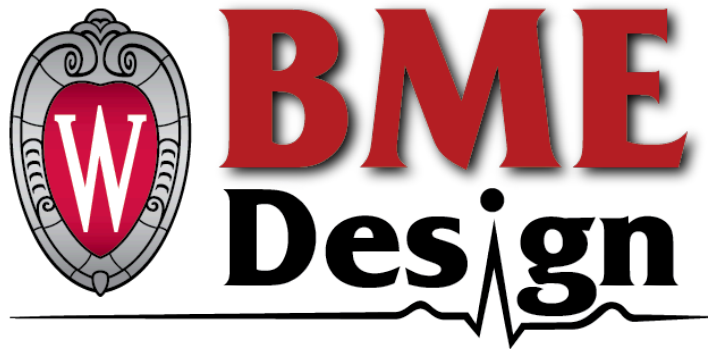




Immunodrop

Preliminary Report

Design Matrix

BME 200/300 Section 312

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Abstract

Prostate cancer and the effect of cancer entering the lymphatic system are among the most studied research topics in microfluidic devices. Cancer's ability to survive for extended periods once it has reached a lymph node makes a reliable, accurate way to model lymph nodes in vitro paramount for testing cutting-edge treatments. One of these treatments involves the injection of B and T cells from lymph nodes into the body, targeting specific regions of cancerous tissue in the patient. To do this, B and T cells are placed within Type I collagen droplets, fabricated using a series of integrated components that form a droplet generator. Many droplet generators exist in laboratories around the world, each tailored to the specific requirements of that laboratory. This project focuses on creating these droplets in bulk using a PDMS chip and a series of syringe pumps, coordinated to pump collagen, surfactants, and fluorinated oils through the system. This will produce collagen beads that straddle the micro- and macrofluidic regimes. The chip and system as a whole are specifically tailored to this implementation. The final system will be tested using the proposed collagen solution with the chosen surfactants and fluorinated oils to evaluate simultaneous flow and autonomous output.

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Introduction

Motivation

Prostate cancer has an 8% metastatic incidence at diagnosis. Metastatic prostate cancer cells survive the longest in the lymph nodes, averaging 31.6 months, compared to 21.3 months in the bones, 19.4 months in the lungs, and 13.5 months in the liver. Despite this primary pathway for metastasis, current 2D lymph node models cannot fully capture the complex structure and cellular interactions necessary to accurately study lymphatic spread. A microfluidic device is required to mass-produce collagen microspheres encapsulating B and T cells to build a realistic, 3D artificial lymph node model for studying this metastasis [1].

Existing Devices and Current Methods

The applications of microdroplets in cosmetics, pharmaceuticals, food, personal care, and household care have resulted in a wide range of fabrication techniques, most notably micropillar sorting designs, rotor-stator bulk emulsion, high-pressure homogenized bulk emulsion, microfluidic droplet generators, spinning conical frustum, and mechanical fragmentation.

Micropillars are being implemented in microfluidics with a variety of impacts. In traditional droplet generators, micropillars can be used as structural support to prevent the delicate surface of microchannels from collapsing under stress. They are also used for flow regulation and sorting as a method of filtration. Finally, they are used to create parallel channels that exponentially increase the droplet generation rate in advanced chip designs [2].

Another competitive alternative is the spinning conical frustum mechanism that has been developed as a centrifugal way of creating extremely unique and ordered droplets without the use of microfabrication [3].

Bulk emulsion is a competitive design that has been used in many fields requiring microdroplets. There are two main emulsion mechanisms used in industry today: Rotor-stator homogenizers and high-pressure homogenizers. These methods create droplets through vigorous shear forces that rely on high turbulence with high pressure/speed or cavities generated by ultrasound. The main implication of these designs is in creating mixtures of nonhomogeneous materials, microdroplet mixtures such as those seen in the explosive industry [4, 5].

Mechanical fragmentation of hydrogels is used in industry in the context of drug delivery, tissue engineering, and scaffolding design. This has some serious advantages that align with needs, including simplicity and speed. A large hydrogel is broken down into small hydrogels that act as polymerized droplets would if created through alternative mechanisms. These do not overcome

the disadvantages that the design faces, as it will make cell encapsulation very difficult and generate irregular gels rather than the spherical droplets that are being generated [6].

Microfluid droplet generators have key advantages that make the mechanism effective for the fabrication of the quantity, size, shape, and uniformity of collagen droplets that the client requires at a low energy threshold. Microdroplet-generating chips offer high repeatability and accuracy by processing directly from unpolymerized collagen solutions without requiring excessive turbulent forces or complex micro-mechanisms [4].

Problem Statement

Prostate cancer (PCa) commonly metastasizes through the lymphatic system. Understanding how cancer cells spread through lymph nodes is therefore critical to developing therapies that can prevent or limit metastasis. However, current lymph node models primarily represent tissue in two dimensions and cannot fully replicate its complex three-dimensional structure and cellular interactions. This project aims to fabricate a microfluidic droplet device capable of producing B- and T-cell-containing collagen microspheres, which will serve as building blocks for a realistic three-dimensional lymph node model to more accurately study cancer metastasis.

Background

Biology and Physiology

Collagen, specifically Collagen Type I, is one of the most abundant proteins in the body, accounting for nearly 90% of the structures within the extracellular matrix. This makes it an ideal host material for cell manipulation and transport [7]. When these hydrogels polymerize, they create structural substrates for cell seeding that facilitate cellular migration [8]. Within microfluidic environments, microscale physics such as laminar flow, capillary forces, and surface/interfacial tension dominate over macroscale physics. This allows for highly predictable fluid behavior [9]. In laboratory settings, droplet generators leverage this predictable behavior by employing syringes and syringe pumps to obtain a constant flow of collagen and fluorinated oil to create exact microdroplets [7, 10].

Collagen Type I undergoes a temperature-dependent sol-gel transition, making its physical state highly sensitive to its thermal environment. At cooler temperatures, the collagen remains in a fluid, unpolymerized state, which is critical for facilitating unhindered flow through microfluidic systems and preventing the viscosity increases that cause channel clogging [11]. Conversely, when exposed to normal physiological body temperatures, the collagen undergoes rapid

polymerization. This thermal transition solidifies the solution into a stable, three-dimensional extracellular matrix, which is essential for successfully encapsulating cells and mimicking the natural biological structures required for in vitro tissue modeling [7, 8].

Prototype Research

This prototype was adapted from a visual representation of the current design presented by Dr. Sanchez De Diego [1]. Channel dimensions were calculated to target the specified droplet volume of 0.5 μL . Resin mold post-processing and device fabrication were informed by published procedures for similar PDMS microfluidic devices [12], with processing conditions adjusted for the materials and equipment used in this project.

Client Information

The client is Dr. Cristina Sanchez de Diego, a Postdoctoral Researcher in the Microtechnology, Medicine, and Biology (MMB) Lab at the University of Wisconsin-Madison. Her primary research involves developing 3D models to study the metastasis of prostate cancer through the lymphatic system [1].

Design Specifications

A comprehensive list of all criteria can be found in the full Product Design Specifications (PDS) document located in Appendix A. The proposed microfluidic device must meet several critical functional and environmental requirements:

- **Performance and Throughput:** The device must operate autonomously without continuous user intervention to generate collagen droplets with a target volume of approximately 0.5 μL . The system requires a 5 to 10-minute run time, efficiently producing between 300 and 600 droplets per cycle.
- **Temperature Regulation:** The collagen solution must be maintained at approximately 4 $^{\circ}\text{C}$ prior to and during droplet generation to limit premature polymerization, which increases viscosity and clogs the microchannels [11]. Cell-containing droplets must then be transferred aseptically to a droplet collection incubator maintained dynamically at 37 $^{\circ}\text{C}$ and 5% CO_2 .
- **Operating Environment and Safety:** The entire device must fit inside a Class II Biological Safety Cabinet and operate under Biosafety Level 2 conditions to maintain sterility and protect the operator from fluidic leaks or pressurized tubing failures [13].
- **Materials:** All fluid-contacting materials, including the PDMS, glass, tubing, and syringes, must be biocompatible and pass ISO 10993-5 in vitro cytotoxicity testing [14].

- **Cost:** The target product cost for the reusable device setup, excluding equipment and materials already supplied by the laboratory, must remain under \$100.

Preliminary Designs

Microfluidic Chip Design

Design 1 (Thin No Outlet): This mold has one inlet and one outlet, with no dedicated ports for biopsy punching. Its 75 μm constriction was designed to produce droplets approximately 100–200 μm in diameter, although the final size will depend on channel height and flow rates. A retaining wall contains the PDMS during casting. The turns lengthen the collagen flow path and add resistance, helping control its flow into the droplet-forming junction.

