MIXING AND INJECTING BIOMATERIALS INTO MICROCHANNELS FOR ORGAN-ON-A-CHIP MANUFACTURING

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MIXING AND INJECTING BIOMATERIALS INTO MICROCHANNELS FOR ORGAN-ON-A-CHIP MANUFACTURING

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LIST OF SYMBOLS AND ABBREVIATIONS

OOC  organ-on-a-chip

2D  two-dimensional

3D  three-dimensional

CFD  computational fluid dynamics

BBB  blood-brain barrier

MBMN  Multiscale Biosystems and Multifunctional Nanomaterials

SHM  staggered herringbone mixer

PA  polyacrylamide

PEG  polyethylene glycol

CAD  computer-aided design

VOF  volume of fluid

TF  transfer function

RPM  revolutions per minute

EMF  electromagnetic force
SUMMARY

Organ-on-a-chip platforms, referring to microfluidic biomimetic systems, combine cell culture, fluid gradients, and material design to simulate the microenvironment of various human organs. This technology has been growing in relevance over the past decade, with multiple companies and start-ups gaining funding to develop such platforms for drug development and personalized medicine. However, there still lies many challenges in manufacturing these complex and semi-living systems. This thesis presents a breakdown of the current engineering obstacles in bringing organ-on-a-chip technology to mass production and presents a partial solution for automated cell mixing and seeding. A device design is presented which sets out to fill a gap in the current production processes for organ-on-a-chip companies. First, a holistic approach to design is considered with overall requirements and specifications set out for a fully automatic hydrogel injecting and cell seeding device. Then, more detailed analysis of the mixing and injecting functions of the device are explored with computational fluid dynamics. A wide range of common hydrogels and their properties are analyzed and considered for maximum compatibility with various organ-on-a-chip designs. Variations of passive mixers are compared and optimized for varying levels of viscosity and shear-thinning properties. A method is presented for injection pressure control to adapt to sensitive organ-on-a-chip burst pressures. Additionally, design details for user usability such as ease of loading cells and compatibility with other lab equipment is considered and implemented in the final design model.
CHAPTER 1. INTRODUCTION

Pharmaceutical research, development, and testing is an enormous field of study and economics with over $83 billion spent on research and development in 2019 alone [1]. Since the 1990s, costs and timelines for bringing a new drug to market have grown while overall productivity and number of new drugs approved per year has decreased [2, 3]. The drug development cycle is a long journey which consists of preclinical research and clinical (human) trials. Though the preclinical phase is relatively short compared to human trials, preclinical in vitro and in vivo research accounts for 46% of these drug research and development costs [1]. Moreover, the incomplete ability of in vitro and animal testing to predict human response during the preclinical phase leads to wasted costs during human clinical trials. Recent estimates show that less than 8% of successful animal trials lead to successful trials in human testing, and over 85% of clinical trials for novel drugs fail [4-6]. There is a huge disconnect between the preclinical data and the drug performance in humans.

Current methods of preclinical testing for drug development are summarized in Table 1. Among the most widely used in vitro platforms are Transwell models (or other two-dimensional (2D) well-plate culture methods) and organoid studies, with the latter rising in popularity due to the importance of the additional functionality from three-dimensional (3D) tissue culture [7]. These in vitro studies are used to screen drugs for potential side effects and efficacy against diseased tissue, which is then followed up with more comprehensive studies in animals. Drugs can often show efficacy in targeted tissue studies but be unsafe for humans; for example, a drug targeting Alzheimer’s may work well on brain tissue when isolated but may be toxic to the liver once it is processed through the body. Animal studies play a critical role in providing a full-body,
multi-organ response to the drug which can present as effective but otherwise be unsafe for humans [8]. However, animal testing remains an expensive process in an ethical gray area, and there are cross-species differences that reduce the predictability of human results [8, 9]. Because of these issues, there is an ever-increasing investigation into other multi-organ, ethically sound, physiologically relevant drug development platforms such as organ-on-a-chips (OOCs).

OOC platforms have immense promise as a more accurate and humane solution for drug development and testing. These devices combine cell culture with microfluidic flow and other actuation methods to mimic biological systems and tissue microenvironments [10]. This technology has the potential to radicalize drug discovery and personalized medicine research by allowing for the observation of physiological functions which closely mimic in vivo counterparts [11]. The physiological relevance of OOCs due to their use of human cells and tissues can reduce late-stage failures in the drug development pipeline by providing more reliable results early on in testing. Experts estimate that this advantage (over the current standard of animal testing or static-culture testing) will save 10-26% of research and development costs for each new drug within 5 years [5]. The resulting demand from pharmaceutical companies has caused the market for OOCs to grow steadily since the field first started gaining momentum in the early 2000s, as indicated by the emergence of at least 28 companies and start-ups within the last 10 years [6, 12]. However, a huge barrier which still stands in the way of organ-on-a-chip production and use is the lack of large-scale manufacturing and operating techniques available for such platforms.
<table>
<thead>
<tr>
<th>Testing Method</th>
<th>Pros</th>
<th>Cons</th>
<th>Reference</th>
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<tr>
<td>2D Culture and Transwell Culture Plates</td>
<td>- Extensive existing characterization</td>
<td>- Reduced expression of cell markers</td>
<td>[13]</td>
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<td></td>
<td>- Detailed protocols for quantifiable assays</td>
<td>- Reproducibility varies</td>
<td></td>
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<tr>
<td></td>
<td>- Scalable and robust</td>
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<td></td>
<td>- Compatible with automation</td>
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<tr>
<td>3D Culture and Organoids</td>
<td>- Genetically stable for patient-specific studies</td>
<td>- Difficult to control culture parameters</td>
<td>[7, 14]</td>
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<tr>
<td></td>
<td>- Organotypic structure and cell expression</td>
<td>- Limited in size</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Lack of mechanical actuation</td>
<td></td>
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<tr>
<td>Organ-on-a-Chip Platforms</td>
<td>- Physiologically relevant culture conditions</td>
<td>- Lack of protocol and precedent</td>
<td>[10-12]</td>
</tr>
<tr>
<td></td>
<td>- Organotypic structure and cell expression</td>
<td>- Difficult to automate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Ability to combine with other “organs”</td>
<td>- Specialized knowledge required</td>
<td></td>
</tr>
<tr>
<td>Animal Testing</td>
<td>- Complete system for drug testing (multi-organ)</td>
<td>- Insurmountable cross-species discrepancies</td>
<td>[6, 8, 9]</td>
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<tr>
<td></td>
<td>- Allows for study of biological functions on which treatment can be</td>
<td>- Expensive</td>
<td></td>
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<tr>
<td></td>
<td>based</td>
<td>- Time-consuming</td>
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<tr>
<td></td>
<td>- Screens unsafe drugs prior to testing in humans</td>
<td>- Ethically controversial</td>
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OOCs require micro-scale precision in both the manufacturing of the chip structure and the seeding of cells into the resulting microchannels. Currently, most OOCs are made by molding polydimethylsiloxane (PDMS) with soft lithography techniques [10, 12, 15]. Though PDMS is great for one-off lab studies due to its transparency, biocompatibility, and flexibility, these manufacturing techniques are slow, expensive, and unsuitable for mass production.
Therefore, there has been more exploration recently into three-dimensional (3D) printing, additive manufacturing, and injection molding techniques for OOC manufacturing, as well as more research into biocompatible materials which are easier to work with than PDMS [12, 15]. This material and manufacturing research of the actual chip platforms has been a recent focus of the field, but an oft-overlooked issue with mass production and usage of OOCs is the burden on the end user to properly perfuse the chips with media and cells [15, 16].

Leading organ-on-a-chip companies rarely, if ever, ship products which already include cells. Instead, they ship cells separately and provide detailed culture instructions for manually seeding their chips with cells [17, 18]. This takes up valuable lab time and introduces the risk of human error when following culture instructions. It also presents a huge obstacle for large-scale studies, since dedicated human attention plus several hours or days of lab time is required to set up each individual platform. Because of the human aspect, there are also knowledge and expertise-based barriers to entry in the field of OOCs which prevents wide-spread adoption and use of these platforms. Whether cells are seeded in OOC manufacturing plants or in the lab space of the end user, the process takes human attention, and there are currently no automated solutions available [19]. After seeding, the OOCs must also be sustained with continually perfused media, which is usually done via a custom syringe pumping system or a gravity-driven flow, both of which require human attention and moderation.

Some applicable technology has been developed in the areas of pipetting robots and bioprinters. These two types of devices effectively bracket the problem presented in this thesis. Pipetting robots, such as offerings from Hudson Robotics, Labcyte, Biotek, and others, are high throughput, have positioning precision of 10-100 microns, and minimum dispensing volumes as low as 0.02 microliters [20]. However, these robots are extremely costly, starting at around
$10,000, work best with liquids, and often take media from a reservoir [21]. Many OOCs use hydrogel encapsulated cell mixture, which is more gel-like in its behavior, and reservoir-style storage can allow cells to settle or hydrogel to set, resulting in an uneven distribution of cells or uninjectable gel. Bioprinters, on the other hand, excel at handling gel-like materials and have accurate placement within +/- 10 microns and volumetric precision around 50 microliters [22, 23]. They are also designed to handle cell-laden gels and therefore do not exert too much stress on the cells. Bioprinters are slower than pipetting robots and are also very costly, on the order of $100,000, and there has not been work done to extend bioprinting technology to microchannel applications [23]. Bioprinters are made to dispense stand-alone material, whereas microchannels on OOCs impart fluidic resistance which will change the desired behavior of the material once it has been dispensed. The science of making bioinks, or injectables, for these devices is also still being understood. These materials must be designed and mixed manually [24], which draws away from the automatic nature of the bioprinter itself. Though bioprinters and automated pipetting robot technology exists, there is critical work to be done in modifying and applying these devices to OOC studies.

This gap in the field of OOC manufacturing can be addressed by a lab bench device to seed purchased OOCs with cells or by a cell seeding machine to be used in OOC mass production lines. The device would straddle the line between pipetting robots and bioprinters, having high throughput and accuracy while still being able to handle gel-like cell-laden material. The goal of this thesis is to design and develop a device to be used in research labs which can build upon available bioprinter and pipetting robot technology to automatically follow protocol and seed cells onto purchased or lab designed OOCs. The device will be flexible to be used with several types of hydrogels and cells, as well as adjust to different configurations of OOC
microchannels and inlets. Various designs for automatic mixing of bioink-like materials were analyzed using computational fluid dynamics (CFD) to select the best option for a wide range of material properties. The mixing of biomaterials and shear stress imparted on cells was studied and tuned using CFD simulations to ensure cell viability after injection into the microchannels.

