# 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. Such a cassette must also be gas impermeable, be able to be visualized on a standard microscope, and deliver even fluid flow over the cell growth area. Previous work included concept development of a reusable cassette prototype and identification/resolution of bubble accumulation in the cassette. Current work includes the development of a single-use cassette which optimizes fluid flow distribution, allows live-cell imaging, minimizes material use, and is ergonomically friendly. These claims are confirmed by dye studies, preliminary cell seeding studies, cost analysis, and ergonomic surveys. Future work includes confirming scale-up of the design and mass-production of the cassette to form a single, continuous piece.

## Background

**Stem Cell Culture**

- Clinical need for regenerative medicine [1]
- Chemical signals direct differentiation [2]
- Requires media supply [3]
- Bioreactor system can automate culture, but needs compartment for cell growth (Fig. 1)

**Importance of Cassette**

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

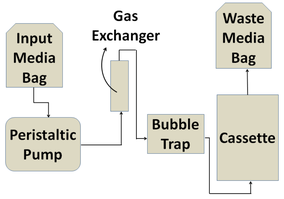



Figure 1: Perfusion bioreactor to connect cassette in parallel for media delivery [2].

## Design Criteria

**Material**

- Gas-impermeable growth plates
- Optically transparent
- No extractables

**Flow**

- Even fluid flow distribution
- Not interrupted by bubbles

**Efficient Culture**

- Even seeding
- Confluent growth

**Able to Image Cells**

**Human Factors**

- Seeding easy/efficient
- Successful bubble removal

## Acknowledgements

- Dr. Derek Hei
- Bill Kreamer
- Julie Johnson
- Diana Drier
- Carol Emler
- Kyle Ripple
- Laurie Larson
- Benjamin Cox
- Nathan Schumacher
- Dr. Rock Mackie and the Morgridge Institute for Research
- MSOE Rapid Prototyping Center
- Dr. Tim Osswald

## Design

### Design Progress

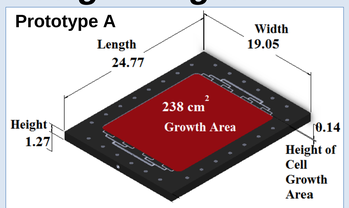



Figure 4: Dimensions of Prototype A in cm.

**Prototype A (Larger Cassette) Description (Fig. 4)**

- "Balanced runner" inlet channels split flow in half 3 times to attempt even distribution of flow
- Parabolic flow patterns (Fig. 5)
- Inlet mirrored to outlet
- Polycarbonate
- Bubbles difficult to remove, only by exterior trap (Fig. 6)
- No success with microscopy.

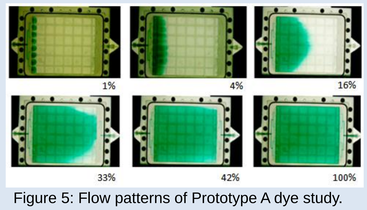



Figure 5: Flow patterns of Prototype A dye study.

**Main Problems**

- Assembly tedious
- Bubble removal difficult
- Difficult to image

Figure 6: Exterior bubble trap

**New Prototype**

**Solutions**

- Growth plate glued
- Bubble port
- Smaller size for microscopy

Table 1: Prototype technical comparison.

| Prototype Comparison | Prototype A: Larger Cassette | Prototype B: Smaller Cassette |
|---|---|---|
| Approx. Cost | ~$150 | ~$30 |
| Cost/Cell Growth Area | ~$0.63/cm² | ~$0.30/cm² |
| Human Factors | >45 min. to assemble | <10 min. to assemble |
| | Difficult to remove bubbles | Easier to remove bubbles |
| | User confusion | More obvious |
| Imaging | Too large for most light microscopes | Sized to fit on microscope stage |
| | Screws make it difficult to sit flat and image | Naturally lies flat |
| | Difficult to focus microscope (no success) | Easy to focus and image |
| Flow Distribution | Good, but parabolic pattern | Very good, appears even |

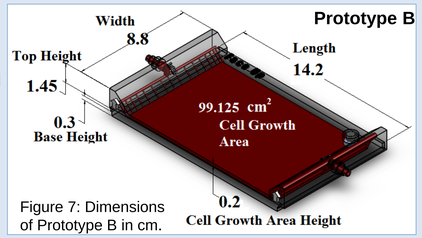



Figure 7: Dimensions of Prototype B in cm.

**Prototype B (Smaller Cassette) Description (Fig. 7)**

- Size-scaled inlet channels split flow based on approximate LOCR[4]
- Excellent flow patterns (Fig. 8)
- 3 size-scaled outlet channels
- Rapid-prototyped with Accura60
- Can directly remove bubbles with port and trap
- Able to be visualized using microscope

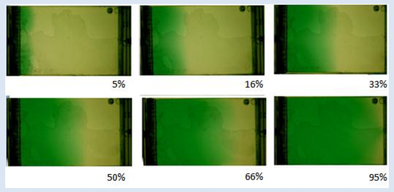



Figure 8: Flow patterns of Prototype B dye study.

