Tissue Biopsy Dissociation

BME 200/300

December 13th, 2017

Client: Dr. Sameer Mathur
University of Wisconsin Hospital

Advisor: Professor Wan Ju Li
University of Wisconsin-Madison Department of Biomedical Engineering

Team Members: Raven Brenneke (Team Leader), Lauren Ross (BSAC), Jamison Miller (Co-Communicator), Nate Richman (Co-Communicator), Victoria Trantow (BWIG), Cory Van Beek (BPAG)

Abstract

Biological research often requires the study of individual cells to gain a better understanding of processes within the human body. The client's research on asthma has focused on the use of tissue biopsy dissociation to obtain individual cells. The research is specifically interested in studying cells before and after an induced asthmatic response. The current device being used for this process, the gentleMACS™ Dissociation Device, did not allow for use of a small tissue sample size. A small tissue sample size, 1-2 mm, was desired to reduce the recovery time and pain of the patient. The team was tasked to create a dissociation device that would successfully dissociate smaller tissue samples and yield viable cells to study. The most important criteria considered in the final design was the ability of the design to perform appropriately and yield at least 50% viable cells. A microfluidic device was used to dissociate this small tissue sample and testing was be conducted to determine the effectiveness of this device. Statistical analysis on the results indicated no difference in cell dissociation when the device was used. Future work proposes methods for increasing cell yield based on the current design.

Table of Contents

I. Introduction

Many doctors and scientists seek to understand different types of medical problems in a greater level of detail than what is currently known. Through careful experimentation and data collection there have been significant gains in knowledge pertaining to different diseases, conditions, and effective treatments.

Biological research often requires the analysis of cells to obtain new knowledge of specific cellular processes. Cells provide structure and function for all living things and they house the biological machinery that makes the proteins, chemicals, and signals for everything that happens within the body. There are about 200 major types of cells and they all function in different ways. Biologists rely on different types of tools to examine these cells and gain a better understanding of how they function. Learning more about how cells work, and what happens when they do not work properly, is imperative in understanding the biological processes that keep humans healthy[1].

Asthma research specifically looks at cells from the lung tissue. The University of Wisconsin Madison has a nationally known research facility for Asthma, Allergy, and Pulmonary Research. Asthma has been studied at UW Madison for over 30 years. Over 400 research studies have been conducted that helped to further explain the role of genetics in asthma, treatment of asthma in children, and how colds affect asthma. This research has helped to develop new asthma medications and guidelines for treatment[2].

An asthma research group at UW Madison currently uses tissue dissociation as a method of studying individual cells to gain an understanding of an asthmatic response. A lung biopsy must be performed to obtain the desired tissue. The biopsy site might be tender and sore for the patient during the recovery process. It is therefore desired to only take as big of a biopsy as absolutely necessary for the research. By taking the smallest possible biopsy, the patient's pain, discomfort, and recovery time will be reduced[3]. In order to be able to take the tiniest tissue biopsy possible for this research, the tissue must be able to be successfully dissociated into viable cells that can then be studied further. This is the problem that the team is seeking to address.

Competing Designs

Figure 1. gentleMACS™ Dissociation device. A dissociation tube is place in the opening at the top, an automatic cycle is then selected with the buttons and display at the bottom.

A well known dissociation device is the gentleMACS™ Dissociator (Figure 1). This benchtop instrument performs a semi-automated dissociation of tissues into single-cell suspensions. A single sample or two samples can be processed at one time. There are two types of unique tubes that can be used with this instrument. Each tube has a series of teeth that perform mechanical grinding with rotation. The instrument offers many programs for a variety of specific applications. Special protocols have been developed by the company for dissociation of specific tissues[4].

Several dissociation protocols are also used. Specific protocols follow the same general steps: tissue is placed in a specific concentration of enzyme solution to break down the extracellular matrix; the solution is then heated to an optimal temperature and then subjected to gentle vortexing or mixing; a filter is used to “strain” the solution and obtain certain types of cells based on filter size.

Problem Statement

Asthma research requires biopsies of lung tissue before and after an induced asthmatic reaction. The tissue needs to be dissociated so that changes in the cells can be studied using flow cytometry. The current method of dissociation requires a tissue sample size of 3-4 square millimeters. The use of a smaller tissue sample size, 1-2 square millimeters, is desired. This smaller tissue sample is unable to break down and dissociate with the current dissociation method. Therefore, the team is tasked with creating a dissociation device that will successfully dissociate a smaller tissue sample and yield viable cells to study.

II. Background

The purpose of the device is to successfully dissociate lung biopsy tissues for asthma research. Though there are different causes of asthma, the client focuses on allergic asthma. Normally harmless airborne allergens trigger an inflammatory response in airways of the lungs, called bronchial tubes. This response is initiated when mast cells release large amounts of histamine to flood the area with extracellular fluid and to attract eosinophils and neutrophils to the affected site. The response is amplified by helper T-cell lymphocytes [5].

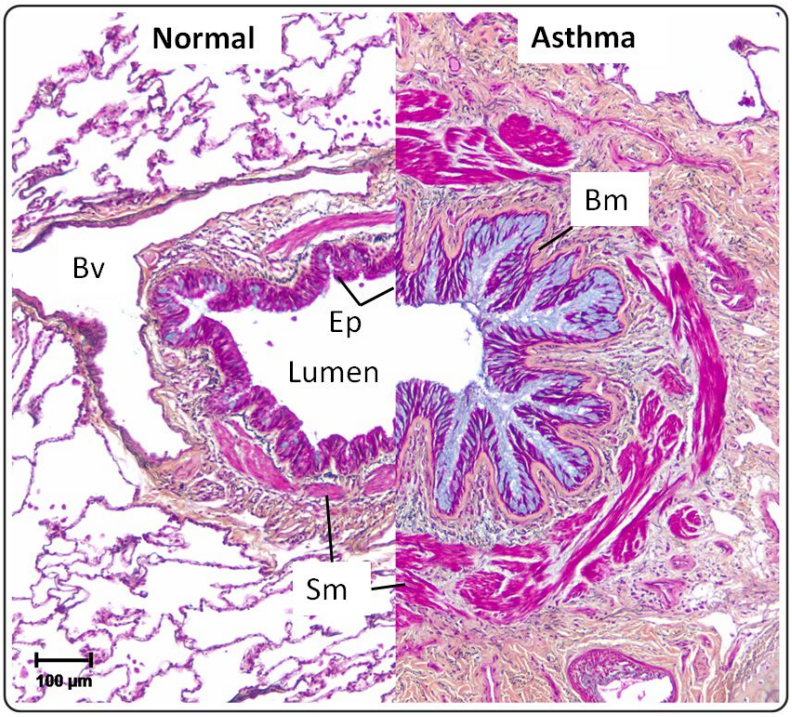


Figure 2. Asthmatic Bronchial Tissue. This visual depicts the cellular level reaction of asthma in a bronchial tube cross section. In comparison to normal tissue, inflammation is present.

To analyze changes in the lung tissue with the allergic response, a biopsy must be performed. There are several biopsy procedures to collect the tissue sample: open, transbronchial, and thoracoscopic. The open biopsy is completed by making an incision in the chest to surgically remove tissue. Similarly, the needle process involves guiding a needle through the chest wall with a CT scan or fluoroscopy. Thoracoscopic biopsies push an endoscope into the chest cavity, and then through the endoscope tools can be inserted to obtain tissue. Nodule removal or tissue lesion may also be performed. Lastly, the transbronchial biopsy, or bronchoscopy, guides a fiberoptic bronchoscope through the nose and into the bronchioles, where the device removes a 1-2 mm sample of tissue [6]. These techniques vary in degree of invasivity, with some requiring anesthesia. The lung biopsy procedure the client uses is the bronchoscopy.

Once the tissue sample is obtained, it must be dissociated into individual cells. Dissociation is the process by which single cells are liberated from a cell aggregate. To achieve this, the extracellular matrix (ECM) must be broken apart without lysing the cells themselves. Two main ways of dissociation include mechanically applying shear forces to the tissue and enzymatically breaking down the extracellular matrix. Unfortunately, many methods of mechanical and chemical dissociation disturb surface markers, nullifying data received from flow cytometry.

Flow cytometry is a method for analyzing the expression of molecules on the cell membrane and within the cell. Fluorescent intensity, emitted by proteins and ligands that bind to cell molecules, is measured by the cytometer. The device has lasers that focus on single stained cells at a time and measure the light scattered and fluorescence emitted [7]. One particular measurement the client desires is the ability to analyze is the activity of eosinophils. Eosinophils are a type of white blood cell, and normally account for only 5% of all white blood cells. High eosinophil counts are related to asthma and allergies, and flow cytometry can detect levels of these cells.

To design the prototype of the dissociation device, several parameters must be fully investigated and determined. Firstly, the shear stresses applied must be sufficient to disturb the extracellular matrix, but not so much as to alter the chemistry of the cells. This requires research of maximum shear strain of the ECM and bronchial epithelial cells. Additionally, the concentration and type of enzyme solution plays a large factor in the success of the device. Dr. Mathur has previously performed testing of various enzymes and found that Collagenase G was least harmful.

Client Information

The client, Dr. Sameer Mathur, is the director of Allergy and Immunology Clinics and the Chief of Allergy at the VA Hospital. He has interests in eosinophil immunoregulatory activity, and performs research on asthma, comparing biopsies before and after an induced reaction.

Design Specifications

The main specification for this project is the development of a device to successfully dissociate lung biopsy tissue samples which are 1-2 mm in size. There must be a minimum of 50% recovery of viable cells. Since the dissociated tissue will eventually be run through a flow cytometer for analysis, there should be no disruption to cell characteristics such as eosinophils. The device's cost should not exceed approximately $10 per use, and if it is reusable, the material must be able to withstand sterilization procedures, either ethanol or autoclaving. A more complete list of design specifications can be found in Appendix A.

