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ORIGINAL ARTICLE

Opening the Gate to the Serism Project: From Earth to Space and Back

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Abstract
In the context of Space Medicine, the aim of "SERiSM" (Role of the *E*ndocannabinoid *S*ystem in *R*eprogramming human pluripotent *S*tem cells under *M*icrogravity) project, selected by the Italian Space Agency, was to study the involvement of the endocannabinoid system (ECS) in the osteogenic differentiation under real microgravity. An innovative and easily accessible stem cell model derived from human blood (human blood-derived stem cells, hBDSCs) was used to this purpose. This model is autologous and possesses a remarkable proliferative and differentiative capacity underground gravity conditions, with high therapeutic potential for bone degenerative diseases. ECS is a fine network of proteins that interact to regulate the endogenous levels of lipid mediators, collectively termed endocannabinoids (eCBs), which in turn are involved in cell communication and in the mechanisms governing the switch between cell life and death. In the frame of the VITA mission, led by European Space Agency (ESA) astronaut Paolo Nespoli, we analyzed the differentiation process also under microgravity condition and evaluated the expression of ECS proteins through immunoassay methods. Our results demonstrate that some elements of the ECS are modulated during the differentiation process and in microgravity, supporting the idea that increased levels of anandamide are indeed need to stimulate type-1 cannabinoid receptor. In conclusion, microgravity could drive endocannabinoid signalling in the former stages of hBDSCs differentiation.

Keywords Endocannabinoid system · Human blood-derived stem cells · Microgravity · VITA mission

1 The SERiSM Concept

Spaceflight is known to induce loss of bone mass, alteration in bone physiology and possibly osteoporosis, thus representing one of the major health risks for astronauts. Endocannabinoids (eCBs) are bioactive lipids that mediate several aspects of human pathophysiology and, because of their ubiquitous activities, they have emerged as promising targets for the development of selective drugs able to modulate their signaling in distinct organs [1]. Among these, eCBs signaling seems to be involved also in the proliferation and differentiation of bone cells, as well as in bone remodeling [2, 3]. Indeed, eCBs metabolic enzymes and their binding targets (i.e., cannabinoid and vanilloid receptors) are expressed in bone cells. In particular, type-2 cannabinoid receptors (CB_2) signaling stimulates proliferation of osteoblast progenitors and favors the bone mineralization process, whereas stimulation of type-1 cannabinoid receptors (CB_1) and of transient receptor potential cation channel subfamily V member (TRPV1) exerts osteoclastogenic effects, restraining bone growth [4]. Accordingly, it has been reported that the lack of CB_2 stimulates bone remodeling but with a net loss of bone mass, and that $cb2^{-/-}$ mice, with a normal phenotype at birth, display over time a phenotype similar to human osteoporosis [5]. On the other hand, genetic inactivation of CB_1 receptor results in higher bone mass in young mice [6] and CB_1 ligand may be used to enhance bone mass

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and prevent age-related osteoporosis. Altogether, these findings suggest that the combined inhibition of CB_1 and CB_2 may be beneficial in preventing age-related bone loss [7], highlighting the therapeutic potential of cannabinoid receptors to re-establish bone homeostasis.

Our previous study, performed in the frame of PromISSe mission organized by the European Space Agency (ESA) in 2011, disclosed an unprecedented engagement of endocannabinoid signaling in lymphocyte apoptosis and immunodepression under real microgravity conditions [8]. Based on these findings and additional literature data [9, 10], we proposed the SERiSM (Role of the *E*ndocannabinoid *S*ystem in *R*eprogramming human pluripotent *S*tem cells under *M*icrogravity) project aimed at investigating whether endocannabinoid signaling might regulate bone loss during space travel; to this aim, we used a human stem cell model derived from peripheral blood (hBDSCs). These cells are autologous and pluripotent, differentiate into an osteogenic lineage by using rapamycin in the presence of suitable scaffolds [11], and could have a remarkable therapeutic potential for the treatment of bone-related disorders.

