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.

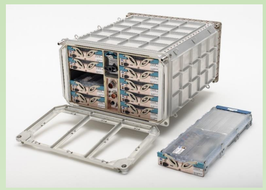
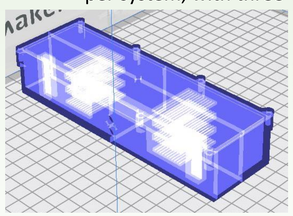


Figure 1. The current Bioculture system with a single bioreactor per cassette. Hart, Dominic. (2016). Biolculture System
https://www.nasa.gov/sites/default/files/atoms/files/bioculture-fs_03oct2016.pdf

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.



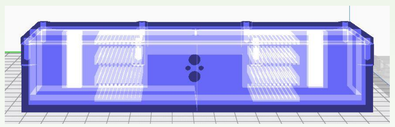
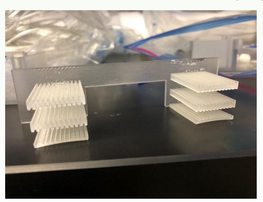


Figure 3 (below). Top racks of the six-bioreactor heat sink broken off and bottom racks show visible warping.

Figure 2. (above and right) The first design for the multi-bioreactor system with space for six bioreactors.

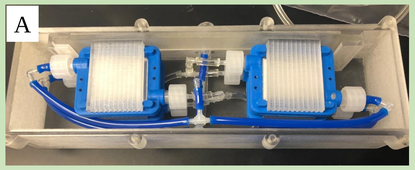


However, there were critical problems with this configuration. The top and bottom racks of the heat sink proved to be too thin as they would bend out of shape and easily break off from the rest of the structure.

After doing a fit test with all six bioreactors, it was clear that there was not enough vertical space unless we made unideal modifications. We decided it was best to modify our design to fit four bioreactors, which would eliminate these issues and allow more room for tubing.

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.



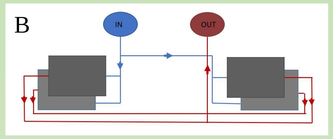


Figure 4. A) Prototype of the four-bioreactor system with plumbing for media. Clear tubing represents intake and blue tubing represents waste.) Diagram of fluid flow through the multi-bioreactor insert.

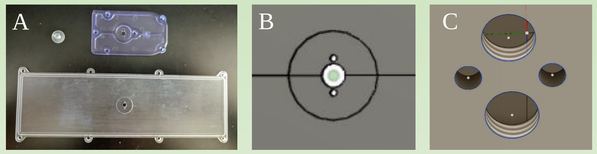
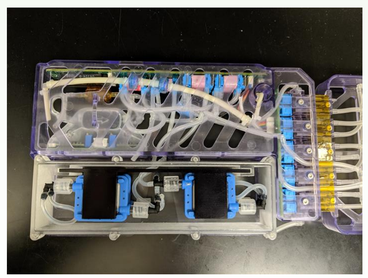


Figure 6. A) top – CO2 gasket for current CellScience module, bottom – lid for the multi-bioreactor system with fitting for CO2 gasket membrane valve. B) shows pressure release flap cutout on the lid. C) shows four access cutouts on the backside of the bioreactor box, one of which is for the gas supply.

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 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.



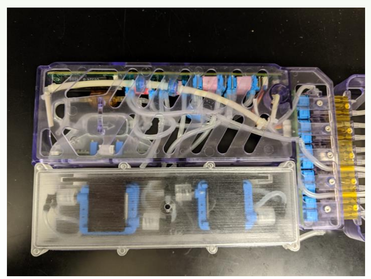


Figure 9. Aerial view of the containment box integrated with the Bioculture system manifold with and without lid and pressure-release mechanism..

Initial Testing

We first wanted to test the viability of the Vitvo bioreactor with automated media exchange. Embryoid bodies (EB) were chosen to test the performance of our flowpath with cell-adherence as well as nonadherence. EBs were cultured for 7 days with no direct interaction. Media was changed twice a day with a preprogrammed microfluidic pump at a flowrate of 0.25ml/min exchanging 750ul per media exchange (total of 1.5ml per day exchanged) through the input valve on the opposite side of membrane to location of EBs. Media would diffuse across the membrane causing no direct mechanical stimulation on the cells and allowing nutrients to be distributed across a gradient evenly.

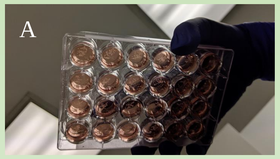
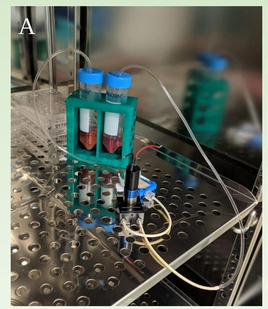
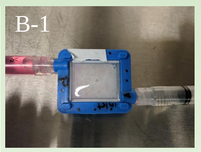


Figure 9. A) shows early development EB cells on low adhesion plates on Day 5 ready to be transferred. B) shows EBs in 3mL syringe being transferred into the Vitvo Bioreactor single test flowpath system.





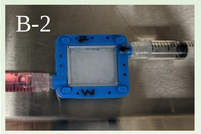


Figure 10. A) shows setup for the single bioreactor testing with two tubes for intake media and waste. B-1) shows fixation of nonadherent EBs which are mostly spherical. B-2) shows fixation of adherent EBs which have visibly flattened.

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.

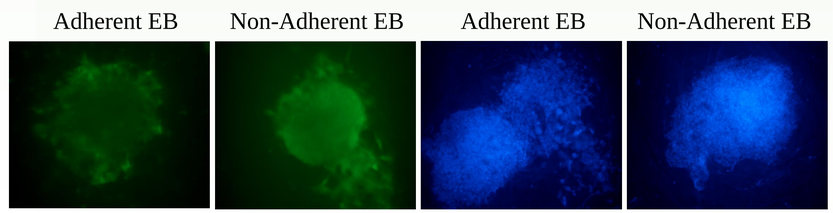


Figure 11. A-1) and A-2) show cells stained with Calcein AM. B-1) and B-2) show nonadherent cells stained with DAPI.