III. Preliminary Designs

Design Idea 1: Modification

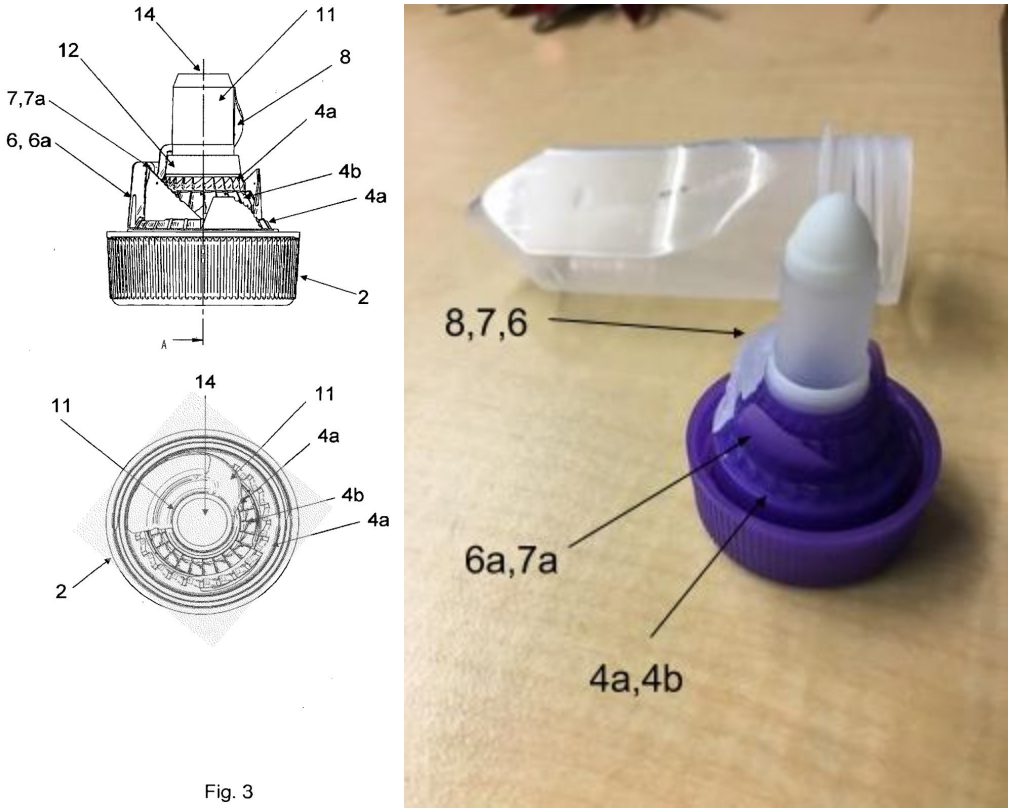


Fig. 3

Figure 3: Illustration image of the current device the client uses. The cap (purple) fits over a tube that fits on top of the genleMACS dissociator. The stator (purple, 2) consists of teeth (4a and 4b) as well as large protrusions 6a and 7a. The rotor consists of a screw to generate flow (8) and an envelope that surrounds the large protrusions on the stator as it spins (7,6). [8]

The first design idea consists of a modification of the client's current device (Figure 3). The client informed us on the issue with the device: it does not dissociate the tissue at all, in contrast to being too harsh and lysing cells (which does not occur). Most likely, parts of the tissue sample are getting stuck in recirculating flows near the teeth, or are able to fit in the gaps between the rotor and the teeth (simplified flow calculations for this design are provided in Appendix B1).

In order to combat this and produce viable cells, from the tissue biopsy, a number of variables would have to be changed. For instance, teeth size, rotational speed and maximal shear forces. Theoretically, it would be a combination of changes in these variables that result in a system that can consistently dissociate small tissue samples.

Design Idea 2: Microfluidic Device

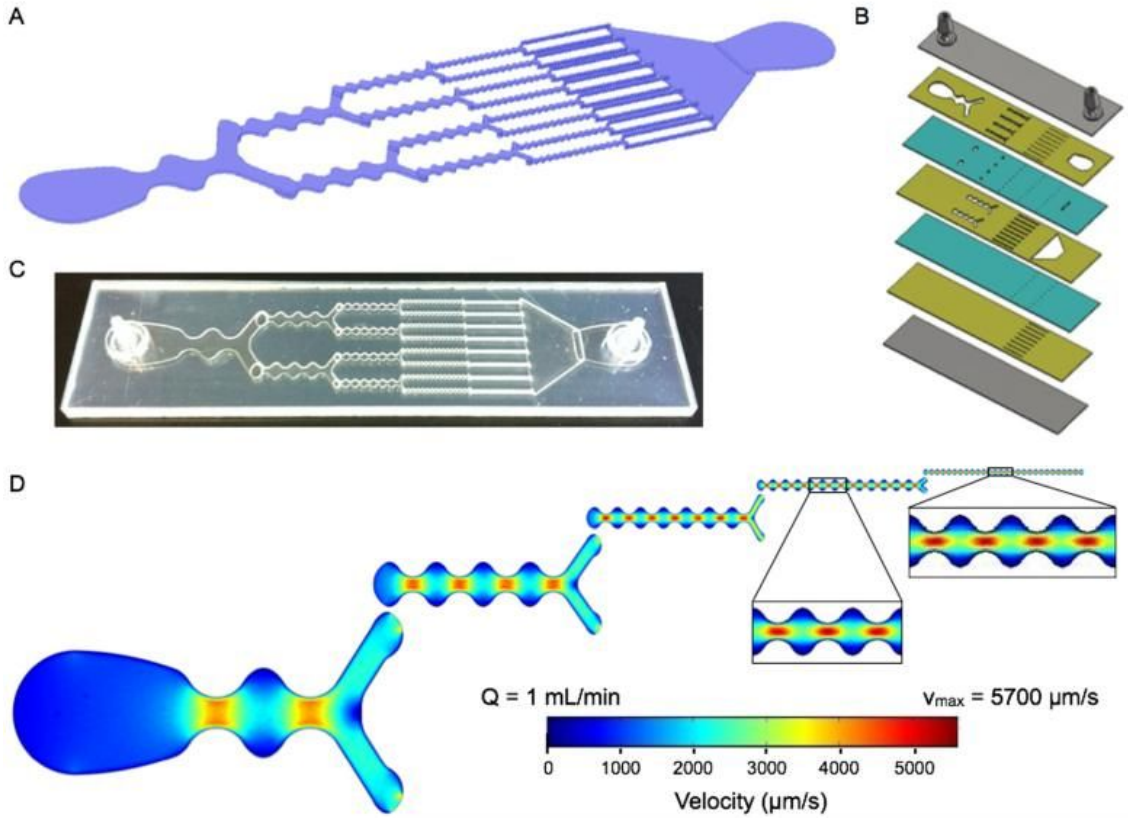


Figure 4: Microfluidic device for dissociation of cell aggregates. (**A)** Schematic of channels with decreasing width. (**B)** Schematic of the individual layers that make up the fabrication of the device. (**C)** Final fabricated product made out of Polyethylene terephthalate (PET). (**D**) Schematic of the flow velocities within the constricting channels. The red portions indicate areas of higher flow velocity and the areas of blue represent low or no flow velocity. [9]

The microfluidic device for dissociation utilizes a network of channels that incrementally decrease in size. The device uses pressurized air to force tissue samples that are in solution through channels with smooth constrictions. Smooth constrictions eliminate the possibility of vortices trapping cells. These channels cause gradients of velocity to form that produce shear forces strong enough to break apart cell aggregates.

This device has been shown to work for tumor cells and cell aggregates. With modification of channel size, pressure and possibly channel pattern design, this device can be modified to dissociate lung tissue samples.

Design Idea 3: Screw Device

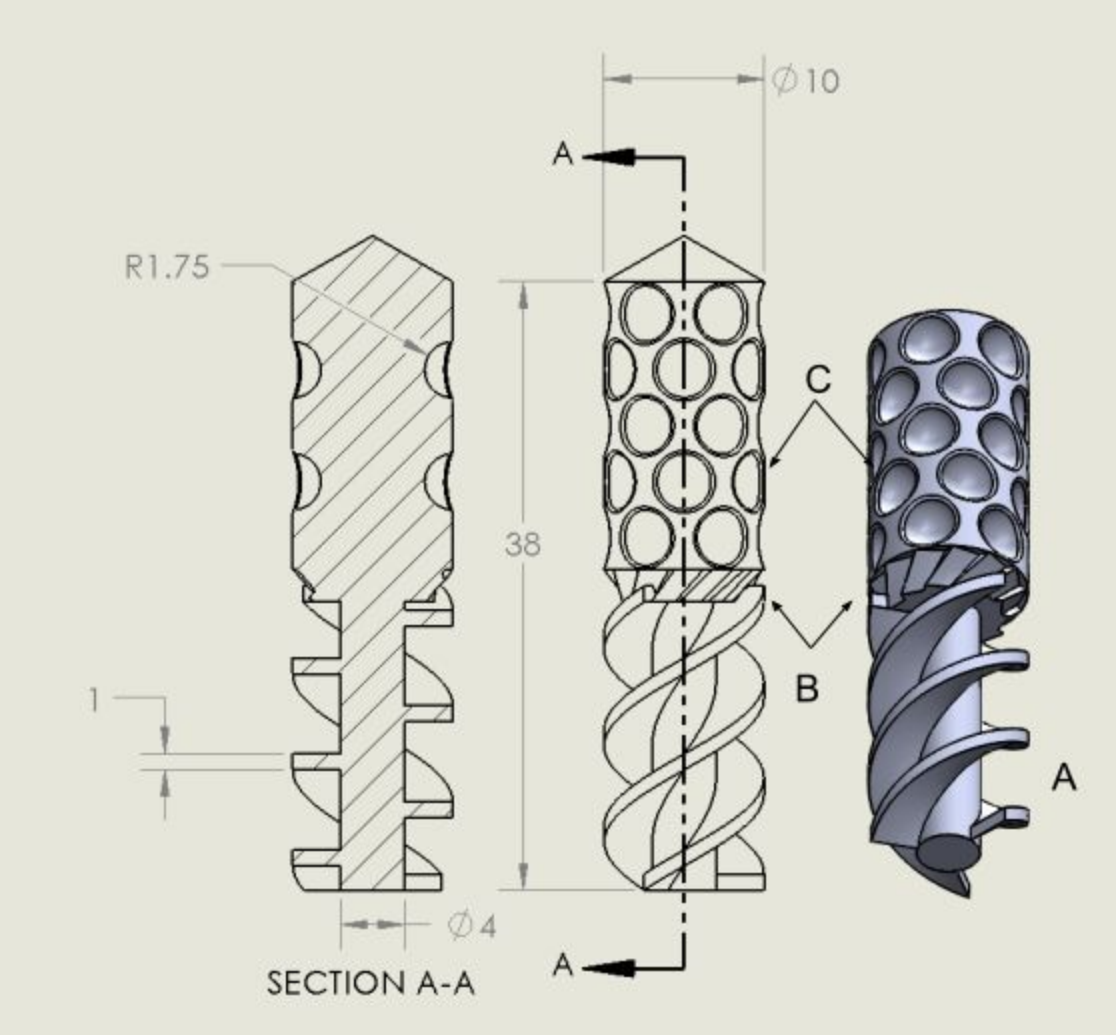


Figure 5: Illustration of screw design, device is shown at the right, while an outline with dimensions is shown in the middle. A section view (A-A) is shown at the left. The device is small and fits inside a chamber with a 100-200 μm gap between the outermost section of the chamber and the inner screw.