The major advantage of this device over similar devices on the market is its flexibility in material usage and protocol. Several different inputs can be given to the mixing portion of the device, and the dynamic nature of passively mixing prior to injection eliminates the settling issue seen with reservoir-style automatic pipettes. The mixing also eliminates the need for the user to carefully concoct bioinks or hydrogel mixtures prior to using the device. A control for injection pressure based on the fluidic resistance of OOC microchannels is also included for robustness. The goal is to reduce the need for human interaction as much as possible, though some limitations still exist due to the delicate and time-sensitive nature of culturing cells.

In this paper, I will first discuss the narrowing of the design problem and overall objectives for seeding cells into microchannels. The requirements and functions of the device will be laid out, and specifications as found in previous literature will be detailed. Then, I will present an overview of the concept generation process and the criteria by which ideas were selected. I will show how an initial design and design alternatives were evaluated using CFD simulations. A case study for the effectiveness of the device will be shown for a blood-brain-barrier (BBB) chip designed by researchers in the Multiscale Biosystems and Multifunctional Nanomaterials (MBMN) lab. Finally, I discuss future work to be done, including improvements to be made for increased throughput, reduced cost, and a scaled-up device to enable mass cell-seeding operations for use in OOC manufacturing plants.
CHAPTER 2. BACKGROUND

This chapter lays out the approach to the design problem, including defining the objective, setting requirements for the design, and exploring design ideas for major functions of the device. Design alternatives are also selected to evaluate and optimize the functionality of the device. The objective for the design was based on previous literature and criticisms on the accessibility of the OOC field, and requirements were drawn from similar devices in bioprinting and pipetting while keeping in mind the needs of OOC users and researchers.

2.1 Defining the Design Problem

OOCs can provide immense value to drug development studies if the technology is made more accessible and uniform. As the field grows in relevance, emphasis is shifting from prototype and single-chip analyses into high throughput biological validation [25]. These wider assays require a more commercial viewpoint of OOC technology that places focus on the user experience, mass production, and ease of use. An ideal future workflow for OOC studies is described in Figure 1. By providing specialized tools which ensure proper set-up and long-term culture of the chips, life scientists can utilize OOCs regardless of their previous knowledge level, or lack thereof. Several devices which provide long-term flow control and monitoring for OOC culturing already exist on the market, including a multi-well high-throughput system from Pfizer and a specialized system from Emulate [26, 27]. Therefore, the ideal controlled mixing and injecting device shown in Figure 1 will be the focus of this paper.
Microfluidic cell seeding and biomaterial injection can be thought of as a specialized application of bioprinting technology. Whereas traditional bioprinting methods aim to create a free-standing 3D structure seeded with cells, organ-on-a-chip devices must be seeded with cells within confined, stiff channels. The differences in structure between the two applications means that bioinks for organ-on-a-chip applications can be less viscous and less quick to set in a solid structure, though they still must be viscous enough to prevent the cells from settling out of suspension. Additionally, the injection process is highly variable due to the confined geometry of the chip and the fluidic resistance imparted by microchannel design. This specific problem has rarely been explored, and an automated solution for seeding cells into channels does not exist in this market. If such a technology were to be developed, the rate of production and ease of use for these platforms would greatly increase. The problem is made more complex, however, since OOC designs vary widely in the method of cell culture. Cell culture in OOCs vary depending on the application. Cells are either seeded onto previously set hydrogel within the chip or
encapsulated in the hydrogel itself, depending on whether the chip is mimicking a membrane-type interface or a 3D tissue structure. Therefore, the functions of the device may be flexible to allow mixing of two or more fluids, injection in two or more phases, or a combination of these parameters to enable use for any style of OOC culture.

An automated solution for OOC cell seeding could either be applied for use in mass manufacturing, i.e., on the manufacturers’ or OOC companies’ side of the supply chain, or for use in research lab settings, i.e., on the end users’ side. To narrow the design parameters, only the research lab setting is considered in this paper. The end user is defined as a research scientist working in OOCs or drug discovery, and the benefit provided by the device is the automation of cell seeding, thereby reducing time in the lab, reducing the need for required expertise, and increasing uniformity of OOC set-ups for multiple trials. Based on these general decisions on the scope of the design, a problem statement was formulated:

*Design a device which can be implemented in a lab setting to reduce the manual burden on researchers using OOCs and increase reliability and uniformity of OOC set-up, while remaining flexible to be used with a wide variety of cell culture styles.*

This statement was kept in mind as more specific design requirements and constraints were brainstormed. Based on other similar lab equipment, common OOC procedures, and the micro-scale nature of these devices, several requirements and constraints were found which are summarized in Table 2. Some requirements, such as volumetric precision, level of force, and speed of operation, would be present whether the device is considered for mass manufacturing or for lab use. Other requirements, like adaptability for various designs of OOCs, convenience, and compact footprint, are unique to the lab setting envisioned for this device. Thus, the design decisions made in this thesis may not be applicable to the future design of an OOC mass-
After setting the requirements for the device overall, the objective of “deliver a range of biomaterials into OOCs” must be further defined. This is a complex action which requires many steps to achieve, so leaving the objective as this single descriptor without further exploration would be misleading. Based on solutions already available on the market and similar processes in the bioprinting and pipetting industries, the design problem can be considered as three major steps: (1) calibrating and moving the injection head to the correct geometric location on the chip, (2) dispensing and mixing the proper ratios of biomaterials, and (3) injecting the mixture into the chip while considering the physical constraints of microchannels and shear stress limits. Each of these challenges will be explored further and ultimately reasoned out through general design.
principles and simulation and CFD analysis while bearing in mind the driving problem statement and context in which this device should exist.

2.2 Exploration of the Design Space

Each of these steps can be achieved in a multitude of ways. To initially explore potential solutions, the challenges were viewed individually. However, the final design must combine ideas together in such a way that solutions were compatible. Though each individual step is subject to its own personal engineering constraints, it also must be coupled with other steps to ensure the functionality and specifications of the final device. For example, choosing an expensive but effective solution for one challenge may limit the appeal of choosing other expensive options for the remaining design sections, since then the overall cost would be too high, and the goal of constraining cost will not be met. Another possibility is that some solutions may be geometrically incompatible, or intrinsically linked, depending on how they are laid out.
Figure 2. Some notable solutions for each major step identified for the device. A survey of existing solutions for these types of problems in other fields or in other applications reveals several types of calibration, passive and active mixing structures, and pump options.

A survey of widely available options and previously researched concepts was taken for the three main steps of the device. The resulting brain map, shown in Figure 2, is by no means exhaustive but provides a useful visual tool to further explore specific solutions. The first step, originally defined as the entire process of calibrating and moving the injection head, was separated into two to explore relevant design properties more clearly. Overall, the movement of the injection head to the designated site is a complex issue involving robotics, controls, and mechanical precision; however, it is also a widely solved issue, with common solutions present already in additive manufacturing devices, bioprinters, pipetting robots, manufacturing robot
arms, and more [28]. Due to its relative complexity along with the availability of common, open-source solutions, the scope of this paper was limited to the calibration, mixing, and flow control portions of the device. Some broad solutions to move of the injection head are still included in Figure 2, and the requirement for the injection head to eventually move will be discussed as other solutions are considered (for example, solutions for mixing or flow control that rely on a stationary injection head are not viable). Specifications were also defined for a theoretical injection head movement system, so the eventual integration of a solution will smoothly work with OOC systems and the rest of the device design.

To evaluate and customize the options available for each step within the device, overarching engineering specifications and targets for the device operation must be defined. The strictness of the engineering specifications will reveal which design options are viable and which are not. The most important targets for the device include viability for in-lab use, i.e., footprint and operation space, preserving cells with proper pressure and fluid flow application, and dispensing the proper volumes of biomaterials. To define these specifications, fully listed in Table 3, common limits in lab usage and OOC design were researched.
<table>
<thead>
<tr>
<th>Function or Constraint</th>
<th>Engineering Specification</th>
<th>Criteria / Target</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Must fit in a lab environment</td>
<td>Footprint fits on a standard-sized lab bench or table</td>
<td>30” x 20” footprint</td>
<td>-</td>
</tr>
<tr>
<td>Account for fluidic resistance of microchannels</td>
<td>Microchannel properly filled with biomaterial</td>
<td>&gt;95% filled</td>
<td>-</td>
</tr>
<tr>
<td>Adjust pressure and mixing speed</td>
<td>&gt;85% cell viability</td>
<td>Shear stress limit</td>
<td>-</td>
</tr>
<tr>
<td>Move injection head to chip inlet</td>
<td>X-Y precision</td>
<td>+/- 10 µm</td>
<td>[20, 23]</td>
</tr>
<tr>
<td>Dispense proper amount of cell and media</td>
<td>Volumetric precision</td>
<td>+/- 100 µL</td>
<td>[17, 18]</td>
</tr>
<tr>
<td>Combine components into a homogenous mixture</td>
<td>Mixing index</td>
<td>&gt;0.90</td>
<td>-</td>
</tr>
</tbody>
</table>

Many specifications were taken from referencing similar devices on the market, i.e., pipetting robots and bioprinters, which are compatible with industry standards (such as well plates) of comparable scale to OOCs. This is due to the current lack of standardization of OOC platforms; because of this limitation, some OOCs may require more precision than these general specifications indicate. Some specifications were also drawn up from general knowledge of OOC studies, cell viability, and goals for the project; since this device has no precedence, it is difficult to put an exact mark on the values that must be achieved to be competitive. Additional experimentation should be done for each of these unknown specifications to determine a threshold which limits the functionality of OOCs, and the values can be adjusted accordingly.
Once the limits and specifications are defined, solutions from Figure 2 can be evaluated under more stringent conditions. The pump or flow control system must have microliter precision while limiting shear stress to preserve the number of cells. Additionally, cells and media are expensive, so waste is undesirable; it is unlikely that a good solution would include lots of complicated tubing where media could be stuck or stagnant after operation, for example.

On one hand, solutions with micro-volume pumps for each biomaterial component, active mixing chambers, and an additional pump to dose the mixed suspension into the OOC allow a very custom approach to each chip with high levels of control over each mixing and injecting parameter. The active mixing chamber could be equipped with a stirrer, magnetic beads, or an acoustic device to impart vibrations into the mixture. The separate pumps for each biomaterial could allow for varying flow rates and ratios of the mixture components to be achieved. An additional pump at the end of the mixing section could help reduce waste and fully utilize the mixed components. A solution like this would most likely be able to achieve the volumetric precision and microchannel fill percentage specifications required of the device, but there are a few downsides. The solution requires multiple pumps which must be accurate and provided power, making it energy intensive as well as expensive [29, 30]. Moving components like the pumps and the active mixer also may require more maintenance over time compared to passive alternatives. Troubleshooting and repairing the device requires knowledge of the components, which is an unwanted barrier to widespread adoption and usage. The size constraint could also become problematic since pumps and active mixing reservoirs take up significant space.

