SPACE LIFE SCIENCES SYMPOSIUM (A1)
Biology in Space (7)

Author: Dr. FABRICE ROTTMEIER
RUAG Space AG, Switzerland, fabrice.rottmeier@ruag.com

FLEXIBLE MEMBRANE CULTIVATION CHAMBER DESIGN FOR THREE-DIMENSIONAL HUMAN CELL STRUCTURE GROWTH

Abstract

In the frame of space medicine experiments funded by the European Space Agency, RUAG Space has been selected for the design, development and testing of dedicated Experiment Units. The experiment, named SPHEROIDS, aims at determining the influence of long-term microgravity exposure on human endothelial cell function, their program of differentiation and apoptosis (programmed cell death) as well as to explore possible blood vessel formation. Studies have shown that the endothelial cells are very sensitive to the physically simulated microgravity environment, which induces the formation of three-dimensional cell aggregates, as well as an up-regulation of several growth factors and of extracellular matrix components, but also initiates apoptosis in the EA.hy926 human endothelial cell line.

The flight experiment planned onboard the International Space Station will enable scientists to distinguish the effects caused by on-ground simulated microgravity experiments on Random Positioning Machine from those due to real microgravity. It will also be investigated if the endothelial cells exposed to such environment can be protected using Vascular Endothelial Growth Factor. The expected cell structures to be formed during the 14 days micro-gravity subset of experiments are spheroids of a diameter of up to 6 [mm] and tubular aggregates of up to 3 [cm] long. In order to analyze the cells and medium after sample retrieval, 80% of the supernatant medium in contact with the cells is automatically extracted from the cultivation chambers, while these are subsequently either fixed by Paraformaldehyde or RNAlater before storage in refrigerated or frozen conditions, respectively.

Here, a novel design of a 15 ml cultivation chamber is presented. The implementation of a transparent and highly flexible chamber membrane enables the extraction of more than 80% supernatant solution at low differential pressures without affecting the morphology of the 3D cell aggregates. The supernatant extraction can be carried out independently of subsequent fixative injection, thereby minimizing the risk of supernatant-fixative contamination. The implementation of a flexible membrane also provides an adaptive volume compensation system for the freezing of biological samples at temperatures down to -130 deg C. In detail characterization of mechanical membrane deformation under cryogenic, ambient, and depressurized conditions are presented. A rigid flat-bed structure at the center of the cylindrical chamber provides a suitable surface for cell-adhesion under regular gravity conditions. The chamber inlet and outlet ports are designed to promote mixing during fixative injection, creating homogenous fixing conditions. Results of biological validation are also presented.