The screw design consists of a small spinning device based on a polymer extruder. The screw at the bottom (A) propels the suspension and tissue towards a small row of teeth (B). The teeth break apart large chunks of the tissue so that they can fit through the small gap in the chamber around the head of the design. The head (A) consists of a series of small dimples (1.75mm in radius) that generate turbulent flows that separates cells from the ECM. This design is based off a distributive mixing head which is meant to break apart particle aggregates in a polymer mix. An adaptation of this design could be used and adjusted to create adequate shear forces to dissociate cells without lysing them.

VI. Preliminary Design Evaluation

Based on the criteria outlined in the preliminary design specifications and the factors particularly stressed by the client, the team established 4 categories on which the prospective designs were judged. According to the client, performance of the design, regarding dissociation of viable cells, is the most important criterion upon which the design should be judged. Therefore, the weight assigned to performance was the highest (40/100). This criteria was difficult to judge due to the fact that the team could not precisely determine the performance until the device had been made. Hence, the team considered outside literature on similar devices as a mean to judge the devices. The next criterion upon which the devices were judged was fabrication. In order to achieve the best performance of the design the team must be able to examine the device's performance after running multiple tests. In order to have the greatest duration of time for testing, the team needed to easily and efficiently fabricate the design; due to this, the criteria of fabrication received the second highest ranking (25/100). The following criteria outlined in the preliminary design matrix demonstrate that the design will be assessed based on cost and usage. The current device used by the client costs roughly $5-$10 per use and the client would ideally prefer the new design to cost the same, if not less. Therefore the criterion of cost/usage received the next highest rating (20/100). The remaining criterion which the design will be evaluated on is ease of use. Ideally, the team would like the design to require a minimized amount of work and time consumption for the lab technician using it. Since the lab technicians are trained in the methods and techniques for common tissue dissociation the device doesn't need to be overly simplistic. This category received the lowest rating for the design matrix (15/100).

Based on the criteria outlined above, the three potential designs were evaluated as shown in Figure 6. The modification of the current design received the highest rating in ease of use since it would require minimal change in the current techniques that the lab technicians already use. This device received low ratings in fabrication since the team had many variables to consider when deciding where the current design fails and due to the difficult task of manufacturing its complex components. Therefore the team also believed its performance would be low since the current device fails and the team lacked confidence that changes they could make would drastically improve its performance. The cost would likely be very similar to the current device, but would most likely not improve the cost/usage; therefore, the design received an average rating in this category.

The microfluidic design received the highest rating in three categories. Based off of the study mentioned earlier, the team predicted that this design would perform very well with the dissociation of the tissue in this project. Due to the team's knowledge of creating a similar device, the team believes this design will be easy to manufacture and therefore ranked it highly. The cost and usage rating for this device was very high due to the inexpensive cost of the material. This device would most likely be used on a one time basis, but nevertheless the team still predicted the device would cost under $1.

The screw design will have a fabrication procedure similar to that of the remodel of the current device, but would be more simplistic and obtainable for the team to create. This device would either be similar in cost to the existing device for a one time usage or would be made to last for multiple uses, ideally at a scaling factor of the cost of one use for the disposable device. This device would require slightly more of the technician to utilise than the modification device, since a few procedural steps would be added. The client seemed slightly opposed to this design, since it resembles something used to lyse cells. While that is possible, the rotational speed and gap sizes would be adjusted to prevent lysing of cells while still dissociating them. However, since this type of mixing head is normally used with viscous fluids to separate particle aggregates, it could be possible that this design (using PBS or another cell medium) would not generate significant enough shear forces at a low rotational speed to dissociate cells without lysing them.

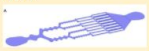
Design Idea:	Modification of current design 	Microfluidic 	Screw Device 
Performance (40)	24 (3/5)	**32 (4/5)**	24 (3/5)
Ease of fabrication (25)	10 (2/5)	**20 (4/5)**	15 (3/5)
Cost/usage (20)	12 (3/5)	**20 (5/5)**	12 (3/5)
Ease of use (15)	**15 (5/5)**	9 (3/5)	12 (4/5)
Total (100)	61	**80**	63

Figure 6. Preliminary design evaluation matrix. This matrix compares the three preliminary designs using criteria outlined in the preliminary design specifications.

Proposed Final Design

After comparing the three preliminary designs using the matrix above and consulting with the client, the team decided that the microfluidic device was best suited solution for the engineering problem. The team believes this design sufficiently meets all the criteria addressed in the preliminary design specifications and can work optimally to suit the client's needs.

V. Fabrication

Materials

It was determined that the most feasible method of fabrication would be to use a 3D printer. Therefore, the material needed to be not only biocompatible, but also 3D printable. Using the normal 3D printing method (fused deposition modeling, or FDM) the material must be melted past its glass transition temperature to be deposited layer by layer. In order for this to be feasible, the glass transition temperature must be relatively low: 100-150° C. Because of this, the design created with traditional 3D printing methods cannot be autoclavable. However, using a different 3D printing method called stereolithography (SLA) the plastic could be cured with UV light, rather than by cooling past its glass transition temperature. With the that printing method, the device could be autoclavable. The SLA process was chosen for this design, since, with a photosensitive resin, the device may be reused, and the plastic would still remain biocompatible. However, in order to make testing more feasible, the prototype utilized by the team was fabricated from PLA. The lid was designed out of an acrylic sheet. A detailed accouning sheet is provided in **Appendix E**.

Methods

The device was primarily fabricated via SLA 3D printing as mentioned above. For prototyping, the design was printed via traditional FDM since it was quicker and had higher availability. During testing, the team realized that a hole for drainage was needed in the end of the device. This was accomplished with a drill press in the student shop. Next, the lid to the design was fabricated out of a sheet of acrylic and cut using a bandsaw in the student shop. The lid was then sanded down to its final size using a belt sander, and the holes were drilled using a center drill and a 13mm drill bit on the drill press.

Once the design was fabricated, a rubber gasket was fitted around the outside of the design. This was done using a rubber splicing kit purchased with the budget. The rubber gasket was sized properly and then superglued on and allowed to dry.

Final Prototype

Figure 7: (Left) Initial device in blue compared with two final prototypes in clear and white. White was used for testing. Lids and adaptor are shown as well. (**Middle**) Final design with tubing adaptor and lid with rubber sealant used for testing. **(Right)** Testing set up with peristaltic pump, clamps for holding device shut, and tubing.

The final design consists of splitting microfluidic channels with decreasing sizes. The tissue is placed in the opening port of the device and the device is hooked up via tubing to a peristaltic pump as seen in Figure 7 and 8. The tissue is then forced through the channels by PBS at a volumetric flow rate of 1mL/s. The combination of the incubation of the tissue in collagenase and the resultant viscous forces in the flow separate the cells from the ECM.

In order to determine the velocity and shear stresses for a given volumetric flow rate, the equations of motion were used, assuming isothermal flow. However, the math was very challenging to solve, so the flow was simplified to parallel plate pressure driven flow, and a macroscopic balance was used for approximation (see Appendix B2). Finally, finite element analysis (FEA) was performed on the device as a whole to find the velocity, shear and pressure distributions across the device.

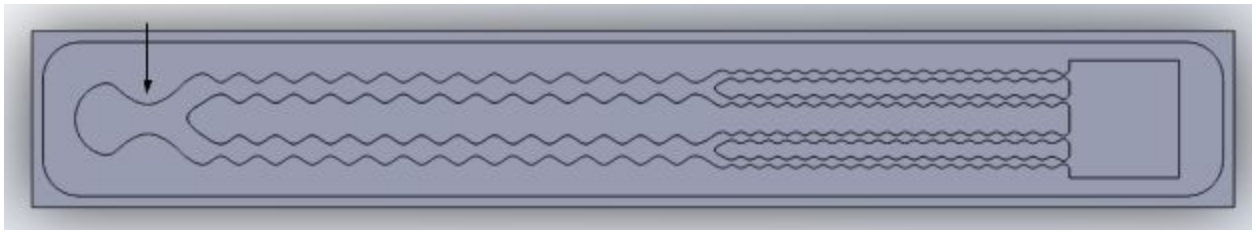


Figure 8: Solidworks model of the final design. Tissue is inserted where indicated by the arrow, then it makes its way from left to right down the progressively skinnier channels until it is collected in the chamber at the end

VI. Testing

In order to analyze the accuracy of the device and fulfill the product design specification of 50% (+/- 10%) cell dissociation and compare with the client's current procedure, the team conducted multiple rounds of testing, both with and without the device and enzymes. Since cell surface markers are altered by the presence of most enzymes, the device would ideally be used without the presence of enzymes or in the presence of a low concentration of an enzyme solution. The client had reported to the team that they were unable to dissociate any viable cells with their current method so the team did not conduct any testing with the gentleMACS™ device.

The team initially tested a procedure that included only enzymatic digestion. The tissue sample was incubated in both a Collagenase G solution and a PBS solution. Both trials were compared side by side with tissue taken from the same lung sample to observe the effectiveness of the enzymatic process in digesting extracellular matrix. As no mechanical dissociation was used, the incubated tissue immediately underwent filtration and the slides were analyzed for presence of effectively dissociated cells, see Appendix C for a complete procedure.

Following the results of enzymatic testing and discussion, the team decided to explore cutting the tissue into smaller pieces before it underwent enzymatic digestion. This theoretically would create a larger surface area with which the enzymes could react. The team tried various methods of cutting via a needle and scalpel but found that the tissue was tacky and difficult to partition. During the process the tissue also started to lose moisture changing its physical properties.

The first round of testing the device proved to be challenging for the team. In conceptual formation of the device, it was thought that a seal was necessary for accurate dissociation. The team began testing the seal for the device by running a PBS solution through the loading site. Since a complete seal had not been developed for the insertion of tissue through the loading site, the team improvised by using a pipette tip cut with a razor blade. Since the rubber seal only lined the outside of the device, the stained PBS solution flooded the empty space between the device and its clear top. The paper clamps the team used to hold the device did not compress the rubber enough to create a tight seal and leaking insued. The PBS solution also did not completely fill the channels as required. The team then refabricated the lid with an appropriate hole size, refabricated the device to accommodate for closer seal to the channels, and 3D printed an adapter to fit in the hole. The adapter was superglued to the hole. A hole was drilled at the base of the collecting duct and an adapter was superglued to the other side. After these modifications to the device, an orange stained PBS solution was run through the device and observed for effectiveness of seal.