To address some of these issues, passive alternatives to each step were considered. Gravity-driven flow is common in OOC operation since it is low-cost and can be optimized for a variety of flow rates [29, 31]. Valves can be added below each reservoir to independently control
the amount of each component added to the final mixture. A passive mixing channel with interfering microstructures can be used to combine the two flows together into a homogenous solution, and only one pump is needed to dose specific volumes to the OOC. This solution is less complex with much fewer moving parts. Therefore, less frequent maintenance or knowledge of microfluidics is required for operation. It also avoids the use of active mixing, which could create increased shear stress (causing cell damage) or undesirably heat the temperature of the cell media [29]. This is critical since gelation is induced in many common hydrogels based on temperature [32, 33]. A pump, such as a syringe or vacuum pump, can precisely control the amount of cell media administered into the OOC’s microchannels [29, 30]. Open-source solutions are available for both syringe and vacuum pumps, which can help lower the cost as well [34, 35]. This solution has some drawbacks in the achievable accuracy and precision of mixing and injection. Valve control and flow rate calculations for the reservoir-driven flow must be very exact to ensure that the ratio of mixture components is maintained throughout operation. Additionally, validation must be done on the mixing channel to ensure the length and microstructure design achieves the target level of homogeneity for a variety of gel viscosities and flow rates.

Specific integrated solutions could be drawn up using combinations of the two ideas discussed above. For example, if the potential inadequacies in passive techniques mentioned earlier (lower homogeneity or accuracy) come to light in testing, the gravity-driven flow or passive mixing channel could be swapped for powered pumps or an active mixing method, respectfully. However, since the aim of this paper is to develop a low-cost and accessible solution for benchtop use, an initial attempt will be made to fully develop the passive solutions to each step in the design problem.
2.3 Detailed Design and Final Concepts

The full design of this device is a complex and multi-faceted problem that most likely requires more analysis and investment from OOC labs and companies. The needs of OOC set-up procedures vary widely depending on the type of gel, type of cells, and device design. To present solutions in this paper that will be useful to OOC labs regardless of the chip design, the detailed designs of this paper will have two areas of major focus: the utility of passive mixing channels as applied to hydrogels, and the control of gel injection into geometrically complex chips.

2.3.1 Passive Mixing Channel

Mixing is a critical step in the operation of this device. Cell culture protocols for OOCs require mixing of various concentrations of cells, cell media, and hydrogel solutions to successfully replicate the in vitro balance of tissue stiffness, nutrients, and cell density [17, 18]. Due to these requirements, the preparation of biomaterials for injection into OOCs is usually a slow process where the necessary components are hand-mixed by researchers. An ideal cell culture initiation device would therefore include a way to select desired concentrations, take in the necessary components, and mix them uniformly in the proper ratios. To achieve this, a review of passive mixing techniques was conducted.

A variety of passive mixing devices were evaluated to determine the best fit for this application. The limiting case in the device’s performance is the injection of encapsulated cells in hydrogel since the gel-like properties are less well understood and behave less ideally compared to cells in a standard liquid solvent. More analysis therefore needs to be done to determine whether widely accepted passive mixing channel designs will still work when applied to shear-thinning hydrogel and bioink-type materials which have high viscosities. The shear force must also be checked to ensure the increased viscosity does not result in cell death.
To select the best candidates for the passive mixing analysis, previous analyses for mixing of nanoparticles in incompressible liquids were summarized [36]. A few of the most promising options are summarized in Table 4. The mixing options considered included intersecting channels, stream splitting, micro ridges (staggered herringbone mixer), hydrophobic surface patterning, embedded barriers or baffles in a variety of geometries, serpentine or zigzag channels, and 3D twisting channel [37-40]. From this previous literature, the passive mixing designs with the highest rate of mixing over the shortest distance were chosen. Several were picked in case results differ due to the varying set-ups of the mixing experiments and the novel nature of viscous hydrogels, which may affect the mixing response in this device specifically. The most promising candidates were zigzag channels, staggered herringbone grooves, and modified Tesla structures. Diagrams are included in Table 3 to further illustrate the differences and document the mixing performances among these designs.

An ideal passive mixer for this application would create a well-mixed solution without imparting excessive shear stress or additional heat to the mixture. Additionally, the performance of many passive mixers is dependent on the flow rate of the liquid; for high throughput capabilities, a mixer design that works well at higher flow rates is desirable. The modified Tesla structure presented by Hong et al. works well (~85% - 95% mixing efficiency) at a variety of flow rates from 1 – 100 µL/min, so was a leading candidate for this device [38]. Baffles, or spaced-out barriers within the body of a straight channel, are also a common passive mixing technique. Rhombic baffles were shown to be the most effective shape compared to circular or rectangular baffles, but the mixing performance was still only up to 83%, which was significantly lower than the other options shown in Table 3 [39]. Therefore, baffles were not considered for use in this device. A combination of options may be combined to achieve good
performance across a wide range of flow rates, for example, SHMs may provide better mixing than modified Tesla structures at low flow rates, so a section of staggered herringbone grooves followed by several modified Tesla structures to combine cells with media regardless of flow rate could be used. One limiting factor in the stacking or extension of these mixers is the fluidic resistance, which causes the pressure drop to increase over the length of the passive mixing structure. If the necessary pressure to drive flow through the channels becomes too high by increasing the length or number of features in the mixing channel, the wall shear stress in the channel could increase to undesired levels. Once a design is chosen, more calculations must be done to ensure that cells will remain viable after mixing.

Table 4. Passive Mixing Methods

<table>
<thead>
<tr>
<th>Mixing Method</th>
<th>Diagram</th>
<th>Mixing Performance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zigzag</td>
<td><img src="image" alt="Zigzag Diagram" /></td>
<td>95%</td>
<td>[36, 41]</td>
</tr>
<tr>
<td>Staggered Herringbone Mixer (SHM)</td>
<td><img src="image" alt="Staggered Herringbone Diagram" /></td>
<td>98%</td>
<td>[37, 42]</td>
</tr>
<tr>
<td>Modified Tesla</td>
<td><img src="image" alt="Modified Tesla Diagram" /></td>
<td>95%</td>
<td>[38, 43]</td>
</tr>
</tbody>
</table>
2.3.2 Mixture Injection and Pressure Control

The injection procedure into OOCs can be very temperamental and the margin for error is low. To better define the constraints of the injection problem, the OOC design to be used was limited to the chip currently in-use in the MBMN lab. This chip is designed to replicate the BBB and is a good example to use since it is complex compared to other common OOC designs, while still using common principles such as multiple adjacent channels, bending channel structures, and permeable membranes. The design described by Ahn et al. will be used for analysis, save for some minor tweaks which were done after the study was published [44]. The chip uses PDMS as its material in addition to its common layout structure. A solution which works to inject material into this OOC would most likely be applicable to other designs as well.

To understand more about the problems experienced when manually injecting hydrogel and cell media into the device, PhD and post-doctoral lab members were interviewed, and I was trained on the procedure for hydrogel injection. The current procedure for filling the chip consists of loading chilled Matrigel into a fixed-volume syringe, then inserting the syringe tip into the channel inlet and carefully manually depressing the syringe until the channel is filled completely (which is checked via visual inspection). The lab members who regularly work with the OOC stated that common problems include leakage into adjacent channels, which is caused by over-pressurizing the flow of hydrogel. Additionally, since each member of the lab has specific quirks when using the syringe, the fill quality can vary depending on who fills the chips that day. If the fill is not done properly on the first try, the OOC must be cleaned extremely well or can no longer be used for experiments. The Matrigel sets quickly at room-temperature and cleaning the OOC can be difficult once the gel solidifies.
Based on these common problems and complaints, as well as the delicate nature of filling the OOC with the proper volume and pressure, it became clear that this process must be made resilient to variations in fill parameters and OOC designs. To make the pressure injection robust to a variety of gels and geometries, the goal was set to create a robust control for the filling pressure applied into the chip. This pressure control will depend on several variables, including the viscosity of the hydrogel, the geometry and fluidic resistance of the microchannels on the OOC, and potentially feedback pressure from within the channel. The necessary precision for proper control will be compared with devices on the market (such as flow control devices, syringe pumps, or other types of pressure application) and a suitable solution can be implemented. Sensors or user inputs for certain variables, such as defining the geometry of the chip to calculate fluid resistance or providing pressure feedback from the outlet of the chip, may also be needed for the control scheme to be extended to a variety of chip designs. First, a theoretical model for pressure-driven flow through a microchannel will be defined. Then, a CFD simulation of the injection process for the given BBB OOC will be constructed to better understand and create a mathematical model for the influence of different parameters. Then, the simulation results combined with theoretical analysis will be used to support the construction of the pressure control design.
CHAPTER 3. METHODS

This section will delve into the techniques and analysis used to validate and tune the design parameters of the device. The two main steps which require further analysis and design are the passive mixing channel and the pressure control for mixture injection. The passive mixing channel must be parameterized and optimized for use with viscous hydrogels while minimizing length and ensuring cell viability. This was achieved by utilizing CFD to evaluate previously published designs for passive mixing under a variety of flow conditions, fluid viscosities, and constrained length. To design the pressure control for injection, a mathematical model for flow control through microchannels was developed. A CFD simulation for injection was conducted to model the properties of injection and pressure fluctuation. Then, this model was used to develop a pressure control loop and increase the robustness of the gel injection. Finally, the usefulness of the results for mixture injection in other OOC designs is discussed.

3.1 Mixing Channel

One of the investigations in this paper is the application of established microfluidic passive mixing techniques to the mixing of non-Newtonian, gel-like fluids such as hydrogel and similar substrates. The goal was to test previously established microfluidic passive mixing methods, shown in Table 4, over a range of flow rates and fluid viscosities to determine the best match for a wide variety of OOC applications. SolidWorks, a computer-aided design (CAD) software was used to create 3D models of the designs. Detailed CAD models of each potential design were created by referencing literature on passive mixer optimizations. In each design, the fluids are first combined using a T-junction with inlet lengths of 2 mm, a 1 mm buffer before the
mixing portion, and a 1 mm outlet after the mixing portion. For the zigzag mixer, angles which were too acute were shown to have inefficient mixing, so an angle of 45 degrees was chosen to ensure the mixer properly functioned [41]. The cross-sectional dimensions were also kept consistent throughout all 3 designs at 0.2 mm by 0.2 mm. A rectangular cross-section was chosen since this more accurately reflects manufacturable microfluidic designs [45]. Finally, a simple T-mixer was also tested as a baseline comparison for the simulation results. In this case, the baseline can help to calibrate the simulation results with other testing or experimental data, since differences in the simulation settings or mesh can lead to distortions in the results.