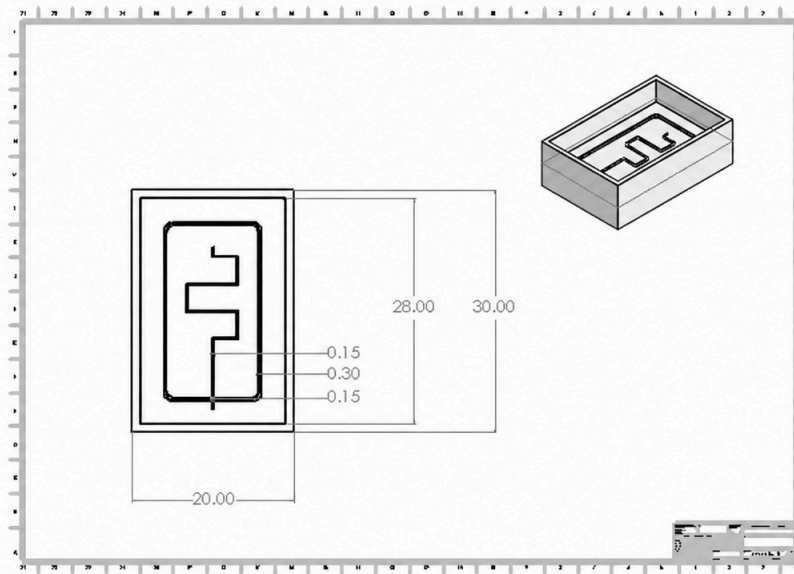


Figure 1: Thin No Outlet Design

Design 2 (Smooth Design): This design uses 750 μm collagen channels and 1,000 μm oil channels, with the goal of producing approximately 0.5 μL volume droplets. Compared with Design 1, its smoother turns provide a more gradual flow path, while dedicated inlet and outlet ports make the device easier to connect and fabricate. The shorter retaining wall also reduces the amount of PDMS needed for each mold.

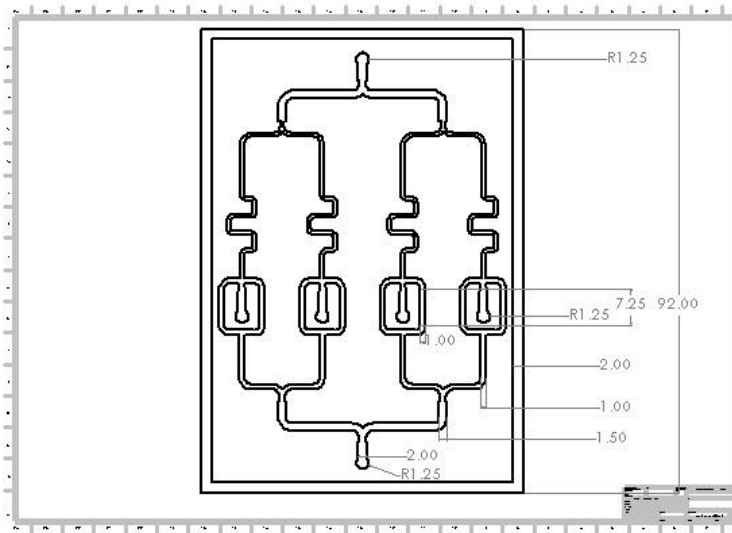


Figure 3: Four-Outlet Design

Cooling Mechanism Designs

Design 1 (Ice Cube Mold): This design aims to cool the collagen stream on the chip in three ways: vapor from the ice, direct contact with the ice, and meltwater flowing onto the chip. The mold has an 8° draft and will be printed in elastic resin to make the ice easier to remove. It will be filled to the brim so that the retaining wall lip forms a flat surface, allowing the ice block to sit flush with the glass. The cylindrical pillars create holes in the frozen ice for the tubing ports.

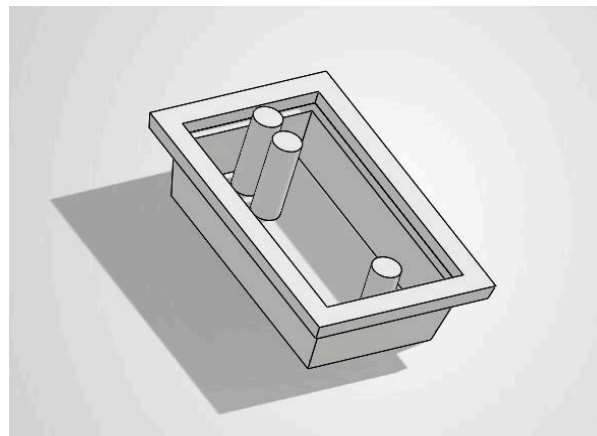


Figure 4: Ice Cube Mold

Design 2 (Water-Glycerol Channel Adjacent): This design builds on the smooth, single-outlet design shown in Figure 2 by adding a cooling channel alongside the collagen channel. A 50:50

water–glycerol solution at approximately $-23\text{ }^{\circ}\text{C}$ would flow through it, drawing heat from the collagen through the $50\text{ }\mu\text{m}$ wall separating the channels. A future version could place a cooling channel on each side of the collagen channel to increase cooling.

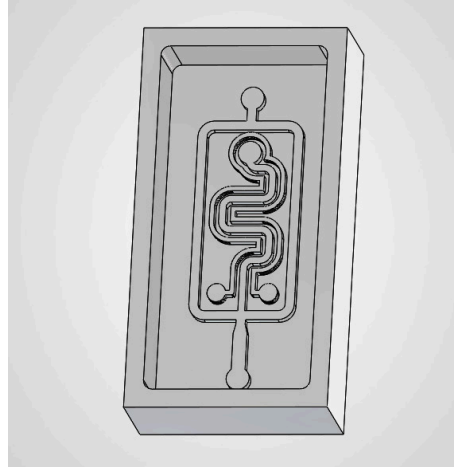


Figure 5: Water-Glycerol Channel Adjacent Design

Design 3 (Water-Glycerol Attached PDMS): This mold design mirrors the smooth, single-outlet design of the collagen and oil channeled PDMS molds; however, it is designed to flow a 50:50 water–glycerol solution at approximately $-23\text{ }^{\circ}\text{C}$ through it. The resulting PDMS design would be bonded to the same glass as the droplet channel PDMS and would flow directly across from it, drawing heat from the collagen through the glass separating the channels. This design could be improved by exchanging the glass platform that both materials are bonded to for a similar but more thermally conductive material.

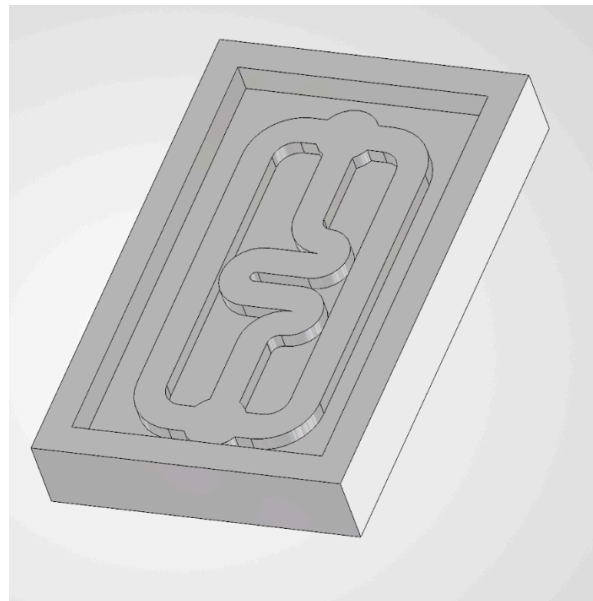
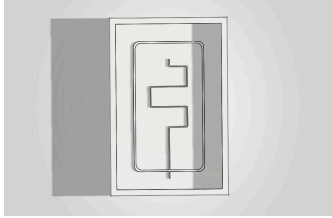
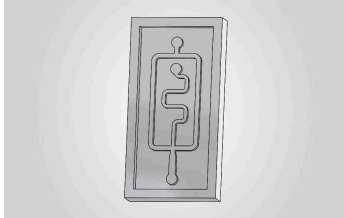
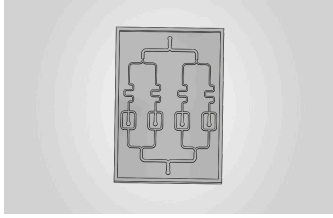


Figure 6: Water-Glycerol Attached PDMS Design

Preliminary Design Evaluation

Design Matrices

Matrix 1: Microfluidic Mold

Criteria (Weight)	Rating	Weighted Score	Rating	Weighted Score	Rating	Weighted Score
Design Sketch	Design 1: Thin No Outlet 		Design 2: Smooth Design 		Design 3: 4-Outlet Design 	
Flow (25)	3/5	15	5/5	25	2/5	10
Accuracy (20)	3/5	12	4/5	16	4/5	16
Usability (20)	3/5	12	4/5	16	3/5	12
Throughput (15)	2/5	6	5/5	15	5/5	15
Reliability (15)	5/5	15	4/5	12	3/5	9
Ease of Fabrication (5)	3/5	3	4/5	4	3/5	3
Total (100)	63		88		65	

Criteria Description and Rating Rationale

Criteria 1 - Flow:

Flow is a critical criterion in the ranking of the microfluidic molds because the flow must be controlled to yield the ideal size droplets at the ideal rate of droplet generation. The designs are ranked based on the ease of controlling the flow rate and the predicted outcome from the variability in channel size and turns.

