Next-Generation Scalable Commercial Biomanufacturing in Low Earth Orbit

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INTRODUCTION

Low Earth Orbit Biosciences will develop and enable a hardware and software technology platform for testing and on-orbit qualification on the ISS U.S. National Laboratory (ISSNL) to address product technology gaps and facilitate applied research and development (R&D) efforts in stem cell biomanufacturing and regenerative medicine. This endeavor incorporates ground-proven, commercial of-the-shelf (COTS), automated technologies to demonstrate a multi-function, low-cost, customizable, and scalable, R&D platform for on-orbit biomanufacturing and regenerative medicine that will expand the capabilities for human cell and tissue culture and provide a novel and sustainable technology platform for growth of the commercial LEO economy that supports clinically relevant terrestrial applications.

METHODS

Bioreactor Continuous Suspension Culture

Cells were thawed from -80 °C frozen suspension and cultured in RPMI +10% FCS, 1% P/S/A filter sterilized media at 37 C, 5% CO2.

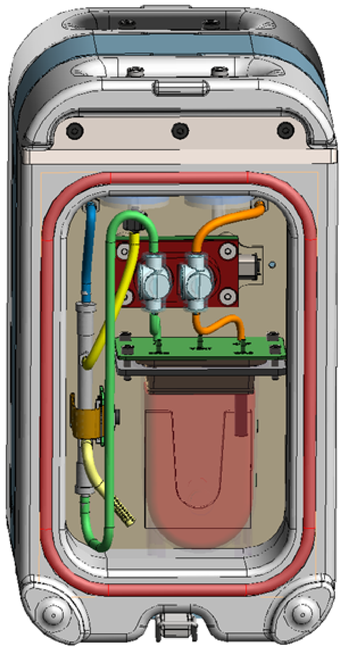
Cells were counted and re-fed weekly to check viability and cell growthAt Passage, cell lines were frozen in 10% DMSO 90% FBS, -80 °C for later reactivation testing. At 2nd passage, cells were transferred to larger bioreactor (0.1L -> 0.5L) and remained in continuous suspension culture for longer than 30 days to date.

Some cells were collected at 2nd passage for testing of cells and culture media for novel intracellular/intravesicular flow cytometric analysis of extracellular vesicle (EV) biomarkers CD63 and ALIX and for the experimental marker of interest to the Khaled Lab: CCT2, a subunit of the Cancer activated chaperonin that is critical for cytoskeletal protein folding and implicated in EV production and cell-cell messaging involved in metastatic potential, migration and possible metastatic site niche development.

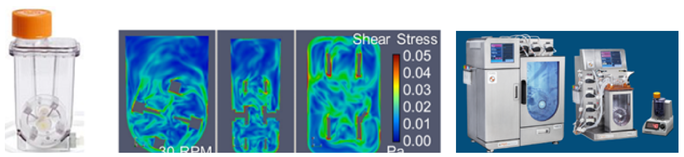
A secondary goal of this testing was to trial a new targeted CCT2 inhibitor preparation, Z-TOP, for use in identifying and binding CCT components in cancer cells and cancer cell EV.

TECHNOLOGY

Scalable Biomanufacturing System



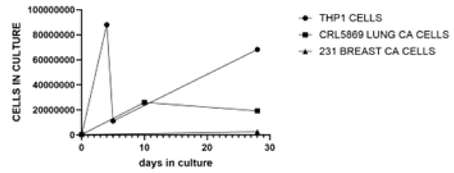
- COTS components
- GMP-compatible, single-use, vertical-wheel bioreactor
- Supports multiple cell types
- Real-time monitoring and control of culture environment
- Automated perfusion media exchange
- Closed-system feeding, sampling and harvest
- Filtration/separation of cells and media
- Bubble management and gas sparging
- Optical sensing of DO, pH
- Contamination resistance
- Remote monitoring
- Live imaging
- Compatible with ISS incubator and on future commercial platforms
- Battery-powered operation available



The PBS Biotech Vertical-Wheel Bioreactor. The unique vertical impeller provides a homogenous, low-shear environment, allowing for seamless process development, scale-up from 0.1L to 80L, and cell therapy product manufacturing. Image Credit: PBS Biotech

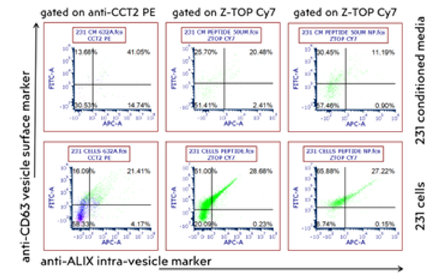
RESULTS

Cell Growth in Vertical-Wheel Bioreactor



Cell type	Culture Passage/ time in culture	Bioreactor Volume	Viable Cells	Non-Viable Cells
THP-1 Human monocyte cell line	Passage 17 to 20 Total of 37 days in continuous culture	80ml-> 2x 80ml-> 300ml->490ml	72%	28%
CRL 5869 Lung adenocarcinoma cell line	Passage 1 to 3 Total of 31 days in continuous culture	80ml->300ml-> 400ml	64%	36%
MDA-MD-231 Breast Cancer cell line	Passage 1 to 2 Total of 30 days in continuous culture	80-> 80ml	63%	37%

Intracellular and Intravesicular Flow Cytometric Analysis



Purpose: 1) Examination of cargo packaged in EV from cancer cell lines grown in suspension culture. 2) Test the ability of new inhibitor peptide drug to enter cells and EV and bind its target protein, CCT2 chaperonin

Findings suggest that a new inhibitor peptide drug can enter and bind its target CCT2 in cells and in cancer cell EV. This is a first step in testing the drug candidate's diagnostic & therapeutic development.

CONCLUSIONS

Demonstrated success with vertical-wheel bioreactor system and protocols using several cell models regarding:

- Cell thawing and seeding
- Suspension cell culture with agitation
- Prolonged cell growth, expansion and passaging
- Cell viability of many cell types, including cells that are typically nonviable in non-adherent culture
- Media exchange, sample collection, and cryopreservation
- Extracellular vesicle recovery and analysis
- Suitability for human cell and gene therapy applications

FUTURE DIRECTIONS

- Optimize conditions for cell recovery, culture and cryopreservation
- Demonstrate success with iPS cells in scalable bioreactor system
- Evaluate new automated technologies for cell thawing and analysis
- Develop gas management and oxygenation protocols
- Proceed with payload integration and flight preparation
- Evaluate technology platform for other biomanufacturing applications

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