Tissue Biopsy Dissociation

BME 200/300

October 11th, 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 cells are then run through flow cytometry to analyze different cellular components. The current device being used for this process, the gentleMACS Dissociation Device, does not allow for use of a small tissue sample size. A small tissue sample size, 1-2 mm, is desired to reduce the recovery time and pain of the patient. The task at hand is to create a dissociation device that will successfully dissociate a smaller tissue sample and yield viable cells to study. The most important criteria considered in the final design is the ability of the design to perform appropriately and yield at least 50% viable cells. A microfluidic device will be used to dissociate this small tissue sample and testing will be conducted to determine the effectiveness of this device.

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 has been a significant gain of 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 smallest 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. 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. 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].

Many 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 then 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 will be to successfully dissociate lung biopsy tissues for asthma research. Though there are different causes of asthma, our client focuses on allergic asthma. Normally harmless airborne allergens trigger an inflammatory response in airways of the lungs, called bronchial tubes. This response is led by T-cell lymphocytes [5].

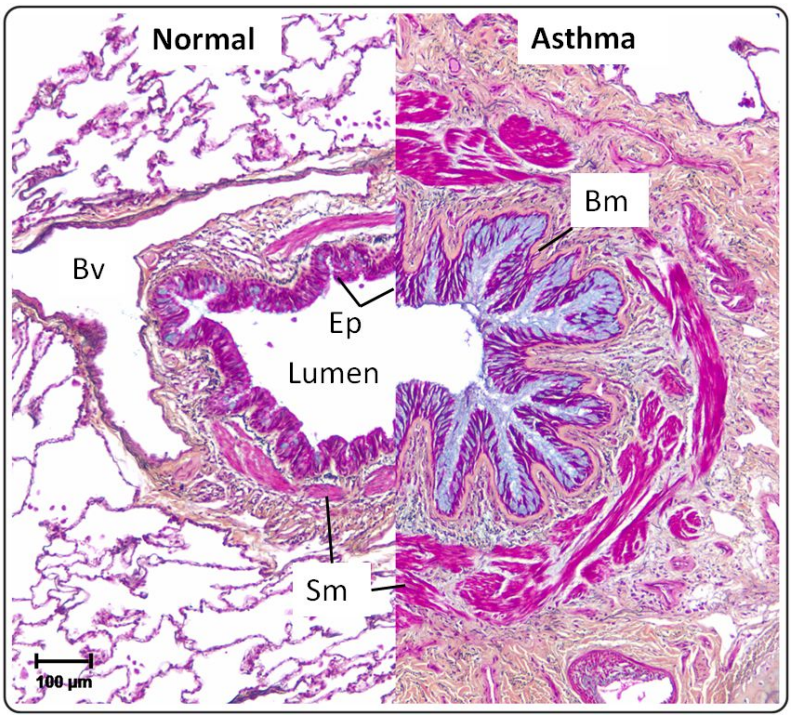


Figure 2. Asthmatic Bronchial Tissue. This visual depicts the cellular level reaction of asthma in a bronchial tube cross section. In comparison to normal tissue, inflammation is present.

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, 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 our 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 are 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 our 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 our 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 our device. Dr. Mathur has previously performed testing of various enzymes and found that Collagenase G was least harmful. Though the material used for the prototype will likely differ from that of the final device, its properties potentially need to allow for 3D printing, micromilling, or autoclaving.

