Bioreactor Cassette for Autologous Induced Pluripotent Stem Cells

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Abstract

Autologous induced pluripotent stem (iPS) cells show great potential for use in patient-specific medical therapeutics. In order for this therapy to be effective, it is necessary to have a bioreactor cassette system capable of culturing multiple iPS cell samples from individual patients without exchanging media between samples. To address this issue, we created and analyzed several different cassette designs with computational fluid dynamics (CFD) to determine the best geometry to achieve consistent flow. We determined that our best design for flow involved a fan into a rectangle with guides, but due to rapid prototyping limitations, this design was modified into two new designs: the straw design and the balanced runner design. These two designs along with the a basic rectangle design were dye-tested to analyze flow characteristics. We determined that the straw design has the greatest potential due to its higher tolerance for bubbles and potential for uniform flow. Future work will further optimize this design and integrate a bubble trap, cell metabolism monitoring system, and ergonomic clamp into the cassette–bioreactor interface.

Background

Induced Pluripotent Stem (iPS) Cells

- Derived from adult tissue [1]
- Pluripotency induced [2]
- Chemical and mechanical signals direct differentiation [3]
- Require media supply [4]

Bioreactor System [3] (Figure 1)

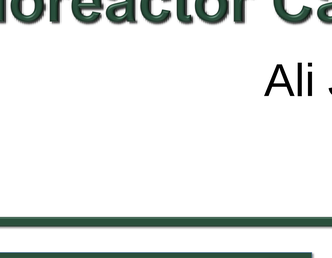


Figure 1: The perfusion bioreactor system will be connected to cassettes in parallel to deliver media without contamination.

Motivation

iPS Cell Culture

- Emerging field – commercially underdeveloped
- Avoids ethical issues associated with ES cells
- Need to optimize stem cell growth, conditions, and monitoring
- Individual samples cannot share media

Goal: To design a perfusion cassette system to efficiently culture independent samples of iPS cells.

Design Criteria

Material

- Steam and gamma sterilizable
- Gas-impermeable growth plates
- Optically transparent
- No extractables

Flow

- Even fluid distribution
- Not interrupted by bubbles

Monitoring

- Metabolism via pH
- Automated flow regulation with feedback

Final Design

Design Concept (Figure 2)

- Linear variation of straw cross-sectional area creates more resistance near center to force flow to edges
- Slanted straws help clear bubbles
- Multiple straws reduce dead zones

Materials

- **Polycarbonate Frame** [5]
  - Autoclave and gamma sterilizable
  - Resistant to weak acid
  - USP–Class VI certified
- **Polystyrene Growth Plate**
  - Commonly used for cell culture

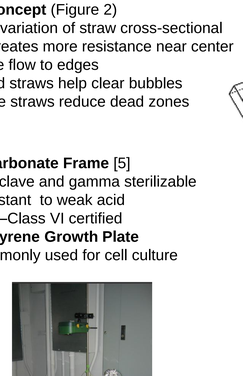


Figure 6: Set up for dye testing.

Testing

- **Prototypes**
  - Rapid prototyped with StereoLithography Apparatus
  - Resin: SLA Huntsman and Watershed
  - Lacquer was used for the rectangle and straw designs to prevent delamination
- **Dye Testing Set Up** (Figure 6)
  - Time-lapse camera
  - Peristaltic pump (0.17–0.3 mL/min)
  - 24 hours (~3 volume exchanges)
  - Dye wavelength 628.5 nm

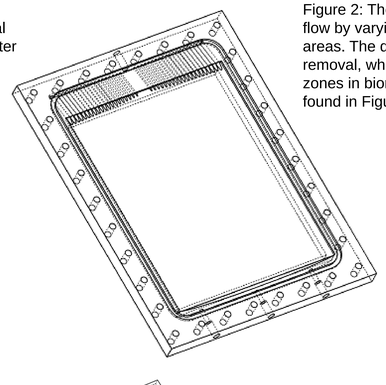


Figure 2: The straw design promotes even flow by varying 40 straw cross-sectional areas. The design is best for bubble removal, which is important to prevent dead zones in bioreactor cell culture. Dimensions found in Figure 3.