Tissue incubated in collagenase G and PBS solution were both run through the device with channels not completely filled dropwise via the peristaltic pump. Tissue incubated in collagenase G and PBS solution were both run through the device with channels completely

filled with PBS and sealed with silly putty. Tissue was run through both the completely filled and partially filled channels at a flow rate of approximately 1ml/3 seconds or 1L/5 min.

In order to analyze the results the team imaged the slides on a fluorescent microscope at 40X magnification and then used thresholding in imageJ to process the images.

VII. Discussion of Results

For each round of testing, slides were created from the cells recovered from each method (See Figures 9-12). The team conducted a two factor study with four treatment conditions; the method without the device using collagenase solution for incubation, the method without the device using PBS for incubation, the method with the device using collagenase solution for incubation, and the method with the device using PBS for incubation. The slides were then analyzed for number of cells using the particle counter in ImageJ. Results of this analysis can be found in Figure 13.

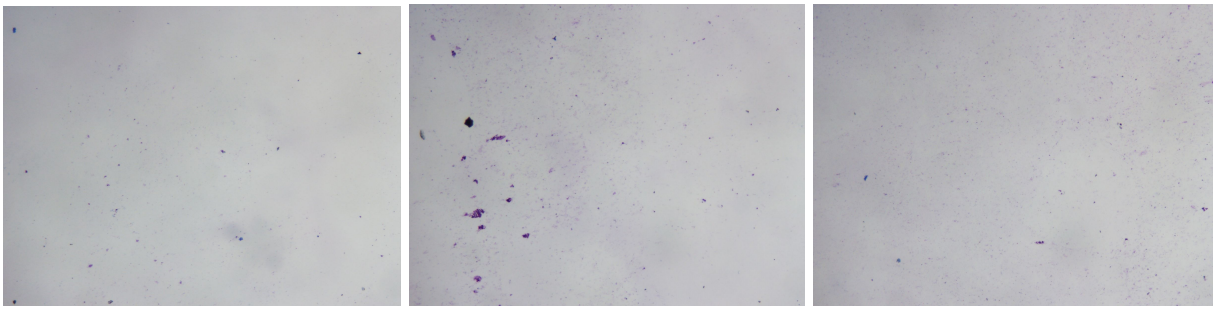


Figure 9: Microscopic images at 40x of slides created from cells recovered from the dissociation attempts using the method *with the microfluidic device after treatment in collagenase solution.*

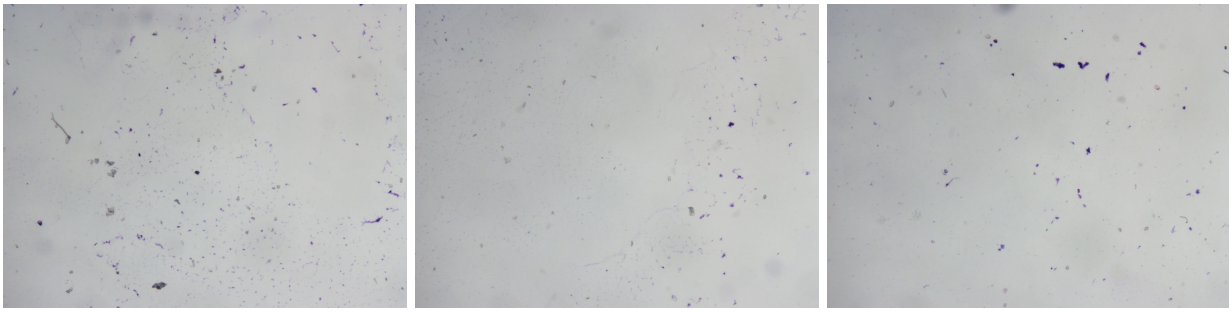


Figure 10: Microscopic images at 40x of slides created from cells recovered from the dissociation attempts using the method *without the device after treatment in collagenase solution*.

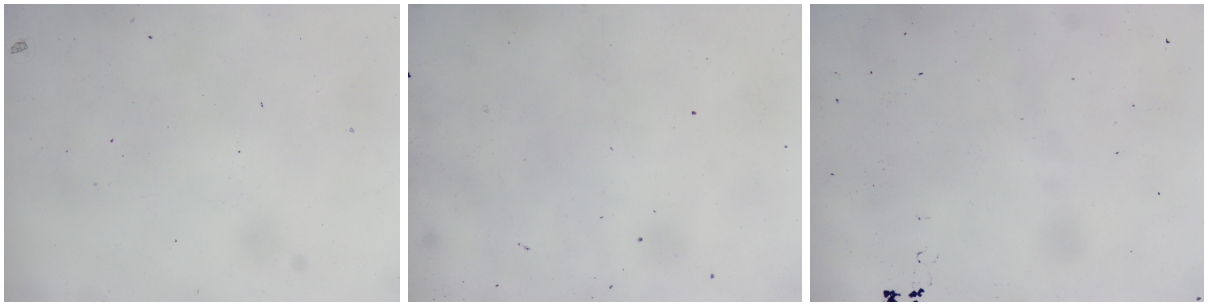


Figure 11: Microscopic images at 40x of slides created from cells recovered from the dissociation attempts using the method *with the device after treatment in PBS solution.*

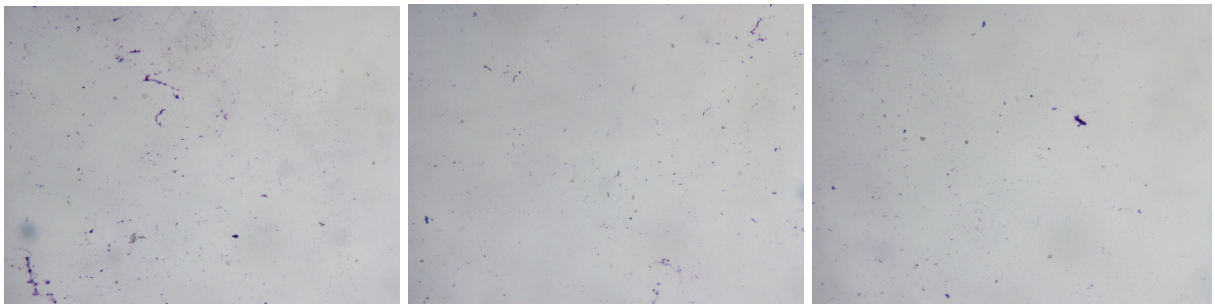


Figure 12: Microscopic images at 40x of slides created from cells recovered from the dissociation attempts using the method *without the device after treatment in PBS solution.*

Cell Count			
Condition 1: Device + Collagenase	**Condition 2: No Device + Collagenase**	**Condition 3: Device + PBS**	**Condition 4: No Device + PBS**
146	410	48	360
502	226	73	237
395	182	89	158
Average: 347.67	Average: 272.67	Average: 70	Average: 251.67

Figure 13: Chart of results of cell counting using ImageJ

ANOVA analysis was conducted on the entire data set. The p-value for this analysis is 0.185, which reveals that the data was not statistically significant. In other words, there is not enough evidence to determine if the use of the device helped dissociate the cells any better than the method without the device.

The team was most interested in comparing the method using the device with collagenase solution (Condition 1) with the method without the device with collagenase solution (Condition 2). Statistical analysis was used to compare Condition 1 and Condition 2, and a graph of these comparisons can be found in Figure 14. A t-test was conducted to determine if the means were statistically significant, however the p-value of 0.595 revealed that they were not. In other words, there was not enough evidence to determine if the use of the method with the device helped dissociate the cells any better than the method without the device for the collagenase condition.

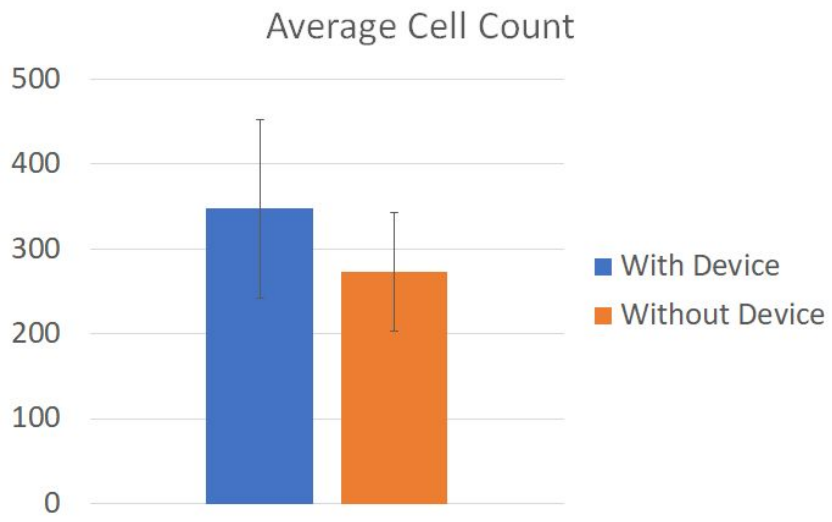


Figure 14: Graphical depiction of the comparison of the means of Condition 1 and Condition 2

Another testing method the team conducted was to try to dissociate tissue without the device. The team tested this method because if successful, it would be simpler and cheaper than any other proposed method. This method involved cutting the sample with a scalpel before putting it in Collagenase and PBS (refer to appendix D). However, this method produced no viable cells or results.

VIII. Future Work

Additional work for the project is needed in order to create a functioning, marketable product to be distributed to customers. These changes will focus in two specific areas: modifications to the current device and further testing to be done under altered conditions. First, focus will be directed to the devices specific changes.

In order to correct the leakage observed during a few of the tests, connection between the device and the lid must be modified. Currently, the rubber seal used is on the outside of the device, and by shifting the rubber closer to the channels, space can be eliminated between the device and cover locations where water was collecting. To fabricate this, the team proposes creating a groove on the device which will wrap around the channels. The device can then be sealed off with a thin length of rubber. The image below shows this proposed design.