To measure the level of mixed-ness or mixing efficiency of each design, Fluent was used to calculate a surface integral of area-weighted uniformity of one of the two mixing phases at the outlet wall. According to the Ansys Fluent Theory Guide, this integral is calculated using Equation 1, where \( \phi \) is the field variable of interest, here defined as the fraction of volume of phase two. This equation is used in conjunction with Equation 2 which calculates the average value of the field variable \( \phi \). In both these equations, \( i \) is the facet index of a surface which has \( n \) facets. If the flow is completely mixed, i.e., uniform, then Equation 1 will yield a value of 1. If the two flow streams are equal but separate with no overlap, the integral will evaluate to 0.5, and if there is no presence of phase two, the integral will equal 0. Therefore, in these mixing simulations where each fluid phase has an equal flow rate, the ideal value would be 1 and the least ideal value would be 0.5.

\[
\gamma_a = 1 - \frac{\sum_{i=1}^{n} [(\phi_i - \bar{\phi}_a) A_i]}{2|\bar{\phi}_a| \sum_{i=1}^{n} A_i}
\]

\[
\bar{\phi}_a = \frac{\sum_{i=1}^{n} \phi_i A_i}{\sum_{i=1}^{n} A_i}
\]
To keep the comparison fair, a set length was chosen for the mixing portion of each device. An additional goal in the mixing design was to minimize mixing length. A shorter mixing length will help achieve the footprint size specification set for the device and help waste less material, since smaller volumes can be mixed. The target length was chosen from a series of simulations with a simple T-mixer, where the mixing length was set to half the length of mixing for the T mixer. The results of this series are shown in Figure 3 and summarized numerically in Table 5. It was determined through checking outlet uniformity that a simple T mixer with a 0.2 by 0.2 mm cross section required 30 mm of mixing length to achieve relatively good mixing (0.921 area-weighted uniformity of phase 2 at the outlet). Since the mixing designs should be much more effective at mixing laminar streams than the simple T mixer, the mixing length to be shared by all designs was set at 15 mm, half of the required length for mixing with the T mixer. As the quantitative results in Table 5 show, a T mixer would not be able to perform well at a length of 15 mm, as the uniformity is only slightly better than the 0.5 minimum value. The quantitative results in Table 5 paired with the qualitative results in Figure 3 also gave insight into the relative “goodness” of uniformity values to expect during mixing. It seems that even for two distinct streams, as is seen in all the outlet contours to varying degrees (besides the 30 mm outlet), the uniformity is still significantly higher than 0.5. Therefore, qualitative checks will be an important supplement to the quantitative uniformity values to measure the mixed-ness of the two streams in further mixing simulations.
Figure 3. Outlet phase contours for increasing lengths of a simple T mixer design

<table>
<thead>
<tr>
<th>Mixer Length (mm)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 2 Area-Weighted Uniformity</td>
<td>0.671</td>
<td>0.660</td>
<td>0.677</td>
<td>0.672</td>
<td>0.629</td>
<td>0.921</td>
</tr>
</tbody>
</table>

In addition to CAD files for the various mixing designs, fluid properties are also needed to run an accurate simulation. The final design should be effective for mixing and injecting into a comprehensive range of OOC designs, so liquids or hydrogels of varying viscosities must be considered. To consider the non-Newtonian features of many hydrogels (pre-gelation), an even wider range of viscosities were tested. Relevant properties of hydrogels were reviewed further to determine a realistic range of viscosities to test.
3.1.1 Investigation of Hydrogel Properties

To determine the fluid properties to be used in the CFD analysis of the mixing passage and injection simulation, a survey of commercially available hydrogels was conducted. The focus in this survey was on biocompatible hydrogels commonly used in cell encapsulation culture. The gelation stiffness, viscosity, and elastic and storage moduli can vary widely even within samples of the same type of hydrogel, depending on concentration, crosslinking factor, and other variables. Additionally, blends of various hydrogels to achieve desired properties are common within the tissue engineering field. To avoid confusion and simplify the design problem, some of the most common hydrogels with publicly documented properties were chosen to be used for analysis. Shear modulus refers to the gel’s reaction to shear stress, which is important for likening hydrogel scaffolds to their in vivo counterparts. The stiffness of the scaffold can affect cell culture quality and maturity, and it is important to choose a hydrogel which provides similar stiffness to the in vivo tissues [45]. Viscosity and formation time are most relevant for the OOC engineering studies presented in this paper, since modelling gel behavior during mixing and injecting relies heavily on those two properties. The extreme cases among these hydrogels (most and least viscous) were chosen to test the device design to reasonable limits. A short summary of these gels can be found in Table 6, with more details provided from specific vendors when available. Because of the variety of tunable characteristics, such as differences in the viscosity or stiffness from increased concentration of gel or addition of crosslinking polymers, some information could not be found. There is a lack of available information on rheological properties of hydrogels pre-gelation, i.e., still in their liquid-like state. When choosing a gel or extrapolating the results from this paper to the mixing and injecting of
different hydrogels, a study on rheological properties such as viscosity, shear modulus, and diffusivity is recommended.

**Table 6. Commercialized Hydrogels and Key Rheological Properties**

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Activation Method</th>
<th>Formation Time (min)</th>
<th>Shear Modulus $G'$ (Pa)</th>
<th>Viscosity (mPa*s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td>Corning Matrigel</td>
<td>Warm to 37° C</td>
<td>30-60</td>
<td>10-50</td>
<td>10-15</td>
<td>[46, 47]</td>
</tr>
<tr>
<td></td>
<td>PureCol – Collagen (0.5%)</td>
<td>Warm to 37° C</td>
<td>&lt; 40</td>
<td>750</td>
<td>32</td>
<td>[32, 48, 49]</td>
</tr>
<tr>
<td></td>
<td>FibriCol – Collagen (1%)</td>
<td>Warm to 37° C</td>
<td>&lt; 40</td>
<td>1,600</td>
<td>1,425</td>
<td>[32, 49, 50]</td>
</tr>
<tr>
<td></td>
<td>Fibrin</td>
<td>Crosslinking</td>
<td>&lt; 1</td>
<td>-</td>
<td>-</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>Pronova UP 100M – Alginate</td>
<td>Crosslinking (ionic or covalent)</td>
<td>20 – 60</td>
<td>3,000</td>
<td>100-300</td>
<td>[32, 51, 52]</td>
</tr>
<tr>
<td>Hybrid</td>
<td>PhotoHA -1% Modified Hyaluronic Acid</td>
<td>Photo-crosslinking</td>
<td>&lt; 3</td>
<td>10,000</td>
<td>-</td>
<td>[32, 53]</td>
</tr>
<tr>
<td>Synthetic</td>
<td>Polyacrylamide (PA)</td>
<td>Crosslinking</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>Polyethylene glycol (PEG)</td>
<td>Crosslinking</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[32]</td>
</tr>
</tbody>
</table>

From the available data and perceived popularity of different options from OOC papers and studies, 1% collagen was chosen to be the high-viscosity option during simulation. The low-viscosity option was water, taken as a substitute for cell media. Cell media rheological properties vary based on the solutes included, but it is generally accepted to be like water, though slightly
more dense and more viscous [54]. The properties of these two fluids as used in simulations are fully described in Table 6. The Schmidt number was calculated using the fluid’s diffusivity constant at 37°C due to availability of data, though mixing would take place at temperatures closer to 10 or 20°C to prevent gelation of the collagen solution [55].

<table>
<thead>
<tr>
<th>Table 7. Mixing Fluids and Relevant Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>Collagen (1% solution)</td>
</tr>
</tbody>
</table>

3.1.2 Computational Fluid Dynamics

Once the mixing design CAD and fluid properties were finalized, Ansys Fluent was used to conduct simulation studies of mixing efficiency with varying mixer designs, fluid viscosity, and inlet velocities. Ansys Fluent uses a finite-volumes method to solve for mass and momentum conservation using the Navier-Stokes equations. Each element in the mesh, created by importing the CAD models into Ansys Fluent Meshing software, is treated as a control volume to solve local balances within the flow. There are several settings and models in Fluent that can be applied to predict the behavior of various types of flow, and one of the first major steps in setting up a Fluent simulation is deciding which type of analysis to perform, along with the corresponding assumptions. For the mixing simulation, the flow was defined as steady, laminar, and incompressible. A steady solution was chosen since the outflow of the mixing channel is the
desired result. In other words, a transient solution is mostly unnecessary since the behavior in the channel up to the outflow is not needed to determine mixing efficiency. If instead, development of the flow was of interest, a transient solution may be chosen. Secondly, liquids are generally taken to have constant densities and are therefore incompressible. Finally, the mixing in this device takes place in microfluidic channels with very small length scales. Specifically, the mixing designs were chosen for their perceived efficacy on mixing flows with low Reynold’s number (Re), as defined by Equation 3.

\[
Re = \frac{\rho v L}{\mu}
\]  

(3)

Because the length scale of flows for OOC applications are so small, even flows with relatively high velocities stay well within the range of Re < 10. This means the flows will always be well within the laminar range, which is defined by Re < 2300 [56]. Laminar flows tend to lead to low mixing efficiency due to lack of chaotic turbulent effects which help to integrate streams of fluid. Laminar mixing (without any mixing channel design) would normally take place via diffusion, which is a slow process which requires a mixing length on the order of thousands of times larger than the channel height [57]. The mixers presented in Table 4 are effective because they are designed such that they split and recombine the fluid streams repeatedly, resulting in chaotic advection [58]. This type of mixing refers to exponential stretching and folding of fluid streams, which helps laminar flows more effectively mix in shorter lengths.

Though a potential design for this type of mixing and injecting device could utilize larger length scales, therefore mixing uniform biomaterials in bulk and opening the doors to turbulent mixing and higher Reynold’s numbers, one of the aims of this device was to minimize waste of biomaterials as much as possible. Therefore, the length scale of the mixer in this device is kept small to mix limited doses at a time, thereby allowing for small batches of OOCs to be prepared
in-lab without necessitating the waste of additional cells or hydrogels. If this device design were to be extrapolated for use in a factory setting, such as in the preparation of OOCs by a start-up or large company, batch mixing could occur and therefore, the mixing design should be re-investigated for optimization under turbulent conditions. However, using the microfluidic dimensions in this device, along with the chaotic advection mixers, a laminar flow condition was deemed acceptable.

