Bioreactor Cassette for Stem Cell Culture

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BME 402
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Client

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Advisor

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Department of Biomedical Engineering

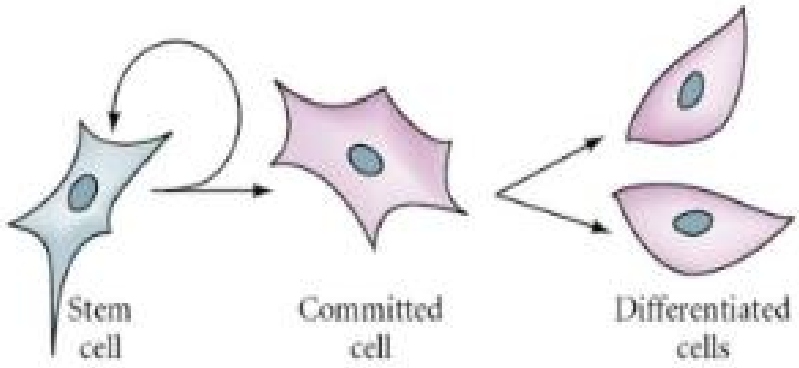
Overview

- Background
- Current Status
- Design Specifications
- Redesign
- Future Work

Problems with Stem Cell Culture

Sensitive to environment

- Daily media change
- Chemical leaching can cause undesired differentiation



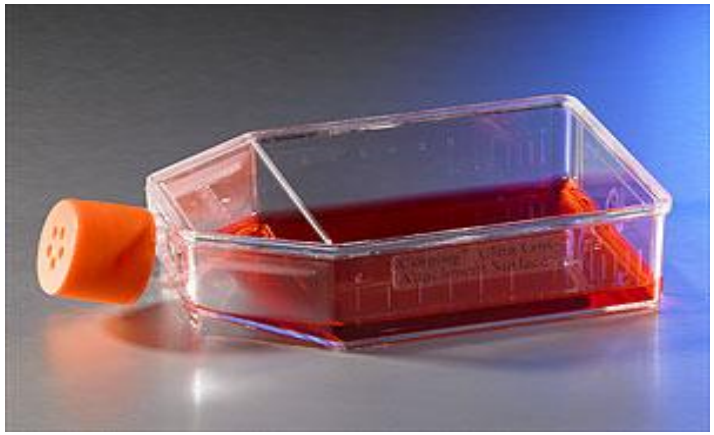
Pluripotent stem cell [1]

Clinical limitations

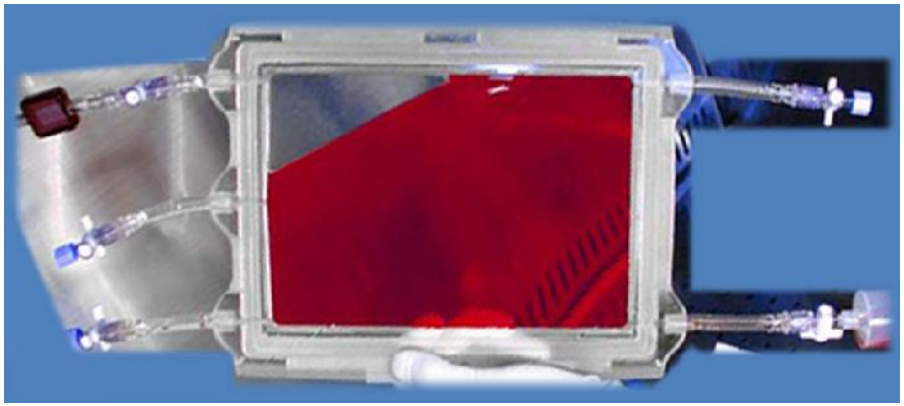
- Desire individualized therapies
- Mass production is not yet feasible

[1] KU Medical Center (2010). "Stem Cell Research 101" *University of Kansas Medical Center. http://www.kumc.edu/stemcell/images.html*

Current Solutions



Static culture[1]



CLINIcell Cassette [2]

[1] Corning (2010). "Corning® Ultra-Low Attachment 75cm² Rectangular Canted Neck Cell Culture Flask with Vent Cap (Product #3814)" *Corning: Life Sciences . http://catalog2.corning.com/*

[2] Innomeditch Technologies. "CLINIcell Cassette". *Innomeditch Technologies. http://www.innomt.com/products/products02_02_04.htm*

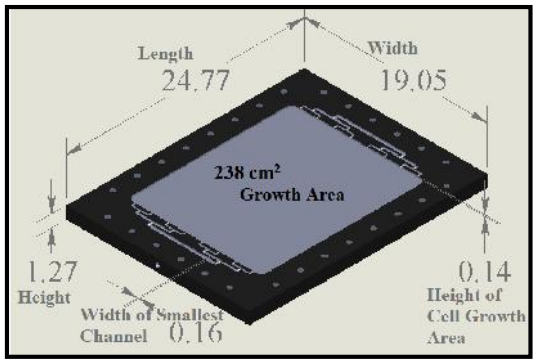
Design Proposal

Design a cassette system that interfaces with a perfusion bioreactor and provides appropriate conditions to culture several different samples of stem cells without exchanging media between them.