SERiSM project was part of the VITA mission organized by the Italian Space Agency (ASI) in 2017. It was launched on August 14th, 2017, from historic Pad 39A at NASA's Kennedy Space Center (KSC) in Cape Canaveral, Florida (USA) onboard the Falcon 9 rocket; it remained onboard the International Space Station (ISS) until September 16th, when it returned back to Earth onboard the SpaceX Dragon capsule. Here, we present the mission profile of SERiSM (Fig. 1) and the experimental data showing the effects of microgravity on cannabinoid receptors expression in the osteogenic process aboard the ISS.

2 SERiSM System Configuration

The SERiSM hardware was developed on purpose by Kayser Italia S.r.l and the flight set included four Experiment Containers (EC). Each EC contained two Experiment Units (EU) that, in turn, was composed of three main components: Cell Bodies (consisting of two identical units), Release System and Electronic Board. Each EU is a brick of biologically compatible plastic (PEEK®) and accommodates two identical CC, and each CC is connected with two reservoirs: one for the activator (rapamycin, Sigma-Aldrich, St. Louis, MO, USA) and the other one for the fixative (RNA*later*, Sigma-Aldrich, St. Louis, MO, USA). The final volume of each CC is 1.78 ml (0.90 ml for fixative; 0.80 ml for sample; 0.08 ml for activator).

The movement of the chemicals and biological samples among the fluid chambers was allowed by eight cylinders machined automatically by the internal microcontroller (Fig. 2).