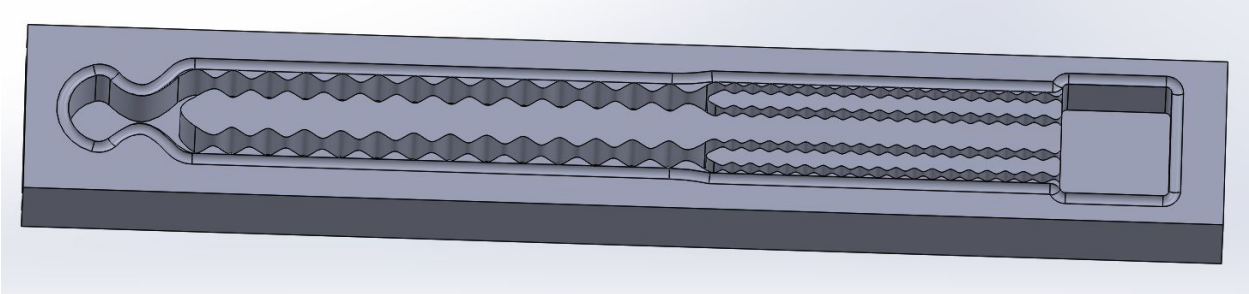


Figure 15: Solidworks model of the proposed next design.

Another modification necessary for the connection of the device to the lid is the design of an ameliorated lid which could straightforwardly attach to or detach from the device. During project testing, the lid was attached with paper clips to create a simple clamp, but this would be modified before the device was integrated in labs. In the future, the lid design should be designed to screw on, clamp, or attach to the device by some other means. The device cover must also be transparent so users can easily view the progress of the fluids and tissue going through.

In order to dissociate small tissues further, the width of the smallest channels should be thinner. Currently, they are printed as 640 micrometers in width. Ideally, to achieve the desired flow rate and shear force to dissociate the small samples, they should be 200 micrometers wide. In this project, the team was limited to the capabilities of the 3D printers in the MakerSpace, which couldn't produce the desired accuracy at that microscale. In the future, the team should explore the use of more accurate 3D printers, or other fabrication methods such as laser printing or micromilling.

The rest of the future work is related to future testing, and the testing environment. The first improvement in this area is to test with a different pump. The peristaltic pump used in testing created a fluctuating flow through the device, which affected the shear forces experienced by the tissue, and therefore the results of the experiment. If the team could find a pump with a consistent flow rate, that would eliminate discrepancy in the shear force.

Further testing should also be done when the project is continued. In order to achieve more statistically significant results, the number of test samples should be increased. Since it is the desire of Dr. Mathur to use the device on both normal and inflamed tissue from patients with asthma, the device should also be tested with the tissue following an induced asthmatic reaction to determine if it dissociates in a similar fashion and with comparable effectiveness to normal lung tissue. Lastly, the device should be tested while running tissue without collagenase. If the device was capable of producing cells without the help of enzymes, it would not only improve the cell-surface markers being analyzed during flow cytometry, but would also save the labs money since they would no longer need to purchase the expensive enzymes.

IX. Conclusions

The use of tissue biopsies is an important aspect in the field of asthma research. Tissue dissociation is used to analyze, via flow cytometry, the cellular makeup of the tissues. The client, Dr. Sameer Mathur supplied the team with the task to create a device that would allow his research team to dissociate tissues of 1-2 square millimeters instead of the standard 3-4 square millimeters. After analysis of design specifications the team was able to develop three possible designs and chose a microfluidic device as the best design to fit the criteria. The microfluidic device utilizes a set of channels progressively decreasing in size as well as pressurized fluid to force tissue samples that are in solution through channels with smooth constrictions. The team believed that this device would allow the client to achieve his 50% viable cell count based on results seen in a similar study. The team moved forward with the microfluidic design and began material selection and fabrication of the design using 3D printers. Prototypes were modified and tested in Dr. Mathur's lab, and the team used a peristaltic pump to generate the flow rate within the device. Biopsy sized tissue samples were taken and tested under a variety of conditions, with and without the device and enzymes. The results of these tests were imaged and analyzed using ImageJ, so that statistical analysis could be run. Although the numerical data was not significantly significant, the device has potential to achieve the original goal of dissociation with several design modifications the team can implement in the future.

X. References

[1] Nigms.nih.gov. (2017). *Studying Cells - National Institute of General Medical Sciences*. [online] Available at: https://www.nigms.nih.gov/Education/Pages/FactSheet_Cells.aspx [Accessed 09 Oct. 2017].

[2] University Of Wisconsin - Department of Medicine. (2017). *Asthma: UW Research and You*. [online] Available at: https://www.medicine.wisc.edu/asthma/asthmamain [Accessed 10 Oct. 2017].

[3] Hopkinsmedicine.org. (2017). *Lung Biopsy | Johns Hopkins Medicine Health Library*. [online] Available at: http://www.hopkinsmedicine.org/healthlibrary/test_procedures/pulmonary/lung_biopsy_92,P07750 [Accessed 10 Oct. 2017].

[4] Miltenyibiotec.com. (2017). *gentleMACS™™ Dissociator - Miltenyi Biotec*. [online] Available at: http://www.miltenyibiotec.com/en/products-and-services/macs-sample-preparation/tissue-dissociators-and-tubes/gentleMACS™-dissociator.aspx [Accessed 10 Oct. 2017].

[5] J. R. Murdoch and C. M. Lloyd, "Chronic inflammation and asthma," *Mutation Research*, 07-Aug-2010. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2923754/. [Accessed: 05-Oct-2017].

[6] "Lung Biopsy," *Lung Biopsy | Johns Hopkins Medicine Health Library*. [Online]. Available: http://www.hopkinsmedicine.org/healthlibrary/test_procedures/pulmonary/lung_biopsy_92,P07750. [Accessed: 05-Oct-2017].

[7] "Introduction to flow cytometry," *Flow cytometry introduction | Abcam*, 06-Oct-2017. [Online]. Available: http://www.abcam.com/protocols/introduction-to-flow-cytometry. [Accessed: 05-Oct-2017].

[8] Dr. R-P. Peters, Dr. E. Kabaha, W. Stoters, G. Winkelmayer and F. Bucher, "Device for fragmenting tissue," European Patent Specification #EP2540394B1, May 05th, 2016

[9] Qiu, X., De Jesus, J., Pennell, M., Troiani, M. and Haun, J. (2017). Microfluidic device for mechanical dissociation of cancer cell aggregates into single cells. [online] PubMed.gov. Available at: https://www.ncbi.nlm.nih.gov/pubmed/25377468 [Accessed 10 Oct. 2017].

XI. Appendix

Appendix A: Product Design Specifications

Microscale Tissue Biopsy Device
Product Design Specifications
2017/12/13

Raven Brenneke, Jamison Miller, Nathan Richman, Lauren Ross, Victoria Trantow, Cory Van Beek

Function: To dissociate cells from small lung biopsy sample. The design must produce a measurable amount of viable cells for flow cytometry.

Client Requirements:

- Dissociate cells from small tissue samples from asthma patients for the duration of the asthma research study
- Must be able to recover cells with minimal disruption so that the cells can be run through a flow cytometer.

Design Requirements:

1. Physical and Operational Characteristics

a. Performance Requirement: The device should successfully dissociate tissue samples with 50% ± 10% cell recovery. The device will be used daily by lab technicians using sterile techniques to load tissue and unload cells.

b. Safety: The device must be sterile and protect the lab tech from possible contamination due to the use of human tissue samples. The device should also be able to withstand spills and drops without shattering or breaking into sharp shards.

c. Accuracy and Reliability: The device must yield at least 50% (+/- 10%) cell recovery from each sample of tissue. It needs to reliably dissociate the tissue sample to completion.

d. *Life in Service*: Life in service will depend on whether or not the device is reusable. If it is reusable it needs to last enough runs so that the cost per use is about $5-$10.

e. *Shelf Life:* Shelf life will also depend on whether the device is reusable or not. If the device is not reusable then the device will be used once and then thrown away. If it is reusable, it should be able to be used as many times as possible to make the cost come to $5-$10 per use.

f. *Operating Environment*: The device will be used in a laboratory setting. During use, the device will be filled with various enzyme-containing solutions including collagenase, sterilization agents, and possible high temperatures and pressures present in an autoclave.

g. *Ergonomics*: The device must be simple for lab techs to control. This includes being able to easily load a sample into the microfluidic device and unload the output from it.

h. *Size*: The device should be capable of dissociating a tissue sample size of 1-2mm. The overall size of the device is not of huge concern as long as it is able to perform successfully.

i. Weight: The weight of the device is currently not applicable to the design criteria given by the client. The microfluidic device is small enough that weight will not be a factor in usability.

j. *Materials*: The material for the device must be cheap enough to obtain the goal of the cost per run being $5-$10. The material should be able to be 3D printed and will need to not induce any inflammatory reaction with the cells. The current material used is PLA and ABS.

k. *Aesthetics*, *Appearance, and Finish*: The device must be simple and not confusing to use. The specific aesthetics and appearance of the final product is not of large concern as long as the device functions properly.

2. Production Characteristics

a. *Quantity*: The client initially requested one device to be manufactured for use, although an additional device may be requested later on.

b. *Target Product Cost*: The initial budget for this project is $300 dollars per device. The cost to manufacture the device on the 3D printer will be determined at later time depending on the type and volume of material selected. The existing device is non-reusable and costs roughly $10 per cap with the tubes accompanying the device costing $6 per tube[1]. The target cost of the microfluidic device is $5-$10 per use.

3. Miscellaneous

a. *Standards and Specifications*: This is a custom device being used in a research setting; there are no international or national standards to abide by.

b. *Customer*: The customer would prefer to have a removable lid on the device in order to remove potentially valuable tissue samples if the device does not run correctly.

c. *Patient-related concerns*: Patients will not be using this device; it will be used in a research setting. There is no storage of patient data incorporated in this device and the devices should be sterile with every use.

d. *Competition*: A current device for tissue dissociation is made by Miltenyi that includes a tube cap with an attached grinding component that is compatible with a machine, gentleMACS™, that initiates the grinding of the tissue. This device is currently used by the client although since their tissue sample size is very small it is unable to be properly dissociated by the larger device [3].

PDS References:

1. Miltenyibiotec.com. (2017). *gentleMACS™ M Tubes - Miltenyi Biotec*. [online] Available at: http://www.miltenyibiotec.com/en/products-and-services/macs-sample-preparation/tissue-dissociators-and-tubes/gentleMACS™-dissociators/gentleMACS™-m-tubes.aspx [Accessed 21 Sep. 2017].
2. Thermofisher.com. (2017). *Nunc™ 15mL & 50mL Conical Sterile Polypropylene Centrifuge Tubes*. [online] Available at: https://www.thermofisher.com/order/catalog/product/339650?SID=srch-srp-339650 [Accessed 21 Sep. 2017].
3. R.-P. D. Peters, E. D. Kabaha, W. Stöters, G. Winkelmayer, and F. G. Bucher, “Device for fragmenting tissue,” EP 2 540 394 B1, 2016.