- **Thin No Outlet:** Being the mold with the smallest channel width (75 μ m), the flow for this device must be more precise since droplet size is set mostly by geometry and flow-rate ratio rather than the absolute flow rate itself. This is advantageous for reproducibility but can cause high shear rates with cells passing through. The sharp turns add resistance intentionally to tune flow into the junction, but may create uneven velocity at each corner. Furthermore, no dedicated biopsy ports lead to more variability in tubing connection, which can cause leaks or bubbles unrelated to the channel design.
- **Smooth Design:** With dimensions of 750/1000 μ m, channels are effectively larger with a much lower flow resistance, which will require higher flow rates to get meaningful shear at the T-junction. This could make the droplets slightly more sensitive to flow, but is not expected to cause significant disruptions. The smooth turns give the mold more uniform flow into the junction than the previous design. Additionally, the ports significantly improve connection repeatability, so the flow rate set is more likely to be the flow rate delivered.
- **4-Outlet Design:** Each individual droplet generator should behave much like Design 2 in isolation, since the dimensions are unchanged. However, flow distribution across the four parallel generators must be considered. Slight differences in channel length, PDMS height, or partial clogging in one T-junction can shift flow rates unevenly. This must be tested rather than assumed. After initial testing, it is clear that small differences between channels will result in unevenly distributed flow, leading to some channels receiving no flow and limiting the throughput that this design is specifically designed to optimize.

Criteria 2 - Accuracy:

Accuracy is a major part of the project, as it dictates the droplet size produced by the microfluidic droplet generator. For this device, a deviation from the 0.5 μ L droplet size is allowed but discouraged. An increase in the size of the drops may cause them to be too big to fit through the body's canals, and smaller drops may not contain enough B and T cells. With these limits in mind, accuracy was assigned the second-highest weighting. Controlling and having a steady size of collagen droplets is paramount to the success of the droplet generator.

- **Thin No Outlet:** The “Thin No Outlet” design was tied for the lowest score within the accuracy criteria. This is mainly due to the thinner and more jagged channels the collagen is pumped through. With less room to flow and sharper turns, the collagen can easily get clogged within the mold itself or flow through the opening at an irregular rate due to fluid mechanics and overlap. Therefore, this mold is more likely to produce irregular, less accurate droplet sizes, giving the design a 3/5 in accuracy weighting.
- **Smooth Design:** The “Smooth Design” is able to get accurate and consistent droplet sizes, giving it a 4/5 score. This design attempts to improve on the “Thin No Outlet” design by using low-friction channels, with walls that are much more conducive to collagen flow.

The wider channels also attempt to reduce the likelihood of blockage, giving the mold geometry less chance to affect droplet size.

- 4-Outlet Design: With this mold, many of the same implementations as the “Smooth Design” are used. For the “4-Outlet Design,” the increased number of channels and outlets can increase the chance for blockages or clogs to form within the channels. The channels in this design are modeled off the “Smooth Design,” which will help the flow of collagen and oil through the mold, but the larger surface area will lead to a higher output of correctly sized beads.

Criteria 3 - Usability:

The ultimate goal of the project is to create a functioning device for Dr. Sanchez de Diego. Keeping that in mind, the usability of all components in the project is a key criterion for consideration. The microfluidic chip designs are ranked based on their projected ease of use in simplifying Dr. Sanchez de Diego’s workflow.

- Thin No Outlet: The first design ranks the lowest in usability, scoring a 3 out of 5, due to the lack of dedicated circular port holes on the chip. This means that Dr. Sanchez de Diego would need to use a biopsy punch to create ports for syringe tubing to enter at a random location. Due to the additional step and uncertainty in port placement, this design ranks lowest.
- Smooth Design: The smooth design excels over the no outlet design because it has ports and outlets. This simplifies the process of inserting syringe tubing and collecting the droplets once they are formed. Additionally, the wider channels make this design more usable, as it allows larger droplets to encapsulate the amount of B and T cells Dr. Sanchez de Diego desires. Therefore, this design scores a 4 out of 5 in usability. It is not a perfect score because feedback from the client is needed to get a perfect score in usability.
- 4-Outlet Design: The 4-Outlet design provides the same usability benefits as described above with the smooth design; the only difference is that the design is repeated four times to allow for a higher droplet generation rate. This increases the usability as it drastically reduces the time for droplet processing; however, the usability may also be reduced as the increased number of channels introduces a greater chance for clogging or variation in droplet sizes. Thus, this design scores a 4 out of 5 in usability as well.

Criteria 4 - Throughput:

The throughput is a measurement of the number of microbeads produced per unit time. Throughput is one of the specific requirements from Dr. Sanchez de Diego and is thus a necessary criterion to evaluate the microfluidic chip designs against. Dr. Sanchez de Diego requires the device to produce at least 300 collagen beads but, more effectively, closer to 600 collagen beads during each cycle.

- Thin No Outlet: The first design provides a simple microfluidic chip layout. The throughput can be changed by manipulating the flow rate of the oil and collagen. The lack of circular ports to be biopsy-punched makes this design score low, as it is susceptible to leaking. This can harm the total throughput of the microfluidic device. This design scores a 2 out of 5 in throughput.
- Smooth Design: The smooth design vastly improves upon the previous design in terms of throughput. The dedicated circular ports for biopsy punching prevent leaks and make tubing connections stronger. The more gradual flow path should contribute to a steadier, higher throughput throughout the device's operation. For these reasons, this design scores a 4 out of 5 in throughput.
- 4-Outlet Design: The 4-outlet design takes all the advantages of the smooth design and builds upon them. This design offers the same benefits in terms of dedicated ports and gradual flow while also multiplying the projected throughput by four. In terms of product specifications, this meets the throughput criteria the best and thus scores 5 out of 5.

Criteria 5 - Reliability:

The criterion of reliability mainly considers the effectiveness of each design over time and how each component will affect the output of each mold. More complexity within a design may lead to an increased number of variables and problems arising within the chip during use. The molding process for each chip may also become increasingly complex as the designs follow the same pattern, leading to an increased number of failed curings and molds that are unusable.

- Thin No Outlet: Because the constriction of the channel is small, droplet size tends to remain relatively consistent once the flow is running, and the geometry does most of the work to keep the droplets uniform. Regardless, the lack of biopsy ports can cause shifts, leaks, or air bubbles to form between runs, which can be harder to control.
- Smooth Design: Here, the droplets are more sensitive to small changes in flow, so it is likely that droplet size will vary, but that is acceptable to the client. That being said, the dedicated ports and smooth turn design make the physical setup sturdier and easier to connect, which allows for more repeatability and reliability for testing.
- 4-Outlet Design: The primary question for reliability for this design depends on test results to see if each channel behaves the same way as the smooth design. Small fabrication differences can cause the design to produce droplets at uneven rates or clog up, which would throw off consistency. Because empirical testing has not yet been conducted on this device, it is fair to say that the 4-Outlet design carries the most uncertainty for reliability.