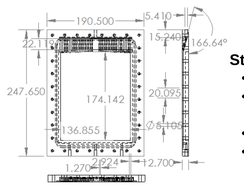
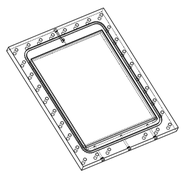


Figure 3: Straw design – dimensions in mm

Straw Design (Figure 3)

- Directs flow outward
- 40 straws (20 each side, symmetrical)
- One output
- Arched collecting trough



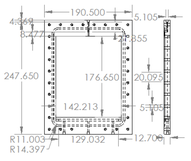


Figure 4: Rectangle design – dimensions in mm

Rectangle Design (Figure 4)

- Control
- One input
- One output
- Rectangular collecting trough

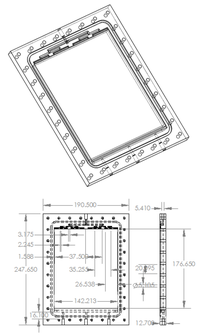


Figure 5: Balanced Runner design – dimensions in mm

Balanced Runner Design (Figure 5)

- Even distribution by dividing the flow in half 3 times
- 3 divisions into 8 branches
- One output
- Rectangular collecting trough
- Thin silicone film seals channels

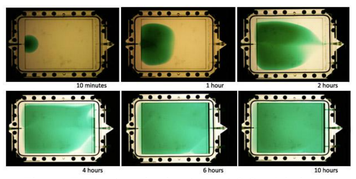


Figure 7: Rectangle dye test at 0.3 mL/min

Rectangle Results (Figure 7)

- 10 hours for complete perfusion
- Similar to CFD analysis (Figure 8)
- Capillary effect from collecting trough
- Poor lateral distribution

Figure 8: CFD analysis of Rectangle design

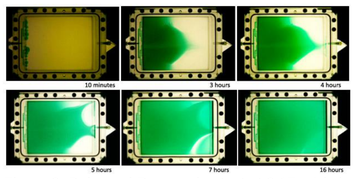


Figure 9: Balanced Runner dye test at 0.17 mL/min

Balanced Runner Results (Figure 9)

- 16 hours for complete perfusion
- Lateral dye distribution
- Reverse flow after reaching trough
- Similar capillary effect and dead zones as the rectangle design

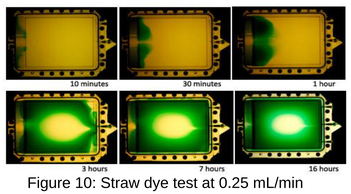


Figure 10: Straw dye test at 0.25 mL/min

Straw Results (Figure 10)

- Results skewed by prototype deformation
- Two fronts with diffusion towards center
- Bubble removal was more efficient than in balanced runner design.

Competition

Static Culture [5] (Figure 11)

- No constant growth factor supply
- Waste buildup

Figure 11: Static cell culture flasks sold by Corning (Product #3814) are frequently used to grow cells. The cells are attached to the bottom and are bathed in liquid media.

Image Courtesy of Corning http://www.corning.com

CLINICell Cassette [6] (Figure 12)

- Perfusion of media
- Gas permeable

Figure 12: The CLINIcell cassette does not have optimized flow distribution, the plates are permeable to oxygen, and the small cassette size is inefficient.

Image Courtesy of INNOMEDITECH http://www.innomt.com/

Future Work

Design Optimization

- Bubble removal
  - Bubble trap (Figure 13)
  - Arched collecting trough
- Straws
  - Increase diameter for center straws
  - Increase number of straws
- Outlet flow

Further Testing with Stem Cells

Monitoring

- pH fiber optic probe to monitor cell metabolism

Manual Assembly Ergonomics

Mass Production

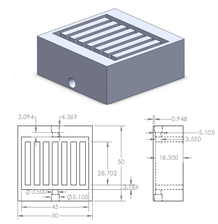


Figure 13: A device to attach proximal to the inlet to remove bubbles in the media caused by oxygenation. Dimensions in mm.

Acknowledgements

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