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Three-dimensional growth of human endothelial cells in an automated cell culture experiment container during the SpaceX CRS-8 ISS space mission – The SPHEROIDS project

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ABSTRACT

Human endothelial cells (ECs) were sent to the *International Space Station* (ISS) to determine the impact of microgravity on the formation of three-dimensional structures. For this project, an automatic experiment unit (EU) was designed allowing cell culture in space. In order to enable a safe cell culture, cell nourishment and fixation after a pre-programmed timeframe, the materials used for construction of the EUs were tested in regard to their biocompatibility. These tests revealed a high biocompatibility for all parts of the EUs, which were in contact with the cells or the medium used. Most importantly, we found polyether ether ketones for surrounding the incubation chamber, which kept cellular viability above 80% and allowed the cells to adhere as long as they were exposed to normal gravity. After assembling the EU the ECs were cultured therein, where they showed good cell viability at least for 14 days. In addition, the functionality of the automatic medium exchange, and fixation procedures were confirmed. Two days before launch, the ECs were cultured in the EUs, which were afterwards mounted on the SpaceX CRS-8 rocket. 5 and 12 days after launch the cells were fixed. Subsequent analyses revealed a scaffold-free formation of spheroids in space.

1. Introduction

Endothelial cells (ECs) form the inner layer of blood vessels (intima) in mammalian organisms and are in direct contact with the blood. They play a key role in functional blood vessel morphogenesis [1] and in the regulation of blood pressure [2].

Experiments performed with a microgravity (μg)-simulating device, the Random Positioning Machine (RPM), revealed that ECs begin to form 3D cell aggregates after short-term RPM-exposure [3-5]. In addition, a part of the ECs of each culture grew as tube-like structures [6]. Proteins significantly altered in their content and involved in the formation of 3D aggregates include β_1-integrin, cytoskeletal proteins, or cell adhesion and extracellular matrix proteins, such as laminin, fibronectin, collagen type I and III, and osteopontin [6-8]. In short-term studies, an increase in apoptosis in adherently growing RPM-exposed cells has been observed [8]. Supplementation with vascular endothelial growth factor (VEGF) attenuated the apoptotic effect of microgravity, but had no obvious influence on the rate of 3D spheroid formation [7,8].

Based on this information, a spaceflight to the International Space Station (ISS) with acronym SPHEROIDS was carried out in 2016 in order to study the EC behavior under spaceflight conditions and to prove the gravity dependency of ECs' tube formation. The requirements encompassed experiment durations of 7 days and 14 days as well as fixation of ECs with RNA*later* and paraformaldehyde (3.4% PFA). A nutrient solution exchange after about 7 days was a prerequisite for the 14-day-experiment. The storage of the fixed samples was intended to take place at 4 °C (PFA) and −80 °C (RNA*later*).

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Since experiments on Earth (i.e., with open liquid handling, plastic consumables or manpower) cannot be replicated in space, the design of an automated experimental unit (EU) was necessitated. The materials intended for the construction of the EU were tested for their biocompatibility before and after construction of the EU (Breadboard Model, Science Model) (Figs. 1 and 2). To prepare the spaceflight, two additional tests, the Science Validation Test and the Experiment Sequence Test, were conducted practicing the sequence of the individual steps with all persons required to elucidate the reliability of the EUs. In addition, the assembly of the EUs was performed considering all quality check marks required by NASA (*National Aeronautics and Space Administration)*, i.e. the assembly of the EUs, time of the upload to the ISS, the powered experiment durations (7 days and 14 days maximal time until fixation) in microgravity with subsequent cool storage of the fixed samples and the download time back to Earth. As the EUs were stored on board the ISS inside the ESA-designed KUBIK experiment container, which can harbor, empower and automatically control the functionality of the EUs, two KUBIK containers available on Earth were used for the Experiment Sequence Test. After this careful preparation, for the first time we showed 3D growth of ECs in space in samples fixed after 5d of ECs-exposure to real μg.

2. Materials & methods

2.1. Cell culture and experimental layout

The human endothelial cell line EA.hy926 is a permanent cell line, which was established by hybridizing human umbilical vein endothelial cells (HUVEC) with the lung carcinoma cell line A549.