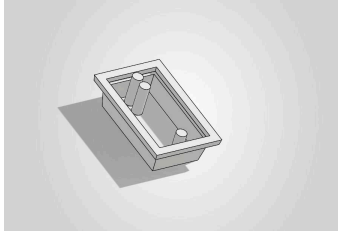
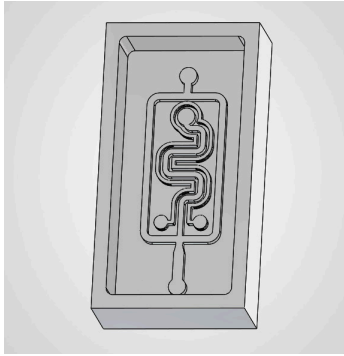
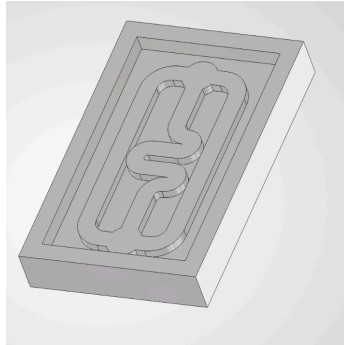
Criteria 6 - Ease of Fabrication:

Since all three designs use similar, relatively straightforward fabrication methods, ease of fabrication carries less weight in the design matrix. It is still worth considering, though, because a new device must be fabricated for each trial. The major manufacturing parameters that must be

taken into consideration are maintaining a viable channel width, establishing precise corner geometry, PDMS wall thickness, and PDMS mold fabrication.

- **Thin No Outlet:** This design is most difficult to fabricate because its narrowest channel and tall, narrow elastomer features are prone to collapse, pairing, and tearing [16]. Sharp corners concentrate stress during peeling, and thin mold ridges can delaminate or break, so the mold itself is at risk as well as the PDMS. The mold also needs thorough post-processing. This narrow channel runs a high risk of collapse after processing.
- **Smooth Design:** This design is the easiest to fabricate because its minimum channel width of 750/1000 μm gives a lower aspect ratio, which reduces the peel stress and risk of channel collapse. Rounded geometry removes the sharp corners where tears start, so the PDMS releases more cleanly from the mold [17].
- **4-Outlet Design:** The individual channels here have the same dimensions as the previous design; however, there are 4 different paths for the fluids to follow, which inherently brings manufacturing complexities. The added difficulty comes from the branching and spacing of the channels, since closely spaced outlets leave thin PDMS walls that can tear at the junctions or when being released from the mold. Furthermore, punching several outlet ports also increases the risk of tearing or failure on one or more channels [17].

Matrix 2: Collagen Cooling Mechanism

Criteria (Weight)	Rating	Weighted Score	Rating	Weighted Score	Rating	Weighted Score
Design Sketch	Design 1: Ice Cube Mold 		Design 2: Water-Glycerol Channel Adjacent 		Design 3: Water-Glycerol Attached PDMS 	
Cooling Effectiveness (25)	4/5	20	4/5	20	3/5	15
Repeatability (20)	3/5	12	5/5	20	5/5	20
Sterility (20)	3/5	12	4/5	16	4/5	16
Usability (15)	4/5	12	5/5	15	4/5	12
Durability (10)	2/5	4	3/5	6	4/5	8
Ease of Fabrication (10)	3/5	6	5/5	10	5/5	10
Total (100)	66		87		81	

Criteria Description and Rating Rationale

Criteria 1 - Cooling Effectiveness:

Cooling effectiveness is necessary as the highest weighted criteria because the entire system must be kept cool to avoid collagen polymerization. If the collagen hydrogel begins polymerization in the microfluidic chip, it can lead to clogging of the channels. Then, the entire

chip would need to be thrown away and remade. To prevent this and make the final product as effective as possible, a sufficient cooling mechanism is necessary.

- Ice Cube Mold: The ice cube cooling method is projected to be an effective method due to the direct contact between the ice cube and the microfluidic chip. This limited barrier between the freezing ice cube and the microfluidic chip allows for high transfer of the cold temperature to the collagen hydrogel in the channels. This design scores a 4 out of 5 in cooling effectiveness because as the ice cube melts, the cooling strength will decrease and eventually disappear. This is not a permanent cooling method.
- Water-Glycerol Channel Adjacent: The water-glycerol channel adjacent to the collagen channel provides a high cooling effectiveness as well. The water-glycerol solution can be kept as cold as -23 °C before it freezes. This provides a much colder ambient temperature than the ice cube. Additionally, this cooling solution is permanent because the water-glycerol solution will stay in the channel and not melt. This design scores a 4 out of 5 in cooling effectiveness because although the cooling power is strong, the cooling channel is separated from the collagen channel by a thin barrier of PDMS.
- Water-Glycerol Attached PDMS: The water-glycerol attached PDMS mold is a similar solution to the adjacent channel above. This design provides a greater area covered in the -23 °C water-glycerol solution; however, it is separated from the collagen channel by the glass that both the microfluidic chip and the water-glycerol PDMS chip will be bonded to. Thus, the cooling effectiveness will be lower due to this greater distance. Research can be done into a material other than glass that can better transmit the cooling effect through to the collagen. Yet, as of now, this design scores a 3 out of 5 in cooling effectiveness.

Criteria 2 - Repeatability:

The repeatability of each design refers to its ability to repeat its cooling effects quickly on the next mold without degradation of the effectiveness of the design or the system itself. This is mainly classified by the way in which each design cools the mold. It is extremely important for the cooling devices to maintain a temperature at or below 4°C, as the collagen gelation process begins affecting the structure and viscosity of the fluid at any temperature higher than 4°C. Repeatability is, therefore, paramount to the success of the overall system and should have a high standard in each design to keep the temperature constant and below the maximum value.

- Ice Cube Mold: The “Ice Cube Mold” has the lowest score within the repeatability criteria because of the liquid that it is using. Water does not stay frozen for a long period of time, especially when kept in a room-temperature space. This shortens the droplet generator’s operating cycle and requires the water to be refrozen often. On top of this, water can be a highly corrosive substance if kept within a confined space, such as the proposed mold. Overall, this gives this design a 3/5 rating when considering repeatability.

- **Water-Glycerol Channel Adjacent:** This design utilizes a Water-Glycerol compound instead of standard water to cool the collagen flowing through the mold. This gives the “Water-Glycerol Channel Adjacent” design a 5/5 rating in the repeatability category. Water-Glycerol is able to maintain lower temperatures than ice and for a longer period of time, making it much more favorable in a situation such as this. The droplet generator can then run for longer periods, generating more collagen beads and providing a greater output volume. The Water-Glycerol will also be situated within the channels surrounding the flow of the collagen, reducing its movement within the mold and reducing stresses on the channels it's held within, increasing the mold's lifespan.
- **Water-Glycerol Attached PDMS:** Once again, Water-Glycerol is used as the focal point of the “Water-Glycerol Attached PDMS” design, giving it a 5/5 score. For repeatability, Water-Glycerol is an extremely favorable fluid to use because of its low freezing point and slower heating than water. Because the fluid doesn't phase-change within the temperature range it will be held in, keeping the fluid steady under the PDMS mold the collagen is flowing through will ensure the integrity of the mold.

Criteria 3 - Sterility:

Sterility refers to keeping the collagen and microfluidic system free from unwanted microorganisms, such as bacteria and fungi, that could interfere with the experiment. Within the bounds of the project, maintaining sterility is especially important because the collagen beads will contain living cells, and any contamination could affect cell survival, immune-cell behavior, collagen gel formation, and the reliability of the lymph-node microphysiological model. Achieving 100% sterility is practically impossible outside of highly controlled laboratory environments, but the goal is to minimize contamination risk through methods such as sterilized materials, closed microfluidic channels, and proper handling procedures. Therefore, the design should focus on creating a system that maintains a highly sterile environment while still allowing efficient collagen droplet generation and cell encapsulation.

- **Ice Cube Mold:** The Ice Cube Mold design has the advantage of being simple and easy to clean due to its open and accessible structure. Since there are fewer enclosed channels, it is easier to remove residues and perform basic sterilization before use. However, because the ice cube is exposed to the surrounding environment during preparation and handling, there is a higher risk of contamination.
- **Water-Glycerol Channel Adjacent:** The Water-Glycerol Channel Adjacent design provides better sterility control because the collagen flow path is enclosed within the PDMS device, reducing exposure to external contaminants. The separate cooling channel also allows temperature regulation without directly interfering with the collagen pathway. However, sterilizing the internal microchannels can be challenging because small channels may trap residues or contaminants, requiring careful flushing and preparation before use.

- **Water-Glycerol Attached PDMS:** The Water-Glycerol Attached PDMS design creates a more controlled and enclosed environment, which helps reduce contamination during collagen processing. The integrated structure is beneficial for maintaining sterility during cell-containing collagen droplet generation because it limits external exposure. However, the more complex channel network makes cleaning and sterilization more difficult, as internal surfaces are harder to access. Additionally, some sterilization methods may affect PDMS properties or bonding.

