Reservoir System for Tissue-on-a-Chip

by

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Executive Summary

Dr. Christopher Heylman’s research laboratory needs an improved reservoir system for their microfluidic device that reduces flow variance, failures, and the physical footprint. Current microfluidic devices have various types of reservoir systems, including vials, 96-well plates, and other cylindrical wells. Several patents and standards must be considered when designing the reservoir system. To develop a prototype the following design process was used: product discovery, project planning, product definition, conceptual design, product development. Dr. Heylman expressed various customer requirements including increased time between cell media changes, reduced leaks and blockages, and reduced flow variance. These were then translated into engineering requirements. Target values were set for these engineering requirements based on the current microfluidic device, other reservoir systems, and other information. Timelines, deadlines, and milestones were outlined for the tasks in each step of the design process. A morphology was used to create concept sketches for our project and a Pugh Matrix was used to evaluate the best concept. A CAD model was created for our concept and fluids calculations were performed to evaluate if the concept would meet the fluidic requirements. A COMSOL model was created to simulate fluid flow through the microfluidic chip and a failure modes and effects analysis was performed. Our CAD model was modified for our final design and detailed drawings were created. Dimensioning, costs, and material selection for our design are discussed, as well as manufacturing instructions and detailed test protocols. Test criteria included 2D surface area, device chamber dimensions, reservoir diameter, volume, devices without leaks, channels with flow, sterilizability, opacity (compared to old device), cell viability, time between media changes, flow velocity, flow velocity standard deviation, and cost. After analyzing the testing results, it was determined that all engineering specifications were met except for devices without leaks and channels with flow. After an explanation of how to use the platform, interpretations from testing were discussed as well as future directions for the project.
Introduction

Dr. Christopher Heylman’s research laboratory, located at California Polytechnic State University, has developed a Tissue-on-a-chip device for preclinical drug testing. It is a microfluidic chip for culturing cells with channels that perfuse biological fluids such as media and drugs. Currently, pipette tips are used as the reservoir system for these fluids. Dr. Heylman needs a new reservoir system that reduces failures, flow variance, and the physical footprint. He needs the system to still be sterilizable and allow for continuous gravity-fed flow.

Our team’s goals with this project were to make the improvements requested by Dr. Heylman, create unique designs that meet both the customer and engineering requirements, create a functional prototype that passes all our benchmarks, and leave Dr. Heylman’s laboratory with a wafer mold and procedure for fabricating and manufacturing the new device platform for future replication. This document discusses background information on the project, objectives, project management, concept generation, conceptual model, detailed design, manufacturing instructions, testing, and conclusions.
Background

Clinical Relevance
The process of approving drugs for clinical use takes over 10-15 years and costs about $1-2 billion. However, 90% of drug candidates that pass preclinical trials fail in clinical trials [1]. Specifically for colorectal cancer drugs, which is of interest to Dr. Heylman’s research group, the attrition rate is 87.0% for drug candidates entering clinical trials. The phase transition probabilities decrease significantly for cancer drugs as they progress through each phase of clinical testing [2]. This further demonstrates the high percentage of drugs that pass preclinical trials but are not proven to be safe and effective in clinical trials.

Preclinical and clinical studies account for 33% and 63% of the overall cost of drug development respectively [3]. There is a significant need to improve the effectiveness of preclinical testing of drugs to better filter out drug candidates and avoid spending valuable time and resources in clinical trials.

Organ-on-Chips
A promising solution to this preclinical drug testing issue is organ-on-chips, which are microfluidic chips with controlled cell microenvironments. Advantages compared with conventional drug testing platforms include higher efficiency in screening time, lower cost, chemical/biological gradient screening, and reduced consumption in costly cell lines and chemical/biological reagents [3]. These devices can be used to culture in vitro vascularized microtumors by supporting physiologic flow and delivering nutrients through microchannels [4]. Many of these devices aim to simulate interstitial flow, which is the movement of fluid through the extracellular matrix of tissues. Interstitial flow velocity ranges from 0.1 and 4.0 µm/s [5]. Some of these devices include two outer microfluidic channels on each side of the cell seeding chamber. The channel with higher pressure acts as an arteriole and the lower pressure one acts as a venule [4]. For some of these devices, fluid flow is driven by pumps, but for others, it is driven by hydrostatic pressure created by wells with different amounts of fluid. The device designs can be complex, having numerous channels [6].

Dr. Heylman’s Device
Dr. Heylman and his student researchers work to create endothelial vessel integration with tumor models within microfluidic devices for low-consumption, cost-effective, reproducible, and standardized preclinical drug screening. These microfluidic devices are composed of polydimethylsiloxane (PDMS) with a glass slide and are batch manufactured by pouring them onto a raised silicon wafer mold. The glass slide seals off the microfluidic channels in the PDMS and supports the device integrity. The device serves the same function as other common cell culturing containers, including 8-well chamber slides and 96-well plates, which is to serve as a vessel for which a cell-gel matrix can be injected and cultured. However, the microfluidic device at Dr. Heylman’s laboratory serves the extra functions of being fully encapsulated and having
two channels with diffusion membranes adjoined to the cell-seeding region. This allows for more unique methods of optimization for endothelial network formation and drug delivery.

The device design has proven to be successful as a vessel for cell culture. However, several issues have arisen with this device design, and Dr. Heylman has reached out to our team to address and solve them. The first is the media delivery/reservoir system. The current system is to insert 200 µL pipette tips with the cell media into the inlets/outlets of the channels. The tips are filled with varying amounts of liquid to create a pressure gradient that drives the flow of the fluids through the device. However, the improper positioning of the tips in the device leads to frequent failures, like leaks and blockages. If the tip is too deep in the hole in the device, a blockage will occur. If the tip is not snug enough, a leak will occur. The depth of the tip in the hole changes the initial cross-sectional area through which the fluid flows, resulting in significant flow variance between devices in an experiment. This method also lacks the ability to change media, so media must be added to maintain gravity-fed flow.

The other major issue with the current device design is the physical footprint of a multi-device experiment. Each 3” glass slide can hold two devices and only two of those glass slides can fit in a petri dish. With many experiments including up to 16 devices, most experiments require 3-4 Petri dishes which not only is difficult to handle for the experimenter but also takes up a large space within the laboratory’s incubator.

Dr. Heylman would like us to solve these issues while making minimal changes to the microfluidic chip design, specifically the dimensions of the cell-seeding region. However, the lengths of the channels can be adjusted to be compatible with the new reservoir system.

Existing Designs
Reservoir systems for six microfluidic devices that are in academic journals are discussed below.

1. UC Irvine microfluidic model system

This system consists of two media reservoirs that have a diameter of 12.5 mm. Two glass vials with the bottom cleaved were then glued to the entrance and exit of the long microchannel. The different heights of liquids create gravity-driven flow through the microchannels. The media levels of the two media reservoirs were adjusted every other day to maintain a constant ΔP [7].

2. InVADE platform

The reservoir system includes a bottomless 96-well plate platform bonded to the base plate. Inlet and outlet wells are directly adjacent to the well that contains the bioscaffold used to culture the cells, allowing for gravity-driven flow. The platform can house 20 endothelialized tissues [8].

3. UC Irvine organ-on-a-chip platform
The reservoir system is a bottomless 96-well plate. The middle layer of the platform contains 12 microfluidic device units, and the bottom layer is a polymer membrane [9].

4. Georgia Tech Lung-on-a-Chip Platform

This multi-layer device was made from a bottomless 96-well plate and two PDMS layers. Collagen membranes are in between the PDMS layers and coverslips are bonded to the bottom. The platform contains 8 microfluidic device units [10].

5. UniChip

This microfluidic device includes open-access ellipse-shaped reservoirs and multiple PMMA layers containing microfluidic channels. The device is placed on a rocking platform to allow flow back and forth between the two reservoirs [11].


The reservoirs for the cell culture medium were punched out of the molded PDMS. The inlet reservoirs of the cell culture medium channels were loaded with EGM-2 medium, and then a vacuum was applied at the outlet reservoirs to fill the hydrophobic channels [12].

Patents

Table 1 displays information about patents that are relevant to our project. This information includes the patent number, patent year, a claim we risk infringing, and plan for addressing this claim.

Table 1. Relevant patents.

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Year</th>
<th>Claim we risk infringing</th>
<th>Plan for addressing</th>
</tr>
</thead>
<tbody>
<tr>
<td>US9874285B2</td>
<td>2013</td>
<td>Each integrated bio-object microfluidics module comprises a reservoir having one or more ports for providing a plurality of solutions [13].</td>
<td>Operating at risk. Make sure our reservoir system has a different design.</td>
</tr>
<tr>
<td>US10954482B2</td>
<td>2018</td>
<td>Microfluidic device with one or more</td>
<td>Operate at risk.</td>
</tr>
<tr>
<td>Patent Number</td>
<td>Year</td>
<td>Claim we risk infringing</td>
<td>Plan for addressing</td>
</tr>
<tr>
<td>---------------------</td>
<td>------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>US10444223B2</td>
<td>2018</td>
<td>A fluidic network having a plurality of fluidic switches, a plurality of fluidic paths in fluid communication with the plurality of fluidic switches, and one or more on-chip pumps coupled to corresponding fluidic paths [15].</td>
<td>Specifying that no fluidic switches are used in our design.</td>
</tr>
<tr>
<td>US20220002646A1</td>
<td>2021</td>
<td>The organ-chip model of claim 1, wherein cell culture media and collection reservoirs are utilized such that passive diffusion can provide the cell culture media to cells without active transport of fluid using a syringe pump [16].</td>
<td>Operating at risk (patent is still pending).</td>
</tr>
<tr>
<td>Patent Number</td>
<td>Year</td>
<td>Claim we risk infringing</td>
<td>Plan for addressing</td>
</tr>
<tr>
<td>---------------</td>
<td>------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>US11248203B2</td>
<td>2022</td>
<td>A method of using a microfluidic device for cell culture, with an open-top microfluidic device comprising a chamber with a lumen and a fluidic cover comprising a microfluidic channel, a porous membrane below said chamber wherein said fluidic cover is configured to detachably cover and close said chamber; seeding cells in said lumen without said fluidic cover covering said chamber; and detachably closing said chamber with said fluidic cover [17].</td>
<td>Specifying that our porous membrane is different from theirs by explaining that our membrane is vertical and allows for horizontal diffusion instead of vertical diffusion.</td>
</tr>
</tbody>
</table>
Standards

A standard that applies to our project is ISO 22916:2022 Microfluidic devices - Interoperability requirements for dimensions, connections, and initial device classification [18]. This is because we will be slightly modifying Dr. Heylman’s microfluidic device so that it will be compatible with the reservoir system. Another standard that applies to our project is ISO 11737-2 - Sterilization of healthcare products — Microbiological methods — Part 2: Tests of sterility performed in the definition, validation, and maintenance of a sterilized product. Our product will be sterilized in the laboratory before being used to culture cells. ISO 10993 — Biological evaluation of medical devices also applies to our project because Dr. Heylman’s microfluidic device is a medical device [19].
Objectives

Problem Statement
The current reservoir system in Dr. Heylman’s lab has too much flow variance, failures due to leaks and blockages, and too large of a physical footprint. A new reservoir system needs to be designed that reduces these issues while still meeting fluidic requirements and being capable of sterilization/operating within a CO2 incubator.