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 1-2 mm in size. There must be a minimum of 50% recovery of viable cells. Since the dissociated tissue will 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

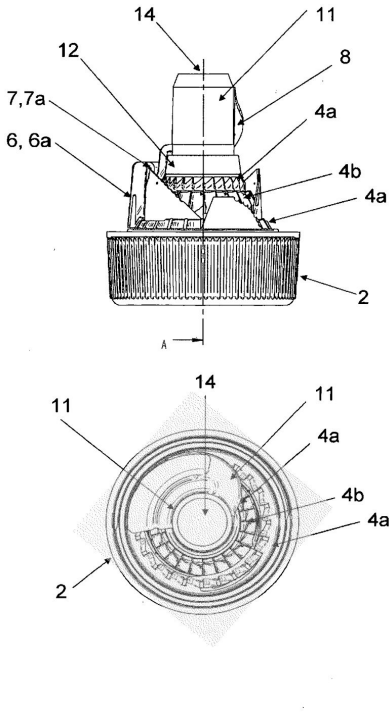


Fig. 3

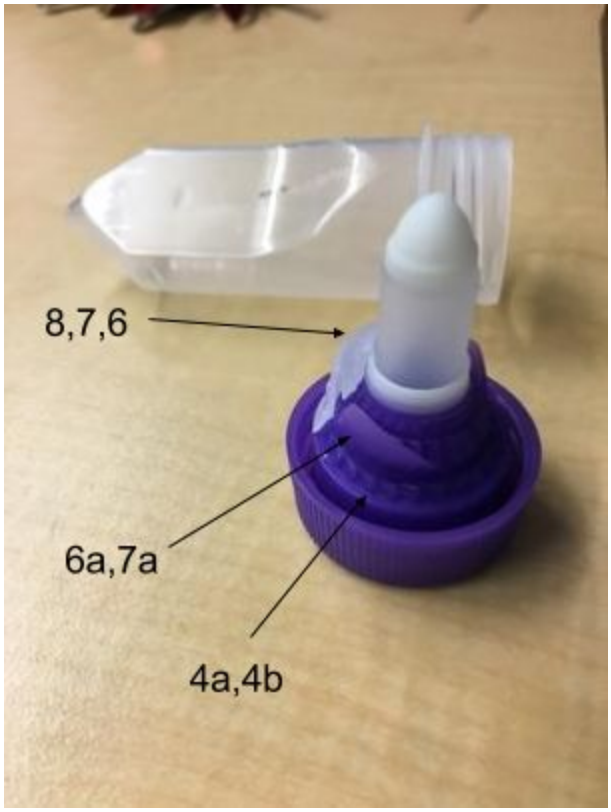


Figure 3: Illustration image of the current device the client uses. The cap (purple) fits over a tube that fits on top of the genleMACS dissociator. The stator (purple, 2) consists of teeth (4a and 4b) as well as large protrusions 6a and 7a. The rotor consists of a screw to generate flow (8) and an envelope 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). While the client does not know exactly what is wrong with the current device (whether it is too harsh and lyses the cells, or whether it is not harsh enough and does not dissociate the tissue), it is suspected that both are happening. 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. At the same time, numerous cells in the sample are probably being lysed when hitting the sharp edges on the stator.

In order to combat this and produce viable cells, from the tissue biopsy, a number of variables will have to be changed. For instance, teeth size, rotational speed and maximal shear forces. Most likely, it will 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