Criteria 4 - Usability:

As with the microfluidic molds above, the ultimate goal of the project is to create a functioning device for Dr. Sanchez de Diego. Therefore, the usability of all components in the project is key. The collagen cooling mechanisms are ranked based on their projected ease of use and ability to make the research workflow as simple as possible. However, usability is weighted slightly lower for these designs due to the higher importance of cooling effectiveness, repeatability, and sterility.

- **Ice Cube Mold:** The ice cube mold is highly usable; however, it is lacking in a few areas. The process of creating the mold is simple, and filling it with water to produce the ice cube is not difficult. The ice cube sits on top of the microfluidic chip and provides cooling to the entire system. However, this design is not perfect in usability because the melting ice is a factor to consider that introduces operational complications. The operator must manage meltwater accumulation within the biological safety cabinet. Therefore, this design scores a 4 out of 5 in usability.
- **Water-Glycerol Channel Adjacent:** The water-glycerol channel adjacent to the collagen channel provides a unique engineering solution and scores a 5 out of 5 in usability. The water-glycerol solution is easily fabricated in bulk and can be stored in a -23 °C freezer when it is not needed. Then, the process is as simple as using another syringe to fill the adjacent channel with solution and leave it there as a stagnant cooling channel.
- **Water-Glycerol Attached PDMS:** The working principle of this design is similar to the water-glycerol adjacent channel design. However, this design adds complexity in that it requires bonding this new PDMS chip to the other side of the glass that the microfluidic chip is bonded to. This makes the entire system bulkier. Therefore, this cooling mechanism design scores a 4 out of 5 in usability.

Criteria 5 - Durability:

The durability of each design mainly depends on each design's ability to withstand wear and tear over repeated use. The material used to make the molds, the bonding mechanisms between pieces, and the fluids being held within each design are considered. A longer-lasting cooling mechanism will greatly improve the ease of use of the overall system and the workflow efficiency, as the operator will not have to refabricate the cooling system between uses of the droplet generator.

- Ice Cube Mold: Though the ice cube mold has a resin mold in which the design would hold its form, since the actual coolant is an ice cube, it will melt over time, which would ultimately be inefficient in terms of long-term durability per trial. The ice cube mold design receives a 2 out of 5 in durability.
- Water-Glycerol Channel Adjacent: The Water-Glycerol Channel is perfectly suited for durability purposes. Since it is adjacent to the primary flow channels, it would simply flow liquid alongside it, minimizing any sort of durability constraints such as melting or cracking. There is a durability concern in the thin wall of the PDMS separating the channels, specifically around the collagen inlet. The high pressure could damage the seal and cause cross-contamination between adjacent channels. Thus, this design scores a 3 out of 5 in durability.
- Water-Glycerol Attached PDMS: Similar to the previous design, since the liquid coolant flows through a channel, there are no other factors that contribute to its durability. However, one durability consideration may be the bonding of this separate PDMS chip to the other side of the glass. This added bulkiness could lead to decreased durability, so this design scores a 4 out of 5.

Criteria 6 - Ease of Fabrication:

Fabrication is an important part of the design matrix. Since each device will likely need to be remade before use because of sterility concerns, the process necessary for each sample must be easy to repeat. Ideally, fabrication should be efficient and produce consistent devices with limited variability.

- Ice Cube Mold: The ice cube mold may be difficult to remove the ice from. If the resin is not flexible enough to release it, the ice could become stuck or crack, making it unusable.
- Water-Glycerol Channel Adjacent: The channel-adjacent water-glycerol design is relatively easy to fabricate, requiring printing and post-processing. Once a working procedure is established, this cooling method should be straightforward to reproduce.
- Water-Glycerol Attached PDMS: Like Design 2, the cooling feature is built into the device, but it is attached to the opposite side of the glass from the collagen and oil droplet generator. The main fabrication concern is connecting inlet ports on both sides of the glass. The device may need to be suspended or use a different tubing connection.

Proposed Final Design

The proposed final design integrates the simple single-channel throughput device with the surrounding water/glycerol channel to create a coherent PDMS mold that will efficiently and reliably generate cooled collagen droplets.

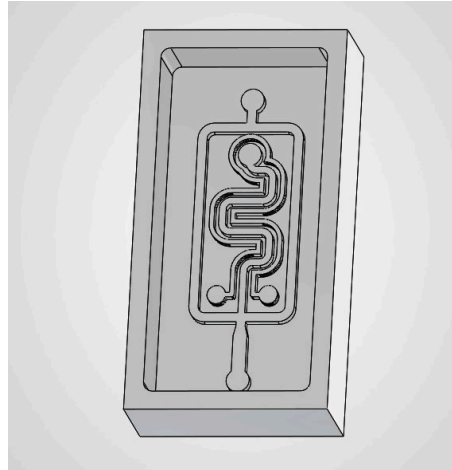


Figure 7: Proposed Final Design

Fabrication

Materials

Resin molds are fabricated using a Phrozen Sonic Mini 8K 3D printer and Phrozen TR300 heat-resistant resin. Isopropyl alcohol (IPA) is used for mold post-processing and PDMS removal. Devices are cast from Sylgard 184 PDMS at a 10:1 base-to-curing-agent ratio and bonded to glass bases using plasma treatment. Fluid connections consist of biopsy-punched ports, double-sided 1/32-inch-to-1/16-inch connectors, hyaluronic acid-coated 1/16-inch tubing, and syringes fitted with tubing adapters. Type I collagen serves as the aqueous phase, while fluorinated oil mixed with Triton X as a surfactant serves as the continuous phase for droplet generation.

Methods

Resin molds are printed from STL files designed in SolidWorks and thoroughly post-processed to support PDMS curing. After removal from the printer, each mold is hand-washed with isopropyl alcohol (IPA) and submerged in approximately 300 mL of IPA in a 1 L beaker. The mold is then sonicated for 30 minutes at 25 °C, dried, and UV-cured at 60 °C for 60 minutes [12].

Following post-processing, Sylgard 184 PDMS base and curing agent are mixed at a 10:1 ratio and poured into each mold to the brim, requiring approximately 4 g of mixture per mold. The PDMS is cured overnight in an oven at 60 °C, then lubricated with IPA to facilitate removal from the resin mold. The molded PDMS and its accompanying glass base are plasma-treated to modify their surface chemistry and promote bonding. Both surfaces receive 12 seconds of high-intensity plasma treatment followed by 108 seconds of medium-intensity treatment. The treated surfaces are then brought into contact and heated at 60 °C for 2 hours to strengthen the bond [15].

Once the device is assembled, the PDMS ports are biopsy-punched to accommodate tubing connections. A double-sided connector compatible with 1/32-inch and 1/16-inch tubing is inserted into each port, with the 1/16-inch connection facing upward. Hyaluronic acid-coated 1/16-inch tubing, intended to reduce surface shear stress, connects the device port to a syringe fitted with a 1/16-inch tubing adapter.

To prepare a collagen solution, keep all reagents and equipment thoroughly chilled on ice to prevent premature gelation. For neutralization, either prepare a 2× PBS/HEPES buffer adjusted to pH 7.7 and mix it 1:1 with collagen before fine-tuning the pH with litmus strips, or neutralize directly using 1 M NaOH. Under the NaOH method, prepare a 10× PBS stock, then combine collagen (target 4 mg/mL), 50 µg/mL fibronectin, 10 µL of 10× PBS, 1 µL of 1 M NaOH, and deionized water up to a 100 µL final volume, mixing gently without vortexing to avoid bubbles. After letting the mixture rest on ice for 10 minutes, verify and adjust the final pH to approximately 7.4. This results in a neutralized, pre-gel collagen precursor solution. This will begin to polymerize into a 3D collagen hydrogel as soon as it is warmed [16].