Appendix B1: Calculations of Shear Forces in gentleMACS™ Device

1 Part Description

The current device which is shown below consists of a rotor and stater, the rotor is rotated by the gentleMACS dissociator. The design of the part with various pieces in the rotor and stater designed to create turbulent flow is difficult to model.

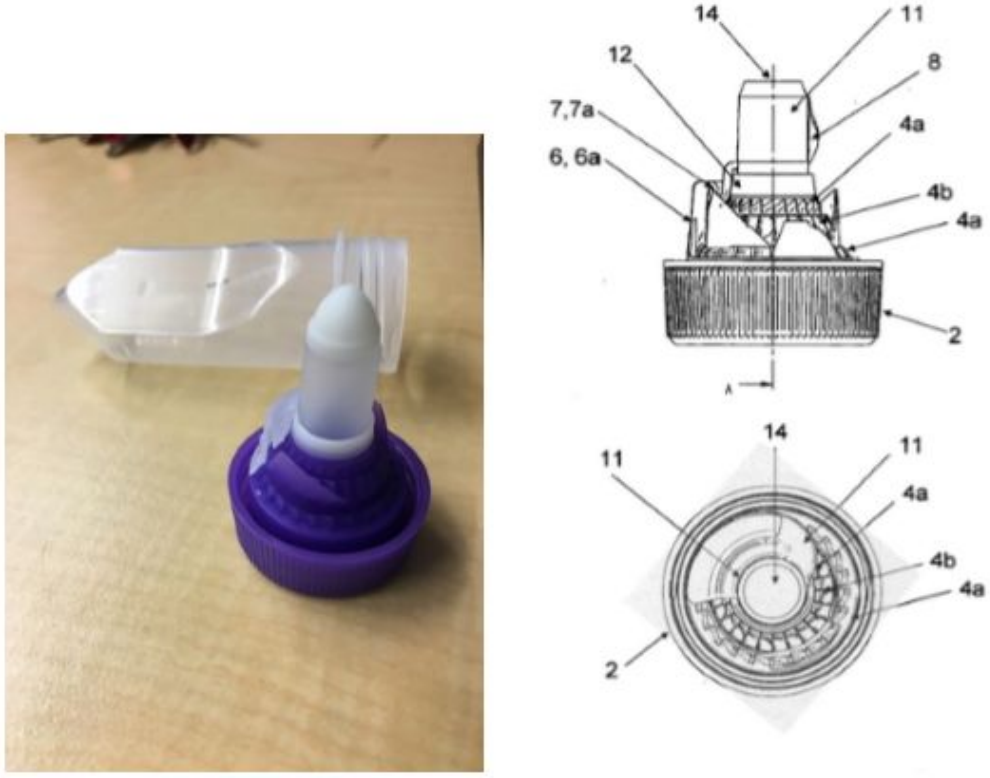


Figure 1: Device and its schematic: **Left**: Image of device. **Right**: Schematic of device. Rotor can be seen as white plastic on the image and 11 on the schematic. Stater is the purple plastic and consists of 2,4ab, 6, 6a, 7 and 7a.

The rotor consists of an agitator that starts turbulent flow (8 in the schematic) and a piece that generates significant shear flow over two taller stators (6,6a and 7,7a). Since shear is the most significant

part of the mechanical dissociation, we will simplify the rotor and stator to two concentric cylinders.

1.1 Simplified Model

In the simplified model, it will be much easier to calculate the shear forces the mechanical dissociator generates.

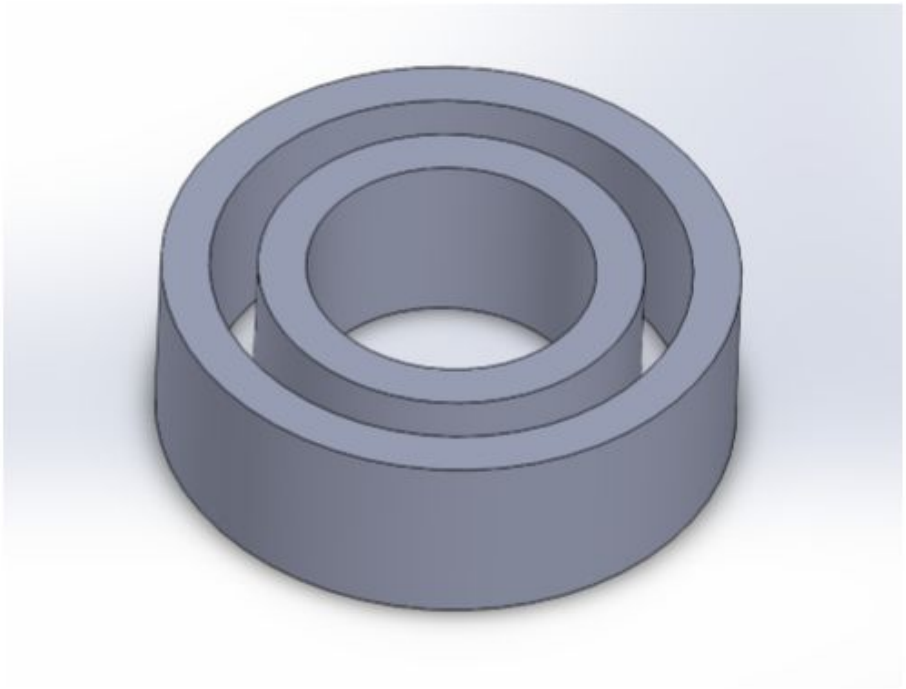


Figure 2: Simplification of device that makes it a simple rotor and stator with one stationary cylinder and one cylinder rotating with a given angular velocity.

2 Calculations

We can assume that v_θ is a function of r and that $v_r = v_z = 0$. Given this we find that the equation of continuity simplifies to 0 on both sides. We can further assume that pressure is a function of z, due to gravity and r, because of centrifugal force. In that case the r, θ and z components of the equation of motion for a newtonian fluid with constant ρ and μ simplify to:

$$-\rho\frac{v_\theta^2}{r} = -\frac{\partial p}{\partial r} \tag{1}$$

$$0 = \frac{d}{dr}\left(\frac{1}{r}\frac{d}{dr}\left(rv_\theta\right)\right) \tag{2}$$

$$0 = -\frac{\partial p}{\partial z} - \rho g \tag{3}$$

for the r, θ, and z components, respectively. We can find v_θ from equation 2 if we integrate:

$$c_1 = \frac{1}{r}\frac{d}{dr}\left(rv_\theta\right) \tag{4}$$

If we then multiply by r and integrate again we get the following:

$$v_\theta(r) = \frac{1}{2}c_1 r + \frac{c_2}{r} \tag{5}$$

If we substitute in the no-slip boundary conditions at $r = \kappa R, v_\theta = \Omega_i \kappa R$ and at $r = R, v_\theta = 0$ we get the velocity and shear stress distribution of:

$$v_\theta(r) = \Omega_i \kappa R \frac{\frac{R}{r} - \frac{r}{R}}{\frac{1}{\kappa} - \kappa}$$

$$\tau_{r\theta}(r) = 2\mu\Omega_i \left(\frac{\kappa^2}{1-\kappa^2}\right)\left(\frac{R}{r}\right)^2$$

These equations can be used to calculate the specific shear felt by the cells in the chamber if we rotate the rotor at a given Ω_i. These will allow us to further develop design idea 1.

Appendix B2: Calculations of Shear Forces in Final Design

1 Microscopic analysis

1.1 Narrow Region

Using the navier-stokes equations, we can simplify the x and y components of the momentum balances in Figure 1 with the assumptions that we are at steady state (1), the velocity is a function of x and y only (2) and that the liquid has a constant viscosity and density (3), i.e. isothermal system:

$$\rho\left(v_x\frac{\partial v_x}{\partial x}+v_y\frac{\partial v_x}{\partial y}\right)=-\frac{\partial p}{\partial x}+\mu\left[\frac{\partial^2 v_x}{\partial x^2}+\frac{\partial^2 v_x}{\partial y^2}\right]$$
$$\rho\left(v_x\frac{\partial v_y}{\partial x}+v_y\frac{\partial v_y}{\partial y}\right)=-\frac{\partial p}{\partial y}+\mu\left[\frac{\partial^2 v_y}{\partial x^2}+\frac{\partial^2 v_y}{\partial y^2}\right]$$

These PDE's are very difficult to solve simultaneously, even with the relations given above by the continuity equation, therefore for particular velocities and stresses, we will need to use finite element analysis.

Simplification Because the above PDE's are too difficult to solve for velocity profiles, we can make more simplifications and look at an area where the flow is approximately pressure driven flow between two plates. Therefore we can assume that the velocity is in the x direction, and that it is a function of y. Pressure however, is still a function of x, but we only need to solve the x-component of the momentum balance, which simplifies to:

$$\frac{dp}{dx}=\mu\left(\frac{d^2 v_y}{dy^2}\right) \tag{1}$$

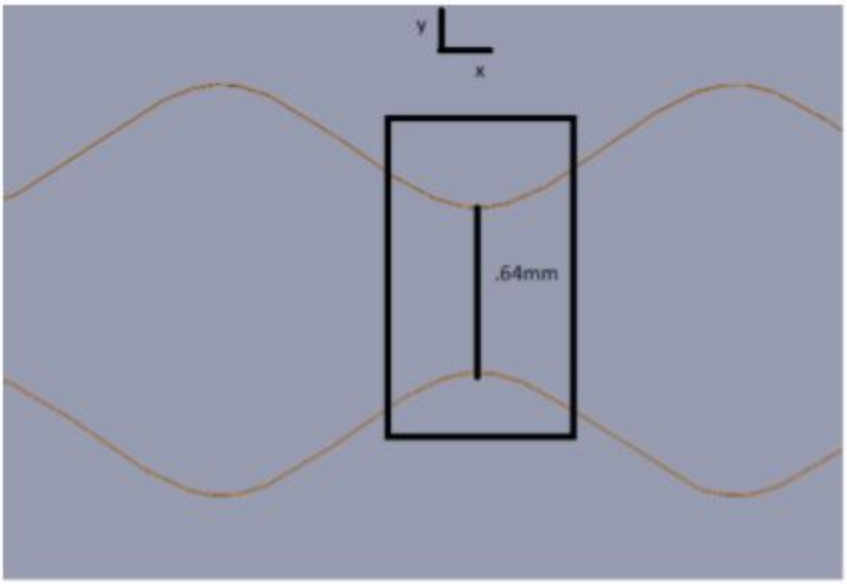


Figure 1: Illustration of narrow section of channel. The curved narrow region can be approximated as a small region of pressure driven flow between two flat plates.