### Imaging Capacity

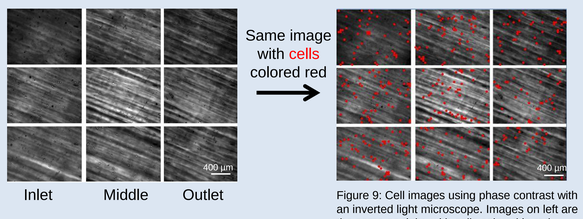



Figure 9: Cell images using phase contrast with an inverted light microscope. Images on left are the same as right, with cells colored in red.

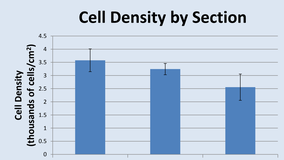



Figure 10: There was no significant cell density difference between the regions at the inlet, middle, or outlet ($F(2,2) = 6.94$, $p=0.08$).

**Imaging Results:**

- Easy to focus microscope
- Cassette fits on stage
- Cell density differences not significant comparing inlet, middle, outlet by 2-way ANOVA (Fig. 9,10)

### Human Factors

Survey and Study

- Subjects: 9 people with cell culture experience
- Study involved asking the participants to prime and remove bubbles from large and small cassette (Fig. 11, 12)
- Survey involved rating the experience and comparing Prototype A and B (Fig. 13)

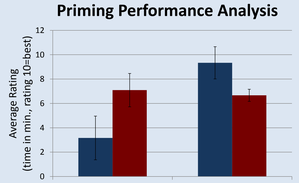



Figure 11: Time to prime (min) and bubble removal success (10 = complete removal) of Prototype A and B ($n=9$, error bars=±1σ, p<< .05 ).




Figure 13: User-rated ease and practicality of priming for Prototype A and B ($n=9$, error bars=±1σ, p<<.05 ).

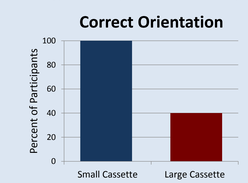



Figure 12: Percent of participants who correctly oriented Prototype A and B after priming ($n=9$).

## Competition

**Static Culture** [4] (Fig. 2)

- No constant growth factor supply
- Waste buildup

Figure 2: Static cell culture flasks

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

**CLINICell Cassette** [5] (Fig. 3)

- Perfusion of media
- Gas permeable

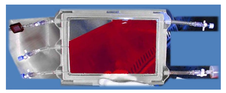


Figure 3: CLINIcell cassette

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

## Future Work

**Perfusion Testing—HEK-293 Cells**

- Viability
- Spatial variation

**Cell Testing—Stem Cells**

- Viability
- Pluripotency staining for OCT4
- Long-term growth

**Mass-Production**

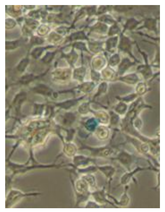
- Two-piece polystyrene injection molding
- Ultrasonic welding for continuous part

**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]

**Research for Optimal Use**

- Optimal media gassing
- Flow rate



## References

[1] Zandstra, P.W. and Nagy, A. Stem cell bioengineering. *Annual Review of Biomedical Engineering.* 2001. 3: 275-305.

[2] Hei, Derek (2010). "Bioreactor Perfusion Design" Waisman Clinical Biomanufacturing Facility, University of Wisconsin-Madison.

[3] NIH (2010). "Stem Cell Information." National Institutes of Health. http://stemcells.nih.gov/info/basics/basics1.asp

[4] Sigma-Aldrich (2010). "Bioreprogramming" Cell Culture: Sigma-Aldrich. http://www.sigmaaldrich.com/life- science/stem-cell-biology.html

[5] CLINIcell 250 cell culture cassette (2010). "User instructions V-2." http://www.innomt.com/products/download/Cell%20therapy_biotechnology/disposable%20bioreactor%20PDF/clinicell/CLINIcell%20250.pdf

[6] Yoon, T., Chang, B., Hyeung-Taek,K., Joo-Hyun, J., Dong-Wook,K., Dong-Youn H. 2010. Human embryonic stem cells (hESCs) cultured under distinctive feeder-free culture conditions display global gene expression patterns similar to hESCs from feeder-dependent culture conditions. *Stem Cell Rev and Rep* 6:425-437

[7] Klim, J., Li, L., Wrighton, P., Piekarczyk, M., Kiessling, L. 2010. A defined glycosaminoglycan-binding substratum human pluripotent stem cells. *Nature Methods* 7: 12: 989-994.

[8] Nagaoka, M., Si-Tayeb, K., Akaike, T., Duncan, S. 2010. *BMC Developmental Biology* 10 (60):1-12