Boundary Definition
This project includes designing a new reservoir system that meets all the customer requirements. The microfluidic chip will not be redesigned but can be scaled up or down to meet the needs of the new reservoir system. The outcomes of this project include performing COMSOL analysis to ensure that the reservoir system meets microfluidic requirements and creating a fully functional prototype of the reservoir system.

Indications for Use
The improved microfluidic device reservoir system is intended for use in medical and education institution laboratory research and preclinical testing. The reservoir system is intended to be used by trained laboratory researchers and laboratory scientists. The reservoir system is intended to be adjoined with the current microfluidic device(s) designed by Dr. Christopher Heylman’s research lab. The initial target tissue/cell types include colorectal cancer cells, human umbilical vein endothelial cells, and human dermal fibroblasts. However, the reservoir system can be used for other cell and tissue types moving forward. The reservoir system is also designed for the following matrices: Matrigel and Fibrin Matrix. Any cell culture media may be used in the microfluidic reservoir system. The microfluidic device reservoir system is intended to be imaged by confocal microscopy and inverted fluorescent microscopy. The reservoir system is intended to remain in a sterile environment which includes incubators, biosafety cabinets (BSCs), and sterile microscope platforms. The reservoir system has not been tested with fluid pumps and is not intended to be used with fluid pumps.

Summary of Customer Requirements
The customers for this project are Dr. Heylman and the research assistants. After talking to both groups, a list of customer requirements was formed and rated by each customer. The most important customer requirements include reducing the failure rate of the reservoir system by reducing the number of leaks and blockages that occur, making sure that the new reservoir system can change out cell media, and confirming that the reservoir system is biocompatible, meaning sterilizable and operatable within a CO2 incubator. Other customer requirements cover the size, shape, and volume of the reservoir system. A list of all the customer requirements can be found in Appendix A.
Description of Quality Function Deployment

After the customer requirements were determined and rated by the customers, engineering specifications were determined. These are quantifiable parameters that align with the customer's requirements. Next, all customer requirements and engineering specifications were given a relationship (strong, moderate, or weak) to determine if the engineering specification was a quantitative test of that customer's requirement. This was done in the House of Quality which can be seen in Figure 1. Once making sure that all customer requirements had a strong relationship with at least one engineering specification, the targets for the engineering specifications were determined based on the current reservoir design and information on other reservoir systems. All the engineering specifications with their target values are shown in Table 2.

Figure 1. House of Quality.
Table 2 lists the engineering specifications, which are based on the customer requirements, as parameters. The table includes a description of each parameter, the target value for that parameter, the tolerance on the target, the risk anticipated in meeting the target value, and the compliance method for that parameter. The risk anticipated in meeting the target value is measured in low, medium, and high. The compliance methods include test, analysis, inspection, or similarity to the existing design.

Table 2. Engineering Specifications Table.

<table>
<thead>
<tr>
<th>Spec. #</th>
<th>Parameter Description</th>
<th>Requirement or Target (units)</th>
<th>Tolerance</th>
<th>Risk</th>
<th>Compliance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2D Surface Area</td>
<td>110 cm^2</td>
<td>150 cm^2 max</td>
<td>L</td>
<td>I</td>
</tr>
<tr>
<td>2</td>
<td>Device Dimensions of Chamber</td>
<td>2.5 mm</td>
<td>+/- 0.1 mm</td>
<td>M</td>
<td>I, S</td>
</tr>
<tr>
<td>3</td>
<td>Reservoir Diameter</td>
<td>7 mm</td>
<td>9 mm max</td>
<td>M</td>
<td>I</td>
</tr>
<tr>
<td>4</td>
<td>Volume</td>
<td>190 uL</td>
<td>80 uL min</td>
<td>M</td>
<td>T, S</td>
</tr>
<tr>
<td>5</td>
<td>Device without leaks</td>
<td>95%</td>
<td>90% min</td>
<td>H</td>
<td>T, A, S</td>
</tr>
<tr>
<td>6</td>
<td>Channels with flow</td>
<td>90%</td>
<td>75% min</td>
<td>H</td>
<td>T, A, S</td>
</tr>
<tr>
<td>7</td>
<td>Sterilizability</td>
<td>0% bacterial growth</td>
<td>0%</td>
<td>L</td>
<td>I, S</td>
</tr>
<tr>
<td>8</td>
<td>Opacity</td>
<td>95%</td>
<td>90% min</td>
<td>L</td>
<td>T, S</td>
</tr>
<tr>
<td>9</td>
<td>Cell Viability</td>
<td>4 times original cells</td>
<td>3-5 times original cells</td>
<td>L</td>
<td>T</td>
</tr>
<tr>
<td>10</td>
<td>Time Between Media Changes</td>
<td>48 hr</td>
<td>24 hr min</td>
<td>M</td>
<td>T</td>
</tr>
<tr>
<td>11</td>
<td>Flow Velocity</td>
<td>2 um/s</td>
<td>+/- 1.9 um/s</td>
<td>L</td>
<td>T, A, S</td>
</tr>
<tr>
<td>12</td>
<td>Flow Velocity Standard Deviation</td>
<td>0.5 um/s</td>
<td>1 um/s max</td>
<td>M</td>
<td>T, A, S</td>
</tr>
<tr>
<td>13</td>
<td>Cost per Reservoir System</td>
<td>$5</td>
<td>$15 max</td>
<td>M</td>
<td>A</td>
</tr>
</tbody>
</table>

- Spec. 1: The 2D surface area of the reservoir system will be measured after production to ensure that it is within the target value, which will help lower the footprint of the reservoir system so allow for multiple devices to fit within the CO2 incubator, a key customer requirement. This is a high-risk parameter as it is one of the main goals of the project.
- Spec. 2: The dimensions of the device chamber must remain the same as the current device chamber.
- Spec. 3: The reservoir diameter should be large enough to allow changing of the media using a pipette. The dimensions chosen are based on the dimensions of the microfluidic chip. This is a critical parameter and one of the main customer requirements.
• Spec. 4: The volume of the reservoir should be able to hold enough liquid for the media to not have to be changed for 24 hours. This is a critical parameter and one of the main customer requirements.
• Spec. 5: Devices without leaks will be tested with a prototype to ensure that media and other biological fluids are not going to be leaking out of the platform. This is a high-risk parameter as the flow of biological fluids is critical to the operation of the platform, and leaks will negatively affect this.
• Spec. 6: Channels with flow will be tested with a prototype to ensure that media and other biological fluids are flowing through the microfluidic channels. This is a high-risk parameter as the flow of biological fluids is critical to the operation of the platform, especially since media helps cells proliferate.
• Spec. 7: The sterility of the device will be tested to ensure that it is sterile, and that no bacteria growth occurs to the device after it is sterilized. This will be a pass-or-fail test.
• Spec. 8: The opacity of the reservoir system will be observed and past testing as long as one can see through the device with the human eye. This ensures that the channels on the microfluidic chip under the reservoir system can be seen.
• Spec. 9: The cell viability of the reservoir system will be tested by growing cells in the device and measuring the number of cells in the cell seeding region.
• Spec. 10: The time between media changes will be tested by measuring how frequently research assistants need to change media to maintain flow through the device. There needs to be minimal scientist intervention.
• Spec. 11: The flow velocity will be tested after a prototype has been built. The goal of this parameter is to ensure that the flow is in the physiological range and the media will not have to be changed for 24 hours.
• Spec. 12: The flow velocity standard deviation will be determined through testing with the goal being little flow velocity variation. This is a high-risk parameter as it is one of the main goals of the project and cannot be tested until a prototype is built.
• Spec. 13: The cost per reservoir system will be determined after a prototype has been created and is limited due to the budget of the project.
Project Management

Overall Design Process

Our team has been following a design process that includes product discovery, project planning, product definition, conceptual design, and product development. Figure 2 shows the flow of the design process.

![Design process diagram](image)

Figure 2. Design process.

Unique Plans

Several of the plans that have been incorporated into our timeline are unique to our project. We have a series of three deliverables from our unique fabrication process for the wafer mold which includes an AutoCAD model, wafer photomask ordering, and wafer fabrication. We also had a couple of unique tests for the microfluidic platform. The first is cell viability testing in which we cultured cells within the platform. We also ran it through a sterility process to ensure that it can be sterilized. Finally, we performed flow velocity testing with FITC powder.

Summary of Gantt Chart

Our team created a Gantt chart to keep track of our deliverables, milestones, and critical path, shown in Appendix B. The deliverables and their timeline are included in Table 3. Our milestones include the Statement of Work, the conceptual design review report and presentation, the critical design review report and presentation, and the final design review presentation. The critical path is mainly dependent on the wafer fabrication process. We were able to successfully complete items within our Gantt chart.

Table 3. Deliverables and Timeline.

<table>
<thead>
<tr>
<th>Deliverable</th>
<th>Date to Begin</th>
<th>Deadline</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Conceptual Model</td>
<td>1/25/2023</td>
<td>2/13/2023</td>
</tr>
<tr>
<td>Project Planning Meeting Slides</td>
<td>1/30/2023</td>
<td>2/1/2023</td>
</tr>
<tr>
<td>Medical Device Recall Assignment</td>
<td>2/1/2023</td>
<td>2/8/2023</td>
</tr>
<tr>
<td>Pugh Chart</td>
<td>2/2/2023</td>
<td>2/8/2023</td>
</tr>
<tr>
<td>Failure Mode and Effects Analysis (FMEA)</td>
<td>2/8/2023</td>
<td>2/15/2023</td>
</tr>
<tr>
<td>*AutoCAD Model</td>
<td>2/14/2023</td>
<td>2/21/2023</td>
</tr>
<tr>
<td>Deliverable</td>
<td>Date to Begin</td>
<td>Deadline</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------</td>
<td>---------------</td>
<td>------------</td>
</tr>
<tr>
<td>^Conceptual design review report and presentation</td>
<td>2/15/2023</td>
<td>2/22/2023</td>
</tr>
<tr>
<td>Peer Evaluations and team health</td>
<td>2/22/2023</td>
<td>2/27/2023</td>
</tr>
<tr>
<td>*Order Wafer Photomask</td>
<td>2/22/2023</td>
<td>2/28/2023</td>
</tr>
<tr>
<td>Hazard and Risk Assessment</td>
<td>2/27/2023</td>
<td>3/6/2023</td>
</tr>
<tr>
<td>*Wafer Fabrication</td>
<td>3/14/2023</td>
<td>3/21/2023</td>
</tr>
<tr>
<td>Spring Quarter Project Plan</td>
<td>3/20/2023</td>
<td>3/24/2023</td>
</tr>
<tr>
<td>*Device and Reservoir manufacturing</td>
<td>4/4/2023</td>
<td>4/17/2023</td>
</tr>
<tr>
<td>Test plan presentation</td>
<td>4/4/2023</td>
<td>4/25/2023</td>
</tr>
<tr>
<td>*Functional Prototype Video</td>
<td>4/18/2023</td>
<td>4/25/2023</td>
</tr>
<tr>
<td>Test plan presentation peer questions</td>
<td>4/25/2023</td>
<td>5/2/2023</td>
</tr>
<tr>
<td>Ethics Reflection</td>
<td>5/4/2023</td>
<td>5/9/2023</td>
</tr>
<tr>
<td>Final Report</td>
<td>5/16/2023</td>
<td>6/6/2023</td>
</tr>
<tr>
<td>*Final Prototype Video</td>
<td>5/23/2023</td>
<td>6/6/2023</td>
</tr>
<tr>
<td>Expo Poster Final</td>
<td>5/25/2023</td>
<td>5/30/2023</td>
</tr>
<tr>
<td>*Senior Survey</td>
<td>6/6/2023</td>
<td>6/13/2023</td>
</tr>
</tbody>
</table>

* Indicates Critical Path Deliverable

^ Indicates Project Milestone
Budget
Table 4 displays the project budget stating the planned cost and actual cost for every item purchased. The finds given consist of the Hannah Forbes Fund ($500) and the Senior Project Fund ($200). After spending a total of $526.19 on the project there is $173.81 left which will be used to purchase a new photomask for ongoing prototype development.