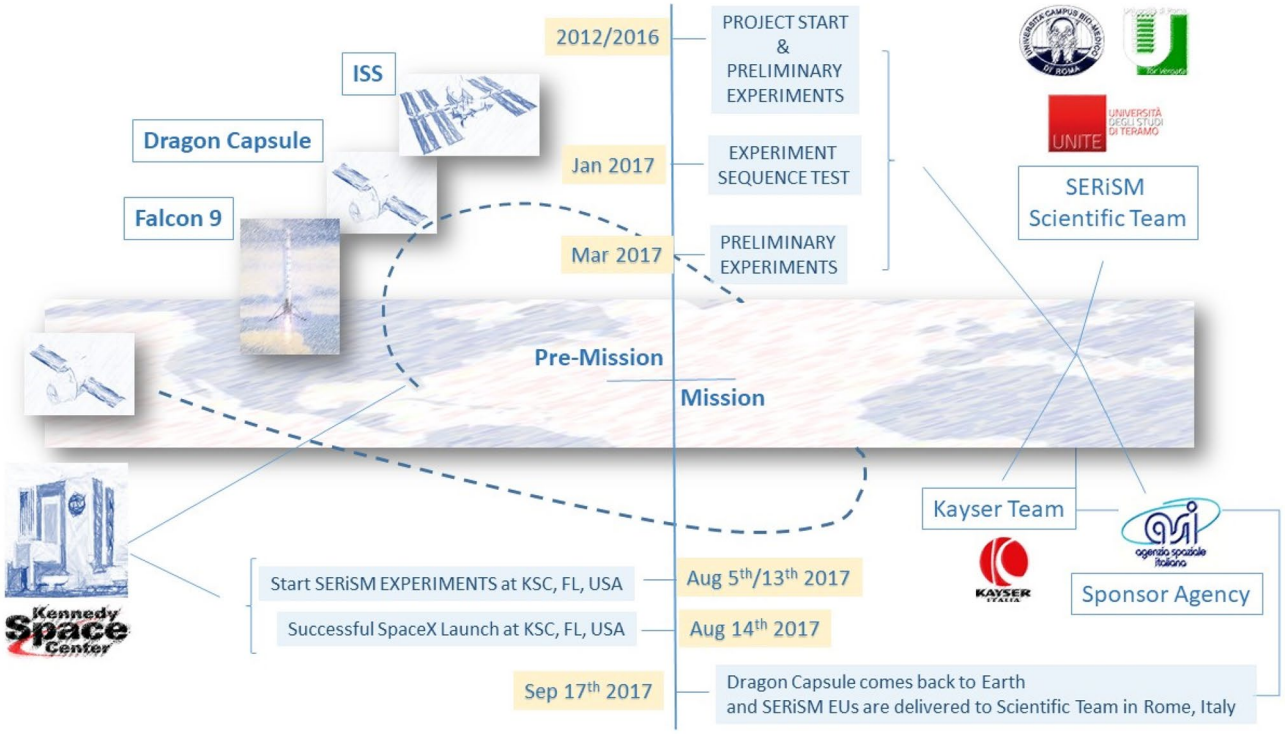

Fig. 1 The timetable of SERiSM project: from ground-based experiments to ISS

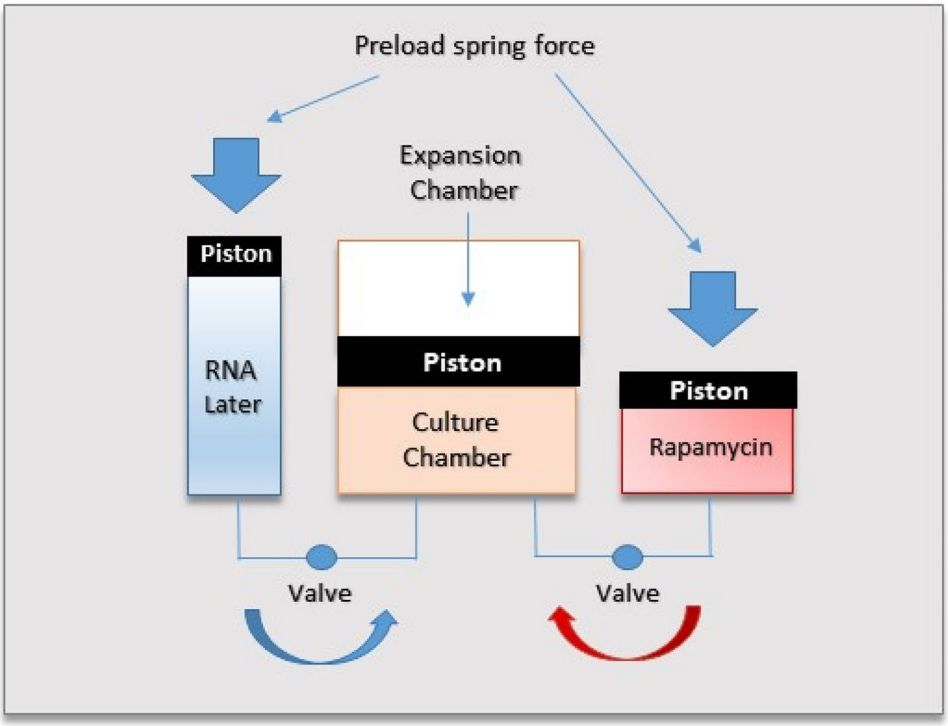

Fig. 2 Pre-flight standby configuration of SERiSM experiment unit

2.1 Pre-light Activities

Pre-flight activities started on August 9th and were carried out in the laboratories of Space Station Processing Facilities of Kennedy Space Center.

Blood was drawn from the antecubital vein of one healthy donor, who gave informed consent to the study; human BDSCs were isolated by ammonium chloride incubation (dilution 1:3 in NH_4Cl 1 M), centrifuged at 1023*g* for 20 min and washed several times with phosphate-buffered saline (PBS), pH7.2 (Oxoid, Hampshire, England), to remove the majority of erythrocytes. Cells were then resuspended in 5 ml PBS and incubated for 72 h at 37 °C in the presence of 50 nM macrophage colony-stimulating factor (Sigma-Aldrich, St. Louis, MO, USA), and 5 µM gentamicin sulphate (BioWest, Nuaillé, France). On August 12th, BDSCs were resuspended in DMEM/F12 medium (Invitrogen, Carlsbad, CA, USA) containing L-glutamine (300 µg/ml), 1% penicillin–streptomycin and 10% fetal bovine serum, all purchased from Sigma-Aldrich (St. Louis, MO, USA), and Bio-Oss scaffold (Geistlich, Switzerland). Then, they were loaded into the CC, for a total of 16 chambers divided into 4 EUs. The SERiSM EU assembled with control electronics was integrated inside the KIC-SL containers (Kayser Italia Containers—Single Level), and then placed inside the Biokit, a passive temperature-controlled experiment container, for the upload onboard launcher.