Specifications

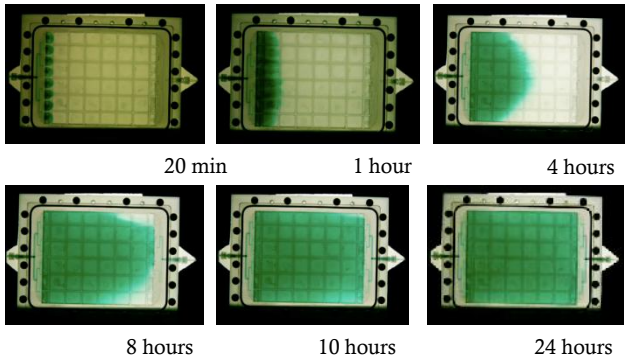
- Use gas-impermeable growth plates
- Be optically transparent
- Ergonomically friendly
- Avoid chemical leaching
- Minimize media use
- Ability to be imaged in a 96-well footprint

Project History



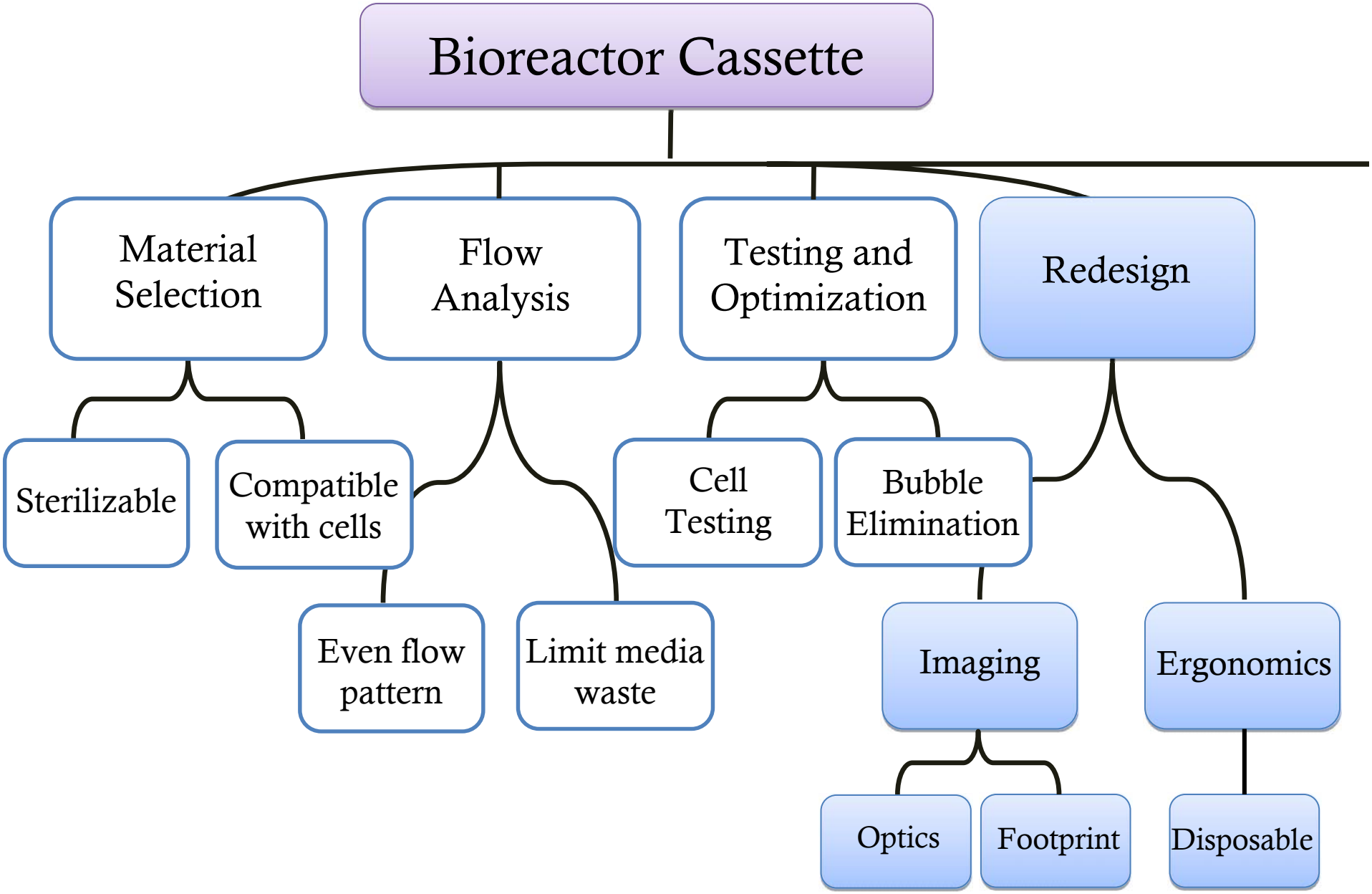
Dimensions in cm

Material Selection, Initial Design

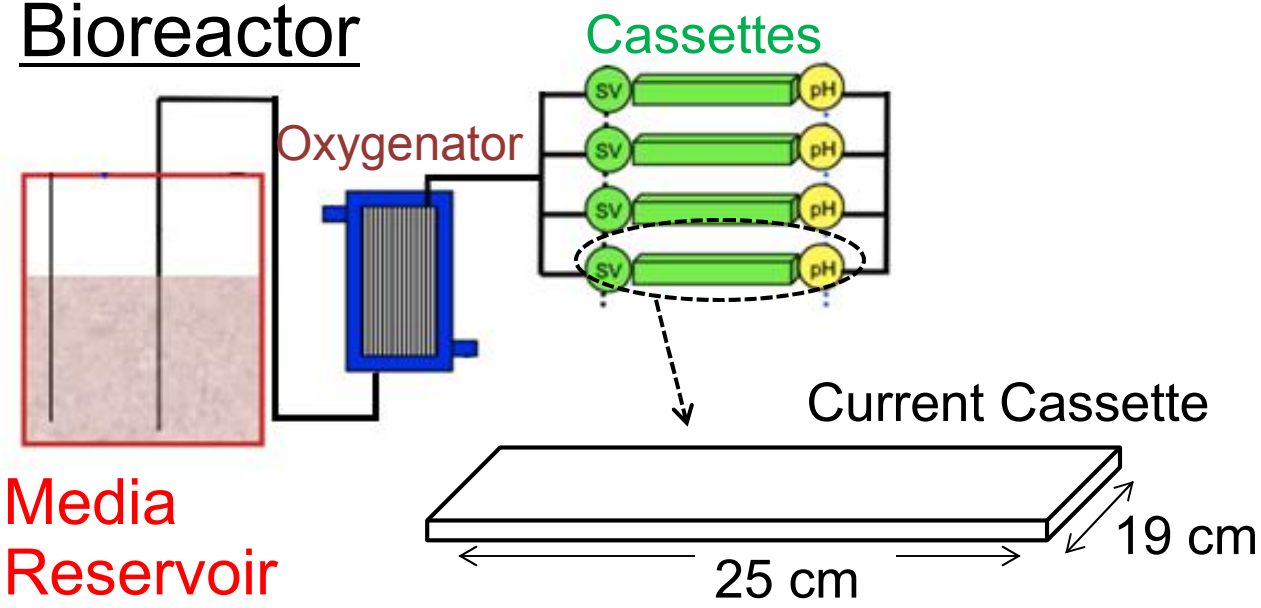


Flow Analysis

Testing and Optimization

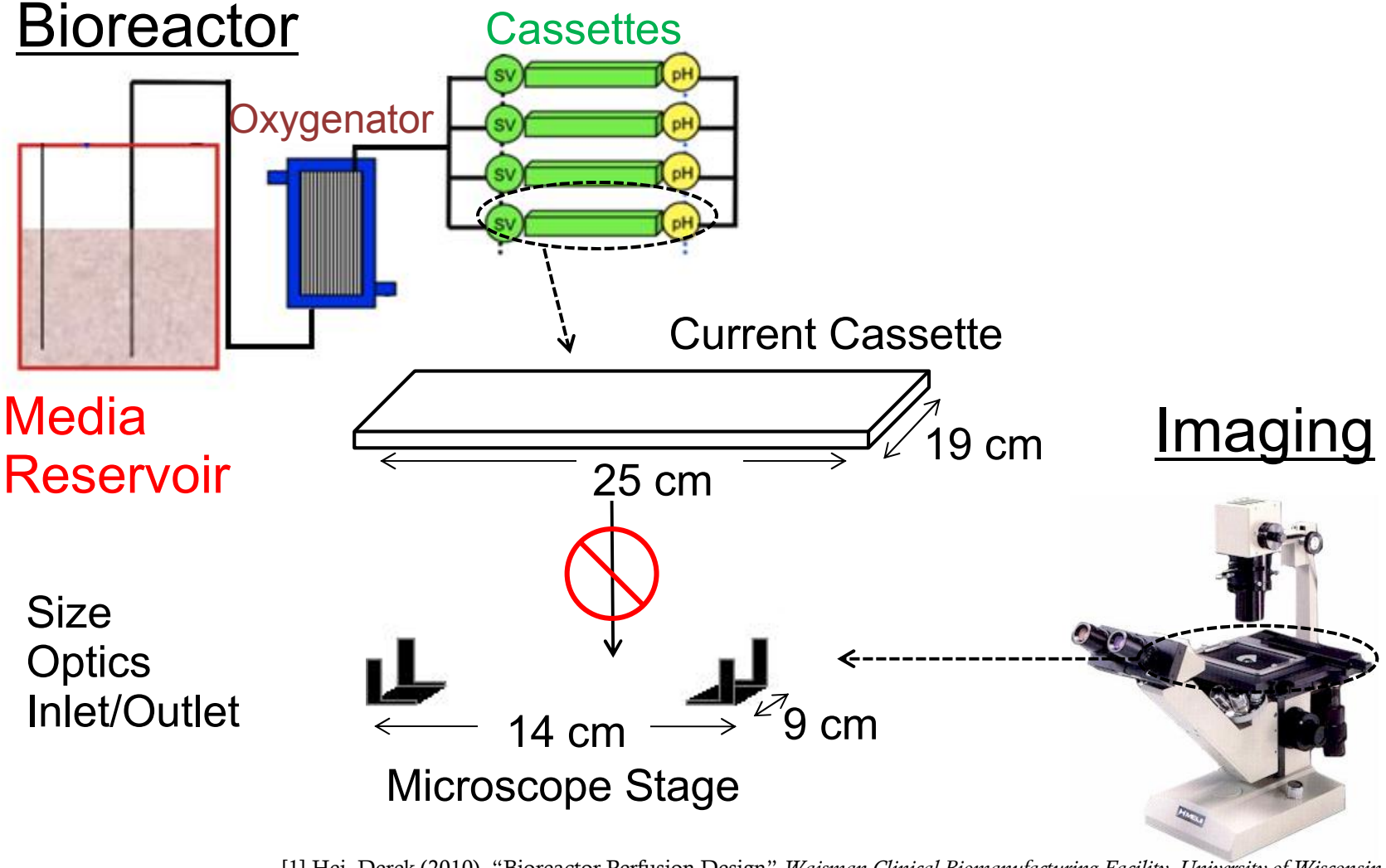

Bioreactor Cassette
Material Selection
Flow Analysis
Testing and Optimization
Redesign
Sterilizable
Compatible with cells
Cell Testing
Bubble Elimination
Even flow pattern
Limit media waste
Imaging
Ergonomics
Optics
Footprint
Disposable

Cassette[1], Microscope Systems[2]



[1] Hei, Derek (2010). "Bioreactor Perfusion Design" *Waisman Clinical Biomanufacturing Facility, University of Wisconsin-Madison.*
[2] http://www.microscope-microscope.org/basic/microscope-types.htm