Table 4. Project Budget.

<table>
<thead>
<tr>
<th>Item Description</th>
<th>Product Number/Company</th>
<th>Purpose</th>
<th>Planned Cost</th>
<th>Quantity</th>
<th>Total Cost</th>
<th>Cost per Unit</th>
<th>Actual Cost</th>
<th>Funds Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottomless 96 Well Plate</td>
<td>Greiner Bio-One</td>
<td>Reservoir System</td>
<td>$273.18</td>
<td>40</td>
<td>$6.83</td>
<td></td>
<td>$208.02</td>
<td>Hannah Forbes</td>
</tr>
<tr>
<td>Large Microscope Slide</td>
<td>Brain Research Laboratories</td>
<td>Bottom of Device</td>
<td>$120</td>
<td>50</td>
<td>$2.40</td>
<td></td>
<td>$140</td>
<td>Hannah Forbes</td>
</tr>
<tr>
<td>Photomask</td>
<td>Artnetpro.com</td>
<td>PDMS Template</td>
<td>$93.50</td>
<td>1</td>
<td>N/A</td>
<td></td>
<td>$107.06</td>
<td>Senior Project</td>
</tr>
<tr>
<td>Cover plates</td>
<td>Greiner Bio-One</td>
<td>Cover Platform</td>
<td>$71.11</td>
<td>100</td>
<td>$0.71</td>
<td></td>
<td>$71.11</td>
<td>Senior Project</td>
</tr>
<tr>
<td>Total Cost</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$526.19</td>
<td></td>
</tr>
</tbody>
</table>
Concept Generation

Morphology

A device morphology was created by decomposing the function of the device and developing concepts for each function. The function of the device was decomposed into the mass arrangement for the multi-device experiment, the glass slide to support device integrity, the cell seeding region and channels, and the shape of the reservoirs. Six concepts were created for each function.

Three concept sketches were created by combining concepts for each function from the device morphology shown in Figure 3.

![Figure 3. Device morphology.](image-url)
The concept sketch shown in Figure 4 is based on the green path. For this sketch, we chose multiple 1ml vials (2), size of 1 device (1), current channel length (1), and narrow cylindrical well shape (3). The 1ml vials in a narrow cylindrical well shape were chosen as they achieve the functions of allowing access to pipette tips and a good gravity-fed flow. With this design, we can also maintain the current device’s cell seeding region and channels, which is a customer requirement.

![Figure 4. Concept sketch 1.](image)

The concept sketch shown in Figure 5 is based on the blue path. For this sketch we chose the 96-well plate (1), big rectangular glass slide (4), elongated channels (2), and wider cylindrical wells (2). This was chosen because the 96-well plate with wider cylindrical wells achieves the functions of multi-device experiments, a small footprint, access to pipette tips, and a good gravity-fed flow. We can maintain the current device’s cell seeding region and only must elongate the channels which is within the customer specifications. We should be able to have 16 devices integrated with this one-plate design.

![Figure 5. Concept sketch 2.](image)
The concept sketch shown in Figure 6 is based on the red path. For this sketch we chose the circular wafer-sized well plate (5), wafer-sized glass slide (4), current channel length (1), and wider cylindrical wells (2). The wafer-sized well plate would eliminate the need to change the length of the channels in the microfluidic chip and the need to cut apart the microfluidic chips once they are fabricated because this well plate would be designed for the current wafer layout. Additionally, this well plate would decrease the footprint of a multi-device experiment. The wider cylindrical wells would allow gravity-fed flow with less flow variance compared to tall and skinny pipette tips.

![Concept sketch 3](image)

**Figure 6.** Concept sketch 3.

**Concept Evaluation**

The three concept sketches were compared using a Pugh Matrix to determine which design met the most key criteria. The key criteria for our Pugh Matrix are based on our customer requirements. After changing the customer requirements into key criteria, we went through them with our sponsor and gave each criteria an importance rating. Then each team member created their own Pugh Matrix, marking each concept as better, same, or worse than the benchmark option, the current reservoir system in Dr. Heylman’s laboratory. Each member’s Pugh Matrix can be found in Appendices C-E. Then a final Pugh Matrix was made based on each team member’s ratings.

The bottomless 96-well plate was chosen as it had the highest rating when comparing customer requirements to the current and other potential designs. When comparing surface areas, the plates both had lower surface areas than the current and vial designs. The plates would be more expensive. All designs will reduce flow rate variance, blockage rate, and leak rate. All designs hit the target values for physiological flow rate, inlet and outlet diameters, cell seeding dimensions, cell death, volume, tensile strength, and time between media changes. The wafer-sized plate would have a potential for autoclave deformation because it would be custom-made
in a sub-optimal manufacturing environment. The vial design would be subject to opacity issues in microscopy because the caps would not be clear and would not allow light through for inverted microscopy. After adding up these comparison values in the Pugh Matrix, the bottomless 96-well plate emerged victorious. This is shown in Figures 7 and 8.

![Figure 7. Final pugh matrix.](image1)

![Figure 8. Pugh matrix summary.](image2)
Conceptual Model

CAD Model

The conceptual CAD model of the microfluidic platform is shown in multiple views in Figures 9, 10, and 11.

Figure 9. Conceptual CAD model isometric view.
Figure 10. Conceptual CAD model top view.
Figure 11. Conceptual CAD model side view.
The microfluidic platform consists of a bottomless 96-well plate (Figure 12), the microfluidic chip (Figure 13), and a glass microscope slide (Figure 14).

**Figure 12.** Model of bottomless 96-well plate.

**Figure 13.** Model of chip.
Figure 14. Model of glass slide.
The bottomless 96-well plate was chosen as the reservoir system after a Pugh matrix comparison. This model excels as it has optimal well volume, a low physical footprint, low-cost, one-piece construction, and the layout allows for multiple devices per bottomless plate. Some considerations had to be made after choosing the bottomless 96-well plate. The first included the spacing and geometry of the devices. The devices need a geometry that will match up to the wells in the reservoir system. Therefore, we needed a new wafer photomask to fabricate new wafer molds with the new geometry. We designed the new wafer photomask in AutoCAD, which can be seen in Figure 15.

![Figure 15](imageurl)  
**Figure 15.** AutoCAD for wafer photomask to be submitted to photomask manufacturer.

The green lines show the outline of the photomask, calibration markers, scale, name, and date. The red 100 mm circle represents the wafer but will not be included in the final submission. The white lines show the microfluidic device array scaled to fit a 96-well plate. The photomask only allows room for half of the total device array for one 96-well plate so we will need to fabricate two wafers from that photomask for one microfluidic platform. This will not be an issue because the photomask is made to be used for multiple wafers. We may even fabricate four wafers to be able to produce two full microfluidic-reservoir platforms per manufacturing cycle.

From creating this CAD model, we learned that we can minimally change microfluidic channel lengths to fit reservoir dimensions. We also learned that we can successfully fit 8 of the microfluidic devices into one microfluidic platform. This will allow for a decrease in the physical
footprint of a multidevice experiment since one of these systems takes up less surface area in the incubator than the current setup for an 8-device experiment.

**Fluid Mechanics Calculations**

We performed fluid mechanics calculations using Bernoulli’s equation. The main locations that were involved in these calculations are the top of the liquid in the source reservoir (Point A), the cell seeding region (Point B), and the bottom of the liquid in the sink reservoir (Point C). The purpose of the calculations was to identify the height of fluid required to drive fluid through the chamber at the appropriate velocity.

First, the velocity of the fluid at Point C was calculated using the flow rate equation, shown in Equation 1. $Q$ is volumetric flow rate, $A$ is cross-sectional area, and $V$ is velocity.

$$Q = AV$$

*Equation 1. Flow rate equation.*

The inputs for solving for the velocity are shown in Table 5. The velocity in the cell seeding chamber was based on values from Dr. Heylman’s current microfluidic device, which have an average of 2.5 um/s. The cross-section at B is a rectangle because it is in the cell seeding chamber. The cross-section at C, the inlet into the chip, is a circle.

**Table 5. Velocity calculation at Point C.**

| Length of Cross section at B | 3.70E-03 m |
| Height of Cross section at B | 1.00E-04 m |
| Area at B                    | 3.70E-07 m^2 |
| Radius at C                  | 1.00E-03 m |
| Area at C                    | 3.14E-06 m^2 |
| Velocity at B                | 1.50E-06 m/s |
| **Velocity at C**            | **1.77E-07 m/s** |

Next, Bernoulli’s equation, shown in Equation 2, was used at Points A and C.

$$\left( \frac{P_1}{\rho} + \frac{\alpha_1(V_1)^2}{2} + g z_1 \right) - \left( \frac{P_2}{\rho} + \frac{\alpha_2(V_2)^2}{2} + g z_2 \right) = \frac{fL V^2}{2D} + \frac{\sum KV^2}{2}$$

*Equation 2. Bernoulli’s equation.*
Table 6 explains what each variable represents in Equation 1.

