SPACEFLIGHT OF HUVEC: AN INTEGRATED EXPERIMENT – SPHINX ONBOARD THE ISS

S. Versari[(1)], J.A.M Maier[(2)], A. Norfini[(3)], V. Zolesi [(3)], S. Bradamante[(1)]

[(1)] CNR-ISTM, Institute of Molecular Science and Technology, Milan (Italy); silvia.versari@istm.cnr.it, silvia.bradamante@istm.cnr.it
[(2)] University of Milan, Department of Clinical Science, Milan (Italy); jeanette.maier@unimi.it
*[(3)] Kayser Italia S.r.l., Livorno (Italy); a.norfini@kayser.it, v.*zolesi*@kayser.it*

ABSTRACT

The spaceflight orthostatic challenge can promote in astronauts inadequate cardiovascular responses defined as cardiovascular deconditioning. In particular, disturbance of endothelial functions are known to lead to altered vascular performances, being the endothelial cells crucial in the maintenance of the functional integrity of the vascular wall. In order to evaluate whether weightlessness affects endothelial functions, we designed, developed, and performed the experiment SPHINX – SPaceflight of HUVEC: an INtegrated eXperiment – where HUVEC (Human Umbilical Vein Endothelial Cells) were selected as a macrovascular cell model system. SPHINX arrived at the International Space Station (ISS) onboard Progress 40P, and was processed inside Kubik 6 incubator for 7 days. At the end, all of the samples were suitably fixed and preserved at 6°C until return on Earth on Soyuz 23S.

1. INTRODUCTION

Orbital space flights clearly demonstrated that the reduction or absence of gravity profoundly affects eukaryotic organisms, including man. In microgravity (µg), astronauts experience long term adaptation with space motion sickness, muscle atrophy, bone demineralization, and cardiovascular deconditioning. Up to now, the underlying mechanisms of vascular dysfunction in µg is still not fully understood. With SPHINX (SPaceflight of HUVEC: an INtegrated eXperiment) we aimed to evaluate whether and how µg affects endothelial cell functions [1, 2]. Here we describe the SPHINX experiment hardware (EH), the experimental procedure and parameter optimization, and the mission profile.

2. MATERIAL AND METHODS

Human umbilical vein endothelial cells (HUVEC) were obtained from the American Type Culture Collection (ATCC) and serially passaged in medium M199 containing 10% FCS, 1% L-glutamine, 1% Penicillin-Streptomycin, 1% sodium pyruvate, heparin (10 U/mL), HEPES (12.5 mM), ECGF (10 µg/ml) on 2% gelatin coated OptiCells™. For the spaceflight experiment cells were seeded on Thermanox™ coverslips (Nunc), kept overnight and then loaded in the SPHINX bioreactors. For proliferation assays cells were trypsinized, stained with trypan blue solution (0.4%) and counted using a Burker chamber. For RNA isolation cells, preserved in RNAlater, were subjected to RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction (Trizol, Invitrogen) followed by a Qiagen RNeasy clean-up procedure. RNA purity was assessed by spectrophotometer (Nanodrop) and RNA integrity was assessed by Agilent Bioanalyzer.

To evaluate the fluid exchange efficacy of the hardware fluids were analyzed by reverse-phase HPLC (Agilent LC 1100) using a Merk Purosphere RP-18 column endcapped 5 µm and a spectrophotometric detector (λ=210 nm). Solvent: 25mM NaH_2PO_4, pH 3.5 with 1 M TPA; flow rate: 0.6 mL/min. Percentage of GSH was identified using a GSH standard solution.

3. RESULTS AND DISCUSSION

The SPHINX EH, derived from STROMA-2 experiment container (EC), was improved by increasing the cell culture support to 2.3 cm^2 and the reservoir volume to 1.4 mL, and by adding PI (protease inhibitor) reservoirs (0.5 mL). SPHINX flight set was made up of 12 EH - each composed of one experiment unit (EU) integrated into the related KIC-SL EC - and 2 soft pouches to upload and download the EH (Fig. 1). Each soft pouch housed 6 EH. Since the EH had to be uploaded at controlled temperature (>27°C), two additional soft pouches (Bio-Kit) with Phase Change Materials (PCM) cartridges were used to provide the necessary thermal control during the upload phase.