The mesh of each CAD model was made in Ansys Fluent Meshing. As a quality check, the orthogonal quality of the mesh was recorded. The orthogonal quality of the mesh indicates how close the angle between elements is to some ideal value, based on the topology of the surface. Generally, it is recommended that the minimum orthogonal quality of the mesh should be no less than 0.1. The number of cells in a mesh can also be helpful in determining the granularity of the simulation results; the more cells there are, the more accurately the fluid can be tracked through the region. The tradeoff is computation time, and especially in microfluidics, small cell size can interfere with the accuracy due to fluid flow “skipping” cells entirely during a timestep (as in the flow both enters and leaves the cell within the same timestep). The mesh inverse orthogonal quality and number of cells for the mesh of each mixer is listed in Table 8. The SHM and modified Tesla structure designs had the most cells, as they were the most complex geometrically and had many more faces and edges than the zigzag or T mixers.
Table 8. Mesh Cell Quantity and Quality for Mixer Designs

<table>
<thead>
<tr>
<th>Mixer Design</th>
<th>Number of Cells</th>
<th>Minimum Orthogonal Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>T mixer</td>
<td>11,197</td>
<td>0.23</td>
</tr>
<tr>
<td>Zigzag</td>
<td>5,096</td>
<td>0.12</td>
</tr>
<tr>
<td>SHM</td>
<td>54,000</td>
<td>0.073 (1 cell)</td>
</tr>
<tr>
<td>Modified Tesla structure</td>
<td>104,668</td>
<td>0.15</td>
</tr>
</tbody>
</table>

The Volume of Fluid (VOF) model in Ansys Fluent was used to simulate the mixing of two phases, which were defined to be either low viscosity fluid or high viscosity fluid (water or collagen, respectively). A three-phase VOF system was used, and air was included to set the mixing device to be full of air at the start of the simulation. Thus, the full VOF model included one primary phase and two secondary phases: phase one (primary) was air, and the two secondary phases were set to be the two fluids being tested. In each simulation, one of the two inlets was set to have a volume fraction of 100% phase two and 0% phase three, and the other was set to the opposite phase. The mixer was initialized with 0% phase two and three, thus being 100% phase one (air). Surface tension and wall adhesion forces were also included in the model, since these phenomena can have a large impact on microfluidic flow.

The flow conditions were chosen to cover a wide range of mixing velocities and viscosities for application to any number of OOC devices. Three flow rates (0.1, 1, and 10 mL/min) and three viscosity combinations (two low viscosity streams, one low and one high viscosity stream, and two high viscosity streams) were tested through simulation. Each combination of viscosity phases and velocity inlet settings is fully described in Table 7. Since
geometric design parameters were chosen based on previous studies, the flow rates were also chosen around the range which the mixers were designed for, with corresponding inlet velocities of 0.0417, 0.417, and 4.17 m/s based on a 0.2 mm by 0.2 mm inlet cross section, respectively. Flow rates below this range are also common, but low flow rates necessitate shorter mixing paths, so it is assumed that if the higher flow rates are well-mixed, so will the low flow rates. By covering a variation in flow rate of two orders of magnitude, plenty of room for custom flow parameterization is given. Though an asymmetric condition for fluid viscosities was tested, the inlet velocities were kept symmetrical to simplify the analysis of mixing uniformity.

Table 9. Inlet Velocity and Fluid Viscosity Conditions

<table>
<thead>
<tr>
<th>Fluid Combination</th>
<th>Inlet Velocities (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water + Water</td>
<td>0.04167</td>
</tr>
<tr>
<td></td>
<td>0.4167</td>
</tr>
<tr>
<td></td>
<td>4.167</td>
</tr>
<tr>
<td>Water + 1% Collagen</td>
<td>0.04167</td>
</tr>
<tr>
<td></td>
<td>0.4167</td>
</tr>
<tr>
<td></td>
<td>4.167</td>
</tr>
<tr>
<td>1% Collagen + 1% Collagen</td>
<td>0.04167</td>
</tr>
<tr>
<td></td>
<td>0.4167</td>
</tr>
<tr>
<td></td>
<td>4.167</td>
</tr>
</tbody>
</table>

Fluent’s steady-state solver for VOF simulations is pseudo-transient, which means that the steady-state solution develops over multiple iterations. To check convergence of each simulation, the scaled residuals were monitored as the solution iterated. The scaled residual of continuity is automatically tracked by Fluent and the condition for manual convergence in this case was defined as below 1e-4. The area-weighted phase uniformity at the outlet was also monitored over each iteration to ensure that the value was approaching some limit. The result was checked by first verifying visually that the phase mixture reached the outflow, then checking convergence of variables of interest, in this case the phase uniformity at the outlet. If the change
in the value of the phase uniformity surface integral over the outlet was 0.01 or less over the last 500 iterations of the solution, the variable of interest was shown to have effectively converged to a final value, and the solution was complete.

3.2 Injection Control

OOC designs often consist of several channels for fluid flow and cell culture laid out parallel to each other. These channels could be separated by permeable membranes or left open to each other with a small gap. The open design is difficult to fill accurately without spilling into side channels, since hydrogel is only held back by surface tension and air-liquid interface energy. To alleviate this problem, other methods of constraining hydrogel are sometimes used, such as phase guides and surface patterning with hydrophilic or hydrophobic materials. Modifications can also be made to the structure of the chip, such as adding tails or narrowing gaps to the solid interface between channels [59]. In the BBB chip used in the MBMN lab, shown in Figure 4, a simple geometric phase guide is used to constrict the hydrogel to the middle channel. The calculations for theoretical injection pressure are therefore relevant to many types of phase guides, as two adjacent rectangular channels is one of the simplest cases. In addition, the channels in the BBB chip are large for a microfluidic device, with a height of 0.1 mm. Micro-posts which were present in previous studies were also removed from the chip to simplify manufacturing. This increases the challenge of filling the channels without leakage, as there is a larger gap which must be constrained by surface tension forces. Overall, the chip has a simple phase guide design which makes theoretical calculation somewhat straightforward, but the phase guide is expected to be sensitive to pressure fluctuations. Therefore, if the pressure control
design can effectively fill the BBB chip without leakage, it would likely work for a wide range of chips with more stringent phase guides. To investigate this chip, a theoretical model of the phase guide structure was first put forth, then a simplified version of the chip channel geometry shown in Figure 4 was modelled in CAD for analysis in Ansys Fluent.

Figure 4. CAD model of the chip used for studies in the MBMN lab. A.) An overall view of the chip design, which consists of 4 identical OOC compartments. B.) A close-up section view of the ports of one OOC compartment, with inlets labelled. Either port can be used as the injection inlet or outlet; the chip is symmetrical, and no distinction is made by lab users when injecting Matrigel. C.) A view of the internal OOC microchannels (section taken at 0.03 mm above the bottom of the chip). Matrigel is injected into the middle channel via the inlets shown in B. D.) A cross section of the OOC microchannels showing the gap between the side and center channels.

3.2.1 Theoretical Pressure-Driven Flow

To determine the maximum pressure that can be used to inject material into the central channel of the chip, microfluidic flow behavior must be more thoroughly investigated. The
phenomena which are particularly relevant to the BBB chip design are capillary action and phase guides, both of which work through the principles of surface tension and surface wetting. Surface tension-related forces have a much higher impact on the behavior of microfluidic flow compared with their macro-fluidic counterparts, since a large portion of the flow is in direct contact with the channel walls. The principle at work keeping the flow constrained to the middle channel is known as a phase guide. Phase guides are variations in the geometric or surface-chemical properties of the channel. These variations affect the wall’s surface-wetting properties, thereby restricting flow through parts of the channel. The narrowed gap between the middle channel and side channels of the BBB chip acts as a geometric phase guide which “pins” the fluid in the gap before it can burst into the side channel. When the hydrogel reaches the edge of the narrowed phase guide, there is a certain amount of pressure it must build up to before it can overcome surface tension effects. This pressure is known as the burst pressure, and it varies with several factors, including contact angles and phase guide dimensions. A more detailed look at this interface is shown in Figure 5.
Work has previously been done in the theory of phase guides and capillary valves [60, 61]. By applying prior knowledge to this specific chip, an understanding of the theoretical injection pressure limit can be achieved. In flow through a rectangular channel under equilibrium, the pressure difference between inside and outside the liquid is given by the Young-Laplace equation, shown as Equation 4.

\[
\Delta P_e = P_i - P_o = -2\sigma \left( \frac{\cos \theta_s}{w} + \frac{\cos \theta_v}{h} \right)
\]  

(4)

Where \( \sigma \) is the surface tension, \( \theta_s \) is the angle between the liquid interface and the side wall, and \( \theta_v \) is the angle between the liquid interface and the top or bottom wall. The interface of interest in this chip design is at the expansion boundary into the side channels. This is where the advancing liquid will be pinned. The problem differs slightly from established literature in that the direction of the advancing liquid is not the direction of the phase guide; rather, the phase
guide is oriented perpendicular to the direction of flow. This means for the cross-section shown in Figure 5, the width (w) from Equation 4 is the dimension into the screen, which is the longest dimension of the chip channel. The height (h) is the height of the narrow gap between the main and side channels.

Equation 4 essentially states that there exist minimum angles $\theta_s$ and $\theta_v$ that the fluid must maintain with the side and top walls to continue advancing through the channel. These two angles must be large enough such that $P_1$ is large enough for the equilibrium pressure difference to be overcome and the liquid to move forward. This angle can be summarized as $\theta_A$, where the condition for an advancing flow is then written as $\theta_s \geq \theta_A$ and $\theta_v \geq \theta_A$. When the liquid reaches the new interface with angle $\beta$ to the old interface (in this case, $90^\circ$) the liquid stops advancing because the angle with the new interface now becomes $\theta_n = \theta_v - \beta$, which is always less than the critical $\theta_A$ needed for the liquid to advance. When the liquid reaches this interface, it bulges until the contact angle with the new surface is sufficient to begin advancing again. The pressure equilibrium equation for this bulging interface is given by Equation 5.

$$P_i - P_o = -2\sigma \left( \frac{\cos \theta_A}{w} + \frac{\cos \theta_1}{h} \right)$$ (5)

In Equation 5, $\theta_1$ represents an ever-changing angle between the continuously growing interface and the new wall. When $\theta_1 = \min(\theta_A + \beta, 180^\circ)$, the interface is under the maximum pressure that can be withstood before bursting into the side channel. This is the condition of interest in this case, where the injection flow rate into the chip is being maximized for device efficiency. Therefore, to find the theoretical maximum pressure for injection, Equation 5 must be solved for the specific chip design and materials of interest.