Since we have a two differential equations with respect to 2 different variables, x and y, they must be equal to a constant of separation, B.

$$\frac{dp}{dx} = \mu\frac{d^2v_x}{dy^2} = B$$
$$\frac{dp}{dx} = B$$
$$\mu\frac{d^2v_x}{dy^2} = B$$

We can then separate the equations and solve the pressure equation to find B:

$$\int_{p_0}^{p_1} dp = B\int_{x_0}^{x_1} dx$$
$$\frac{(p_1 - p_0)}{L} = B$$

We can then substitute B back into the equation for v_x

$$\frac{d^2v_x}{dy^2} = \frac{(p_1 - p_0)}{\mu L}$$
$$v_x(y) = \frac{(p_1 - p_0)}{\mu L}y^2 + c_1y + c_2$$

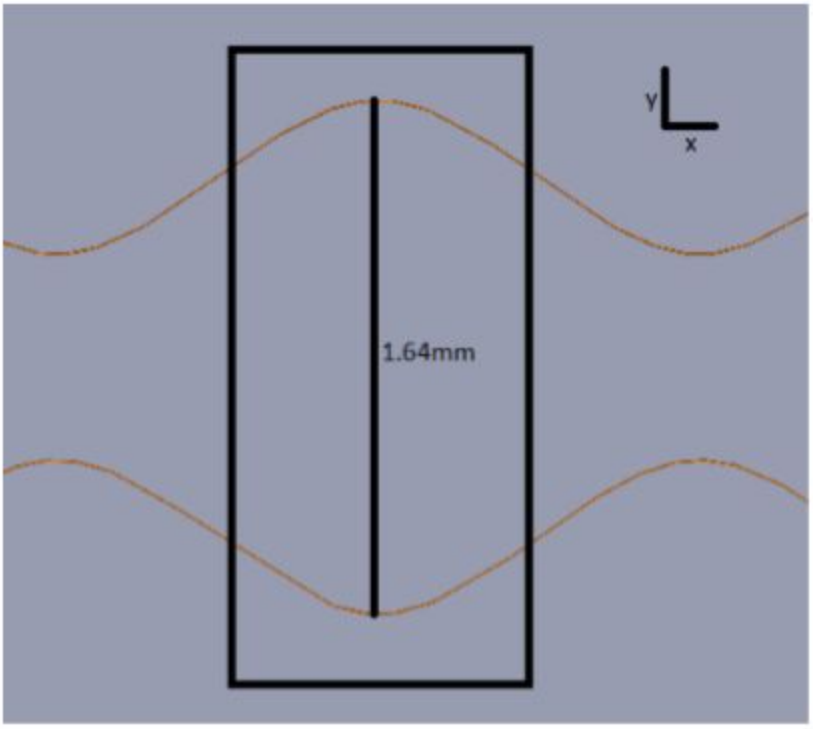


Figure 2: Illustration of narrow section of channel. The curved narrow region can be approximated as a small region of pressure driven flow between two flat plates.

Applying the boundary conditions $v_x(-h) = 0$ and $v_x(h) = 0$, where thickness is 2h we can solve for the velocity profile. We can then apply Newtons law of viscosity $\tau_{yx} = -\mu\frac{\partial v_x}{\partial y}$ to solve for the shear stress distribution:

$$v_x(y) = \frac{(p_0 - p_1)h^2}{2\mu L}\left(1 - \left(\frac{y}{h}\right)^2\right) \tag{2}$$

$$\tau_{yx} = \frac{(p_0 - p_1)}{L}y \tag{3}$$

1.2 Wide Region

The same problem fan be formulated for the wide region of the channel, Figure 2 The only difference in solution would be the value of h in the above equation.

1.3 Comparison

In order to compare the wide and small sections we need to find their volumetric flow rate. The flow rate can be found by integrating the velocity

profile along a characteristic cross-sectional area.

$$Q = \iint v_x(y)dA = 2 * \int_0^D \int_0^h \frac{(p_0 - p_1)h^2}{2\mu L}\left(1 - \left(\frac{y}{h}\right)^2\right) dydz \tag{4}$$

$$Q = \frac{2D\Delta p h^2}{2\mu L}\left[y - \frac{y^3}{3h^2}\right]_0^h \tag{5}$$

$$= \frac{2D\Delta p h^2}{2\mu L}\left[h - \frac{h^3}{3h^2}\right] \tag{6}$$

$$= \frac{2D(\Delta p)h^3}{3\mu L} \tag{7}$$

With a Q of 1mL/s we can then analyze the pressure needed to create these flows, as well as the maximum velocities and shear stresses:

Wide section:

$$1 * 10^{-6}m^3/s = \frac{2 * .010m * (\Delta p) * (.00082m)^3}{3 * 1 * 10^-3Pa * s * .001m}, \Delta p = .272Pa \tag{8}$$

$$v_{max} = \frac{(\Delta p)h^2}{2 * \mu L} = \frac{.272Pa * (.00082m)^2}{2 * 1 * 10^-3Pa * s * .001m} = .182m/s \tag{9}$$

$$t_{xz}^{max} = \frac{\Delta p * y}{L} = \frac{.272Pa * .00082m}{.001m} = .223N/m^2 \tag{10}$$

Thin section:

$$1 * 10^{-6}m^3/s = \frac{2 * .010m * (\Delta p) * (.00032m)^3}{3 * 1 * 10^-3Pa * s * .001m}, \Delta p = 4.577Pa \tag{11}$$

$$v_{max} = \frac{(\Delta p)h^2}{2 * \mu L} = \frac{4.577Pa * (.00032m)^2}{2 * 1 * 10^-3Pa * s * .001m} = .234m/s \tag{12}$$

$$t_{xz}^{max} = \frac{\Delta p * y}{L} = \frac{4.577Pa * .00032m}{.001m} = 1.46N/m^2 \tag{13}$$

The thin section requires a much larger pressure difference to create the same volumetric flow rate, which is expected. This causes the v_{max} to be larger as well as the maximum shear stress.

2 Macroscopic balance

We can approximate flow rate and velocities at entrance and exit by using macroscopic balances. The macroscopic mass, momentum and mechanical energy balances are shown below for an isothermal system:

$$\sum\left(\rho_1 \langle v_1 \rangle S_1\right) - \sum\left(\rho_2 \langle v_2 \rangle S_2\right) = 0 \tag{14}$$

$$F_{f\to s} = -\Delta\left(\frac{\langle v^2 \rangle}{\langle v \rangle} + pS\right)\underline{u} + m_{tot}g \tag{15}$$

Figure 3: Macroscopic balance set up. We pick two planes that have the same cross sectional area S. We can then do macroscopic balances on this section of the channel to find forces of the fluid on the solid to approximate shear forces and flow rates.

$$\Delta\left(\frac{1}{2}\frac{\langle v^3\rangle}{\langle v\rangle}\right) + g\Delta h + \int_1^2 \frac{dp}{\rho} = \hat{W}_m - \hat{E}_v \tag{16}$$

Since there are no moving surfaces in this system, $\hat{W}_m$ can be neglected. Secondly since our solution will be water based, we can assume incompressible flow, so that ρ is not a function of pressure, then the integral term becomes $\frac{p_2-p_1}{\rho}$, and finally, since our design does not change in height, we can exclude the gravitational potential energy term in the mechanical energy balance. We assume that the flow is isothermal and that the density does not change. Since we picked planes with equal cross sections, we can see from the mass balance that $\langle v_1\rangle = \langle v_2\rangle = \langle v\rangle$ Then we can further simplify the mechanical energy balance to the following:

$$\frac{\Delta p}{\rho} + \hat{E}_v = 0 \tag{17}$$

We can assume that the shapes in the channel can be approximated by using friction factor terms for sudden contractions and sudden expansions:

$$\hat{E}_v = \sum^{i} 1/2v^2 * (.45(1-\beta)) + \sum^{j} 1/2v^2 * (\frac{1}{\beta} - 1)^2 \tag{18}$$

where β is the ratio of the small to large cross sectional area: $6.4mm^2/16.4mm^2 = .3902$. We can get $\langle v\rangle$ from the analysis above. For the sudden expansions, $\langle v\rangle$ is just the spacially averaged velocity from the thin section, whereas for

the sudden contractions it is the spacially averaged velocity from the wide section. Where $\langle v \rangle$ is given by $2/3 v_{max}$

$$\hat{E}_v = i\left(\frac{1}{2}(\frac{2}{3} * .182\frac{m}{s})^2(.45(1-.3902))\right) + j\left(\frac{1}{2}(\frac{2}{3} * .234\frac{m}{s})^2(\frac{1}{.3902}-1)^2\right)$$
$$= i * .00202m^2/s^2 + j * .0297m^2/s^2$$

In the smallest channel of our design, we have 18 sudden contractions and 18 sudden expansions, we can now use this to calculate the pressure drop along one of these channels.

$$\frac{\Delta p}{1000kg/m^2} = 18 * .00202m^2/s^2 + 18 * .0297m^2/s^2 \tag{19}$$

$$\Delta p \approx 570.96Pa = .083lb_f/in^2 \tag{20}$$

Thus with a flow rate of 1ml/s, the smaller channels would have a pressure drop of about .083 psi

3 Finite Element Analysis

We used FloXpress simulation in SolidWorks to do flow analysis with an entrance volume flow rate of 1ml/s. After choosing appropriate boundary conditions, we can inspect the analysis for velocity and pressure distributions, as well as plots of the surface shear stresses.

3.1 Comparison to my calculations

As can be seen in the figure, I overestimated both the velocities (only slightly) and also the pressure (because it depended on the velocities and I overestimated the friction). The pressure is obviously the biggest difference, from about 500 that I calculated to about 130 that FEA found. This was expected because I approximated each wave shape as a sudden contraction and expansion, but they are not sudden, they are gradual. Therefore I overestimated the friction generated, and therefore the pressure drop.

3.2 Whole design

While the calculations I did earlier are useful for getting a general idea of what flow might be like in the device, the FEA analysis is much better for practically designing the device. We can simulate other flow rates as well and see how that affects the forces and velocities in the channels inside the device.