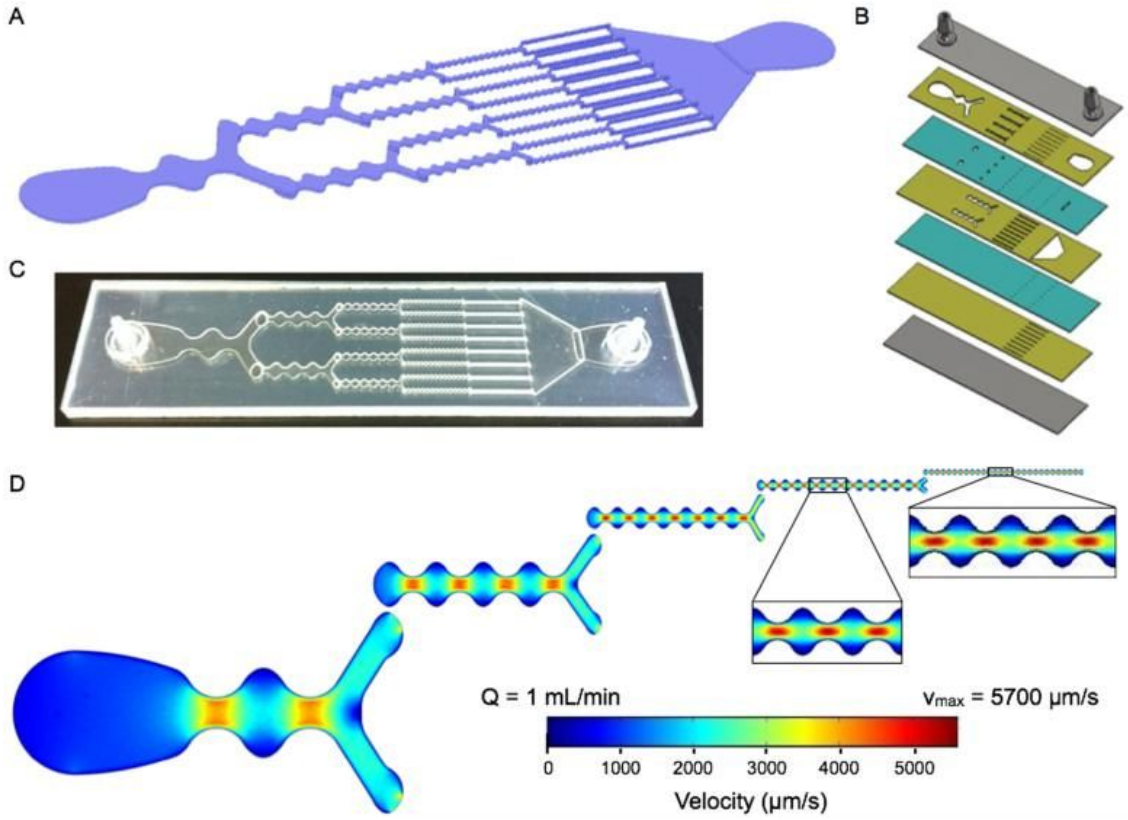


Figure 4: Microfluidic device for dissociation of cell aggregates. (**A)** Schematic of channels with decreasing width. (**B)** Schematic of the individual layers that make up the fabrication of the device. (**C)** Final fabricated product made out of Polyethylene terephthalate (PET). (**D**) Schematic 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