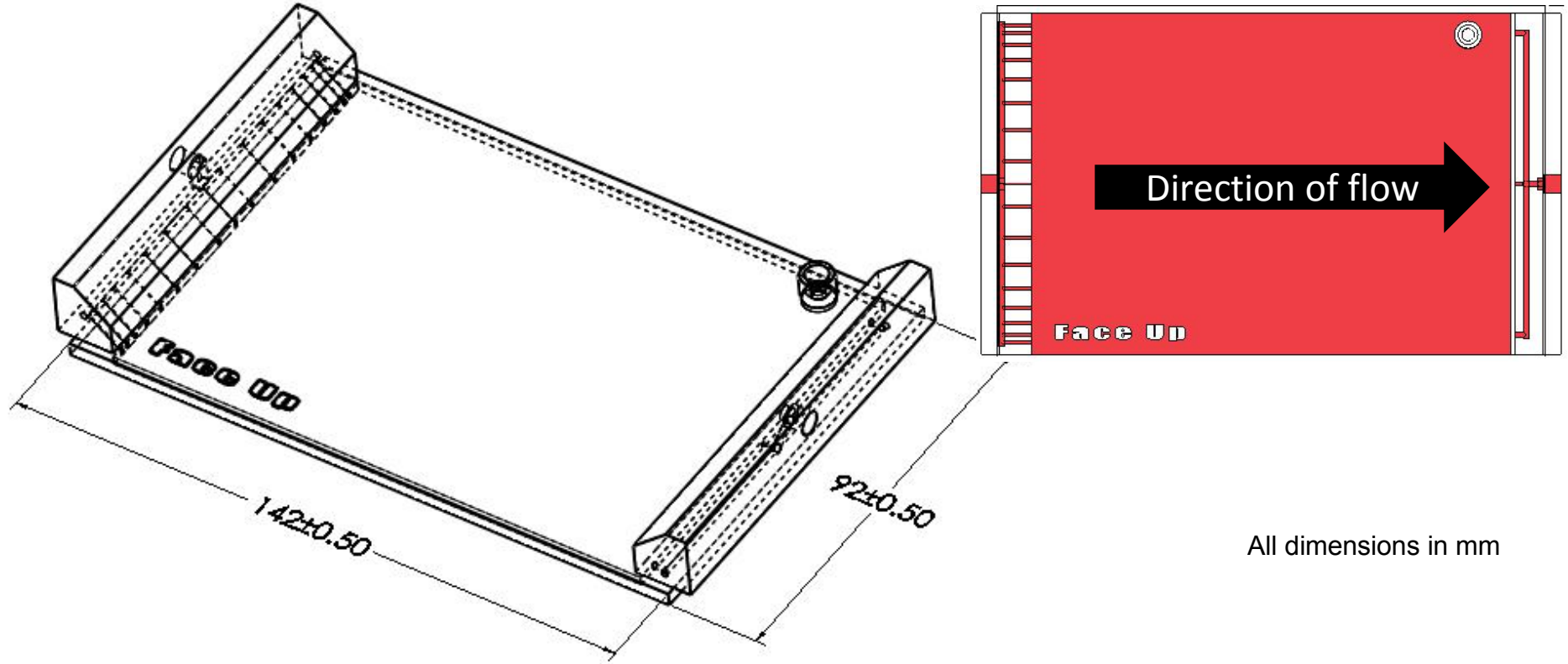
Cassette[1], Microscope Systems[2]



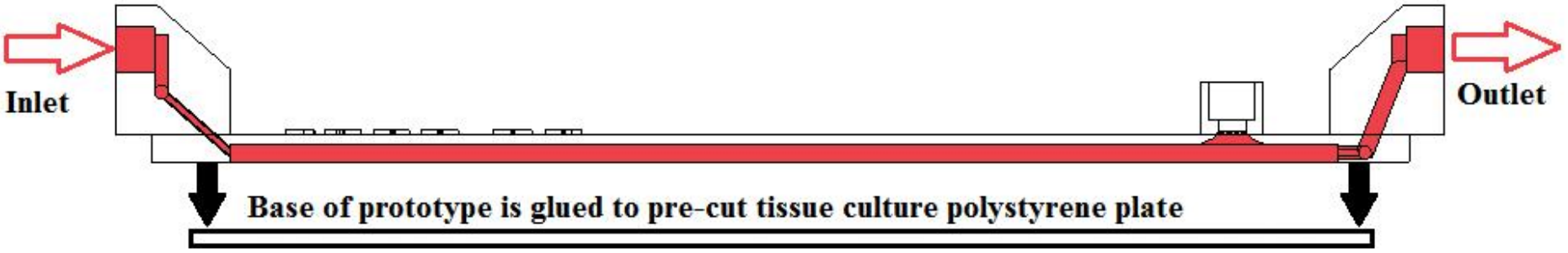
- Size
- Optics
- Inlet/Outlet

[1] Hei, Derek (2010). "Bioreactor Perfusion Design" *Waisman Clinical Biomanufacturing Facility, University of Wisconsin-Madison.*
[2] http://www.microscope-microscope.org/basic/microscope-types.htm

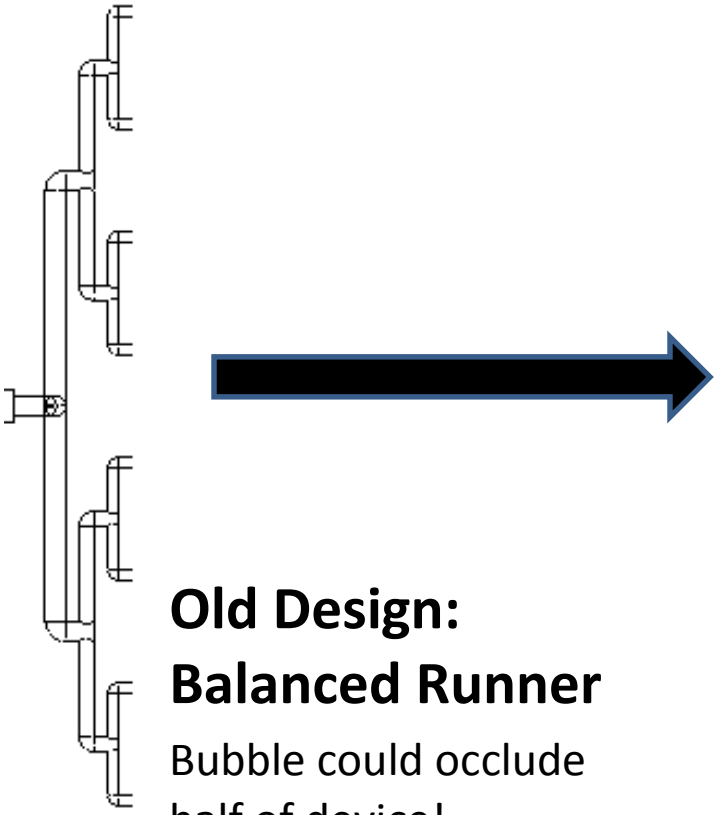
Design



All dimensions in mm

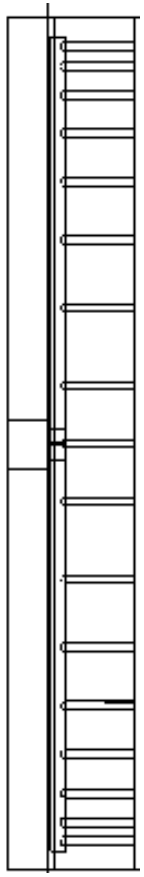


Inlet



Old Design:
Balanced Runner

Bubble could occlude half of device!