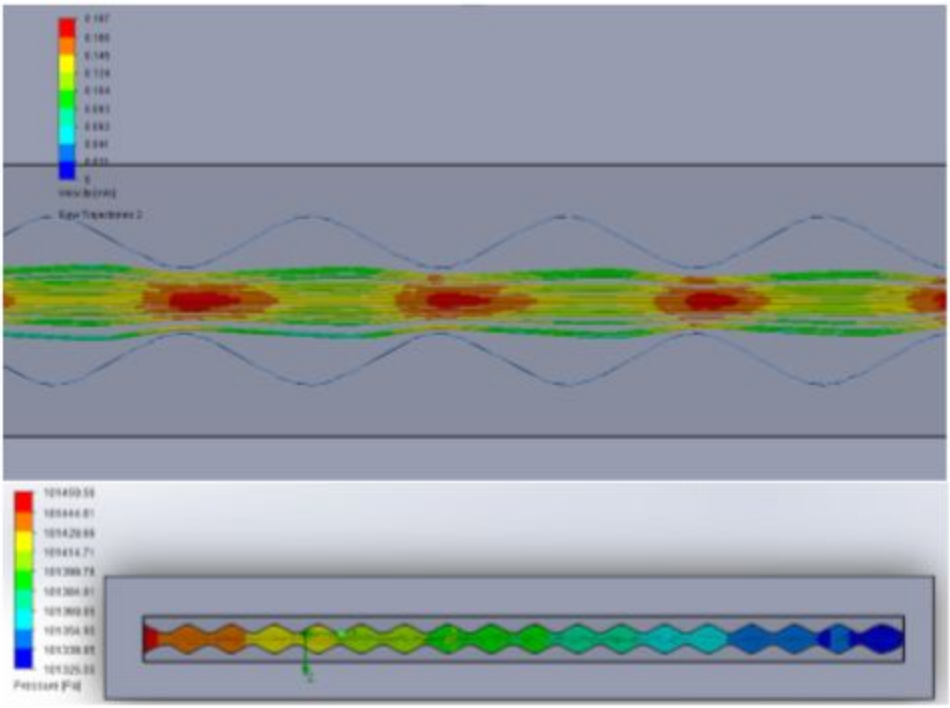


Figure 4: Pressure along flow (top) ranges from 101459.56 Pa to 101325 Pa (atmospheric pressure) making a pressure drop of about 134.5 Pa. This makes sense because my calculations overestimated the friction effects and therefore caused the pressure drop to be larger. Velocity profiles (bottom) shows the velocity in the constriction being about .187m/s and in the wide part about .09m/s. It also makes sense that these values are lower than my predicted values since I also overestimated.

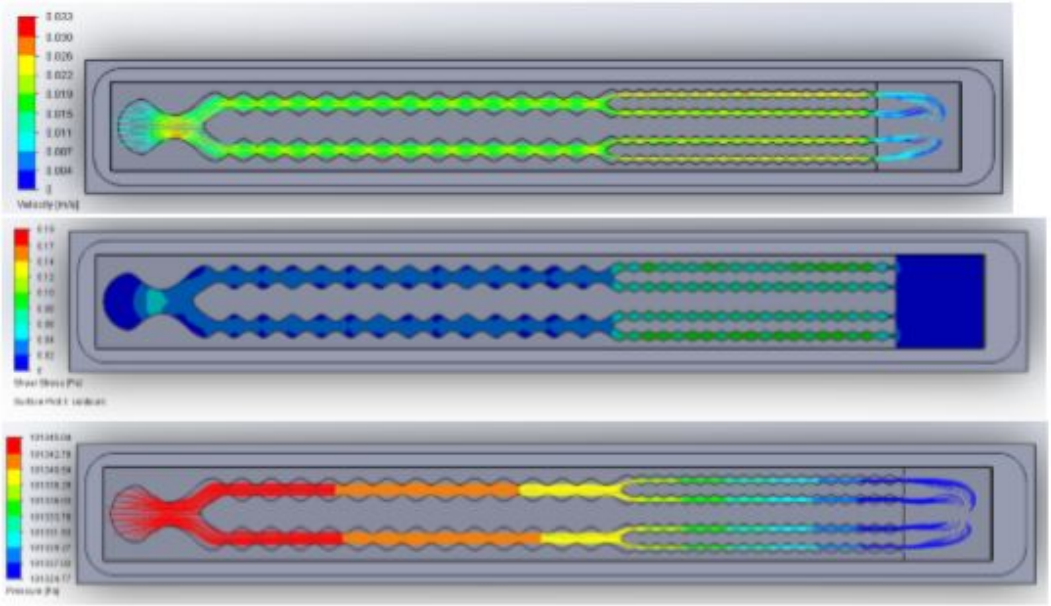


Figure 5: Velocity (top), shear stress (middle) and pressure (bottom) along length of device with 1mL/s flow rate. Velocity ranges from about .03 m/s down to about .01 m/s. Shear stress maximums occur in the final stage of the device with the narrowest channels, with a max of about .11 Pa. And finally, the pressure drop across the device is about 21 Pa

Appendix C: 3D Printing Protocol

1. Open Solidworks file
2. Save as .STL file.
    a. File Save As
    b. Save as *.STL instead of *.SLDPRT
3. Open in either CURA for Ultimaker or PreForm for SLA printer
4. Prepare for printing
    a. Automatically orient design, make adjustments if design in is red, this means it won't fit in the printer.
    b. Automatically generate supports. Density about .8.
5. Send to printer
    a. Either save to flash drive in CURA
    b. Or plug USB into computer and send to printer.

Appendix D: Testing Protocol

Testing Protocol without Device

1. Obtain sample of tissue
    a. Sample is approximately 1.5 inches in diameter (dramatically larger than normal sizes)
2. Take 4 small biopsies with a biopsy tool
3. Prepare two 50 mL conical tubes
    a. The first tube is 1x PBS solution
    b. The second tube has 1x PBS, and 20x Buffer S, Enzyme A, Enzyme D from the dissociation kit that comes with the gentleMACS™ device
4. Add two biopsy samples to each tube
5. Place tubes in an incubator for 30 minutes on a rocking device.
6. Vortex conical tubes for 5 seconds
7. Filter solution with a 50 micron filter
8. Centrifuge tubes
    a. Room temperature, 1300 rpm for 10 minutes
9. Remove supernatant
10. Re-suspend cell solution from the bottom in PBS
11. Load into cytospin device
    a. Centripetal force forced cells onto glass slide
12. Stain slide with HEMA 3

Modified Testing Protocol without the Device

1. Obtain portion of inflamed human lung tissue
2. Retrieve 8 small biopsies of the tissue
3. Prepare four 50 mL conical tubes
    a. The first two tubes are 1x PBS solution
        i. Place two biopsy samples, whole, into one tube (PBS OG)
        ii. Take two biopsy samples and cut them as finely as possible by hand ** before placing in another tube (PBS Mod)
    b. The second two tubes contain 1x PBS (2.28 mL), and 20x Buffer S (120 uL), Enzyme A (15 mL), Enzyme D (100 uL) from the dissociation kit that came with the gentleMACS™ device
        i. Place two biopsy samples, whole, into one tube (Col OG)
        ii. Take two biopsy samples and cut them as finely as possible by hand ** before placing in another tube (Col MOD)

4. Incubate samples in conical tubes for 30 minutes on a gentle rocking device
5. Filter solutions using a 50 micron filter
6. Centrifuge tubes
    a. Room temperature, 1200 rpm for 10 minutes
7. Remove supernatant
8. Re-suspend cell solution from bottom of tube in 200 uL of Hank's Buffer
9. Set aside 50 uL from each tube for viability test
10. Load into cytospin device
    a. Centripetal force forces cells onto glass slide

** Partitioning the biopsy samples

1. Put buffer on petri dish with the samples
2. Using razor blade and needle tip, slice samples with fine precision
3. To transfer cells, use p5000 pipette with wide tip
4. Collect tissue and liquid and transfer to conical tube

Testing Protocol with Device

1. Obtain sample of tissue
    a. Sample is approximately 1.5 inches in diameter (dramatically larger than normal sizes)
2. Retrieve 4 small biopsies with a biopsy tool
3. Prepare two 50 mL conical tubes
    a. The first tube is 1x PBS solution
        i. Add two biopsy samples
    b. The second tube contains 1x PBS (2.28 mL), and 20x Buffer S (120 uL), Enzyme A (15 mL), Enzyme D (100 uL) from the dissociation kit that came with the gentleMACS™ device
        i. Add two biopsy samples, whole
4. Incubate samples in conical tubes for 30 minutes on a gentle rocking device
5. Transfer samples into the port of the microfluidic device using a pipette
6. Set up tubing
    a. Connect tubing from peristaltic pump to the adapting connector on the device
    b. Be sure the device is sealed
7. Run PBS through the device with peristaltic pump at 1L/5 minutes (500 Rabbit setting)
    a. Dissociation process is complete when all fluids and materials are collected in the retrieving well on the opposite end of the device
    b. Optional: run solution multiple times for further dissociation

8. Transfer solution to a conical tube
9. Vortex conical tubes for 5 seconds
10. Filter solution with a 50 micron filter
11. Centrifuge tubes
    a. Room temperature, 1300 rpm for 10 minutes
12. Remove supernatant
13. Resuspend cell solution from bottom of tube in PBS, then load into cytospin device
    a. Centripetal force forces cells onto glass slide
14. Stain with HEMA 3

Appendix E: Project Finances

Date	Item	Description	Merchant	Part #	Qty	Cost Each	**Total**
11/08/17	1/4" x 10' Latex Hose	Connection for device to pump	Menards	6841250	1	$14.99	**$14.99**
11/15/17	Silicon Tubing 3mm x 5mm x 3m	Used for connecting new device to parasteltic pump	Gilson	F117975	1	$49.00	**$49.00**
11/15/17	PVDF Tubing Connectors 10 pk.	Used for connecting new device to parasteltic pump	Gilson	F1179951	1	$38.00	**$38.00**
11/15/17	3mm Silicon Tubing 4 pk.	Used for connecting new device to parasteltic pump	Gilson	F1825114	1	$60.00	**$60.00**
11/19/17	Metric Splicing Kit	Used to create rubber seal	Grainger	1RHA6	1	$66.71	**$66.71**
12/3/17	Silly Putty	Used to seal device to lid	Walmart	552976190	1	$9.41	**$9.41**
12/3/17	Sugru Glue	Used to seal device to lid	Menards	5629019	2	$8.43	**$16.86**
12/3/17	Kwikseal Ultra Clear	Used to seal device to lid	Menards	5638893	2	$5.03	**$10.06**
	Total						**$265.03**
	Note: 3D printing would have cost an additional $15.19 for the semester, but it was free at the MakerSpace.						