Table 6. Bernoulli’s equation variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>ρ</td>
<td>Density</td>
</tr>
<tr>
<td>g</td>
<td>Gravity constant</td>
</tr>
<tr>
<td>P1</td>
<td>Pressure at point 1</td>
</tr>
<tr>
<td>α1</td>
<td>Kinetic Energy Factor at point 1</td>
</tr>
<tr>
<td>V1</td>
<td>Velocity at point 1</td>
</tr>
<tr>
<td>z1</td>
<td>Height at point 1</td>
</tr>
<tr>
<td>P2</td>
<td>Pressure at point 2</td>
</tr>
<tr>
<td>A2</td>
<td>Kinetic Energy Factor at point 2</td>
</tr>
<tr>
<td>V2</td>
<td>Velocity at point 2</td>
</tr>
<tr>
<td>z2</td>
<td>Height at point 2</td>
</tr>
<tr>
<td>f</td>
<td>Friction factor</td>
</tr>
<tr>
<td>L</td>
<td>Length</td>
</tr>
<tr>
<td>V</td>
<td>Average velocity</td>
</tr>
<tr>
<td>D</td>
<td>Diameter</td>
</tr>
<tr>
<td>K</td>
<td>Loss coefficient</td>
</tr>
</tbody>
</table>

The inputs for the right-hand side (RHS) of the equation, which accounts for head loss, are shown below in Table 7. The major head loss accounts for friction and the minor head loss accounts for bends and geometry changes in the channels.

Table 7. RHS of Bernoulli’s equation inputs.

<table>
<thead>
<tr>
<th>Major Losses</th>
<th>Minor Losses</th>
<th>RHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>f</td>
<td>2.18E-02</td>
<td>0.75 4</td>
</tr>
<tr>
<td>L</td>
<td>0.0151 m</td>
<td>0.75 2</td>
</tr>
<tr>
<td>D</td>
<td>6.86E-04 m</td>
<td>10 2</td>
</tr>
<tr>
<td>v</td>
<td>1.50E-06 m/s</td>
<td>0.5 2</td>
</tr>
<tr>
<td>e</td>
<td>2.50E-02 m</td>
<td>1 30</td>
</tr>
<tr>
<td>e/D</td>
<td>3.64E+01</td>
<td></td>
</tr>
<tr>
<td>nu</td>
<td>1.00E-06 m^2/s</td>
<td></td>
</tr>
<tr>
<td>Re</td>
<td>1.21E-03</td>
<td>555</td>
</tr>
<tr>
<td>Head Loss Maj</td>
<td>5.40E-13 m</td>
<td>Head Loss Min 6.2438E-11 m^2/s^2</td>
</tr>
<tr>
<td>RHS total</td>
<td>6.30E-11 m^2/s^2</td>
<td>Head Loss Pump 0 m^2/s^2</td>
</tr>
</tbody>
</table>
The Reynold’s Number was calculated using the equation shown in Equation 2. The density of cell media is 1000 kg/m^3 and the viscosity is 8.48E-04 Pa*s. We assume laminar flow because Reynold’s Number is small.

\[
Re_D = \frac{\rho V D}{\mu}
\]

Equation 3: Reynold’s number equation

Table 8 explains what each variable represents in Equation 3.

**Table 8. Reynold’s number equation variables.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re_D</td>
<td>Reynold’s number</td>
</tr>
<tr>
<td>(\rho)</td>
<td>Density</td>
</tr>
<tr>
<td>V</td>
<td>Velocity</td>
</tr>
<tr>
<td>D</td>
<td>Diameter</td>
</tr>
<tr>
<td>(\mu)</td>
<td>Viscosity</td>
</tr>
</tbody>
</table>

Table 9 displays the inputs for the left-hand side (RHS) of the equation.

**Table 9. RHS of Bernoulli’s equation inputs.**

<table>
<thead>
<tr>
<th></th>
<th>LHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Point (A)</td>
<td>Second Point (C)</td>
</tr>
<tr>
<td>(P_1)</td>
<td>(P_2)</td>
</tr>
<tr>
<td>(v_1)</td>
<td>(v_2)</td>
</tr>
<tr>
<td>(z_1)</td>
<td>(z_2)</td>
</tr>
<tr>
<td>(\text{alpha}_1)</td>
<td>(\text{Laminar})</td>
</tr>
<tr>
<td>(\text{alpha}_2)</td>
<td>(\text{Laminar})</td>
</tr>
<tr>
<td>LHS Total</td>
<td>(6.30\times10^{-11} \text{ m}^2/\text{s}^2)</td>
</tr>
</tbody>
</table>

The pressure at Points A and C is atmospheric pressure, so there are set to 0. The height at Point C is 0 because it is the datum. Velocity at Point A is assumed to be zero because the diameter of the well is significantly larger than the diameter of the inlet. The Goal Seek function in Excel was used to set the right-hand side of the equation equal to the left-hand side by changing \(z_1\), the height of the fluid.

According to our calculations, the height of the fluid required is extremely small and is not anywhere near the fluid heights of the current reservoir system for Dr. Heylman’s device. Thus, it is likely that the large number of assumptions we had to make while performing these calculations have led to inaccurate results. We had to make assumptions about the fluid properties of the biological fluids, including the cell media and the Matrigel. The Matrigel is especially difficult to get fluid properties for since it will change as the cells grow inside it. Additionally, we had to simplify the device geometry during several parts of the calculations.
We learned from performing these calculations that we needed to create a COMSOL simulation for our experiment. The fluid mechanics of this device are extremely complicated, so COMSOL is more suitable for modeling the device's fluid flow.

**COMSOL Simulation**

A COMSOL simulation was created to measure the velocity of the fluid in the device, specifically the cell seeding region. A 3D model of the fluid channels in the device was created in SolidWorks and imported to COMSOL. A mesh with about 460,000 elements was created, shown in Figure 16.

![Microfluidic channel mesh](image)

**Figure 16.** Microfluidic channel mesh.

The “Free and Porous Media Flow” fluid flow setting was used to set the material properties for the media and the Matrigel and simulate fluid flow. The pressure at the inlets below the source wells was set assuming the height of the source liquid reservoir was 10 mm, which is the maximum starting height of the liquid of the reservoir. Figure 17 displays the velocity of the media at various cut slices in the channels. The fact that velocity is larger in the smaller channels than in the cell seeding region is visual confirmation that the simulation appears to be accurately modeling fluid flow.
A cut line was positioned in the center of the cell seeding region to measure the velocity, shown in Figure 18.

A parametric sweep was performed, changing the height of the source liquid reservoir to from 10 to 6 mm in 1 mm increments. We expected fluid heights in the source well above 5 mm to be the working range for our system. Once the fluid height drops to 5 mm, the sink well will be
at the same height as the source well, so there would be no pressure to drive fluid flow. Figure 19 displays velocity in the cell seeding region along the cut line for each of the 5 heights.

![Line Graph: Velocity magnitude (µm/s)](image)

**Figure 19.** Velocity vs. position along cut line in cell seeding region for various media heights in the source well.

The results of the simulation display that the flow velocity will be within our engineering requirements of 0.1 to 3.9 µm/s in most of the cell seeding region from maximum reservoir height to near the halfway point. Additionally, the flow velocity range is near 1 µm/s since the maximum velocity in the cell seeding region is about 1.02 µm/s and the minimum is about 0.03 µm/s. This indicates a small flow variance. Thus, the design of our device should meet the fluidic requirements.

**FMEA**

Our failure modes and effects analysis are shown in Table 10. OCC is the likelihood of occurrence, DET is the ability to detect, and SEV is the severity of the effect. All three are weighted on a ten-point scale. RPN is the risk priority number which is equal to OCC x DET x SEV. The PDMS being misshapen has the highest RPN value, as this failure would lead to improper fluid flow meaning the device is not functional. To prevent this, we ensured that the wafer has good integrity post-fabrication. Additionally, the polystyrene deforming has the next
highest RPN value as this failure would also lead to improper fluid flow. To prevent this, we ensured that our sterilization technique will not deform the polystyrene. There are several different ways that a leak could occur, such as poor bonding and cracks. The hardest failure to detect would be if the PDMS doesn’t properly solidify and is deformed. Recommend actions for addressing these failures are shown in Table 10.

Table 10. Failure Modes and Effects Analysis.

<table>
<thead>
<tr>
<th>Function Affected</th>
<th>Potential Failure Mode</th>
<th>Potential Effect(s) of Failure</th>
<th>OCC</th>
<th>DET</th>
<th>SEV</th>
<th>RPN</th>
<th>Cause of Failure</th>
<th>Recommended Actions</th>
<th>Responsible Person</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid Retention</td>
<td>Leak</td>
<td>Improper flow of media, not enough media delivered</td>
<td>3</td>
<td>1</td>
<td>8</td>
<td>24</td>
<td>Plasma bond failure</td>
<td>Ensure that the reservoir is correctly bonded to the chip</td>
<td>Ryan A.</td>
</tr>
<tr>
<td>Microscopy</td>
<td>Poor image quality</td>
<td>Difficulty making experiment measurements</td>
<td>10</td>
<td>1</td>
<td>8</td>
<td>80</td>
<td>Crack in bottom glass</td>
<td>Ensure good integrity of the bottom glass</td>
<td>Kyle C.</td>
</tr>
<tr>
<td>Fluid Retention</td>
<td>Leak</td>
<td>Improper flow of media, not enough media delivered</td>
<td>10</td>
<td>1</td>
<td>8</td>
<td>80</td>
<td>Crack in bottom glass</td>
<td>Ensure good integrity of the bottom glass</td>
<td>Olivia C.</td>
</tr>
<tr>
<td>Fluid flow</td>
<td>PDMS being misshapen</td>
<td>Improper fluid flow</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>38</td>
<td>defect in wafer fabrication</td>
<td>Ensure good integrity of wafer post fabrication. Ensure level and consistent SU-56 hardening.</td>
<td>Ryan A.</td>
</tr>
<tr>
<td>Fluid Retention</td>
<td>Leak</td>
<td>Improper flow of media, not enough media delivered</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>56</td>
<td>Crack in bottomless 96-well plate</td>
<td>Ensure good integrity of bottomless 96-well plate</td>
<td>Olivia C.</td>
</tr>
<tr>
<td>Fluid retention and flow, microscopy</td>
<td>Polystyrene deforming</td>
<td>Improper flow of media, blockage of inlets</td>
<td>9</td>
<td>1</td>
<td>10</td>
<td>90</td>
<td>Deformity from sterilization</td>
<td>Sterilization technique will not deform polystyrene. No high heat.</td>
<td>Kyle C.</td>
</tr>
<tr>
<td>Microscopy</td>
<td>Polystyrene deforming</td>
<td>Unnatural curvature and obstruction of view</td>
<td>9</td>
<td>1</td>
<td>10</td>
<td>90</td>
<td>Deformity from sterilization</td>
<td>Sterilization technique will not deform polystyrene. No high heat.</td>
<td>Ryan A.</td>
</tr>
</tbody>
</table>
Detailed Design

Figure 20 displays a SolidWorks model of the microfluidic platform, consisting of a bottomless 96-well plate, two microfluidic chips, and a glass microscope slide.

Figure 20. SolidWorks assembly.

There are four devices per chip, which is the maximum number of devices that can fit on a single photomask while still having the appropriate spacing to align with the wells in the 96-well plate. There are still 8 devices per microfluidic platform like our conceptual CAD model. The chips will have a gap of about 1 mm in between them in case the chips need to be slightly rotated to align the inlet holes with the wells.
Figure 21 shows an assembly drawing of the microfluidic platform.