Mirrored on right and left

New Design: Straws

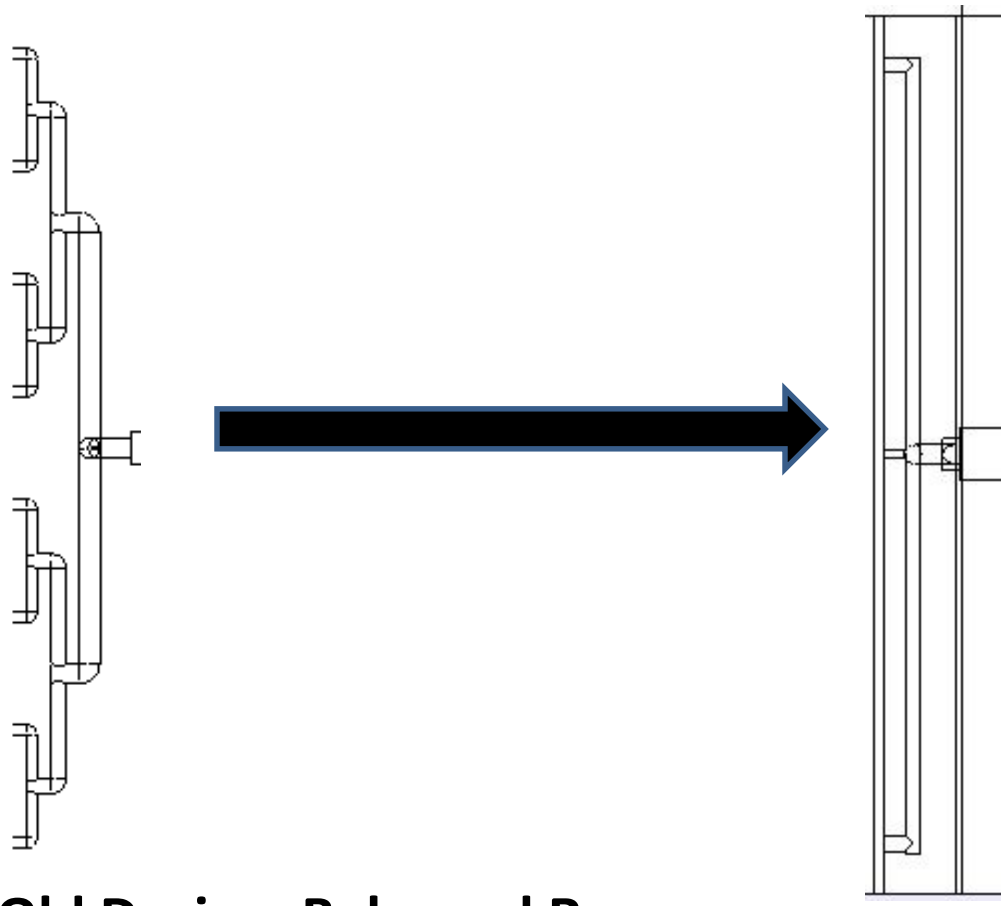
Designed for better management of bubbles

Inlet is raised to accommodate stage size

Hagen–Poiseuille Equation to approximate straw size

$$L \propto R^4$$

Outlet



Old Design: Balanced Runner

-Unnecessarily complicated

Outlet is raised to accommodate stage size

$$L \propto R^4$$

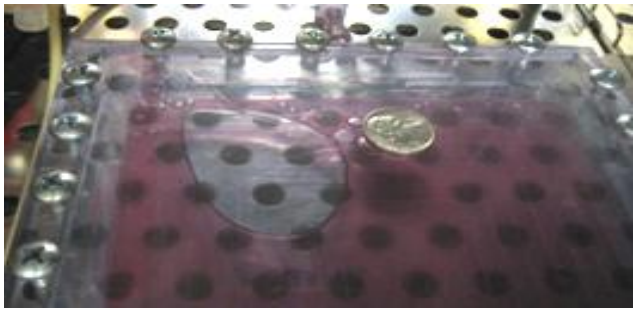
New Design: 3 collecting tubes

-Simplified

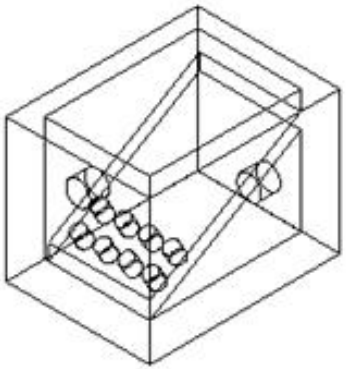
-Encourages flow to all three outlet ports