Final Prototype

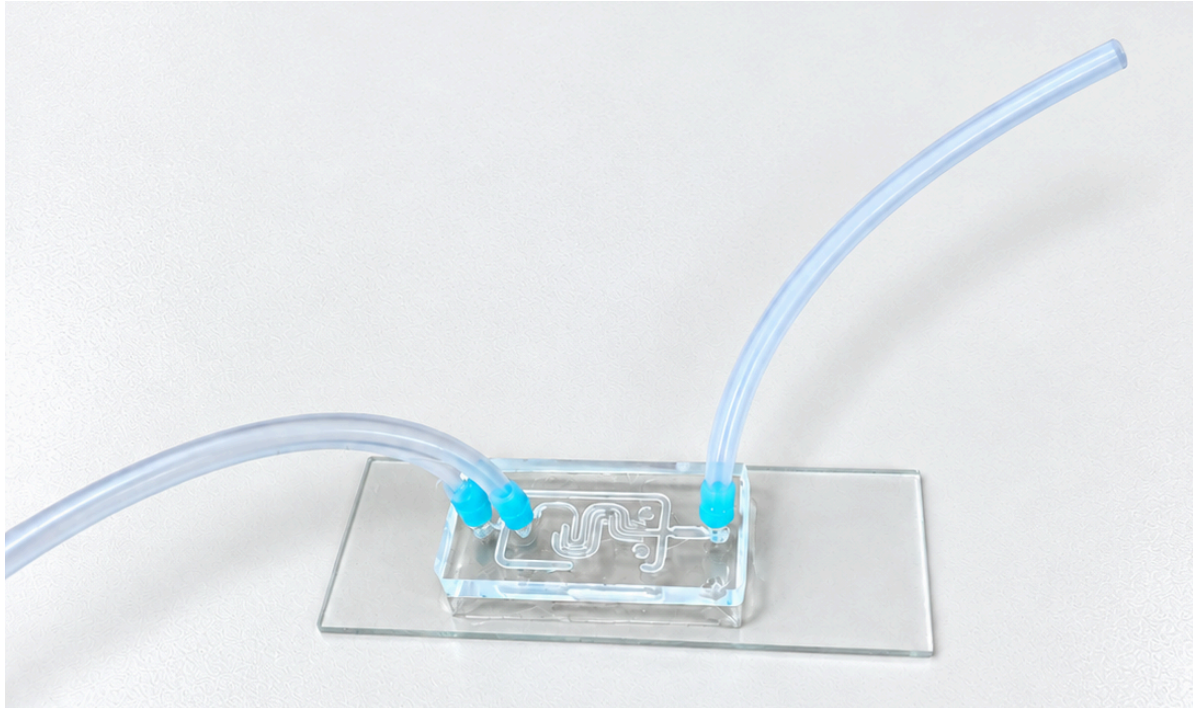


Figure 8: Fully assembled prototype chip featuring an integrated adjacent cooling channel and connected fluidic tubing

Figure 8 shows a physical prototype of the current microfluidic chip with the adjacent cooling channel design. This prototype has biopsy punched holes with the tubing connected to begin preliminary testing.

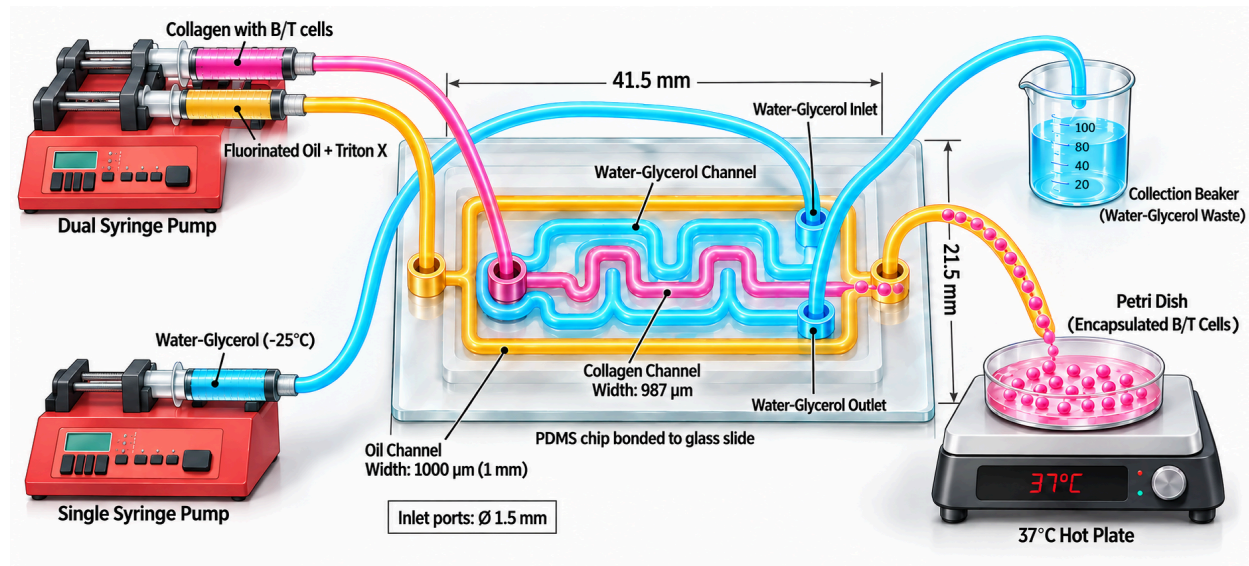


Figure 9: Graphical Representation of the Final Microfluidic Droplet Generating System [17]

Figure 9 is a representation of what the total system will encompass. All of these components are being researched and considered in the final design because they are used in Dr. Sanchez de Diego's laboratory.

Testing and Results

Microchip testing: As an initial testing solution, water was run through the collagen and oil channels of the design and the combined mixture into a catch basin. The solutions representing the oil and the collagen were indicated as different using food coloring, and their mixing point was observed to indicate the successful combination of solutions at the point of droplet generation. The oil and collagen channels successfully transported flow through the PDMS mold and into the output tubing initially very successfully. After multiple reuses of the chip, as well as removal and reinsertion of the input tubing, there was breakdown in the area surrounding the collagen and oil wells. This led to mixing of the mixtures through failure points in the PDMS chip and the diversion of oil flow through one individual channel rather than following both paths around the chip.

Collagen Testing: Based on Dr. Sanchez's collagen-making procedure, collagen was made and used to test its polymerization properties and its ability to form hydrogels. For testing purposes, the formula would have to be scaled, as it would be using a lot of collagen for trials. Sterility is currently not taken into consideration since live cells have not been introduced, which would be at risk under unsterile conditions.

Collagen was used in syringe pumps and measured at 15-minute intervals with three different batches of collagen droplets. Initially, the collagen was placed in an ice bath, and if it stayed outside for longer, it was expected to reach thermal equilibrium with the room temperature and begin its fibrillogenesis. The objective was to see if the time taken for the collagen to be pumped out of the syringes would increase as the collagen solution was left out at room temperature in the syringe pumps. This is crucial for design because collagen will polymerize when it contains the cells; however, it cannot polymerize before it forms the droplets, or else it would clog up the syringe, increasing the internal pressure (especially at such high pressures, which are needed for the rapid generation of the droplets) and resulting in the syringe or microfluidic chip exploding. Initially, the temperature of the collagen was 2 °C, and as the 45 minutes elapsed, it reached 19°C. As mentioned above, 3 trials were run with the syringe pump at 15 minutes, 30 minutes, and 45 minutes intervals. At 15 minutes, it took 4 seconds for the collagen to leave the syringe and fall onto the Petri dish at an infusion rate of 20 µL/min. At 30 minutes, it took 5 seconds, and at 45 minutes, it took 8 seconds.

The margins for error in this trial were high; however, collagen testing must continue to see if the time difference will affect the results and, if so, by how much. References to the research paper “Rheology and Confocal Reflectance Microscopy as Probes of Mechanical Properties and Structure during Collagen and Collagen/Hyaluronan Self-Assembly” can be used in future trials.

This study is essential as it looks at how collagen changes over time when forming a hydrogel. The researchers used rheology and microscopy to study how temperature affects collagen gelation and its physical properties. Based on this research, further testing can be done on how the temperature and time spent outside the ice bath affect collagen flow and polymerization. This would help understand whether collagen starts forming fibers before the droplets are generated, potentially clogging the syringe or microfluidic chip. In the future, trials can be repeated under more controlled conditions to measure changes in viscosity and gelation to better understand how these factors affect droplet formation [18].

Discussion

The goal of the project is to be within the proposed tolerance limits of a previously manufactured droplet cited by the client. Carrying over the measurements from this specific microfluidic system, the aim of the setup within this project is to obtain droplets at a volume of $0.5\mu\text{L} \pm 0.23\mu\text{L}$ ($0.99\text{ mm} \pm 0.16\text{ mm}$ diameter) [19]. This measurement is essential to the functionality and accuracy of the lymph node model, as collagen beads that are too small will not have enough volume to hold enough B and T cells to have an effect on a system. B and T cells are often 8-10 micrometers in diameter, and a relevant source of therapeutic effect will likely require 10-15 of these cells in each droplet at the low end of its range [20, 21]. In addition, the client set a minimum of about 300-500 collagen beads per cycle of the droplet generator. This

will allow multiple batches and samples to be taken from the same microfluidic run without constantly rerunning the device.