![Figure 21. Assembly drawing.](image)

Table 11 displays our bill of materials.

**Table 11. Bill of Materials.**

<table>
<thead>
<tr>
<th>Item #</th>
<th>Test</th>
<th>Quantity</th>
<th>Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Microfluidic Chip</td>
<td>2</td>
<td>PDMS</td>
</tr>
<tr>
<td>2</td>
<td>Bottomless 96-well Plate</td>
<td>1</td>
<td>Polystyrene</td>
</tr>
<tr>
<td>3</td>
<td>Glass Slide</td>
<td>1</td>
<td>Glass</td>
</tr>
<tr>
<td>4</td>
<td>Photomask</td>
<td>1</td>
<td>Fused Silica</td>
</tr>
<tr>
<td>5</td>
<td>Cover plate</td>
<td>1</td>
<td>Plastic</td>
</tr>
</tbody>
</table>

Figure 22 shows the bottomless 96-well plate. Bottomless 96-well plates are only made of polystyrene, so no other materials could be considered. Polystyrene is a good material for 96-well plates because it is cell compatible and has high temperature and chemical resistance [20].
Because the plate is made of clear polystyrene and the cell seeding regions are positioned directly under empty wells, imaging cells and measuring flow velocity throughout the chip will not be hindered. The height of the wells is 10.9 mm. According to our COMSOL simulation, this is tall enough to create a pressure gradient to drive interstitial flow velocities. Additionally, the diameter of the wells is 6.58 to 6.96 mm, which is larger than the average diameter of a 200 μL pipette tip. This means that the new reservoir system will have less flow variance than the current reservoir system because the height change will be less for the same volume of media that flows through the microfluidic device. Additionally, the wide diameter will allow for easy media changes using a pipette. A bulk order of 40 units was purchased from Greiner Bio-One for around $270 [21].

Figure 22. Greiner Bio-One bottomless 96-well plate [21].

Figure 23 shows the drawing of the PDMS microfluidic chip. PDMS is an ideal material for the chips because it is cell compatible and clear. The inlet holes go completely through the PDMS to allow media to be transported from the 96-well plate to the microfluidic channels in the
devices. The overall chip dimensions are 66 x 53 x 4 mm, which allows for the bottoms of the wells that are in use to be sealed while still being able to form the gap between the two chips in the microfluidic platform. Distance between inlets is in increments of 9 mm, which allows for 96-well plate compatibility since the wells are 9 mm apart. The microfluidic channels are 0.1 mm in depth, which is the depth of the device channels in the current microfluidic device. The cell seeding chamber is 2.5 mm across, which is the same as the current microfluidic device. This meets our engineering requirement for the device dimensions of the chamber. The photomask used to create the microfluidic chip costs $94 [22]. Dr. Heylman’s lab has PDMS, so we do not expect to have to buy this material. A bulk order of 1000 g of PDMS will be purchased for about $185 if we run out of PDMS.

![Figure 23. Drawing of microfluidic chip.](image-url)
Figure 24 displays a single microfluidic device on the microfluidic chip.

The glass slides are 102 x 127 x 1.2 mm. Glass is an ideal material for the base because it is strong and cell compatible. This will make the overall surface area of the microfluidic platform about 130 cm^2, which meets our engineering specification of a maximum surface area of 150 cm^2. Because the glass slides are clear, the process for imaging the cells and measuring flow velocity throughout the chip will not be hindered. 50 units were ordered for around $120 [23].

Cover plates are for keeping the media from getting contaminated while it is being transferred from the BSC to the incubator. They are made specifically for covering 96-well plates. 100 units were ordered for around $71.
Prototype Manufacturing

Our prototype manufacturing process can be broken down into two parts: wafer fabrication and microfluidic platform manufacturing.

Wafer Manufacturing

The wafer fabrication process is a complex multi-step process that requires the use of hazardous reagents and the use of a microfabrication laboratory.

Materials:

- Class 1000 Microfabrication Laboratory
- Negative Epoxy Photoresist SU-8 2050
- Hydrofluoric Acid (HF)
- Buffered Oxide Etch (BOE)
- Negative Photoresist Spin Coater
- Programmable Hot Plate
- 2 100mm wafers
- Spin washer/cleaner
- UV photoresist curing machine
- Custom photomask
- SU-8 developer
- Petri dishes
- Dry air nozzle

Procedure:

1) Wear appropriate PPE and follow all safety precautions
   a. Gloves
   b. Gown
   c. Safety glasses
   d. Mask
   e. Shoe covers
   f. Hair net
   g. Close toed shoes
   h. Pants
2) Repeat all of the following steps for two wafers
3) The process begins with the cleaning of the 100 mm wafer through a process of acid baths and washes
   a. HF for 10 min
   b. Water wash
   c. BOE solution for 2 min
   d. Water wash
e. Spin wash

4) That wafer is transferred to a hot plate to be baked at 200°C for five minutes.

5) SU-8 50 for our negative epoxy photoresist should already be pulled out and warmed to room temp.

6) For a device thickness of 100um, we need a spin speed of 1000 rpm.
   a. For the spread cycle, ramp to 500 rpm at 100 rpm/second acceleration. Hold at this speed for 5–10 seconds to allow the resist to cover the entire surface.
   b. For the spin cycle, ramp to the final spin speed at an acceleration of 300 rpm/second and hold for a total of 30 seconds.

7) After spinning on the photoresist, soft-bake the wafer at 65°C for 10 min followed by 95°C for 30 min.

8) Next, we will use the UV photoresist curing machine with the photomask to cure the portions of the photomask that correspond to the device(s) using a wavelength of roughly 425 nm.

9) Following exposure, bake the wafer(s) at 65°C for 1 min followed by 95°C for 10 min.

10) Finally, develop the wafer(s) in SU-8 developer for roughly 10 min, rinse with developer, and spray dry with air.

11) Store upright in an airtight petri-dish container.

Microfluidic Platform Manufacturing

For the microfluidic platform, all steps should take place in the metrology room in the microfabrication laboratory.

Materials:

- PDMS and curing agent
- Plastic cups
- Vacuum chamber
- Petri dishes with master mold wafers
- Oven
- 2 mm syringe hole punch
- Plasma cleaner
- Scotch tape

Procedure:

1) Wear appropriate PPE and follow safety precautions
   a. Gloves
   b. Pants
   c. Closed-toed shoes

2) Start by mixing the PDMS and curing agent
   a. 10:1 PDMS and curing agent
   b. 35g:3.5g for each wafer
c. Pour and stir in plastic cup

3) Remove all the air bubbles in vacuum chamber
   a. Takes about 30 min

4) Pour out the PDMS into the petri dish with the wafers and place in an oven at roughly 60°C overnight.

5) Cut out the PDMS devices at the border of the wafer and petri dish.

6) Cut out the devices according to the template that we have premade.

7) Poke out the vertical channels using a 2 mm syringe hole punch.

8) Plasma bond the glass slide, PDMS devices, and bottomless 96-well plate together by placing them together after they’ve been in the plasma bonder exposed at high Rf for 20 seconds at 2 torr.
   a. Make sure surfaces are cleaned with scotch tape
Detailed Test Protocols

The summary of the test plan for each engineering specification is shown in Table 12. This summary includes the engineering specification the test will satisfy, the test type, the sample size, where the testing will take place, and the equipment used for the testing. The test plan schedule with dates and times is shown in Table 13.

Table 12. Summary of Test Plans.

<table>
<thead>
<tr>
<th>Test #</th>
<th>Test</th>
<th>Engineering Specification(s)</th>
<th>Sample Size</th>
<th>Facilities</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Device Measurements Study</td>
<td>2D Surface Area, Device Dimensions of Chamber, Reservoir Diameter</td>
<td>3</td>
<td>Building 192 Room 330</td>
<td>Digital Caliper</td>
</tr>
<tr>
<td>2</td>
<td>Volume Test</td>
<td>Volume</td>
<td>1</td>
<td>Building 192 Room 328</td>
<td>Pipette</td>
</tr>
<tr>
<td>3</td>
<td>Failure Test</td>
<td>Devices with leaks, Channels with blockages</td>
<td>5</td>
<td>Building 192 Room 328</td>
<td>Pipette, food dye</td>
</tr>
<tr>
<td>4</td>
<td>Sterility Test</td>
<td>Sterilization</td>
<td>3</td>
<td>Building 192 Room 328</td>
<td>Microscope, media, UV Crosslinker</td>
</tr>
<tr>
<td>5</td>
<td>Opacity Inspection</td>
<td>Opacity</td>
<td>5</td>
<td>Building 192 Room 328</td>
<td>Inverted Microscope, ImageJ</td>
</tr>
<tr>
<td>6</td>
<td>Observational Study</td>
<td>Cell Viability, Time between Media Changes</td>
<td>3</td>
<td>Building 192 Room 328</td>
<td>Digital Timer, Cells, Cell Media, Cell Counter, BSC</td>
</tr>
<tr>
<td>7</td>
<td>Velocity Test</td>
<td>Flow Velocity, Flow Velocity Standard Deviation</td>
<td>3</td>
<td>Building 38 Room 134</td>
<td>FITC, Inverted Microscope</td>
</tr>
<tr>
<td>8</td>
<td>Cost Test</td>
<td>Cost per Reservoir System</td>
<td>1</td>
<td>Building 192 Room 330</td>
<td>Computer</td>
</tr>
</tbody>
</table>
Table 13. Test Plan Schedule.

<table>
<thead>
<tr>
<th>Test</th>
<th>Personnel</th>
<th>Date</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Device Measurements Study</td>
<td>Olivia Corvelli</td>
<td>4/26/23 – 4/27/23</td>
<td>10 min</td>
</tr>
<tr>
<td>Volume Test</td>
<td>Kyle Cekada</td>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>Failure Test</td>
<td>Kyle Cekada</td>
<td></td>
<td>20 hours</td>
</tr>
<tr>
<td>Sterility Test</td>
<td>Olivia Corvelli</td>
<td>5/1/23 - 5/6/23</td>
<td>10 hours</td>
</tr>
<tr>
<td>Opacity Inspection</td>
<td>Olivia Corvelli</td>
<td>5/1/23 - 5/4/23</td>
<td>45 min</td>
</tr>
<tr>
<td>Observational Study</td>
<td>Ryan Adams</td>
<td>5/5/23 - 5/11/23</td>
<td>10 hours</td>
</tr>
<tr>
<td>Velocity Test</td>
<td>Kyle Cekada</td>
<td>5/5/23 - 5/10/23</td>
<td>18 hours</td>
</tr>
<tr>
<td>Cost Test</td>
<td>Olivia Corvelli</td>
<td></td>
<td>20 min</td>
</tr>
</tbody>
</table>

Test 1 – Device Measurements Study

Materials:

- Digital Calipers

Procedure:

1) Record the dimensions of the device chamber, as shown in Figure 25.