Bubble Port

Problem #1: large bubbles entering cassette

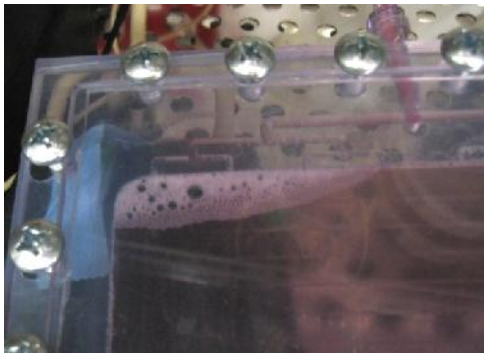


Solution



Bubble trap to place prior to inlet

Problem #2: small bubbles of gas formed within the cassette



Bubble Port

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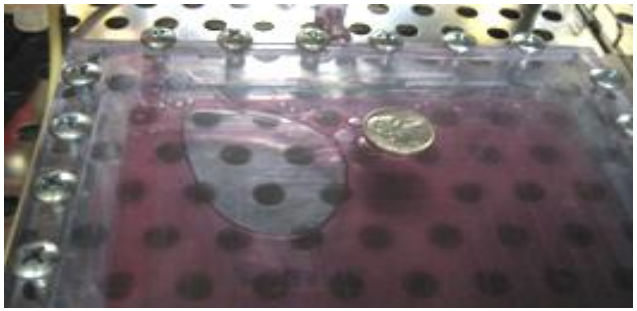
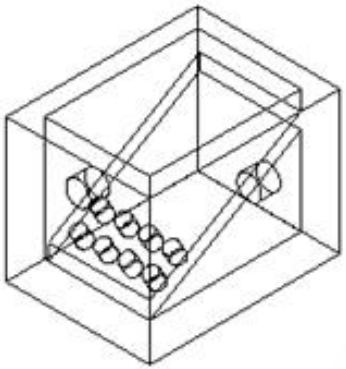


Solution



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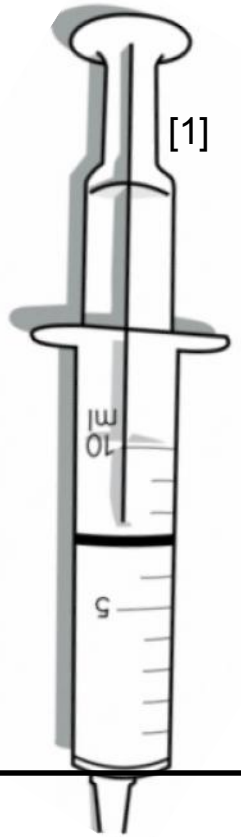
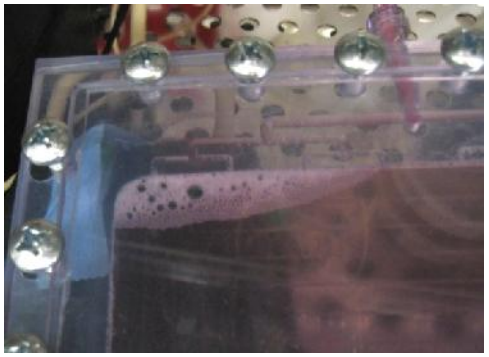
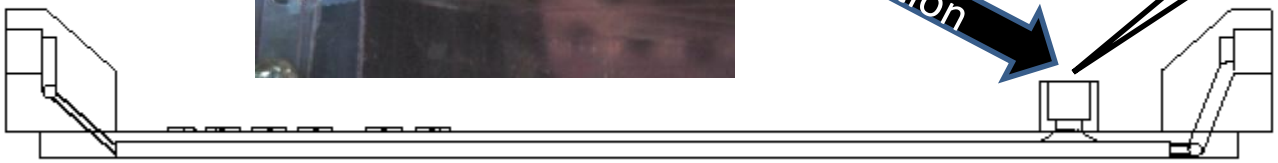


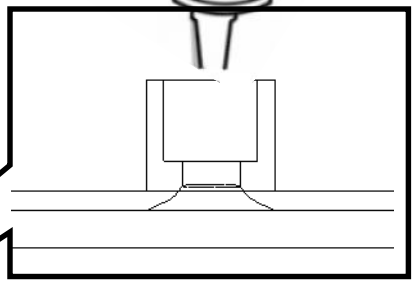
[1]