Many ethical considerations come with the use of human cells within medical research. This project is being done under the jurisdiction and within the guidelines of the Wisconsin Institute for Medical Research, an institute that commits to and prides itself on the research ethics and integrity it holds itself to. Guidelines and requirements set by both it and federal agencies are strictly followed, documented, and trained upon to ensure an ethical standard is upheld while doing work within its limits [22, 23]. The device itself will also not be handling human cells, and will instead be creating and storing the vessels in which trained professionals will be inserting and monitoring the B and T cells.

The project and system itself has many moving parts, meaning changes and revisions can be easily made and will most likely have to be made more often. Optimal syringes, connectors, tubing, syringe pump settings, PDMS chip designs, and cooling apparatuses will all need to be prototyped and tested in combination with each other. Isolating variables and finding quantitative results for each measurable test will be essential for choosing the best materials and resources for the device. Each of the formerly listed components may also be sources of error. The resistance, viscosity, and harsh tendencies of the collagen, surfactant, and fluorinated oils used in the procedure can each affect the components differently. However, with the tested optimal collagen solution and a proposed chip and syringe pump setup rooted in research, the foundation of the initial evaluations is sound.

Conclusions

Prostate cancer metastasis remains a significant clinical challenge due to the lack of physiological three-dimensional models capable of mimicking the cellular architecture of lymph nodes. Current two-dimensional cultures fail to capture the complex spatial interactions required to study cancer cell spread, creating a direct need for an automated microfluidic platform capable of producing uniform, cell-laden collagen microspheres. To address this challenge, the proposed final design incorporates a single-junction PDMS microfluidic chip featuring 750 μm collagen channels, 1000 μm fluorinated oil channels, and an integrated, adjacent water-glycerol cooling channel maintained at $-23\text{ }^{\circ}\text{C}$. This integrated configuration targets an output volume of approximately 0.5 μL per droplet while preventing premature collagen polymerization prior to droplet formation.

Preliminary evaluations demonstrated the core feasibility of the fabrication and fluid delivery processes, while also identifying critical mechanical and thermal boundaries. The 3D-printed resin mold fabrication and PDMS curing procedures successfully produced well-defined, transparent microchannels that bonded durably to glass substrates via plasma treatment. Initial fluidic testing with dye solutions verified that the microchannels could guide

flow to the junction without leakage during early trials. Furthermore, time-dependent testing of collagen without cooling confirmed that room-temperature exposure significantly increases fluid transit time under constant pump rates. This highlights the necessity of active thermal regulation to avoid channel clogs and pressure-induced failure. Additionally, repeated manual reinsertion of tubing connectors into the biopsy-punched ports led to structural breakdown around the inlet wells, causing cross-channel fluid leaking and uneven oil flow distribution.

For future prototypes, structural revisions will be implemented to strengthen the fluidic interfaces and improve process consistency. Rather than subjecting single-use chips to repeated port re-insertions, rigid barbed connectors or reinforced inlet collars will be evaluated to preserve the PDMS boundary around the wells. Future work will center on validating the full droplet-generation setup using the active adjacent water-glycerol cooling line to quantify droplet monodispersity and throughput within the client's 5-to-10-minute cycle target. Subsequent testing will incorporate fluorescent beads as cell analogs, advance to live B and T cell encapsulation trials under BSL-2 conditions, and integrate an automated downstream collection vessel to transition formed microspheres directly into 37 °C incubation.

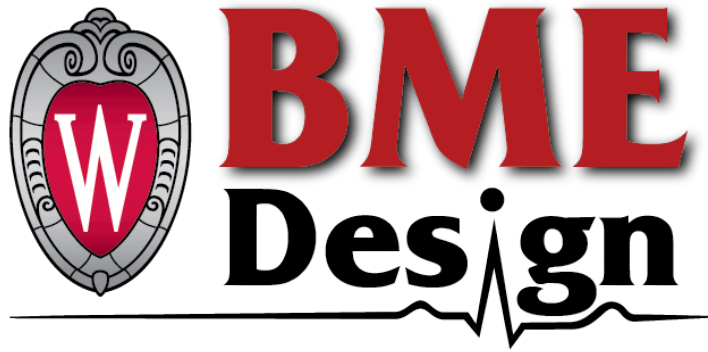
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Appendix

Appendix A - Product Design Specifications



Immunodrop

Product Design Specifications

Product Design Specifications (PDS)

BME 200/300 Section 312

September 17, 2026

Client

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Function

Collagen, particularly Collagen Type I, is the primary structural protein of the extracellular matrix (ECM) and serves as an ideal scaffold for cell manipulation and 3D culture [1]. While microfluidic droplet generators paired with continuous oil phases reliably generate collagen beads to model physiological environments [1,2], prostate cancer commonly metastasizes through the lymphatic system, where understanding cellular transit requires accurate representations of lymph node architecture. However, current in vitro lymph node models rely primarily on two-dimensional cell cultures that fail to replicate the complex 3D tissue architecture and cellular interactions essential for studying metastatic spread. Therefore, this project focuses on fabricating a specialized microfluidic droplet generator for Dr. Cristina Sanchez de Diego to produce uniform collagen microspheres encapsulating B and T lymphocytes, providing the foundational building blocks for a biomimetic 3D lymph node germinal center to investigate cancer metastasis.

Client requirements

The client has the following requirements:

- Produce collagen droplets with a target volume of approximately 0.5 μL .
- Maintain the collagen solution at approximately 4 $^{\circ}\text{C}$ during droplet generation to reduce premature gelation and channel clogging.
- Fill the collagen gels with B and T cells.
- Ensure that the collagen gel fully polymerizes at approximately 37 $^{\circ}\text{C}$.
- Generate droplets automatically after activation without requiring continuous user intervention.
- A five- to ten-minute run-time was requested in order to keep up with the quick-paced nature of the lab.

Design requirements

1. **Physical and Operational Characteristics**
 - a. **Performance requirements**

- i. The device should be autonomous in its operation, with the pumps being activated and the collagen droplets being produced and stored on the other end. New syringes of fluorinated oil and collagen should be easily accessible and replaceable when needed. The flow of each fluid used in the device- fluorinated oil, collagen, and a mixed-in or external surfactant- should all be simultaneous and at similar rates to properly produce collagen beads.
- ii. Bead collection should also be an automatic process, with a holding chamber designed to immediately initiate the process of incubation required prior to experimental use of the collagen microspheres. This will take place at 37 °C and should be implemented into the flow of the device naturally.
- iii. The device should operate at a rate and duration in accordance with the needs of the client currently specified to produce at least 300 collagen beads but more effectively closer to 600 collagen beads during each cycle. Additionally, this production cycle must take place within a timeframe that increases the efficiency of the bead production process beyond the current timeframe required to individually hand-curate each microdroplet.

b. Safety

- i. All fluid-contacting components must be sterilized before experiments involving live cells to prevent culture contamination and user exposure to biological materials.
- ii. Syringes, tubing, and connections must remain below their manufacturer-specified pressure limits to prevent leakage, disconnection, or component failure [3].
- iii. The glass substrate must be inspected for cracks and handled carefully to prevent breakage, cuts, and exposure to sharp edges.
- iv. Heating and cooling units associated with the survival and incubation of B and T cell collagen beads should be diligently tested to remain within safe limits.
- v. All heated surfaces must be appropriately labeled.
- vi. Users must wear appropriate personal protective equipment and follow applicable laboratory and biological-safety procedures.

c. Accuracy and Reliability

- i. The accuracy of the droplet generator will be within a variable range of the 0.5 μ L specified value for collagen beads. Using tolerances and deviations stated in a previous publication from the client detailing a microfluidic droplet process, the predicted tolerance of this project is about 0.5 μ L $\pm$ 0.23 μ L (0.99 mm $\pm$ 0.16 mm diameter). This could be

caused by varying sizes of microfluidic channels or differing pressures calibrated within the device.

d. Life in Service

- i. The entire device should run for approximately 5-10 minutes, producing 300-600 collagen droplets to fulfill the number of B and T cells used in each of the client's experiments. Molds need to be switched out after each use. Any leftover collagen in the device will eventually gel and clog the tubing, even at 4 °C, so each microfluidic chip will only be used once. This will also happen to any excess collagen not used in the first batch, so a new batch of collagen must be made each time as well. This all leads to a life-in-service time of approximately one use.

e. Shelf Life

- i. With this device, failing parts, such as clogged syringes, damaged tubing, or faulty pumps, must be switched out easily without disassembling the entire system. These components have a wide range of lifespans, with syringes remaining sterile for 3–5 years [4], while tubing used in the system remains usable unless a leak or breakage occurs. More expensive components, such as the cooling apparatus and pumps, should be able to withstand 8–12 years of use before requiring maintenance [5]. The PDMS microfluidic chips can also be fabricated in batches and kept in reserve for weeks to months when stored at room temperature and protected from sunlight and dust. The exact shelf life of the chips will need to be confirmed through testing, with the number kept in reserve depending on experimental demand.