![Device geometry with red indicator showing device chamber.](image)

Figure 25. Device geometry with red indicator showing device chamber.

2) Record the reservoir diameter for 1 device well
3) Record the platforms 2D surface area
4) Repeat for all devices in 3 platforms

Analysis:

1) Find the total average value for each measurement
2) Compare average value for each measurement to the state tolerances in Table 2

Expected Outcome:

- All dimensions should be within the stated tolerances in Table 2
Test 2 – Volume Test

Materials:

- Pipette
- Water

Procedure:

1) Pipette water 100 uL at a time until the well is at maximum capacity
2) Record volume

Test 3 – Failure Test

Materials:

- Pipette
- Food dye
- Water

Procedure:

Setup

1) Mix water with food dye

Priming

1) Draw up 200 uL of dyed water
2) Put pipette tip in the inlet hole of the left source well
3) Twist top of pipette to inject water into channels until water starts appearing in a different well
4) Repeats steps 1-3 with the left sink well
5) Repeats steps 1-3 with the left cell seeding region inlet
6) Plug the two cell seeding region holes with plastic plugs

Testing

1) Fill source wells to the top as shown below in Figure 26.
2) Check on platform every 24 hours
   a. Record devices with leaks
       i. If device has a leak, stop testing that device
   b. Record channels without flow
       i. If source well remains the same height, it’s channel doesn’t have flow
       ii. If sink well remains empty, it’s channel doesn’t have flow
   c. If the channel remains without flow for 2 days, reprime the device
3) Repeat until source and sink wells have been equilibrated or 6 days have passed

Expected Outcome:
- Devices without leaks are within the stated tolerances in Table 2.
- Channels with flow are within the stated tolerances in Table 2.

Test 4 – Sterility Test
Materials:
- UVP CL-1000 Ultraviolet Crosslinker
- ECM
- Warm water bath
- Pipettes
- Incubator
- Inverted microscope with camera
- ImageJ

Procedure:
1) Wear the appropriate PPE and use aseptic technique throughout the procedure
2) Setup ultraviolet (UV) crosslinker
   a) Verify the preset exposure setting is 120,000 microjoules per cm²
      i) On the crosslinker touch pad, select the preset button
      ii) Select the exposure button
      iii) The display should show 1200, if not input 1200 using the touch pad
      iv) Select the enter button
   b) Verify the preset ultraviolet time exposure setting is 2 minutes
      i) On the crosslinker touch pad, select the preset button
      ii) Select the time button
      iii) The display should show 2.0, if not input 2.0 using the touch pad
      iv) Select the enter button
3) Insert device to be sterilized into the UV crosslinker as shown in Figure 27

![Figure 27. Loading platform in crosslinker.](image)

4) On the crosslinker touch pad, select the start button
   a) The green UV light should turn on, as shown below in Figure 28

![Figure 28. UV light sterilizing platform inside crosslinker.](image)

   b) At the end of the exposure cycle the crosslinker will beep 5 times to signal exposure is complete
5) Warm EGM in the water bath until temp of 37 C is obtained
6) Transfer EGM into BSC using sterile technique
7) In BSC, transfer EGM to 15ml conical
8) Fill top side channel wells of all devices with 300 μL
9) Place device in incubator for 48 hours
10) After 48 hours, check on microscope at 10X for fungal or bacterial growth using sterile technique
   a) If fungal or bacterial growth is present, take images to quantify growth in ImageJ
11) Discard device
12) Repeat for 3 platforms

Expected Outcome:
• No bacterial or fungal growth after 48 hours of incubation.

Test 5 – Opacity Inspection
Materials:
• Inverted microscope
• ImageJ

Procedure:

Operating Microscope

1) On the inverted microscope find the cell seeding region on a new device
2) Focus the image properly by adjusting the focus knob and the light intensity
3) Take note of the light intensity, make sure to not change it for the rest of the test
4) Capture an image
5) Repeat with 4 more of the new devices
   a. Make sure to not change the light intensity
6) On the inverted microscope find the cell seeding region on an old device
7) Focus the image properly by only adjusting the focus knob
   a. Make sure the light intensity is NOT adjusted
8) Capture an image
9) Repeat with 4 more of the old devices
   a. Make sure to not change the light intensity

ImageJ

1) Open the image in ImageJ by clicking File, Open and locating the image
2) Using the Straight tool, draw a line across the center of the cell seeding region as shown in Figure 29
3) Select the Analyze button, then Plot Profile
4) In the window that pops up select Data, then Copy All Data
5) Paste the data in an Excel spreadsheet
6) Find the average of all the Grey Values given
7) Repeat for every image captured
   a) Should be 5 images total

T-test

10) Perform a T-test to compare old vs. new device

Expected Outcome:

- There should be no significant difference between old and new device

Test 6 – Observational Study
Materials:

- ECM
- BSC
- Pipettes
- Centrifuge
- Inverted microscope with camera
- ImageJ
- Warm water bath
- 3T3’s
- UV crosslinker
- T75 Flasks
- Aspirator
• Trypsin
• Cell counting slide
• Matrigel
• Incubator
• Calculator
• Minitab

Procedure:

1) Wear the appropriate PPE and use aseptic technique throughout the procedure
2) Put ECM in water bath
   a) When warm, pass into BSC
3) Open 2 flasks in BSC using sterile technique and label the flasks
4) Thaw a vial of 3T3’s from liquid nitrogen in the water bath until all ice is melted.
   a) Transfer into the BSC using sterile technique
5) Add 11 mL of ECM to each flask, and pipette the full 1 mL of 3T3’s into flask
   a) Sterilize device in UV Crosslinker
6) Transfer device to BSC
7) Warm media and trypsin in water bath:
8) Count cells in flask
   a) Aspirate off media of cells in a T75 flask
   b) Rinse cells with DPBS & aspirate off
   c) Add 3mL of trypsin & let cells detach
   d) Quench Trypsin with 3 mL of media (V_{suspend} = 6 mL)
   e) Triturate and take 100μL sample to microcentrifuge tube (V_{mix} = 100μL)
   f) Add 100 μL of Trypan Blue to the microcentrifuge tube (V_{TB} = 100μL)
   g) Triturate cell-media solution with the Trypan Blue thoroughly
   h) Put a coverslip on hemocytometer over the grid
   i) Inject 20μL TB solution between the gap of coverslip and hemocytometer
   j) Count cells under upright white light microscope to count cells
      i) Live – White, Dead - Blue
   k) Calculate volume of desired number of cells in wells
      i) Do serial dilution as necessary, aim for 10 μL
9) Take Matrigel out fridge. Remove microcentrifuge tubes, pipette tips, and stainless-steel holder from freezer.
10) NOTE: Reverse pipette to avoid bubbles, spread gel into all four corners/flatten
11) Dispense ~10 μL of cell solution for desired cell density in microcentrifuge tubes (8 for 8 device 96 well plate platform)
12) Plate Matrigel-cell solution
   a) Place gel into frozen stainless-steel holder
   b) Put 40 μL of Matrigel into one microcentrifuge tube with cells
c) Swirl pipette tip to mix Matrigel in or lightly triturate but AVOID BUBBLES
d) Reverse pipette 40 uL of well-mixed solution to designated chamber well
e) Repeat a-d for remaining tubes and plate into devices

13) Cure device platform with gels in incubator for 40 minutes
14) Plug cell seeding region holes
15) Once gels are set, add 180 uL media
   a) Each well should contain 180 uL
16) Look to see if there is any leaks in the devices
   a) Count the number of leaks/failures
      i) Be specific in recording
17) Confirm presence of cells on microscope
   a) Take a picture of the entire cell seeding region
18) Incubate for 36 hours
   a) Change media at 18 hours
19) Take another image of entire cell seeding region at 36 hours
20) Use image J to count cells in both images
   a) Use the cell counter plugin shown in Figures 30 and 31

![Figure 30. ImageJ cell counter plugin window.](image-url)
21) Do a T test for the expected 3T3 proliferation
   a) 3T3’s double every 18 hours [24]
   b) Use minitab

22) Discard device

Expected Outcome:
- The cell viability levels are within the stated tolerances in Table 2
- The time between media changes is within the stated tolerances in Table 2

Test 7 – Velocity Test

Materials:
- FITC powder
- Scale
- Spatula
- Weight boat
- Graduated cylinder
- Beakers
- Cell media
- Inverted microscope with fluorescent light

Procedures:
*Devices must pass Failure Test prior to Velocity Test.

Matrigel Loading

1. Take Matrigel out fridge. Remove microcentrifuge tubes, pipette tips, and stainless-steel holder from freezer.
2. Plate Matrigel solution
   a. Place gel into frozen stainless-steel holder
   b. Put 40 uL of Matrigel into one microcentrifuge tube
   c. Swirl pipette tip to mix Matrigel in or lightly triturate but AVOID BUBBLES
   d. Reverse pipette 40 uL of well-mixed solution to designated chamber well
   e. Repeat a-d for remaining tubes and plate into devices
3. Cure device platform with gels in incubator for 40 minutes
4. Plug cell seeding region holes

FITC solution preparation

1. Create a 2.4 mM solution of Fluorescein isothiocyanate (FITC) in water
   a. Scoop out FITC powder using spatula and put in weigh boat on scale
   b. Pour FITC powder into beaker and add water
   c. Mix thoroughly

Flow velocity test

1. Turn on fluorescent microscope
2. Set Olympus fluorescent light source to setting 3
3. Set fluorescent exposure to 250 ms and gain to 1.00 in Infinity Analyze
4. Fill both source wells with 350 uL of FITC solution as shown below in Figure 32.

![Figure 32. Fluorescein isothiocyanate (FITC) solution in source wells.](image)

5. Place platform under microscope so that cell seeding region is visible under microscope
6. Capture initial image
7. When the dye appears in the media channels right next to the cell seeding region, take an image.
8. Take images every 2 minutes until fluorescent color is uniform across cell seeding region
   a. Turn light on before the picture is taken.
   b. Turn off after picture is taken.

Analyzing images

9. Using ImageJ, draw line across the one of the sides of the cell seeding region from top to bottom as shown below in Figure 33.

![Figure 33](image)

**Figure 33.** Using ImageJ to analyze dye distribution across cell seeding region.

10. Calibrate ImageJ by setting this distance to 1050 um.
11. Click “Analyze”, then “Plot Profile”. A plot will appear like the one below in Figure 34.
12. Copy data over to Excel
13. Repeat for each image for that device.
14. Import Excel data into MATLAB
15. The MATLAB script will start at the top channel (which should be the dark end) and identify the distance where the grey value is above equals 65520, which is white and is the highest possible grey value.
16. Record these distances and the time points for the images.
17. Using this data, calculate the velocities at each time point by using the equation below.

\[ V = \frac{x_{n+1} - x_n}{t_{n+1} - t_n} \]

Equation 4. Velocity equation.
18. Calculate velocity standard deviation by using Excel function.
19. Perform these steps with 3 devices in one platform.