2.2 In-flight Activities

On August 16th, samples were transferred from the Dragon capsule to ISS and were loaded into the Kubik facility, already set up at 37 °C, in a static position. Human BDSCs were activated for different times (0, 48, and 72 h) by automatic injection of 10 nM solution rapamycin in each CC. At the end of each incubation time, cell suspensions were fixed with RNA*later* (900 µl/culture chamber), and were immediately moved by the astronaut into the minus 80 °C laboratory freezer for ISS (MELFI) facility.

Then, samples were removed from MELFI and wrapped into the double cold bags for return to Earth. On September 16th, SpaceX's CRS-12 Dragon unberthed from the ISS ahead of a return to a Pacific Ocean splashdown.

2.3 Post-Flight Activities

After recovery, samples were shipped still frozen to Livorno and then delivered from Kayser Italia team to the University of Rome for post-flight analysis. The scientific team was responsible for the samples collected from EUs. A flowchart representing the main activities of mission, as well as the facilities on ISS, is shown in Fig. 3.

Each sample was centrifuged at 14,000*g* for 5 min for fixative removal and was frozen for the planned biochemical analysis. Protein expression of cannabinoid receptors was analyzed through immunoblotting by using the rabbit

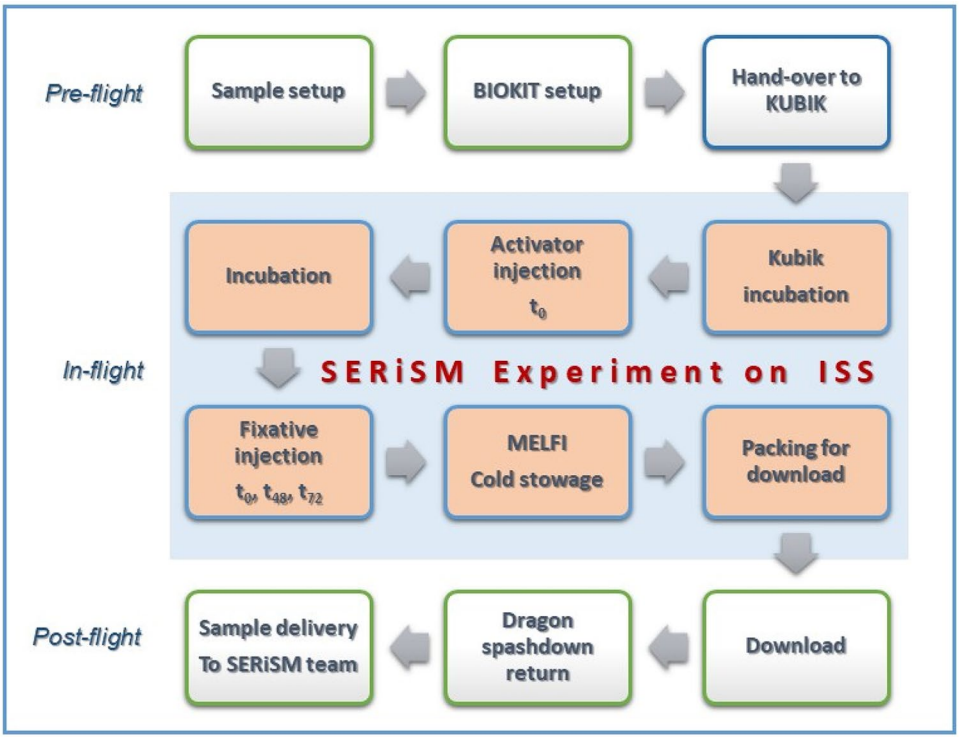

Fig. 3 Experimental procedure of SERiSM project during pre-flight, on orbit and post-flight operations

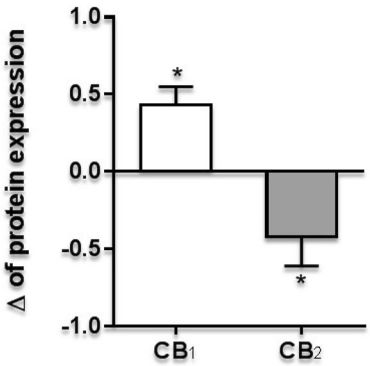

Fig. 4 Relative expression of CBRs after 72 h on ISS vs t0 $*p < 0.05$

polyclonal antibodies specific for CB_1 (1:200 dilution) or CB_2 (1:200 dilution) receptors (both from Cayman Chemicals, Ann Arbor, MI, USA).

