Design, Prototyping, and Testing of a Novel Flowpath With an Array of four 3D Matrix Vitvo Bioreactors for the NASA Bioculture System

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Introduction

The NASA Bioculture System is an advanced cell culture closed-loop system containing highly automated flowpaths designed to conduct long term biology experiments on ISS with earth remote controllable medium flow, temperature, gas composition, medium exchange, cell sampling and fixation. This technology was already demonstrated with successful cardiomyocyte and osteocyte cultures experiments onboard the ISS and is now supporting NASA PI science. The Bioculture System, however, can only support 10 cassettes with disposable flowpaths, each containing a single hollow fiber bioreactor with a culture capacity of about 2ml.

This constraint not only severely limits the number of investigators that can conduct experiments in space, but also subjects the experiments to limitations in the number of replicates and conditions that can be studied. To address these limitations, we sought a novel design solution to maximize the number of separate bioreactor cultures and volume that can be conducted simultaneously. To this end we designed, prototyped, and are now testing a Vitvo 3D Matrix 2ml bioreactor insert that replaces the conventional Bioculture System hollow fiber bioreactor. This design will allow the Bioculture System to support up to 40 different bioreactors at once.

First Iteration with Six Bioreactors

In the first iteration of our multi-bioreactor system we attempted to fit six bioreactors per system, with three Vitvo matrices stacked on each side.

Second Iteration with Four Bioreactors

The current version of the multi-bioreactor inserts will be able to support up to 40 samples. Specifically, the novel gas-tight containment housing insert contains four Rigenerand Vitvo bioreactors stacked on each side of a heat sink powered and controlled by the existing heating element and pair of temperature sensors.

Future Work - Integration with CellScience

We will proceed with more extensive testing in order to optimize the integration of this multi-bioreactor containment system with the CellScience Bioculture system. This goal was in mind through the entire prototyping process. The bioreactor containment structure was built to fit into the rectangular slot of the CellScience Bioculture manifold originally holding a single bioreactor. Each bioreactor will have direct access to media through tubing that comes through via a luer to thread cutout at the backside of the box. Media waste will be expelled from the other end of the bioreactors and taken away through the same mechanism. We will continue working towards optimizing a fully-functioning multi-bioreactor cell culture system in hopes that it will allow for more necessary opportunities to conduct biological experimentation in space.

Results

We used Calcein AM stain and DAPI nuclear stain to visualize EB viability. Calcein AM stain showed extensive viability of the EBs and an initial morphologic shift to an adherent phenotype for adherent EBs. DAPI nuclear stained showed cellular outgrowth from adherent EB spheroids and similar cell count between adherent and nonadherent cells. We conclude that Eb culture adhesion has no effect on proliferation when cultured with the Vitvo bioreactor flowpath, verifying that the matrix surface can be suitable for automated cell culture.