Expected Outcome:
- Pass criteria
  - For all 3 devices:
    - Velocity is in between 0.1 and 4 um/s
    - Velocity standard deviation is between 0 and 1 um/s.
Test 8 – Cost Test

Materials:

- Computer

Procedure:

1. Determine the cost per unit for
   a. Bottomless 96 well plate
   b. Cover plate
   c. PDMS
   d. Glass slide

2. Total the cost per unit for all the components of a platform to determine the total cost per device

Expected Outcome:

- The cost per device is within the stated tolerances in Table 2
Testing Data and Analyses

Summary of Test Results

Table 14 displays a summary of our testing results. The last column shows which engineering specifications were met.

*Table 14. Testing results for engineering specifications.*

<table>
<thead>
<tr>
<th>Spec. #</th>
<th>Engineering Specification</th>
<th>Test</th>
<th>Measurement</th>
<th>Pass criteria</th>
<th>Pass/Fail</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2D Surface Area</td>
<td>Device Measurements Study</td>
<td>127 cm²</td>
<td>150 cm² max</td>
<td>Pass</td>
</tr>
<tr>
<td>2</td>
<td>Device Dimensions of Chamber</td>
<td>Device Measurements Study</td>
<td>2.6 mm</td>
<td>2.5 +/- 0.1 mm</td>
<td>Pass</td>
</tr>
<tr>
<td>3</td>
<td>Reservoir Diameter</td>
<td>Device Measurements Study</td>
<td>7 mm</td>
<td>9 mm max</td>
<td>Pass</td>
</tr>
<tr>
<td>4</td>
<td>Volume</td>
<td>Volume Test</td>
<td>350 µL</td>
<td>80 µL min</td>
<td>Pass</td>
</tr>
<tr>
<td>5</td>
<td>Devices without leaks</td>
<td>Failure Test</td>
<td>68%</td>
<td>90% min</td>
<td>Fail</td>
</tr>
<tr>
<td>6</td>
<td>Channels with flow</td>
<td>Failure Test</td>
<td>18%</td>
<td>75% min</td>
<td>Fail</td>
</tr>
<tr>
<td>7</td>
<td>Sterilizability</td>
<td>Sterility Test</td>
<td>0%</td>
<td>Bacterial/Fungal Growth</td>
<td>Pass</td>
</tr>
<tr>
<td>8</td>
<td>Opacity</td>
<td>Opacity Inspection</td>
<td>118%</td>
<td>90% min</td>
<td>Pass</td>
</tr>
<tr>
<td>9</td>
<td>Cell Viability</td>
<td>Observational Study</td>
<td>3 times original cells</td>
<td>3-5 times original cells</td>
<td>Pass</td>
</tr>
<tr>
<td>10</td>
<td>Time Between Media Changes</td>
<td>Observational Study</td>
<td>24 hr</td>
<td>24 hr min</td>
<td>Pass</td>
</tr>
<tr>
<td>11</td>
<td>Flow Velocity</td>
<td>Velocity Test</td>
<td>1.33 µm/s</td>
<td>2 +/- 1.9 µm/s</td>
<td>Pass</td>
</tr>
<tr>
<td>12</td>
<td>Flow Velocity Standard Deviation</td>
<td>Velocity Test</td>
<td>0.32 µm/s</td>
<td>1 µm/s max</td>
<td>Pass</td>
</tr>
<tr>
<td>13</td>
<td>Cost per Reservoir System</td>
<td>Cost Test</td>
<td>$14.75</td>
<td>$15 max</td>
<td>Pass</td>
</tr>
</tbody>
</table>
Test 1 – Device Measurements Study
Table 15 displays the results of the Device Measurements Study. All tested engineering specifications were met. The percentage difference between the recorded device chamber dimension and the pass criteria was 0%. The 2D surface area was within the tolerance range. The percentage difference between the recorded device 2D surface area and the minimum pass criteria was 14%. The percentage difference between the recorded reservoir diameter and the pass criteria was 0%.

Table 15. Device Measurements Study Result.

<table>
<thead>
<tr>
<th>Recorded Dimension</th>
<th>Device Chamber</th>
<th>2D Surface Area</th>
<th>Reservoir Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stated Tolerances</td>
<td>2.6 mm</td>
<td>127 cm^2</td>
<td>7 mm</td>
</tr>
<tr>
<td>Pass/Fail</td>
<td>Pass</td>
<td>Pass</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Test 2 – Volume Test
The volume of the 96-well plate wells was found to be 350 uL. Because the measured volume was higher than the pass criteria of 80 uL, the volume engineering specification was met. The percentage difference between this measurement and the pass criteria was 126%.

Test 3 – Failure Test
Table 16 displays the results of the Failure Test. 68% of the devices on the platforms were without leaks. Because the proportion of devices without leaks was lower than the pass criteria of 90%, the “Devices without leaks” specification was not met. The percentage difference between the average measurement and the pass criteria was 29%.

18% of the channels on the platforms had flow during the Failure Test. Because the proportion of channels with flow was lower than the pass criteria of 75%, the “Channels with flow” specification was not met. The percentage difference between the average measurement and the pass criteria (75%) was 122%.

Table 16. Failure Test results.

<table>
<thead>
<tr>
<th>Platform #</th>
<th>Devices without leaks (% of total devices tested)</th>
<th>Channels with flow (% of total channels tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>
Test 4 – Sterility Test

Table 17 displays the results from the Sterility Test. No bacterial or fungal growth was seen over a 48-hour period within all tested platforms.

**Table 17. Sterility Test Results.**

<table>
<thead>
<tr>
<th>Platform #</th>
<th>Time Elapsed</th>
<th>Fungal/Bacterial Growth Present</th>
<th>Pass/Fail</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48 hours</td>
<td>0</td>
<td>Pass</td>
</tr>
<tr>
<td>3</td>
<td>48 hours</td>
<td>0</td>
<td>Pass</td>
</tr>
<tr>
<td>4</td>
<td>48 hours</td>
<td>0</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Test 5 – Opacity Inspection

Table 18 displays the results from the Opacity Inspection. It was found that the new devices had a grey scale average or brightness level 118% greater than the old device. Additionally, a two samples paired t-test was performed with a p-value of 0.05 to determine statistical significance between the new device and old device grey scale averages. A p-value of 0.0165 was found, showing that the new device grey scale average was statistically greater than the old device grey scale average.

**Table 18. Opacity Inspection Results.**

<table>
<thead>
<tr>
<th>Device #</th>
<th>New Device Grey Scale Average</th>
<th>Old Device Grey Scale Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>132.531</td>
<td>106.098</td>
</tr>
<tr>
<td>2</td>
<td>111.848</td>
<td>108.211</td>
</tr>
<tr>
<td>3</td>
<td>130.332</td>
<td>105.108</td>
</tr>
<tr>
<td>4</td>
<td>132.313</td>
<td>105.255</td>
</tr>
<tr>
<td>5</td>
<td>128.371</td>
<td>116.685</td>
</tr>
<tr>
<td>Average</td>
<td>127.078</td>
<td>108.271</td>
</tr>
</tbody>
</table>

Test 6 – Observational Study
Figure 35 displays the results of the Observational Study. It was found that after 48 hours there was an average increase in cells of 143.09% for all devices (n=4). Data was separated for devices with flow (n=2) and devices without flow (n=2). For devices with flow, there was an average increase of 64.48%. For devices with flow, there was an average increase of 220.43%. 2 sample paired T-tests were used to compare 0 and 48 hour groups for all three conditions. There was a statistically significant increase in cells from 0 to 48 hours for all devices and those with flow. A two sample paired T-test with the null being $\mu_1 - \mu_2 = \text{two times the original cell count}$ had a P-Value = 0.326. We must accept the null, which means that the observational study passed the criteria.

![3T3 Cell Count at 0 and 48 Hours](image)

**Figure 35.** Observational study results.
Test 7 – Velocity Test

Figure 36 displays the average velocities for each of the devices tested. The error bars display the standard error. All three devices tested had measured velocities between 0.1 and 3.9 µm/s, which means they were acceptable velocities.

![Flow Velocity for Devices Tested](image_url)

**Figure 36. Flow velocity for devices tested.**

Table 19 displays the results of the Velocity Test. The average velocity measured in the cell seeding region was 1.33 µm/s. Because the average velocity was in the range of 0.1 to 3.9 µm/s, the velocity specification was met. The percentage difference between the average measurement and the target velocity (2 µm/s) was 29%.

The standard deviation of the velocity measured in the cell seeding region was 0.32 µm/s. Because the velocity standard deviation was lower than the pass criteria of 1 µm/s, the velocity standard deviation specification was met. The percentage difference between the average measurement and the target velocity standard deviation (0.5 µm/s) was 44%.

**Table 19. Velocity Test results.**

<table>
<thead>
<tr>
<th>Device #</th>
<th>Velocity (µm/s)</th>
<th>Standard Deviation (µm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.458</td>
<td>0.462</td>
</tr>
<tr>
<td>2</td>
<td>0.196</td>
<td>0.058</td>
</tr>
<tr>
<td>3</td>
<td>2.326</td>
<td>0.442</td>
</tr>
<tr>
<td>Average</td>
<td>1.326</td>
<td>0.320</td>
</tr>
</tbody>
</table>
Test 8 – Cost Test

Table 20 displays the results from the Cost Test. The cost per device was found to be $14.75, lower than the stated tolerance of $15 per device, meaning that the engineering specification was met.

*Table 20. Cost Test Results.*

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost per Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottomless 96 Well Plate</td>
<td>$5.20</td>
</tr>
<tr>
<td>Coverplate</td>
<td>$0.71</td>
</tr>
<tr>
<td>Large Microscope Slide</td>
<td>$2.80</td>
</tr>
<tr>
<td>PDMS</td>
<td>$6.04</td>
</tr>
<tr>
<td>Total Cost per Device</td>
<td>$14.75</td>
</tr>
</tbody>
</table>
Instructions for Use (Operation manual)

Materials:

- ECM
- BSC
- Pipettes
- Centrifuge
- Inverted microscope with camera
- ImageJ
- Warm water bath
- 3T3’s
- UV crosslinker
- T75 Flasks
- Aspirator
- Trypsin
- Cell counting slide
- Matrigel
- Incubator
- Calculator
- Minitab

Manual:

1) Wear the appropriate PPE and use aseptic technique whenever handling the device
   a. Gloves
   b. Pants
   c. Closed-toed shoes
2) Put ECM in water bath
   a. When warm, pass into BSC
3) Open 2 flasks in BSC using sterile technique and label the flasks
4) Thaw a vial of 3T3’s from liquid nitrogen in the water bath until all ice is melted.
   a. Transfer into the BSC using sterile technique
5) Add 11 mL of ECM to each flask, and pipette the full 1 mL of 3T3’s into flask
   a. Sterilize device in UV Crosslinker
6) Transfer device to BSC
7) Warm media and trypsin in water bath:
8) Count cells in flask
   a. Aspirate off media of cells in a T75 flask
   b. Rinse cells with DPBS & aspirate off
   c. Add 3mL of trypsin & let cells detach
   d. Quench Trypsin with 3 mL of media ($V_{suspend} = 6 \text{ mL}$)
e. Triturate and take 100μL sample to microcentrifuge tube ($V_{mix} = 100\mu L$)
f. Add 100 uL of Trypan Blue to the microcentrifuge tube ($V_{TB} = 100\mu L$)
g. Triturate cell-media solution with the Trypan Blue thoroughly
h. Put a coverslip on hemocytometer over the grid
i. Inject 20μL TB solution between the gap of coverslip and hemocytometer
j. Count cells under upright white light microscope to count cells
   i. Live – White, Dead - Blue
k. Calculate volume of desired number of cells in wells
   i. Do serial dilution as necessary, aim for 10 μL

9) Take Matrigel out fridge. Remove microcentrifuge tubes, pipette tips, and stainless-steel holder from freezer.