Our results showed that, after 72 h on the ISS, CB_1 and CB_2 proteins were expressed with an opposite trend: CB_1 increased, whereas CB_2 decreased (Fig. 4). Instead, nor the other eCB-binding receptor TRPV1 nor any of the eCB metabolic enzymes were affected.

3 Conclusions

In conclusion, the SERiSM project has highlighted the modulation of cannabinoid receptors in a model of osteogenic differentiation of BDSCs induced by rapamycin, as schematically depicted in Fig. 5.

In this context, we have recently published the proteomic changes and epigenetic modifications occurring during stem cell differentiation in the microgravity environment [12]. Further studies are needed to better understand the relationship between these two events to ascertain the potential of endocannabinoid signaling in bone remodeling. In particular, it will be interesting to compare the data obtained under the same experimental conditions used onboard the ISS (and using the same CC), also on Earth.

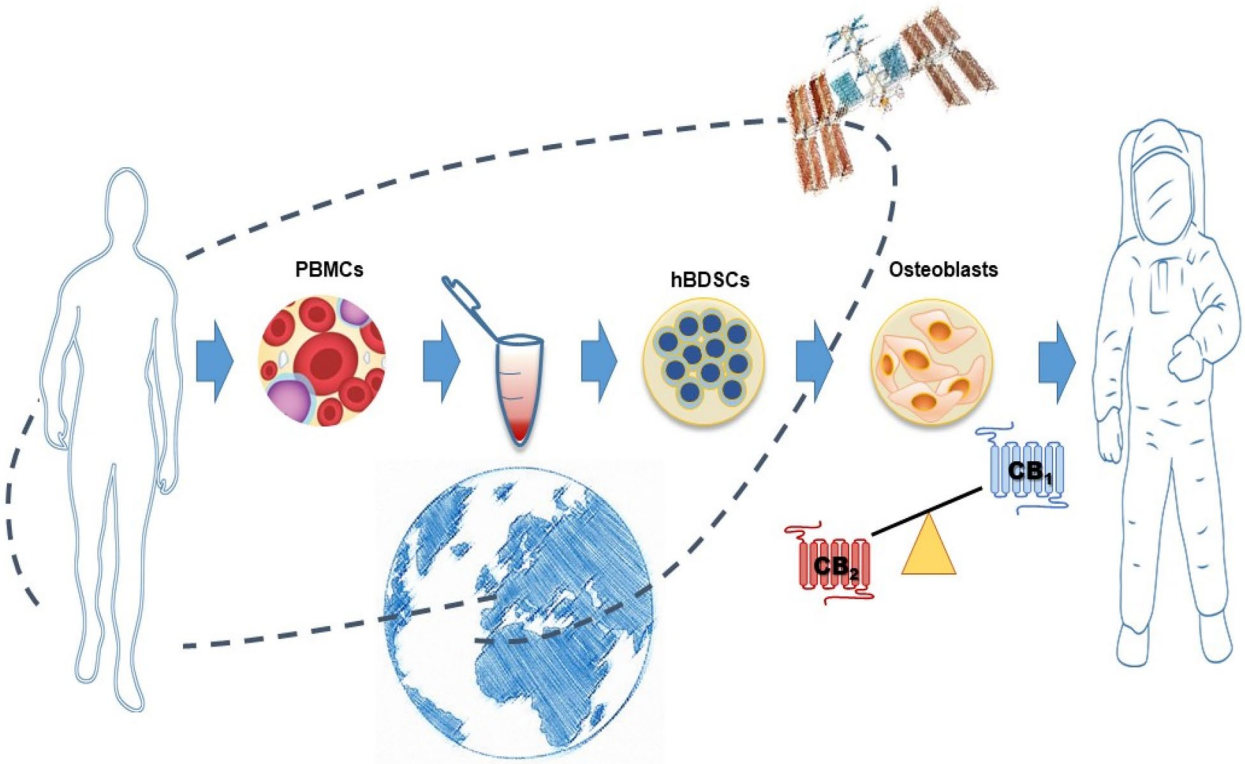

Fig. 5 Graphical abstract of the SERiSM mission profile

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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