Bone Marrow Microenvironment Culturing System for Mesenchymal Stem Cells:

Product Design Specifications

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Function: The Bioreactor will continuously deliver oxygen (in %) at user-specified hypoxic concentrations while continuously monitoring and adjusting O_2% to meet user-specified O_2%. The Bioreactor will also deliver stable temperature and humidity, as well as exhibit impermeability to outside air pollutants and other sources of cell culture contamination.

Client Requirements: The Bioreactor will provide a scalable and cost-effective platform to study the effect of hypoxic conditions on the quiescence of human mesenchymal stem cells (hMSCs). The Bioreactor must fit within Professor Li's available lab space by maximizing limited bench space or the existing Bioreactor area.

Design Requirements:

1. **Physical and Operational Characteristics**
    - ***a. Performance Requirements:*** The Bioreactor should be developed with the aim of standardizing a repeatable, scalable method for medium- to small-scale experiments to study the effects of hypoxic conditions on cell populations. To meet this goal, it should manipulate oxygen tension (pO_2, %) of the hMSC culture environment by controlling O_2% in the culture chamber.
    - ***b. Safety:*** Pure O_2 is a flammable gas, so handling gas cylinders and lines must follow OSHA guidelines and standards. Since the Bioreactor is also a platform to culture human or animal cells, it must withstand sterilization techniques and culture cells without risk of contamination.
    - ***c. Accuracy and Reliability:*** The Bioreactor must control O_2% of the cell culture environment within ± 1% while continuously monitoring and adjusting the measured O_2% to meet user-specified concentrations. The Bioreactor should also maintain a record of the air quality and oxygen levels to ensure system functioning, as well as allow the user to detect adverse events (i.e. power outage or leaving the door open).
    - ***d. Life in Service:*** The Bioreactor must be able to sustain cell culture environment and deliver reliable gas flows over a period of at least 5 years.
    - ***e. Shelf Life:*** The Bioreactor will not require maintenance for regular cleanings and possible replacement of air/gas filters or O_2 sensors.
    - ***f. Operating Environment:*** The Bioreactor will operate in a standard cell culture laboratory. The chamber will be placed inside a CO_2 cell culture incubator, while the

oxygen sensors and controls will be placed outside. The gas lines, which connect the Bioreactor to the gas sensor and controls, will enter the CO_2 incubator through a fitted pre-existing back port.

g. ***Ergonomics:*** The Bioreactor will be modeled after traditional cell culture dishes to ensure user familiarity and ease of access for cell care. The Bioreactor should also be operable without excessive force, as well as have appropriate handles, grips, and wheels for convenient transportation.

h. ***Size:*** The Bioreactor should not be larger than the maximum interior of a ThermoFisher Heracell CO_2 culture incubator (80 cm length, 700 cm width, 100 cm height). Ideally, the Bioreactor should fit on one shelf of the incubator. The Bioreactor also must be large enough to house at least nine Corning Costar six-well cell culture plates (each 12.8 cm length, 8.5 cm width, 2.0 cm height) for a total internal Bioreactor volume of at least 2,611.2 cm^3.

i. ***Weight:*** The Bioreactor must not exceed a total weight of 50 lbs so that it can be easily carried by two people.

j. ***Materials:*** The Bioreactor, its gas lines, and housing of gas sensors/controls must be composed of materials that can withstand sanitization by a 70% ethanol/water solution and an autoclave.

2. Production Characteristics

a. ***Quantity:*** One Bioreactor.

b. ***Target Product Cost:*** The Bioreactor should be less than the cost of current market competitors ($1000+ average). The target cost for our Bioreactor is $200.

3. Miscellaneous

a. ***Standards and Specifications:*** Will be determined upon product fabrication.

b. ***Customer:*** The target customer are researchers in the Li lab who will study variable oxygen tensions and its effect on hMSC quiescence. Other collaborators who wish to perform hypoxic experiments on a small scale may also be customers.

c. ***Competition:*** Several competitors exist for this product, ranging in cost and scalability.

i. The most direct competition is the Biospherix C-Chamber Hypoxia Chamber for cell culture [1]. This design consists of a small plexiglass chamber which is able to house plates at a total surface area of 12.75” x 10.25” and up to 2” in height. A manifold in the back of the chamber allows for gas lines to connect to the chamber, which flow past a controller which is able to monitor and control the flow of gases into the chamber based on the gas flow out of the chamber. While this product is comparable in terms of the intended size and function of our Bioreactor, its estimated price is in the range of $1000 which is expensive for smaller scale studies.

ii. Another competitor is the Stemcell Technologies Hypoxia Incubator chamber, which is a portable chamber which operates within an existing laboratory incubator to deliver variable hypoxic conditions [2]. It is likewise extremely scalable and can be used within any existing cell culture incubator, but the price is high at around $600 and requires additional proprietary components, such as a flow meter and additional tubing and setup. The largest issue with

this design is that it does not provide dynamic control of gas flows; the chamber is first flooded with oxygen, then purged to the correct hypoxic concentration and sealed. This implies that long-term experiments may require excessive hands-on maintenance to maintain the oxygen tension at the correct levels.

iii. The high-end competition to this solution is embodied in the Baker Ruskinn Invivo$_2$ Hypoxic Cell Culture Workstation [3]. This solution provides constant control of Oxygen tension, but is expensive with the cost in the thousands of dollars. This provides a more permanent solution to long-term cell culture focused on the effects of hypoxia in particular.

References:

1. BioSpherix. (2017). *Hypoxia Chamber for Cell Culture.* Retrieved from: http://www.biospherix.com/cell-culture-equipment/hypoxia-chamber-c-chamber.html
2. STEMCELL Technologies. (2017). *Hypoxia Incubator Chamber.* Retrieved from: https://www.stemcell.com/hypoxia-incubator-chamber.html
3. Baker. (2017). *INVIVO2.* Retrieved from: https://www.bakerco.com/products/invivo2