3.1 Fluid exchange efficacy

To measure fluid exchange efficacy, the first medium reservoir (1.4 mL) was filled with 5 g/L GSH standard solution. The culture chamber (CC), the space over the valves and channels (1.7 mL) and the PI chamber (1.9 mL) were filled with ultrapure water. After first piston activation, GSH content inside CC and inside all of the PI chambers was measured using HPLC. Results in terms of GSH content indicated 74% exchange efficacy.

3.2 Bio-Kit thermal performance tests

As Progress Vehicle had no power to warm up the system we tested a number of possible solutions to guarantee an internal temperature to the SPHINX EH greater or equal to 27 or 32°C for at least three days (from launch to installation in Kubik). Different combinations of PCM (Environmental Process Systems Ltd) were taken into account, depending on number and size. Tests were performed in a thermal chamber,

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simulating the worst environmental temperature (0°C). In details, 12 SPHINX EH were inserted in two SPHINX soft pouches (6 EH each), then each soft pouch was inserted in one Bio-Kit transport container filled with the PCM pre-heated at 37°C for 48 h. Although all of the considered combinations tested worked properly, the 6 PCM PlusIce S27 were chosen because of the best compromise between box mass and thermal performance.

Fig. 1. Top left) SPHINX EU. Each EU is composed of a brick of biological compatible plastic (PEEK®) where five cylinders, a culture chamber (CC) and connecting channels are machined. Five small valves separate the different fluids and the CC. Each cylinder has a piston for the fluid injection into the CC; once the piston is released by a preloaded spring, the fluid contained into the cylinder flows into the CC and the waste medium is recovered behind the activated piston and suitably preserved with a protease inhibitor cocktail. Top right) SPHINX EU and integrated electronics. Bottom left) SPHINX integrated into the KIC-SL experiment container (EC). Bottom right) SPHINX EH in the soft pouch.

3.3 Cell viability and optimal seeding density

We evaluated the feasibility of the proposed spaceflight protocol in terms of cell viability (27°C from launch to installation in Kubik) and optimal seeding density for post-flight analyses. In details, cells were seeded at different densities in medium M199 added with 12.5 mM HEPES and cultured simulating the proposed spaceflight protocol: 4 days at 27°C followed by 7 days at 37°C. Cell number was determined at selected time points. Results indicated that cells survived 4 days at 27°C and that the optimal seeding density was 40.000 cells/cm^2, avoiding either too soon cell confluence or too high cell mortality that could spoil post-flight analyses.

3.4 RNA yield optimization

We performed some experiments aimed at the optimization of RNA yield for post-flight analyses. Since we observed interference (flocculation) between medium and RNAlater with significant reduction in RNA yield, we evaluated the improvements produced by two protocols: 1) two PBS washes followed by one RNAlater injection (hypothetical concentration: 74% RNAlater, 19% PBS, 5% culture medium) or 2) one PBS wash followed by two RNAlater injections (93% RNAlater, 5% PBS, 2% culture medium). We found that protocol 2 significantly increased RNA yield. We finally optimize the RNA extraction protocol doubling Trizol content with significant increase in RNA yield.

3.5 SPHINX loading and mission profile

12 SPHINX EUs were assembled, the electronic board mounted, and each EU integrated into its KIC EC. The EH were installed into 2 soft pouches 13 hrs before launch and kept at 37°C until hand over to NASA. SPHINX was launched with Progress 40P and, as soon as the docking was completed (4 days after launch), was operated inside KUBIK incubator, previously stabilized at 36.5°C. During the 7 days' experiment all of the medium exchanges and fixation operations were automatically executed according to a predefined timeline. After experiment completion, the EH were maintained in Kubik at 6°C before re-entry with Soyuz 23S. In parallel with the flight experiments, 12 duplicate control experiments, prepared in Baikonur in identical EH, were kept on ground and submitted to the same experimental protocol of the in-flight experiments.

The SPHINX experiment has been completely successful: all the activation steps, temperature control, and chemical fixatives worked properly. The retrieved samples were in good shape: cells were well attached to the cell culture support, indicating that they were still alive when fixed with RNAlater, and culture media were clear and well preserved. For post-flight analyses, cells were treated with Trizol and frozen at -80°C, and culture media were stored at -20°C.

4. ACKNOWLEDGEMENTS

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5. REFERENCES

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