The fluid of interest is Matrigel solution, and the material for the chip is taken to be PDMS. Although the real injection material is Matrigel, and most likely in other OOCs would be
some type of hydrogel such as collagen or alginate (as previously discussed), water was chosen for theoretical calculations. This is due to availability and documentation of surface tension forces of water. For more detailed calculations with specific fluid properties or on different polymer substrates, contact angle tests can be performed to determine surface tension forces and angles. Substituting $\theta_s \approx \theta_v = 140^\circ$ between PDMS and water, $\sigma = 0.072$ N/m for water, $\theta_1 = 180^\circ$, $w = 4.2$ mm, and $h = 0.1$ mm into Equation 5 yields a maximum pressure differential of 1,137.39 N/m² [62]. If the pressure within the main channel passes this threshold, the fluid will burst into the side channels. If a factor of safety of 1.2 is included to ensure no leakage into side channels, then the target pressure for injection should be around 945 N/m². To double check whether injection would be possible with this pressure, Equation 4 was used to see the pressure differential needed to advance the fluid through the main channel ($w = 0.3$ mm, $h = 0.3$ mm). The pressure differential using the same contact angle and surface tension properties of water is 735.4 N/m². This leaves a very small margin of error where the liquid can still advance through the main channel but does not burst into the side channels. However, the main channel increases in height at the middle section where the side channel phase guides are located. In this part of the channel ($h = 1$ mm), the advancing pressure differential is 478 N/m². If a sensitive control is developed which can detect liquid advancement and slowly increase or decrease based on sensed pressure, it could help to prevent bursting in this critical area.

These theoretical calculations provide a critical foundation off which to base the injection pressure setpoint and control loop tuning. However, the phase guide properties change depending on the OOC design, and many fluids, like Matrigel, suffer from a lack of documentation related to surface tension forces and contact angles. The burst pressure of any given chip can be tested systematically by shifting a reservoir of fluid higher and higher to produce incrementally
increasing static pressure within the chip, then taking note of the burst condition. Alternatively, the contact angle and surface tension properties of the fluid of interest can be tested using existing techniques, and the OOC dimensions can be plugged into Equations 4 and 5 as presented above. Either way, finding the pressure setpoint involves some level of manual work, which is not ideal but may be necessary until documentation of hydrogels improves.

Once the target injection pressure has been determined, the problem turns to developing a control scheme and operating specifications for a syringe pump system that is precise enough to track the pressure within small ranges of 200 to 400 N/m².

3.2.2 Control Design

A syringe pump was selected as the tool to inject gel into the chip. Syringe pumps operate by using a motor to turn a lead screw which drives a syringe in at a constant rate, creating flow. These types of pumps can accurately and repeatedly dispense microliter volumes which makes them a common fixture in OOC labs and a natural starting point for this device’s design. A basic layout of the device parts was sketched out in Figure 6 to facilitate understanding of the interactions between each component. To apply the proper amount of pressure into the OOC, a desired pressure set limit can be determined from either experimental results or the mathematical model discussed previously. Then, a microcontroller will handle the logical processing to give the motor a command and generate fluid flow into the OOC via the syringe pump. A pressure sensor at the outlet is included to close a feedback loop, which allows the controller to consider any disturbances or miscalibration within the system and correct the pressure set limit. A basic control loop that can be used to execute this logic was drawn out in Figure 7 to realistically apply the calculated pressure to the chip. There are two main components which must be designed in the control loop: the transfer function and the feedback gain settings.
The transfer function must be used in the controller to translate the desired pressure into commands that the motor can understand. To achieve this, the syringe pump volume, lead screw design, motor revolutions per minute (RPM), and stepped voltages must be understood and the relationships between those characteristics must be calculated. Second, the gains for the pressure feedback must be tuned to adjust the pressure set limit quickly and accurately. There are several relevant metrics that can be used to measure a control loop’s performance, including rise time, settling time, overshoot, and steady-state error. The gains must be tuned to maximize performance, or at least achieve a level that will not impede the device’s performance or cause pressure bursting.

![Diagram](image)

Figure 6. Simplified setup to be used in the device to inject hydrogel into the device. The pressure sensor is placed at the outlet of the OOC, where changes in the internal fluid pressure can be monitored. A micro-controller and motor driver execute the control of the syringe pump.
To design the control loop and properly model its performance, the specifications and behavior of the syringe pump must first be known. To achieve this and minimize footprint and cost of the device, an open-source syringe pump design was used. The parametric open-source design allows parts of the pump to be customized for this particular use case, and the accuracy for a pump constructed with a 10 mL syringe was shown to be +/- 1% [35]. The open-source library is widely cited and provides a good basis off which to work. The original design uses a NEMA11 motor and an M5 threaded rod. An M5 thread has a pitch of 0.8 mm, meaning the threaded rod will move 0.8 mm inwards for every revolution of the motor. The amount of fluid pressure this movement generates is dependent on the syringe size used. A smaller syringe size provides more precision over fluid flow but has a smaller fluid flow limit than a large syringe. Therefore, the syringe size should be carefully chosen to not be grossly oversized, but still comfortable able to achieve the target 950 Pa of fluid pressure. Since this pressure value is relatively small, it should not be difficult to achieve; the main concern is in the precision.
To calibrate the generated pressure within the syringe, the linear force of the pump must first be calculated. Equation 6 can be used to calculate the linear force of the pump. The force depends on the generated force of the motor as well as the frictional force working against the motor, represented here as efficiency.

\[ F_{\text{linear}} = \frac{2\pi \tau_{\text{motor}} \eta}{\text{pitch}} \]  

(6)

To solve this equation, the torque curve for the NEMA 11 stepper motor from the library’s bill of materials as well as the 0.8 mm pitch value of the threaded rod were referenced. The motor torque at RPMs below 200 was 6.5 N·cm, and the efficiency of the motor is assumed to be around 0.7. This yields a linear force of 35.7 N. To get the pressure from this value, the force can simply be divided by the internal area of the syringe. For a 10 mL syringe with an inner diameter of 14.5 mm, the pressure is 2,210 Pa [63]. This is over 20 times more than the target pressure of 950 Pa. To adjust this as much as possible, the smallest stepper motor available from the same supplier was selected. This motor had a torque of 0.8 N·cm on average at lower speeds, which yields a linear force of 4.4 N. The corresponding pressure for a 10 mL syringe is 2,700 Pa, which is much closer to the target pressure. Considering that high speeds will also decrease the torque due to increased back-electromagnetic forces, this motor was selected for powering the syringe pump. However, this exercise in selecting a motor and syringe revealed that as the target pressure is extremely small, a syringe pump may not be the best option for the operation of this control loop. The syringe pump was still chosen for analysis since it is a common tool in labs and low cost, but in future iterations of the device, a more precise pressure control such as pneumatic valve control would be desirable.

With the parameters of the pump defined, the control loop was constructed in MathWorks Simulink to tune the design. Though a rough model of the pump, summarized by Equation 6,
Initially used to size the motor and syringe, a more detailed transfer function was derived to model its behavior. The stepper motor was approximated as a direct current (DC) motor for the purpose of deriving this function. A DC motor can be modelled electrically and mechanically as represented by the diagram shown in Figure 8. From this diagram, equations can be written to derive the motor TF between input voltage \( V_{in} \) and output torque \( \tau_m \).

![Diagram of DC motor](image)

Figure 8. A diagram of the relationships between the electrical and mechanical components of a DC motor.

Initially, Kirchhoff’s loop rule can be applied to establish a relationship among the electrical components, as shown in Equation 7. An additional equation can be written to describe the rotational dynamics of the rotor, which is shown as Equation 8. Lastly, the two equations can be linked together using the relationships in Equation 9 and 10, which use the motor constants to relate the back-electromagnetic force (EMF) voltage to rotational velocity, and the motor torque to the circuit’s current.

\[
V_{in} - R_m I_m - L_m \frac{dI_m}{dt} - V_{em.f} = \Sigma V = 0 \quad (7)
\]

\[
J \frac{d^2 \theta_m}{dt^2} = \Sigma \tau = \tau_m - D \frac{\theta_m}{dt} \quad (8)
\]

\[
\tau_m = K_t I_m \quad (9)
\]
\[ V_{em.f} = K_v \frac{d\theta_m}{dt} \] (10)

After taking the Laplace of these equations, rearranging, and substituting where appropriate, a TF between \( \theta_m \) and \( V_{in} \) can be written. The TF, assuming static initial conditions, is shown in Equation 11.

\[ \frac{\theta_m(s)}{V_{in}(s)} = \frac{K_t}{(Js^2 + Ds)(R_m + L_m s) + K_v K_t s} \] (11)

To get the desired TF between input voltage and torque, the relationship between torque and \( \theta_m \) from Equation 8 can be used. By multiplying both sides of Equation 11 by \((Js^2 + Ds)\), the new TF shown in Equation 12 comes into view.

\[ \frac{T_m(s)}{V_{in}(s)} = \frac{K_t Js + K_t D}{(L_m s + R_m)(Js + D) + K_v K_t s} = \frac{K_t Js + K_t D}{L_m Js^2 + (R_m Js + L_m D + K_v K_t) s + R_m D} \] (12)

Equation 12 was used in Matlab in the model for the syringe pump. All variables are known from the motor specification sheet: \( R_m = 24 \, \Omega, \, L_m = 8 \, \text{mH}, \, K_v = K_t = 8 \) [64]. Before tuning, to check the theoretical setpoint and desired behavior of the pump, a study on the chip was conducted in Ansys Fluent.

3.2.3 Computational Fluid Dynamics

To determine whether a linear control design would suffice, a CFD model for the injection process into the chip was developed. The gels used in cell culturing are non-Newtonian in nature, but at mostly constant flow rates (beyond the initial flow acceleration when injection begins), it was predicted they would behave linearly based on the shear rate they experience within the channels. To cover the full extent of possible gel behaviors, models were made using a wider range of viscosities beyond biomimetic materials. The starting point for this analysis was chosen to be Matrigel, as that is the actual material the MBMN lab uses in their BBB chip.
The inner portion of the BBB chip was measured and modeled in CAD, again using SolidWorks, resulting in the structure shown in Figure 8. The inlet was made with an artificially smaller diameter and deeper insertion point to mimic the pipette tip used to inject the hydrogel. The main outlet (highlighted in red) was set to the bottom face of the outlet well. This was done to prevent the material from building up in the outlet and bursting into the side channels regardless of inlet pressure. Currently, this is prevented by visually monitoring the hydrogel as it flows through the middle channel and stopping the pipetting action once the outlet well is reached. In a control design, this could be done automatically by sensing outlet pressure. The red outlet was set to 0 Pa of gauge pressure, since the outlet is open to the atmosphere, and the orange vents were set to 200 Pa of gauge pressure to help the solution converge. This vent gauge pressure should be lower than the surface tension forces calculated previously in section 3.2.1, so it should have little effect on the behavior of the simulation compared to real-life experimental results. Trials were done without this vent pressure added, but surface tension forces and wall adhesion modelling in Fluent is largely a black-box process and it was difficult to tune the parameters to prevent bursting into side channels, even at very low inlet pressures on the order of 100-200 Pa.
Figure 9. Inner structure of the BBB chip with inlets and outlets marked.

