Bioreactor Cassette for Stem Cell Growth

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Abstract

Stem cells show great potential for use as patient-specific medical therapeutics. In order for this therapy to be effective, a bioreactor cassette system is required that is capable of providing conditions for growing multiple stem cell samples from individual patients without exchanging media between samples. Last semester we focused on basic design concepts, material selection, and flow analysis. This semester we collected data regarding cassette function and cell behavior within a simulated bioreactor system and found that the formation of bubbles (3.6 ±1.1 mL of air after 3 days operation) within the system prevented optimal performance of the bioreactor cassette. To address bubble accumulation, we designed a bubble trap better suited to our needs than similar commercially available products, characterized its performance, and confirmed its usability in the bioreactor system with ergonomic testing. Initial studies with HEK-293 cells in the bioreactor cassette system and bubble trap show improved cassette performance, but revealed several areas for ergonomic improvements in the design. Future work includes replicating the initial cell study with the bubble trap in the bioreactor system and improving the ergonomics of the cassette's design.

Background

Stem Cell Culture
- Clinical need for regenerative medicine [1]
- Signals direct differentiation [2]
- Require media supply [3]

Importance of Cassette
- Avoids manual daily media change
- Individualized therapy is practical with automation

Previous Semester Work
- Designed cassette housing
- Optimized geometry for flow

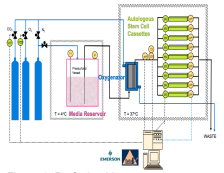

Figure 1: Perfusion bioreactor to connect cassettes in parallel for media delivery [2].

Motivation for Bubble Trap

Early Studies Indicated Bubble Problem

Possible sources
- Hollow fiber gas exchanger cleaning
- Media off-gassing
- Oxygenator
- Improper priming

Goal: To design a perfusion cassette system to efficiently culture independent samples of stem cells, including removal of bubbles

Design Criteria

Material
- Steam and gamma sterilizable
- Gas-impermeable growth plates
- Optically transparent
- No extractables
- No induction of differentiation

Flow
- Even fluid flow distribution
- Not interrupted by bubbles

Efficient Culture
- Even seeding and confluent growth
- Facilitate controlled differentiation

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Design

Previous Work

Redesign from last semester:
- Included balanced runner design at outlet
- Dye study demonstrated proficient flow patterns
- Reduced size to < 60 mL

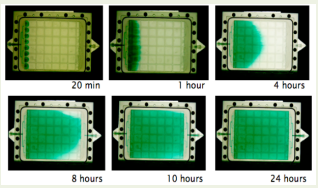

Figure 7: Flow patterns of mirrored balanced runner cassette

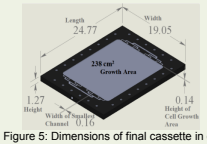

Figure 5: Dimensions of final cassette in cm

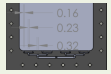

Figure 6: Diameter of balanced runner channels, ranging from 0.16 to 0.32 cm

Figure 8: Bubble formation after 3 days of perfusion

First cell trial indicated bubble problem:
- Size of bubble: 3.6 ±1.1 mL (Figure 8)
- Needed immediate attention to keep cells bathed

Bubble Trap

Figure 9: Bubble trap with bubbles in the upper chamber

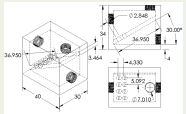

Figure 10: Bubble trap dimensions in mm

Bubble trap was 100% efficient in removing bubbles (~70 µL) under a total air volume of 15 mL.

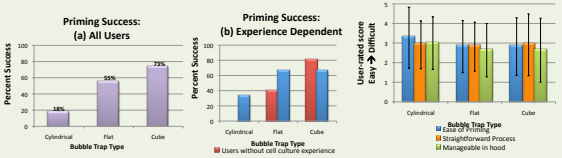

Figure 11: Priming Success (a) Participants primed our bubble trap (cube) with the greatest success rate (73%). (b) Breakdown based on cell culture experience shows a success rate of 0% for the cylindrical trap among those without experience.

Figure 12: There was no significant difference for ease of priming, straightforward nature or manageability in the hood of each trap ($F(2,2) = 2.11$, $p=0.24$).

Cell Study

Cell Study Results:
- Setup error prevented acquisition of cell results from cassette
- Successful Trypan protocol
- Crystal violet protocol led to cell delamination

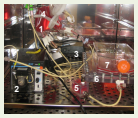

Figure 13: The cell study setup has many components: (1) media, (2) pump, (3) oxygen pump, (4) hollow fiber, (5) bubble trap, (6) cassette, and (7) control flask.

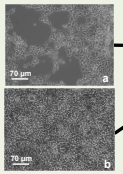

Figure 14: Phase contrast microscopy of control flask (a) 60% confluent (b) 100% confluent.

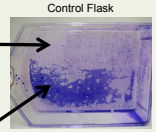

Figure 15: Crystal violet stain of control flask shows uneven cell densities.

Competition

Static Culture [4] (Figure 2)
- No constant growth factor supply
- Waste buildup

CLINICell Cassette [5] (Figure 3)
- Perfusion of media
- Gas permeable

Cylinder Trap (Baxter) (Figure 4a)
- Causes pressure buildup
- Difficult to remove bubbles

Flat Trap (Baxter) (Figure 4b)
- Incorrect priming destroys trap
- Difficult to remove bubbles

Figure 2: Static cell culture flasks
Image Courtesy of Corning
http://www.corning.com

Figure 3: CLINIcell cassette
Image Courtesy of INNOMDISTECH
http://www.innomt.com/

Figure 4: (a) Cylinder trap (b) Flat trap

Future Work

Cassette Redesign
- Manual assembly ergonomics
  - Remove screws
  - New housing design (Figure 16)
    - Decrease setup time and priming
    - Improved sterility
    - Better for mass production
- Flow characteristics

Bubble Trap Redesign (Figure 17)
- Increase overwhelm volume
- Easier bubble removal

Cell Testing—HEK-293 Cells
- Viability
- Spatial variation

Cell Testing—Stem Cells
- Viability
- Pluripotency staining for OCT4
- Long-term growth

Xeno-free Culture
- Remove animal origins for safety
- Use mTeSR1 medium [6]
- Test different substrates
  - Recombinant peptides with RGD [7]
  - Recombinant E-cadherin and igG-Fc protein [8]

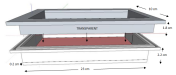

Figure 16: New cassette housing design

Figure 17: Modified bubble trap design

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