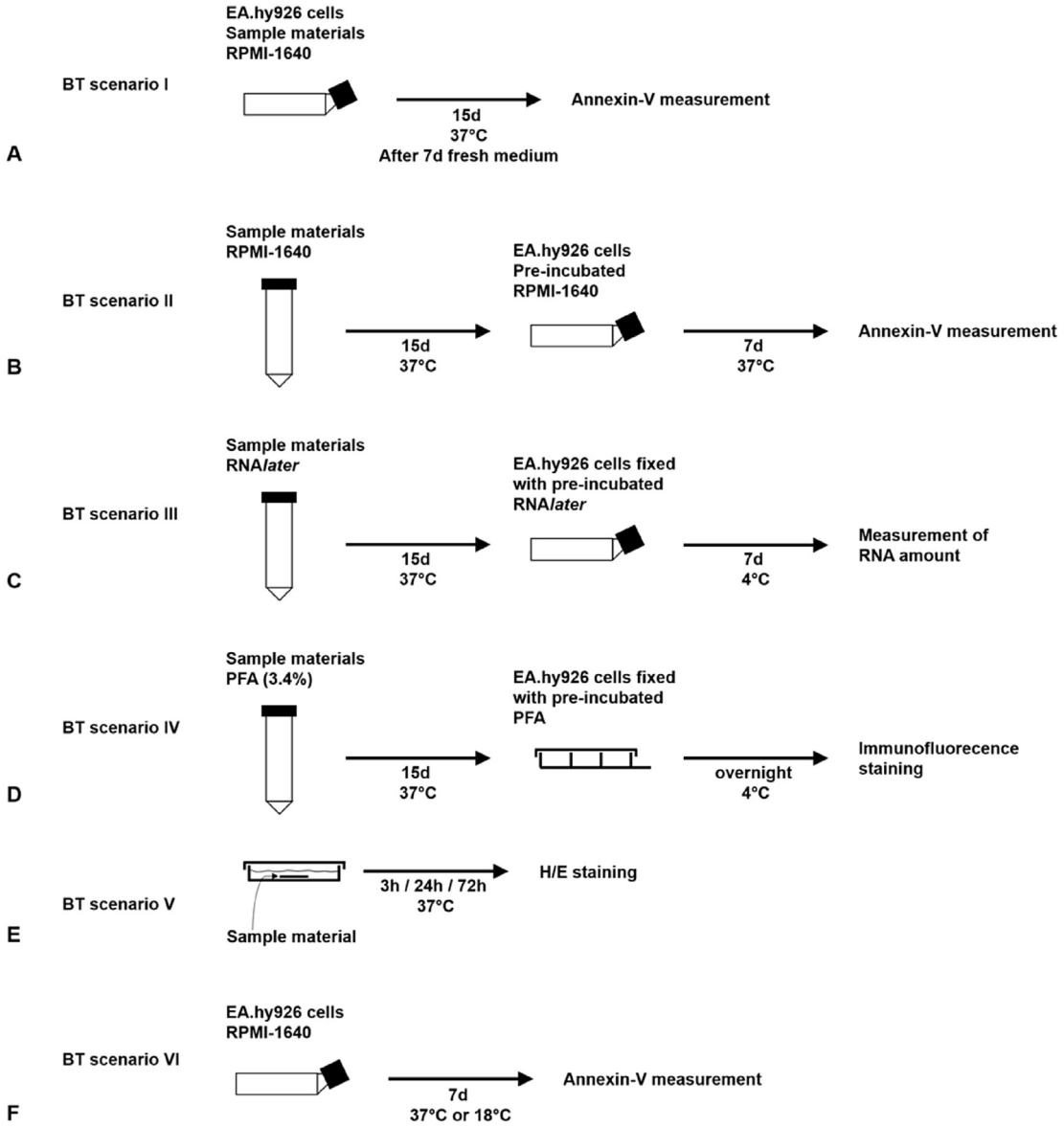

Fig. 1. Flowchart of the biocompatibility tests performed according to six different scenarios. Test procedures and schedules of the scenarios I to VI are shown in **A**–**F**. When a T25 cm^2 cell culture flask was required for a test (**A**,**B**,**C**,**F**), 10^6 EA.hy926 cells suspended in 15 ml corresponding complete culture medium were seeded into a flask one day prior to the start of the experiment. Pre-incubation of the various pieces of material was done in 50 ml tubes filled with about 20 ml of either medium, RNA*later* or PFA solution (**B**,**C**,**D**). Cells seeded into four-well cell culture slides (BD Bioscience) were used for immunofluorescence staining (scenario IV, **D**). Fixed samples were stored at 4 °C prior RNA isolation and measurement of the amount of RNA (**C**) or staining (**D**). For tests according to scenario V (**E**), the material sample was placed in the middle of a petri dish and the EA.hy926 cells were seeded directly on the surface. After inoculation, the petri dish was carefully filled with medium trying to avoid the excessive stirring so that the cells were not washed of the material. After 3, 24 and 72 h of incubation cells adherently growing to the materials were detected by HE staining.