f. Operating Environment

- i. All procedures involving live cells must be performed aseptically in a biological safety cabinet by trained personnel.
- ii. Cell-free fabrication and proof-of-concept testing may be performed in an appropriate laboratory space by trained personnel.
- iii. The collagen solution must be maintained at approximately 4 °C before and during droplet generation to limit premature polymerization, which could increase viscosity and clog the microchannels [6].
- iv. Cell-containing droplets must be transferred aseptically to an incubator maintained at 37 °C and 5% CO₂. The cells will survive for over 72 hours under these conditions, far longer than is required for droplet single-generation sessions [7].

g. Ergonomics

- i. The device should stay isolated from other laboratory equipment so as not to interfere with other protocols or testing. A collection dish, the syringe loading spots in the pumps, and the mold should all be easily accessible

and independent of each other's places in the system. Overall, the system should be one piece of equipment, able to be moved by one person without any loose-hanging or free parts that need to be moved later. Because of this, weight is the largest ergonomic consideration, followed by the size of the system.

h. Size

- i. For the droplet generator, Ali et al.'s design may be used as a starting point rather than choosing the dimensions randomly. Their device used a 100 μm -wide collagen channel, 300 μm -wide oil channels, and a 75 μm nozzle where the droplets formed. This setup produced collagen droplets ranging from about 100–1400 μm , depending on the pressure and operating conditions [8].
- ii. Another study by Hong et al. used a T-junction to generate collagen microspheres ranging from approximately 50–300 μm , with about 2% variation in size. Based on these studies, 100–300 μm is a reasonable starting range for the microspheres, but the channel dimensions should be finalized after deciding how many B or T cells the client wants per microsphere. This is important because the droplet size and cell concentration will determine the number of cells in each microsphere [9].
- iii. The device as a whole must fit inside a Class II Biological Safety Cabinet per the Centers for Disease Control and Prevention and National Institutes of Health "Biosafety in Microbiological and Biomedical Laboratories," which states that all immune cells must be handled under Biosafety Level 2 conditions. This will help maintain sterility and protect the operator from fluidic leaks or pressurized tubing failures. This requires that the device is operable within a 1.2m x 1.8m x .78m working area [10].

i. Weight

- i. 1–3 mg/mL of collagen [8].
- ii. The device must be able to be operated and maneuvered by one user. This gives the system a maximum weight of around 50 lb, so it is able to be moved by anyone.

j. Materials

- i. Materials contacting the cell suspension, including PDMS, glass, tubing, and syringes, must be biocompatible.
- ii. Autoclavable components should be autoclaved at 121°C and 15 psi for a 20-minute exposure cycle, when appropriate, and opened inside the biological safety cabinet.
- iii. Non-autoclavable components must be disinfected or sterilized using a method that does not damage the material or leave potentially harmful chemicals on the material surface.

- iv. Printed resin molds must be washed, dried, and UV post-cured according to the resin manufacturer's specifications before PDMS casting [11].
- v. Finished PDMS components must not contain uncured resin or other residues that could inhibit PDMS curing or harm the cells.
- vi. The inside of the chip will be hydrophilically coated via plasma treatment [12].

k. **Aesthetics, Appearance, and Finish**

- i. The generator should have a clean, transparent, and well-finished PDMS structure, with smooth and clearly defined microchannels so that observers can easily see the flow and droplet formation during testing. PDMS is commonly used for microfluidic devices because it is optically transparent, biocompatible, flexible, and relatively easy to fabricate [13].

2. **Production Characteristics**

a. **Quantity**

- i. One functional device is required for the client.
- ii. Because each microfluidic chip will be single-use, the finalized design must allow chips to be fabricated repeatedly with consistent channel geometry and droplet-generation performance.

b. **Target Product Cost**

- i. The target cost for the reusable device setup, excluding equipment and materials already supplied by the laboratory, is less than \$100.

3. **Miscellaneous**

a. **Standards and Specifications**

- i. The device is not considered a medical device under section 201(h) of the Food, Drug, and Cosmetics Act.
 - 1. The act states a device is "an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including any component, part, or accessory, which is (1) recognized in the official National Formulary, or the United States Pharmacopeia, or any supplement to them, (2) intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or (3) intended to affect the structure or any function of the body of man or other animals, and which does not achieve its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of its primary intended purposes" [14].
- ii. The incubation device will require being heated to an appropriate incubation temperature, so ISO 7010-W017 must be considered.

1. ISO 7010-W017 mandates the use of a standardized warning symbol to identify equipment presenting hazardous high-temperature surfaces and to support consistent risk communication across laboratory and industrial environments. Proper implementation requires evaluating the equipment's operating temperature, the likelihood of inadvertent contact, and the visibility and durability of the applied symbol. Thermal burn hazards and physical configurations that may impede recognition must be considered during risk assessment. ISO 7010 states that "the purpose of standardized safety signs is to draw attention rapidly and unambiguously to objects and situations affecting safety," and requires application in accordance with ISO 3864-1 and ISO 3864-4 to ensure uniform color, shape, and meaning [15].
- iii. The device must comply with ISO 22916, a standard on interoperability requirements for dimensions and connections of microfluidic devices.
 1. The minimum distance of any hole from the side of the chip is 3mm. The total chip thickness can range from 0.8mm to 4mm. All ports must be at least 0.4mm in diameter. For ports 1.5mm apart center-to-center, the maximum port diameter is 0.7mm. For ports 3mm apart center-to-center, the maximum port diameter is 2.0mm. For ports 4.5mm apart center-to-center, the maximum port diameter is 3.5mm [16].
- iv. The device must fit inside a Class II Biological Safety Cabinet per the Centers for Disease Control and Prevention and National Institutes of Health "Biosafety in Microbiological and Biomedical Laboratories".
 1. This states that all immune cells must be handled under Biosafety Level 2 conditions. This will help maintain sterility and protect the operator from fluidic leaks or pressurized tubing failures [10].
- v. The device and materials used must not kill the immune cells before testing or research occurs. Thus, the device must pass ISO 10993-5 in vitro cytotoxicity testing.
 1. This testing involves soaking the device's materials, such as PDMS and tubing, in cell culture media at 37°C for 24 to 72 hours. Next, expose a standard cell line to this liquid and measure cell health using an MTT or XTT assay. The passing score is 70% cell viability. If the materials reduce cell survival by more than 30% compared to a control group, they are considered toxic and fail the test [17].

b. Customer

- i. The client wants the device to maximize usable collagen-droplet output while minimizing operating time and user involvement.
 - 1. This includes vertically integrating the collagen and device cooling procedure, manual droplet fabrication process, and completed collagen bead incubation into a single automated cycle.
 - 2. A single control will initiate this cycle, which then runs independently, producing the desired number/volume of beads.
- ii. The device is intended for use by trained personnel in the client's research laboratory.

c. Patient-related concerns

- i. N/A: The device is intended for laboratory research and will not involve patients. Sterilization requirements are addressed under Safety, Materials, and Operating Environment.

d. Competition

- i. Micropillars are being implemented in microfluidics with a variety of impacts. In traditional droplet generators, micropillars can be used as structural support to prevent the delicate surface of microchannels from collapsing under stress. They are also used for flow regulation and sorting as a method of filtration. Finally, they are used to create parallel channels that exponentially increase the droplet generation rate in advanced chip designs. [18]
- ii. Another competitive alternative is the spinning conical frustum mechanism that has been developed as a centrifugal way of creating extremely unique and ordered droplets without the use of microfabrication. [19]
- iii. Bulk emulsion is a competitive design that has been used in many fields requiring microdroplets. There are two main emulsion mechanisms used in industry today: Rotor-stator homogenizers and high-pressure homogenizers. These methods create droplets through vigorous shear forces that rely on high turbulence with high pressure/speed or cavities generated by ultrasound. The main implication of these designs is creating mixtures of nonhomogeneous materials, such as microdroplet mixtures, as seen in the explosive industry [20, 21].
- iv. Mechanical fragmentation of hydrogels is used in industry in the context of drug delivery, tissue engineering, and scaffolding design. This approach offers significant advantages, including simplicity and rapid processing speed. A large hydrogel is broken down into small hydrogels that act as polymerized droplets would if created through alternative mechanisms. These do not overcome the disadvantages that the design faces, as it will

make cell encapsulation very difficult and generate irregular gels rather than the spherical droplets that are being generated. [22]

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