10) Setup ultraviolet (UV) crosslinker
   a. Verify the preset exposure setting is 120,000 microjoules per cm$^2$
      i. On the crosslinker touch pad, select the preset button
      ii. Select the exposure button
      iii. The display should show 1200, if not input 1200 using the touch pad
      iv. Select the enter button
   b. Verify the preset ultraviolet time exposure setting is 2 minutes
      i. On the crosslinker touch pad, select the preset button
      ii. Select the time button
      iii. The display should show 2.0, if not input 2.0 using the touch pad
      iv. Select the enter button

11) Insert device to be sterilized into the UV crosslinker

12) On the crosslinker touch pad, select the start button
a. At the end of the exposure cycle the crosslinker will beep 5 times to signal exposure is complete
13) Transfer devices to the BSC
14) NOTE: Reverse pipette to avoid bubbles, spread gel into all four corners/flatten
15) Dispense ~10 uL of cell solution for desired cell density in microcentrifuge tubes (8 for 8 device 96 well plate platform)
16) Plate Matrigel-cell solution
   a. Place gel into frozen stainless-steel holder
   b. Put 40 uL of Matrigel into one microcentrifuge tube with cells
   c. Swirl pipette tip to mix Matrigel in or lightly triturate but AVOID BUBBLES
   d. Reverse pipette 40 uL of well-mixed solution to designated chamber well
   e. Repeat a-d for remaining tubes and plate into devices
17) Cure device platform with gels in incubator for 40 minutes
18) Plug cell seeding region holes
19) Once gels are set, add 180 uL media
   a. Each well should contain 180 uL
20) Look to see if there is any leaks in the devices
   a. Count the number of leaks/failures
      i. Be specific in recording
21) Confirm presence of cells on microscope
   a. Take a picture of the entire cell seeding region
22) Incubate for 36 hours
   a. Change media at 18 hours
Figure 38. Example of device after 24 hours of simulated incubation. Blue represents media.
Yellow represents Matrigel.

23) Take another image of entire cell seeding region at 36 hours
24) Use image J to count cells in both images
   a. Use the cell counter plugin
25) Do a T-test for the expected 3T3 proliferation
   a. 3T3’s double every 18 hours [24]
   b. Use Minitab
26) Discard device
Discussion and Overall Conclusion

The aim of this project is the preliminary development of a new reservoir system for the microfluidic chip used in Dr. Heylman’s lab for pre-clinical drug testing. The prototype that was created incorporated all the customer requirements shown in Figure 14. To implement the bottomless 96 well plate as the new reservoir system the microfluidic chip had to be magnified, meaning the channels were lengthened to allow the inlets and outlets to line up with the wells in the bottomless 96 well plate.

The prototype provides proof of concept for using a bottomless 96-well plate as the reservoir system. Additionally, tests were performed to prove the functionality of the device. These tests included a velocity test to confirm interstitial velocity within the cell seeding region, an observational study to confirm cell viability and the functionality of the device, and a sterility test to confirm that the device can be sterilized. The device passed every test except the failure test, which was a water test performed after every device was manufactured to test for leaks and blockages. It was found that 32% of devices tested experienced a leak and 82% of devices tested had channels without flow.

A continuation of this project would involve reducing the number of leaks and blockages. To address the channels without flow, the diameter of the channels leading to the cell seeding region should be increased. This would decrease flow resistance. The velocity test measured the velocity until the FITC solution had filled the cell seeding region, which is in the initial stages of the equilibrium process for the source and sink wells. Because of this, the velocities measured in the velocity test are the maximum velocities that will occur from the initial filling of the source wells until equilibrium has been reached between the source and sink wells. Thus, the ideal velocity within the cell seeding region during the velocity test would be around 4 um/s, which is the upper end of the acceptable range for flow velocity. Then, as the pressure gradient decreases over time as the source and sink wells equilibrate, the velocity would decrease and remain in the desired range.

Additionally, to try to reduce the number of blockages, a more thorough device priming technique should be used before every test. Priming the device properly would mean there are no air bubbles present within the device. Air is a compressible fluid, so having air bubbles in the channels absorbs the pressure created by the media head height. This pressure is necessary to drive the flow, so air bubbles will stop the flow. Within the manufacturing process, more thorough chip cleaning to ensure particles are removed.

To address the leaks one PDMS chip should be used instead of two. To plasma bond the 96-well plate to the 2 chips, the chips must be level with each other so that the 96-well plate has sufficient contact with both chips. It is extremely difficult to make two chips of the same thickness because it requires being extremely precise during the PDMS pouring process. Having one chip would make the PDMS easier to plasma bond to both the glass slide and bottomless 96 well plate because it is more likely to be a flat surface.
The objective of this project is the preliminary development of a new reservoir system for the microfluidic chip Dr. Heylman uses in his lab. To achieve this, the next step will be implementing the talked-about suggestions to reduce the number of leaks and blockages. Overall, the prototype developed for this project showed promise of being an effective device.
References


Appendices

APPENDIX A. Customer Requirements: Wants/needs for both customers, Dr. Heylman and the research assistants, with the customers rating of importance (1-min, 10-max).

<table>
<thead>
<tr>
<th>Customer Requirements</th>
<th>Dr. Heylman</th>
<th>Research Assistants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower the footprint of a multi-device experiment</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Reduce flow variance</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Reduce failures due to leaks and blockages</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Minimize changes to current device design (cell seeding area)</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Ability to change out cell media</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Allow ability for continuous gravity fed flow</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Clear (usable in microscopy)</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Ability to add liquids to reservoirs</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Similar cost to current reservoir system</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Sterilizable</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Biocompatible</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>One-time use</td>
<td>10</td>
<td>7</td>
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<tr>
<td>Minimal scientist intervention</td>
<td>9</td>
<td>10</td>
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</table>
APPENDIX B. Gantt Chart.
## APPENDIX C. Kyle’s Pugh Matrix.

<table>
<thead>
<tr>
<th>Key Criteria</th>
<th>Importance Rating</th>
<th>Benchmark Option</th>
<th>Dr. Heyman Current Reservoir System</th>
<th>Cummins water-spray well plate</th>
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<tbody>
<tr>
<td>Smaller surface area</td>
<td>4</td>
<td>+</td>
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<tr>
<td>Low cost</td>
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<td>-</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Low flow rate variance</td>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Physiological Flow rate</td>
<td>9</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Low Geometric Deformation Post-Autoclave</td>
<td>4</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>High time between media changes</td>
<td>10</td>
<td>S</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>High tensile strength of bond</td>
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<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Low blockage failure rate</td>
<td>7</td>
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<td>+</td>
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<tr>
<td>Target volume</td>
<td>10</td>
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<td>S</td>
<td>S</td>
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<tr>
<td>Low Cell Death</td>
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<td>S</td>
<td>S</td>
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<td>High Opacity</td>
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<tr>
<td>Low Leak Failure Rate</td>
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<td>+</td>
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<tr>
<td>Same cell seeding dimensions as current design</td>
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<tr>
<td>Target inlet reservoir diameter</td>
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<tr>
<td>Target outlet reservoir diameter</td>
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</tbody>
</table>

**Sum of Positives**: 9, 7, 9  
**Sum of Negatives**: 1, 1, 1  
**Sum of Sames**: 5, 7, 5  
**Weighted Sum of Positives**: 50, 48, 56  
**Weighted Sum of Negatives**: 5, 4, 6  
**TOTALS**: 45, 44, 51
## APPENDIX D. Ryan’s Pugh Matrix.

<table>
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<th>Key Criteria</th>
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<th>Benchmark Option</th>
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<th>1 mL Wells</th>
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<td>High tensile strength of bond</td>
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<tr>
<td>Target volume</td>
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<td>-</td>
<td>+</td>
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</tr>
<tr>
<td>Low cell death</td>
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<tr>
<td>High Opacity</td>
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<tr>
<td>Target inlet reservoir diameter</td>
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<tr>
<td>Target outlet reservoir diameter</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

| Sum of Positives                                 | 11                | 5                | 8                         |            |                              |
| Sum of Negatives                                 | 1                 | 2                | 2                         |            |                              |
| Sum of Sames                                    | 3                 | 8                | 5                         |            |                              |
| Weighted Sum of Positives                        | 57                | 31               | .49                       |            |                              |
| Weighted Sum of Negatives                        | 5                 | 14               | 9                         |            |                              |
| TOTALS                                           | 52                | 17               | .40                       |            |                              |
### APPENDIX E. Olivia’s Pugh Matrix.

<table>
<thead>
<tr>
<th>Key Criteria</th>
<th>Importance Rating</th>
<th>Benchmark Option</th>
<th>Bottomless 50-well plate</th>
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<td>Low flow rate variance</td>
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<td>Physiological Flow rate</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>S</td>
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<tr>
<td>Low Geometric Deformation Post-Autoclave</td>
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<td>S</td>
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<tr>
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<td>+</td>
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<td>High tensile strength of bond</td>
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<td>+</td>
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<td>+</td>
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<tr>
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<td>5</td>
<td>+</td>
<td>-</td>
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| Dr. Heyman Current Reservoir System       |                   |                  |                          |            |                             |
|                                          |                   |                  |                          |            |                             |

<table>
<thead>
<tr>
<th>Concept Selection Legend</th>
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<th>Benchmark Option</th>
<th>Bottomless 50-well plate</th>
<th>1 mL vials</th>
<th>Custom water-reservoir plate</th>
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</thead>
<tbody>
<tr>
<td>Better</td>
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<td>+</td>
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<tr>
<td>Same</td>
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<tr>
<td>Worse</td>
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<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

| Sum of Positives                          | 9                 | 7                | 5                        |
| Sum of Negatives                          | 1                 | 4                | 1                        |
| Sum of Sames                              | 5                 | 4                | 9                        |
| Weighted Sum of Positives                 | 54                | 53               | 29                       |
| Weighted Sum of Negatives                 | 6                 | 13               | 5                        |

| TOTALS                                    | 49                | 40               | 24                       |