Problem #2: small bubbles of gas formed within the cassette



Solution

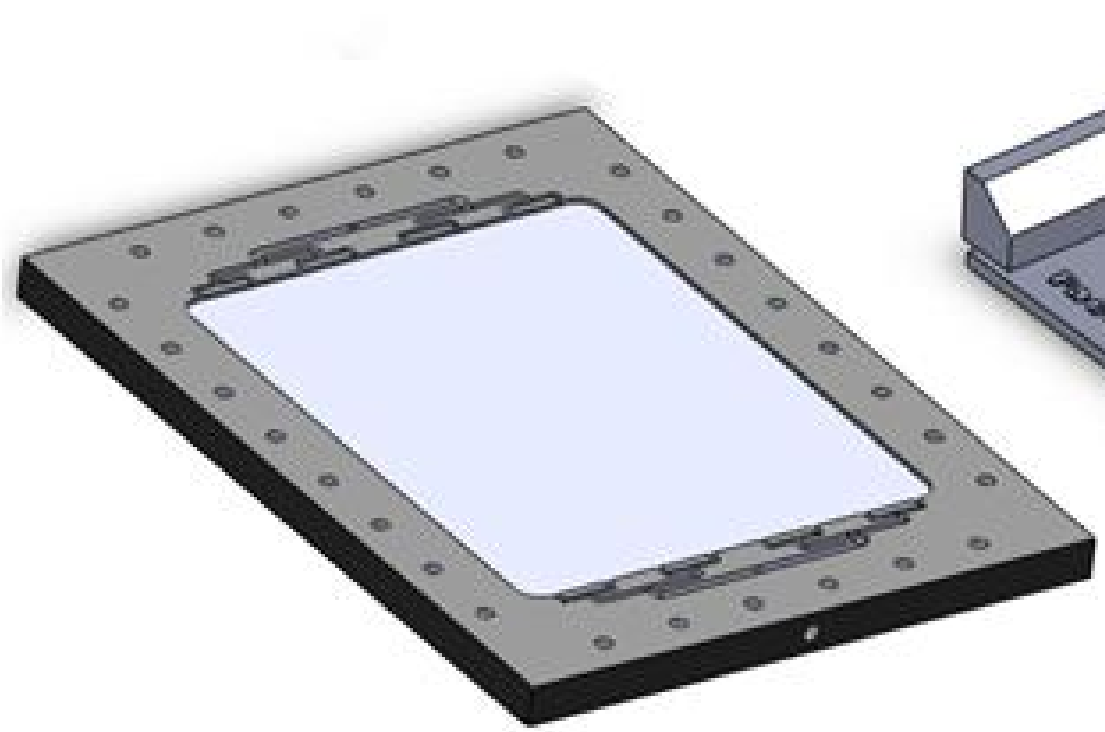




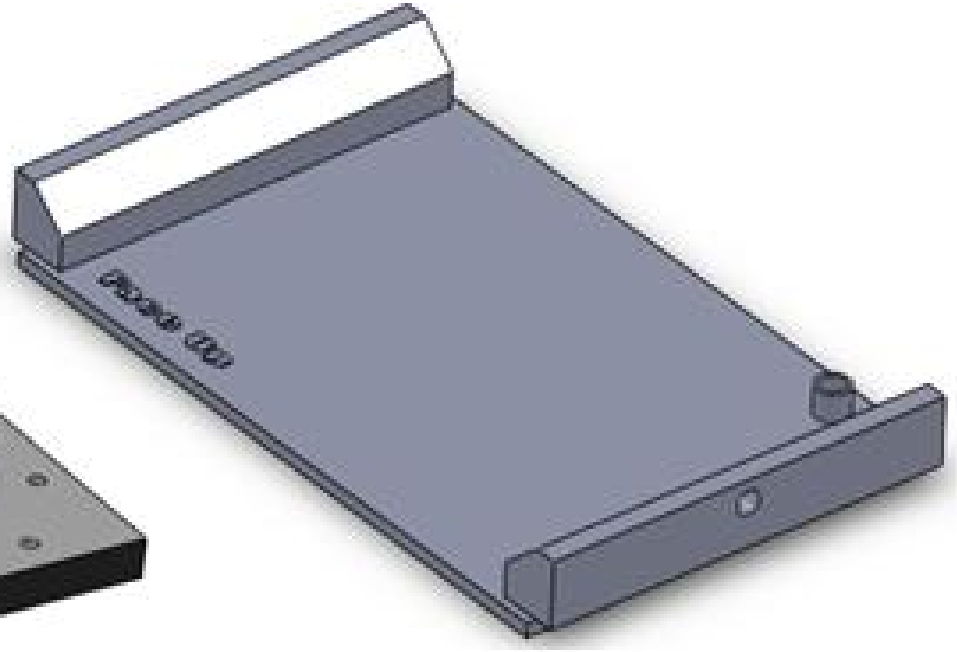
-Manually remove air.
-Curvature helps to coalesce bubbles

[1] http://www.bohrkronen.co.cc/syringe-bevel.html&page=5

Material Reduction



369.28 cm^3/238.00 cm^2
=1.55 cm^3/ cm^2

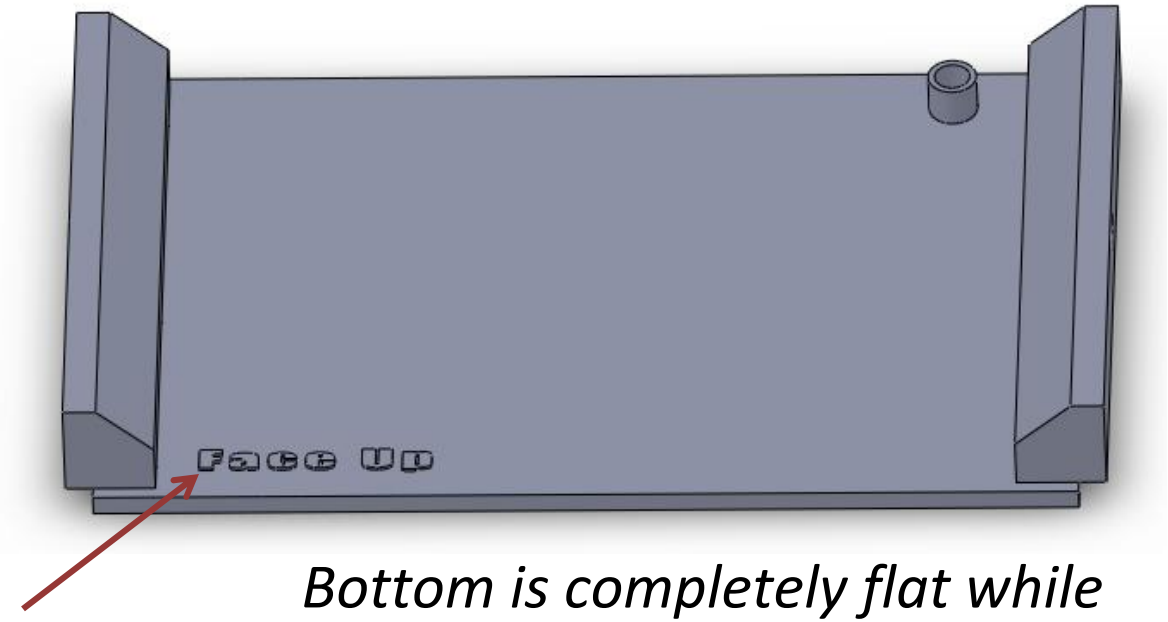


45.15 cm^3 / 111.76 cm^2
=0.40 cm^3/ cm^2

~4-fold reduced material cost per growth area

Ergonomics: Avoiding Setup Error

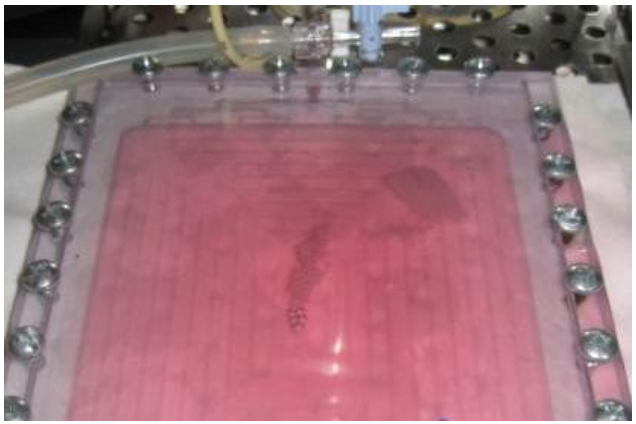
- "Top" vs. "bottom" becomes more obvious to avoid mistakes in setup
- Arrow to indicate intended direction of media flow