The mesh of the CAD model was constructed using Ansys Fluent and checked for orthogonal quality, like the meshes created for the mixer designs. The initial surface mesh had some issues around the fillet from the side channel phase guides to the main channel due to stretched aspect ratios at the sharp yet curved interface. However, the polyhedral volume mesh created had good properties, and no issues with convergence were found. The number of cells in the mesh was 40,497 and the minimum orthogonal quality was 0.11.

The simulation runs were done using a steady, pressure based, VOF solver like the settings used for the mixing simulations performed previously. The chip was initialized with air, and water was injected into the inlet with a given pressure. Results were qualitatively monitored in the form of phase pathlines, and iterations were done until the pseudo-transient solution had at
least one water pathline reach the red outlet. If side channel bursting occurred before this happened, the inlet pressure was marked as too high. These simulations were done to ensure that behavior within the chip was like the expected behavior based on phase guide theory. To meet this goal, four inlet pressures were chosen: 600 Pa, 850 Pa, 1000 Pa, and 1500 Pa. The results are summarized in Table 9 and further described by Figures 9 through 12. If the fluid successfully reached the outlet without bursting into the side channels, the trial was marked as a pass. Otherwise, it was marked as a failure.

Table 10. Simulation Results for Chip Injection

<table>
<thead>
<tr>
<th>Injection Pressure (Pa)</th>
<th>Expected Result</th>
<th>Simulation Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>No advancement through channel; pressure too low</td>
<td>Failure</td>
</tr>
<tr>
<td>850</td>
<td>Successful injection</td>
<td>Pass</td>
</tr>
<tr>
<td>1,000</td>
<td>Successful injection (borderline case)</td>
<td>Pass</td>
</tr>
<tr>
<td>1,500</td>
<td>Burst into side channels; pressure too high</td>
<td>Failure</td>
</tr>
</tbody>
</table>
Figure 10. Pathline diagram for injection at 500 Pa.

Figure 11. Pathline diagram for injection at 850 Pa.
Figure 12. Pathline diagram for injection at 1000 Pa.

Figure 13. Pathline diagram for injection at 1500 Pa.
As demonstrated by the simulation results, the behavior of three of the cases closely matches what was expected based on the previous theoretical calculations. Both the 850 Pa and 1000 Pa injection pressure allowed the water to reach the outlet without overflowing, but the 1500 Pa injection pressure caused bursting into the side channels. The only unexpected result was the pathline development under 500 Pa of injection pressure. Instead of being unable to advance through the channel due to lack of sufficient pressure, it did proceed through the first portion of the channel. This is most likely due to accumulating water at the inlet area which increases the pressure and pushes the water through the channel. However, eventually the water seems to build up more in the injection area, causing overflow into the side channels before the water can reach the outlet. Though the development was unexpected, the case still fails as predicted and shows a different type of error that could occur if the pressure is set too low. The 1000 Pa injection pressure also qualitatively performed slightly better than the 850 Pa injection pressure case. There were fewer straying pathlines, and of course under higher pressure, the flow rate was faster, so the outlet was reached in fewer timesteps. These results show that lowering the pressure setpoint to be safe and avoid bursting may lead to new problems if taken too far. Overall, these test cases corroborate the calculated injection behavior based on phase guide pressure equilibrium.
CHAPTER 4. RESULTS AND DISCUSSION

4.1 Mixing Channel

The mixing channel simulations were conducted on the nine viscosity and velocity combinations shown in Table 8 to determine which design, or combination of designs, would best apply to a wide range of custom mixing parameters. A baseline test using a T mixer was also performed to check the overall efficacy of the simulation at predicting real-life mixing results. The overall uniformity results for each combination are shown in Figure 13. As shown by the uniformity measures, every design outperformed the simple T mixer by a good amount. The most effective designs overall were the modified tesla mixer and the SHM. While the zigzag mixer performed well at low velocities, it didn’t mix the asymmetrical viscous streams very well and underperformed compared to the SHM at higher velocities.
Figure 14. Mixer uniformity results with varying flow rates and fluid combinations.

The results are somewhat unexpected in that they don’t show a clear trend downwards or upwards for mixing efficiency at different flow rates. It is difficult to tell what this is due to since the spread of data is very wide. For example, for the data water mixing using the SHM, the variation in data could be centered around some constant mixing uniformity value of about 0.85, with differences due to the manifestation of chaotic advection effects in each trial. Since convergence was equally strict for all simulations, more data points may be necessary to pick out individual trends. One pattern that can be seen from the data is that almost all the mixing designs
(bar 0.1 mL/min flow in the modified Tesla mixer) were inefficient at uniformly distributing asymmetrical flow (i.e., mixing a stream of water into collagen or vice versa). It’s possible that the differences in the viscosities caused one flow to be dominant and hindered interaction between the streams. The splitting and recombination of these streams could also be affected by the differences in diffusivity. Based on previous studies done on each mixer design, it was expected that performance would distinctly change based on flow rates. The SHM and zigzag mixer were designed for slower flow, whereas the modified tesla structure mixer performed better under faster flow. One potential cause is that the area-weighted uniformity measure used to test mixing varies from the measures used in the experiments from previous literature. References for the mixer designs either used computer vision analysis of color or pH testing to determine whether mixing was achieved. The area-weighted uniformity method has its merits, such as being easily calculable and an objective measure for analysis, but it is also an incomplete data point on its own. Two streams with 0.8 area-weighted uniformity of phase 2 could have very different distributions of phase 2 molecules. This problem with using area-weighted uniformity to measure mixed-ness was also discussed in section 3.1.2, where the nature of this calculation prevented mixed-ness from ever being 0. This decreases the granularity of the results and could lead to overall “flattening” of the output data, as seen in Figure 13.

Despite these problems, it was still shown through simulation that SHM is most likely the leading candidate for a passive mixer design in this device. The minimum area-weighted uniformity for that design was 0.803 for the asymmetrical mixing at 1 mL/min flow rate. Based on patterns observed in the phase contours for each run, an example of which is shown in Figure 14, the mixing quality may benefit from a slightly more extended outlet. The straightening of the channel allows the effects of chaotic advection to disperse through the fluid, and this mixing may
finalize into a more uniform stream. With a slightly longer outlet and additional movement from entering the syringe pump and being injected into the chip, the current 0.803 outlet uniformity will most likely improve more.

Figure 15. Contour of SHM phases with 1 mL/min flow rate

The original target for mixed-ness was set in the device specifications in Table 3 as greater than 0.9. None of the mixing designs investigated were able to reach this metric as measured through Fluent by area-weighted uniformity. Even if the SHM mixer improves through an extended outlet and passive diffusion in injection, the worst-case scenario may still not reach that level of mixing. An additional consideration, though, is that there is a lack of research surrounding this specification. This device design is entering a novel space in the OOC market, and there are no direct competitors yet. Therefore, it is difficult to tell what a proper estimate for necessary mixed-ness would be, and what lower bound would prevent the device from achieving uniform cell culture. Obviously, 100% mixing efficiency is desired, but that is an unrealistic
goal. To that end, additional experimentation is needed under the supervision of biologists and OOC experts to determine realistic expectations for a device of this type. Areas of focus should include mixing non-homogenous streams, viscous hydrogels, and varying flow rate to determine which mixing outcomes result in usable OOCs and which mixing levels hinder cell growth or hydrogel formation.

4.2 Injection Control

After calculating a theoretical target pressure and using that to create a control loop design and validate a CFD model for analysis, the model and control were combined to test the sensitivity of the pressure input. Some preliminary results for the CFD simulation with only a single static pressure input were already shown in section 3.2.3. Based on these results, the pressure setpoint was chosen to be slightly higher than previously defined; it was moved from 850 Pa, which was based on theoretical phase guide pressure, to 950 Pa, which is still within the theoretical pressure bound but may be more robust to gel accumulation and fill the chip more quickly.

To tune the feedback control loop, feedback data points are required. To achieve this, a “pressure sensor” was added to the inner chip CAD model. At first, it was thought that adding the sensor to the outlet would be sufficient. The edited pressure sensor CAD is shown in Figure 15. The pressure sensor consists of an extruded cut into the outlet well of the chip, which is in the bottom right. The outlet is now set to the ring which exists around the pressure sensor at the top of the outlet well, and the pressure sensor operates by recording the pressure on the bottom face of the extruded cut. This configuration is
consistent with the vision that the pressure sensor would be inserted into the chip outlet, but not in such an attached way that the outlet becomes pressurized and inhibits flow.

Figure 16. Edited CAD model with pressure sensor

An initial simulation was run to obtain an outlet pressure profile. The inlet pressure was set to 950 Pa, which was previously set as the goal for successful biomaterial injection based on section 3.2.3. The facet value average of pressure was measured on the bottom face of the extruded cut, as described previously. This value considers the measured pressure on each face of the cells which contact the selected plane, then averages that value across the number of cells counted. The simulation resulted in an outlet pressure profile shown in Figure 16. To get a closer look at specific artifacts within this graph, the simulation was run again to specific iterations (200, 600, and 1900) to see what event within
the chip caused the peaks and valleys. These changes in the pressure signal could potentially be used to monitor the progress of the water through the chip and give feedback to the syringe pump. The results are shown in Figure 17 and 18. At 200 iterations, the water reaches the bottom of the inlet well. At 600, it has progressed through the main channel and has nearly reached the outlet branch. After this point, the pressure measured by the outlet sensor is nearly 0 as the fluid progresses through the chip. Then, at 1900 iterations, the fluid has built up within the middle channel and reached the outlet well, and the outlet pressure dips. The pressure then builds up again as the fluid continues to be injected into the channel.

Figure 17. Outlet pressure measurement from Fluent simulation
Figure 18. Chip pathlines at 200 iterations

Figure 19. Chip pathlines at 600 iterations.
An interesting note on the developing chip pathlines in Figures 17 through 19 is the changing color of the pathlines. These colors represent the volume fraction of the fluid phase, with 1 (red) being completely water and 0 (blue) being completely air. The pathlines are released from the inlet surface which is 100% water, so it is logical that no pathlines are completely air. As the iterations develop, though, the pathlines transition from a green to a light yellow, then to a full red. Based on the results, fully red pathlines are a feature of importance. If the pathlines show less than 100% volume fraction of water, there could be air bubbles or voids left in the chip channel.

From the results seen in Figure 16, it may be difficult to realistically control the fluid flow based off the pressure value at the outlet. For one, the pressure values are extremely small, even when there are spikes. The readings are on the order of 5 to 10 Pa, so a sensor
for the outlet would have to be extremely precise and therefore expensive. The bigger problem is that during the filling of the main channel, i.e., the part of the injection process which can cause problems, there are little to no pressure feedback cues. The pressure at the outlet hovers around 0 Pa, which makes sense since the fluid is in the largest connected part of the chip and is likely pushing air out through the vents rather than through the designated outlet. Even if the fluid were to burst into the side channels, it is unlikely that the outlet pressure would change. Therefore, the purpose of the feedback loop is lost; the sensor provides no way to determine whether a failure has occurred or not.

