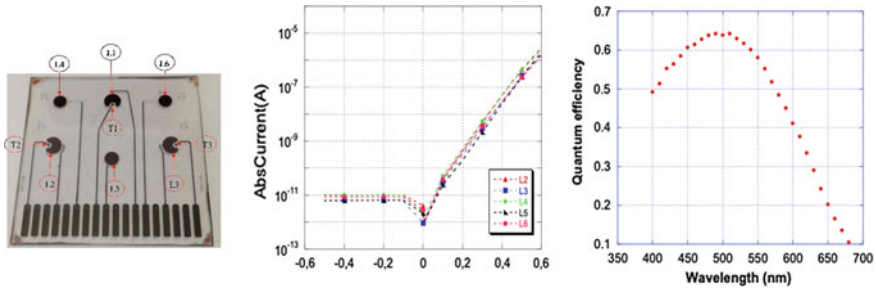


Fig. 4 Left: sample of the smart-glass sensors (the combined large C-shaped photodiode with the small temperature sensor are visible as T1-L1, T2-L2 and T3-L3). Center: current-voltage characteristics in dark conditions of five out of six on-chip a-Si:H photodiodes. Right: spectral quantum efficiency of one of the a-Si:H photodiodes of the smart-glass device

The a-Si:H diodes are deposited on the bottom glass side and implement both the temperature sensors and the photosensors. The sensors are p-type, intrinsic, n-type a-Si:H stacked structures deposited by Plasma Enhanced Chemical Vapour Deposition (PECVD). The difference between the two types of sensor is just in the front contact layer (on glass surface), which is metallic for the temperature-diodes while it is made of transparent ITO for the photodiodes. In order to allow the monitoring of the temperature of the incubation chamber and ensure optical measurements of the cell culture, a particular shape of the devices has been designed, with a large C-shaped photodiode and small temperature-diode, as reported in Fig. 4.

Current-voltage characteristics have been measured for all the a-Si:H diodes to verify both the dark current and the diode ideality factor which give indication of the overall process quality (center in Fig. 4). Spectral quantum efficiency measurements in reverse bias conditions have been performed on the photodiodes as shown in Fig. 4 (right).

Temperature sensors have been characterized by measuring the variation of the forward voltage drop at constant forward bias current of 10^{-5} A/cm² by varying the temperature on a hot-plate. A sensitivity of 3.3 mV/°C has been found.

3 Conclusions

This work describes the design, fabrication and test of a lab-on-chip device for the execution of experiments on cell cultures on nanosatellite missions. It features an incubation chamber for cells with a-Si:H optical sensors for the monitoring of the cell culture and a-Si:H temperature sensors for the active control of the environmental parameters using thin-film heaters. Preliminary device design, fabrication and test has been reported. Future steps include the use of the microincubator with bacterial cultures, the test of the on-chip generation of the carbon dioxide and finally the test with mammal cells cultures.

References

1. Jeffery JPS, Chancellor C, Scott GBI (2014) Space radiation: the number one risk to astronaut health beyond low earth orbit. *Life*
2. Barcellos-Hoff MH, Blakely EA, Burma S, Fornace AJ, Gerson S, Hlatky L, Kirsch DG, Luderer U, Shay J, Wang Y et al (2015) Concepts and challenges in cancer risk prediction for the space radiation environment. *Life Sci Res* 92–103
3. Abragall P, Gue A (2015) Lab-on-chip technologies: making a microfluidic network and coupling it into a complete microsystem a review. *J Micromechanics Microengineering* 17
4. Caputo D, de Angelis A, Lovecchio N, Nascetti A, Scipinotti R, de Cesar G (2015) Amorphous silicon photosensors integrated in microfluidic structures as a technological demonstrator of a true Lab-on-chip system. *Sens Bio-Sens Res* 3:98–104
5. Halldórson S, Lucumi Moreno E, Gómez-Sjöberg R, Fleming R (2015) Advantages and challenges of microfluidic cell culture in polymethylsiloxane devices. *Biosens Bioelectron*
6. DePalma A (2017) Microfluidics for cell culture and analysis
7. Young LEWK, Beebe DJ (2010) Fundamentals of microfluidic cell culture in controlled microenvironments. *Chem Soc Rev*