Words "Face Up" make top even more obvious

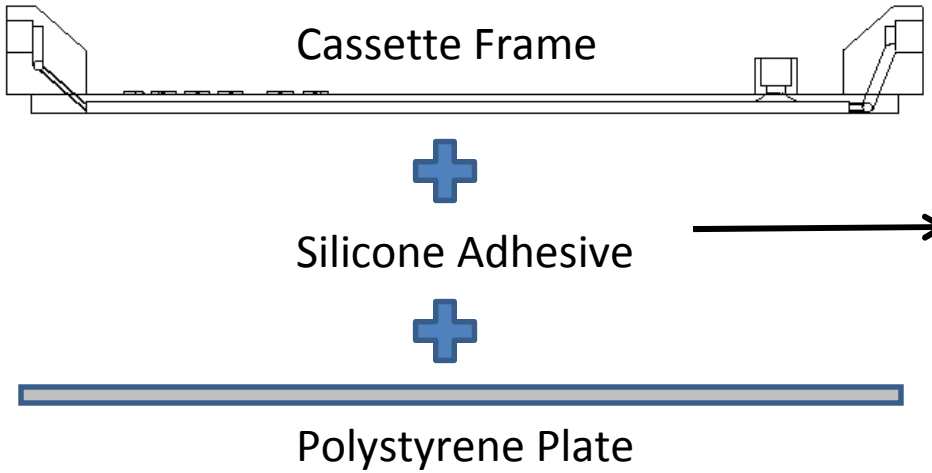
Bottom is completely flat while top has protrusions

Ergonomics: Faster & Easier Assembly



Old Design

- 28 screws
- Difficult to set up
- Time-consuming
- Sits on screws: non-flat surface

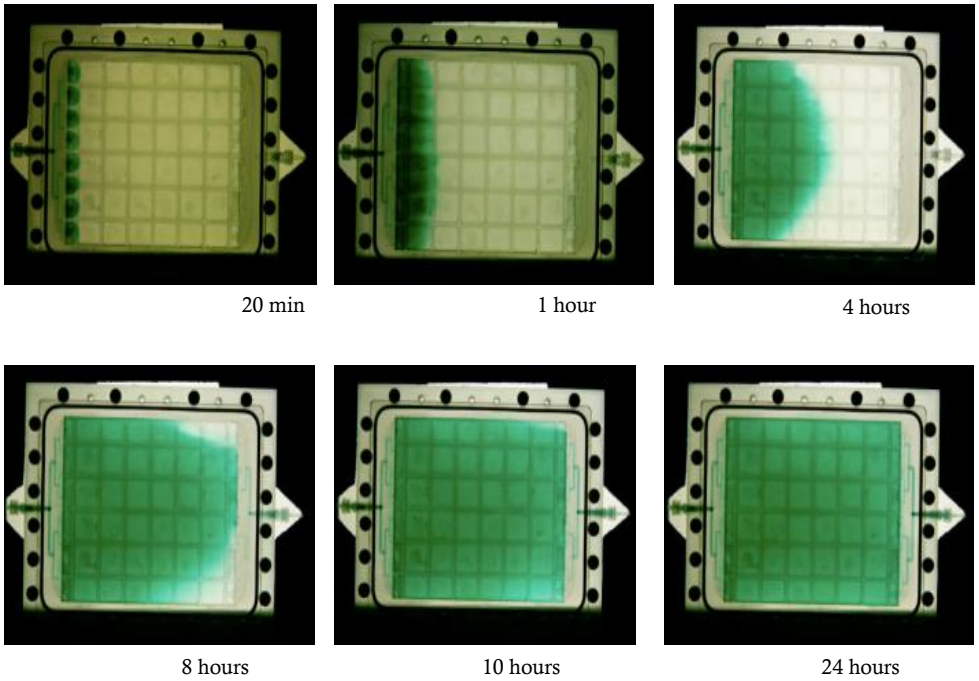


New Design

- No screws
- Faster to set up
- Sits flat on polystyrene

Future Plans

- Flow test on new prototype using dye studies
- Cell growth tests using HEK-293AD
  - Trypan blue viability stain
  - Crystal violet stain



Acknowledgements

- Dr. Hei and WCBF team
- Nate Schumacher, Ben Cox
- Dr. Mackie, Dr. Eliceiri

Questions?

Redesign Matrix

Component	Feature	Present in Old Cassette?	Effective?	Incorporate into New Design?	Justification
Inlet	Balanced Runner	X	No		
	Same Plane (Cell Growth)	X	Yes		
	Straws			X	
	Elevated Plane (Cell Growth)			X	Microscope optics
Outlet	Mirrored Inlet	X	No		
	Same Plane (Cell Growth)	X	Yes		
	Single Outlet				Eddy Formation
	3-Port Outlet			X	Simplification
	Elevated Plane (Cell Growth)			X	Microscope Optics
Cell Growth Area	Permanently Encased	X	?	X	
	>1.5mm material thickness	X			
	Accessible				Beyond fabrication abilities
	<1.5mm material thickness			X	1.27mm thickness in 96 well plate
Bubble Trap	Exterior Gravity Trap	X	Partially		Degassing
	Sterilizable	X	No	X	
	Gravity Trap Incorporated Before Inlet				
	Filter Trap, Incorporated		No		
	Manual Release Trap, Incorporated			X	
Disposability	Multiple-Use	X	No		Wear affects growth patterns
	Single-Use			X	
Ergonomics	Assembly >30 min	X	No		
	Assembly < 30 min			X	Disposability, Gluing
	Labeled Top			X	