To rectify this, a modified feedback loop is proposed. Previously, in calculating the pressure generated from a syringe pump, there were several unknowns (such as motor friction force) and concern over the precision of a syringe around such small pressure values. A feedback loop of value could be created by connecting tubing to the outlet of the syringe pump, then installing a pressure sensor between the syringe pump and the chip inlet. The pressure sensor could provide feedback to ensure the setpoint of the syringe is as accurate as possible to the theoretical setpoint which should prevent bursting. This solution is viable since the phase guide calculations made in section 3.2.1 match closely with the behavior of the fluid in simulation. This suggests that theoretical calculations may be enough to guide the fluid inside the chip, and the real concern is with executing the inlet pressure setpoint as accurately as possible. The modified control block diagram to represent this change is shown in Figure 21.
Figure 21. Modified control block diagram. Additional tubing and feedback using a pressure transducer was added to control syringe pump performance.

Based on the edited pressure setpoint, sensor feedback location, and properties of the chosen motor, a control loop and pump model were constructed in MathWorks Simulink. The TF derived in Equation 12 was used to model the torque of the syringe pump motor. The torque was then converted to a linear force using the pitch of the screw, which was divided by the inner syringe area to calculate pressure, as shown in Equation 6. The efficiency was chosen as a randomly generated number in the range of 0.6 to 0.8. An outlet line was also included in the model, which carries a pressure drop calculated in Equation 13 based on 10 mm of 0.5 mm diameter tubing. The tubing was assumed to be smooth, with a laminar flow velocity given by the linear progression and diameter of the syringe pump.

\[ \Delta P = \frac{\lambda \rho v^2 L}{2D} \]  

The model then takes the output pressure from the end of the tubing close to the chip inlet, where the sensor is planned to be located. There is a proportional-derivative (PD) feedback loop in place to tune the voltage input to the syringe pump based on the measured pressure at the chip inlet. The overarching control loop as described in Simulink is shown in
Figure 22. There are two subsystems in this control loop; one describes a simple controller which has a gain relating the input pressure to a voltage, and the other is a more complex model of the syringe pump which includes the motor TFs previously derived. A close-up of the motor model is shown in Figure 23.

Figure 22. Overarching control loop for injection pressure in Simulink
The tube and syringe subsystem uses laminar pressure loss equations to calculate the pressure drop over the delivery tube, and Equation 13 is used to calculate the exerted pressure from the motor torque via the syringe. These two values are then used to obtain the inlet pressure at the chip.

The control loop was tuned to have a quick settling time and low overshoot. The settling time and rise time are critical, since it was shown in the simulations that a low injection pressure could cause fluid build-up in the inlet well which affects the flow dynamics during the injection process. Overshoot is also a chief concern, since any pressure outside the bounds set could cause irreversible bursting into the side channels of the chip. The targets were chosen to be 5% maximum overshoot and 1 second settling time. Based on the block diagram constructed in Simulink, the system was tuned to meet these
requirements. The resulting gains are shown in Table 11. A transducer gain was also included, as the voltage input to the DC motor had to be tuned to a realistic value. The transducer parameter was tuned to make the feedback voltage on the order of 1.

<table>
<thead>
<tr>
<th>Gain Type</th>
<th>Proportional</th>
<th>Integral</th>
<th>Derivative</th>
<th>Transducer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuned Value</td>
<td>15</td>
<td>110</td>
<td>2</td>
<td>4e-7</td>
</tr>
</tbody>
</table>

Once the control loop response was tuned in Simulink, the control output was graphed and analyzed to determine the step response of the system. The response is shown in Figure 24 with a step input of 950 Pa. The performance of the control in response to this step input is summarized in Table 12.
Overall, the performance of the control loop met the targets set. The main concern when tuning the control gains was the percentage overshoot. The chip filling procedure is very sensitive to spikes in pressure, and bursting is irreversible. Therefore, it is of utmost importance to make sure that even with overshoot, the inlet pressure stays below 1,137 Pa which was calculated as the phase guide burst pressure. This control loop meets that target, but the system was observed overall to be very sensitive, and overshoot tended to be high. When the step response is initially fed into the loop, the motor current (and therefore the syringe pressure) spikes instantaneously. This is most likely due to a lack of capacitance in the motor model and the fact that motor acceleration is unlimited. A real system would have some capacitance, either in the physical system components or in the electric circuitry, and motor acceleration would be limited by real factors like friction and circuit response time. This would reduce the spike and further limit the overshoot of the system, which helps achieve the target behavior.

One concern in the control response is the steady state error of 7.56 Pa. Though an integral gain was implemented, this steady state error remained throughout tuning. The value of the error is extremely small, though, so it is unlikely that this would be a problem during in-lab testing. Another control response was recorded with a step input of double the original pressure (1900 Pa) to determine whether this error would become more significant

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Table 12. Performance of Tuned Control Loop

<table>
<thead>
<tr>
<th>Metric</th>
<th>Target</th>
<th>Actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Overshoot</td>
<td>5%</td>
<td>1.68%</td>
</tr>
<tr>
<td>Settling Time</td>
<td>1 s</td>
<td>0.67 s</td>
</tr>
<tr>
<td>Steady-state Error</td>
<td>0</td>
<td>7.56 Pa</td>
</tr>
</tbody>
</table>
through an increase in input magnitude, but the error only increased to 9.3 Pa. Based on this result, the steady state error remains at a relatively small value in comparison to the input pressure and the overshoot pressure. Therefore, it is acceptable for the operation of the control loop.
CHAPTER 5. CONCLUSION

This paper focused on critical first steps for addressing a major obstacle in the development and widespread adoption of OOC testing platforms. OOCs show great promise in their potential to revolutionize the pharmaceutical testing pipeline, thereby saving companies time and preventing increasing drug development costs from being passed on to the consumer. However, these platforms are largely non-standardized, suffer from high manufacturing costs and slow manufacturing times, and have a high barrier of entry for non-specialized lab workers. To overcome these barriers, OOC platforms must be designed with large-scale manufacturing in mind, and devices and techniques must be created or modified to accommodate these unique chips. This paper delves into the theoretical design of one such manufacturing device. The device presented in the introduction and background of this paper aims to solve the problem of uniformly and quickly filling OOC platforms with hydrogel and cell-laden biomaterials. The goal is to improve the quality of OOC studies and make the relevant technology accessible to all lab workers, regardless of previous experience. Several major areas of focus were laid out for this type of device, and two critical functions were chosen to receive detailed design and analysis: mixing of biomaterials and injection into the chip.

A passive mixer was selected to mix biomaterials due to its low cost, low maintenance, and ease of manufacturing. Several popular designs were modelled and analyzed via simulation on equal footing. The designs were tested over a wide range of flow rates and fluid viscosities. The SHM was chosen as the most consistent and effective mixer design. It produced a flow with an area-weighted uniformity of 0.8 or greater over the outlet for all flow variations, and its design is concise and easy to manufacture.
For injection control, testing was done on the BBB chip used by the MBMN lab. In the current chip filling procedure, there is a high probability for errors, and the process is very sensitive to human input. Filling the chip properly requires skill, time, and results still vary from person to person. To determine the proper injection pressure, phase guide theory was examined and applied to the geometric design of the chip. A CAD model of the channels within the BBB chip was created and calibrated to check the accuracy of the theoretical model. Finally, two options for pressure sensing feedback were explored, with sensors either at the outlet or before the inlet. Pressure readings at the outlet indicated that the sensor would need to be extremely precise and accurate in order to act as a control, so the inlet pressure sensor was used instead to more reliably achieve target input pressures. The control loop was tuned to quickly reach the target pressure while being resistant to unknowns or changes within the syringe pump model.

The injection of hydrogels into chips is a necessary part of the OOC manufacturing process, but prior to this paper, not much work has been done in this area. This paper considers the variables which go into the injection process and how to account for changes in viscosity and fluid properties in the control design.

### 5.1 Limitations

Though this work presents many ideas which could be of use in the design of a novel microfluidic manufacturing device, there are notable limitations in the work. Most prominently, there is a lack of experimental results obtained by testing the injection and mixing of real chips and hydrogels. The conclusions presented in this paper are based on theoretical calculations and simulation results. Experimental results were difficult to obtain for several reasons, most notably due to in-person limitations from the COVID-19 pandemic. To alleviate this problem,
suggestions for laboratory experiments are made where possible; general outlines of how to test certain unknowns are provided, and acknowledgements to the limits of simulation are made. For future development of this device and especially for application of this technology to new chip designs which are not explored in this paper, experiments and calculations should first be run to determine the operation settings.

Another notable limitation is the user requirement of theoretical calculations or experimentation to determine the burst pressure for unique chip designs. The target value was found by hand using phase guide theory and validated using a simulation model. If the device design is to be extended to a wide range of OOCs, as is the vision, then this is less than ideal. Depending on the OOC design and resulting target pressure, the pump hardware may even have to be changed and re-specified for the proper values. This puts a large burden on the end user, and a real device would either need very straightforward guidelines or contain an automatic pressure calibrator to handle new OOC designs. Also, if the pressure limit calculated for other chip designs is on the same order of magnitude as the limit calculated for the BBB chip in this paper, a pneumatic valve pressure control can be used instead of a syringe pump to achieve small pressure values more precisely and easily.

Lastly, the passive mixing channel is effective but must still be validated for use in mixing real cell-laden material for OOC culturing. If it is found that the uniformity achieved by the passive mixing channel is not sufficient to initiate proper cell culture within OOCs, then it may need to be supplemented or replaced with an active mixing component instead.
5.2 Future Work

Much remains to be done to achieve the vision which was set out in this paper. Two of the several major functions of an ideal OOC injection device are explored, but the design is far from complete. For the mixing channel and the injection control, protocols and real testing must be drawn up and executed to ensure viability. Future work to bring to fruition a completely autonomous design includes mechatronics, structural design, and component selection. Much like the open-source syringe pump design referenced in this paper, parametric and customizable components should be prioritized as OOC platforms are not standardized. Overarching specifications such as temperature control, sterile handling of biomaterials, and user interface also need to be considered for inclusion in the complete device. The design work presented in this paper could also be extended to large-scale manufacturing techniques, which could help OOC start-ups and companies attract customers. If these results are validated using in-lab techniques and biomaterials, then a large step forward could be taken in the manufacturing and design of OOCs. Usability, reliability, and uniformity are issues which are holding the field back from wider adoption, and any device that helps alleviate those issues will surely be valuable.
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