Dual-Chamber Autonomous Bioreactor for the growth of 3-D Cell Cultures in Microgravity

Austin James, Kevin King, Isaac Phillips, Cody Tapscott

Oshman Engineering Design Kitchen

Problem Statement

The goal of this project is development and testing of an autonomous bioreactor for use in microgravity. We created a proof of concept that showed successful operation of twelve autonomous chambers and creation of the critical air-liquid interface needed for cell growth.

Background and Motivation

Long duration spaceflight poses multiple hazards to human health, including increased exposure to radiation, nutritional deficiencies, and physiological changes due to exposure to microgravity. The use of unique three-dimensional (3-D) organotypic cell culture models that faithfully recapitulate the morphological features, differentiation markers, and growth characteristics observed in normal human epithelial tissues offer promise for use as a platform for research into these risks of manned space flight and possible countermeasures for risk reduction.

In a microgravity environment, the lack of gravitational forces to retain the media at the bottom of the cultures and prevent it from floating to the top to cover the epithelial layers poses a problem of maintaining of the air-liquid interface. There is currently no 3-D biological culture chambers on board the ISS for research into the effects of microgravity.

Testing Results

Microgravity Testing

Our prototype went through two major states of testing. The first occurred in Microgravity, where our team performed various fluid flow processes in the NASA parabolic aircraft. We constructed a twice watertight clear flight chamber in which we could observe the testing of chamber processes by Bluetooth application control.

Observed Results in Microgravity:
- Some leaking in chamber seals
- Mostly successful filling and fluid flow
- High capillary forces compared to the gravitational force as expected

Full Scale Ground Testing

The second round of testing occurred at the Oshman Engineering Design Kitchen after we had developed our final prototype to address issues found during microgravity testing and to expand the number of cell culture chambers from three to twelve. We also expanded the scope of the code to allow for direct control of individual valves and pumps, specifics chambers, or the whole twelve chamber system.

Observed Results in Earth Gravity:
- Complete reliability and full functionality of the upper chambers for both filling and emptying
- Successful filling, emptying, and flushing of bottom chambers
- Successful app interface for valve testing
- Successful app control of pumps and valves
- Successful processes of fill, empty, flush for specific chambers with some limitations on timing

Design Solution

Overall Design

- Block design for organization
- 6 Units with 12 total chambers
- 48 Solenoid Valves for independent control
- 6 Micro pumps
- Control via application over Bluetooth

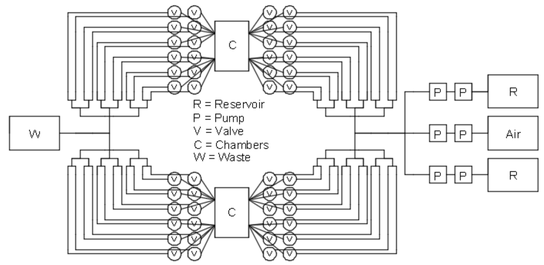

Figure 1. Simplified 2D block diagram of the overall design

Chambers

- Dual chamber design allows for critical air-liquid interface
- Three parts: Top half, bottom half, and removable insert
- Each half has a well cut into them
- Top half well is all the way through for application of membrane
- Bottom half has extra groove for removable insert
- Both halves have an O-ring groove to prevent leaking
- Removable insert contains trans-well mesh that the 3D culture is glued to.
- Filling pathways are different depending on inlet/outlet and top/bottom chamber to optimize filling and emptying

Electronics

Arduino-based actuation and timing control system

- Arduino directly interfaces with the valves and pumps
- Allows for automatic filling and emptying based on empirically determined timings
- Implements basic communication protocol with the control software
- Sends control signals to valves and pumps

Cordova-based smartphone application

- Provides a user interface to test and control the device
- Communicates with Arduino via Bluetooth
- Allows for easy independent control of all valves and pumps
- Can perform predetermined functions or be operated manually

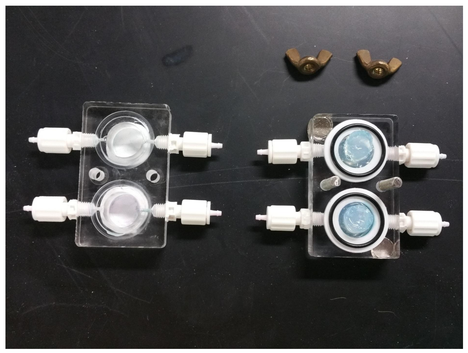

Figure 2. Chambers disassembled with cell culture substitute glued in

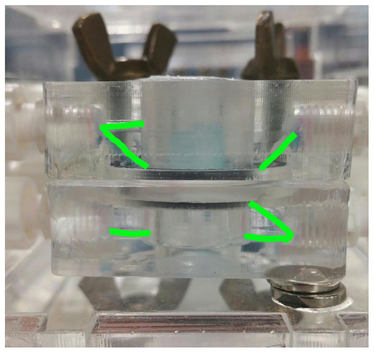

Figure 3. Inlet and outlet pathways highlighted in green

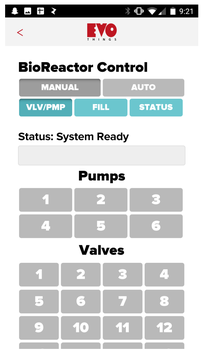

Figure 4. VLV/PMP Controls

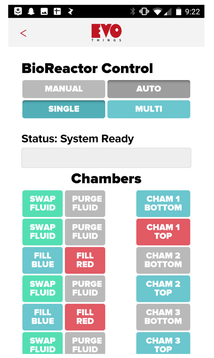

Figure 5. SINGLE Controls

Figure 6. MULTI Controls

Conclusion and Future Plans

Our team has improved the functionality in all aspects of the bioreactor design on which we started work last January. The cell culture chamber shape and design has been tested extensively, and consolidated and improved. The app interface allows for efficient use and testing of the cell culture chambers' air-liquid interface.

Our final design allows for our collaborators at Wiley to test the chamber design, system functionality, and code interface. We have also provided a fully coded function process for the automatic growth of cell cultures of all twelve chambers. The next step is the in-depth testing and timing development of the system processes and possible future testing in microgravity.

Acknowledgements

We would like to thank Dr. Zarana Patel for the opportunity to collaborate on this project and for the great opportunities which she has provided throughout the completion of our design. We would also like to thank our mentor Dr. Wettergreen for overseeing our progress throughout the whole design process. Finally, we would like to thank everyone who has provided assistance, help, and expertise on the project including William Nelson, Dr. Janice Huff, Joe Gesenhues, Carlos Amaro, and all the staff at the OEDK.