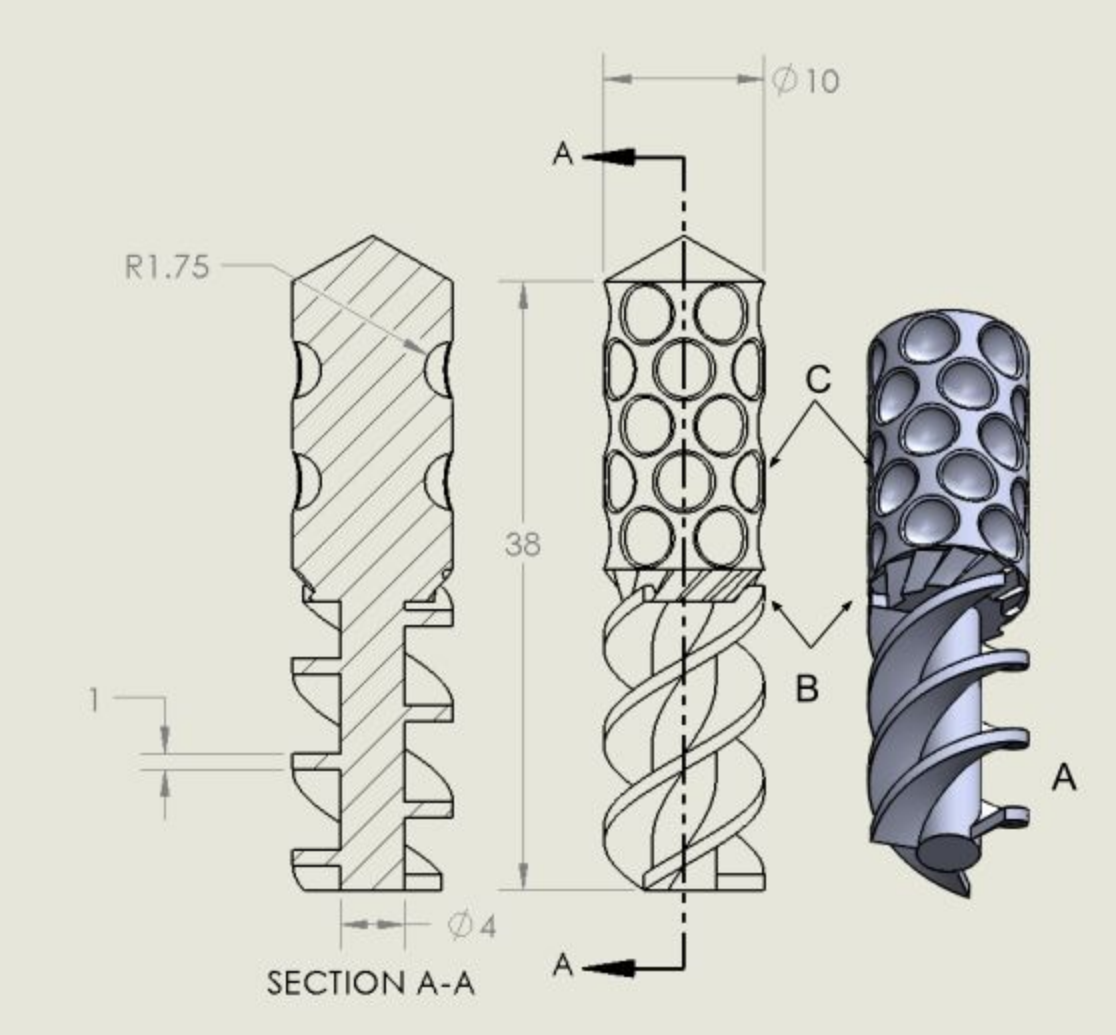


Figure 5: Illustration of screw design, device is shown at the right, while an outline with dimensions is shown in the middle. A section view (A-A) is shown at the left. The device is small and fits inside a chamber with a 100-200 μm gap between the outermost section of the chamber and the inner screw.

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 will be judged. According to the client performance of the design, regarding dissociation of viable cells, is the most important criteria upon which the design should be judged. Therefore the weight assigned to performance was the highest (40/100). This criteria is hard to judge due to the fact that we can not precisely judge its performance until the device has been made. Therefore the team considered outside literature on similar devices as a mean to judge the device. The next criteria upon which the device is judged is fabrication. In order to achieve the best performance of the design the team must be able to judge the device's performance after running multiple practice tests. In order to have the most amount of time for testing of the device the team needs to be able to easily fabricate the design, due to this the criteria of fabrication received the next highest rating (25/100). To suffice other criteria outlined in the preliminary design matrix the design will be judged based on cost and usage. The current device used by the client costs roughly $5-$10 per use and the client would ideally like the new design to cost the same if not less. Therefore the criteria cost/usage received the next highest rating (20/100). The last criteria on which the design will be judged on is ease of use. Ideally we would like the design to require minimal amount of work for the lab technician using it as well as the least amount of time. Since the lab technicians are trained in the methods and techniques for common tissue dissociation the device doesn't need to be too 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 has 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 believes its performance will be low since the current device fails and the team lacks confidence that changes they could make will 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 predicts that this design will perform very well with the dissociation of the tissue in this project. Due to the teams access to a precision micromill and knowledge of creating a similar devices the team believes this design will be easy to manufacture and therefore gave it a high rating. The cost and usage rating for this device is very high due to the cheap cost of the material. While this device would most likely be used on a one time basis, the team still predicts 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 will 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 if not cheaper. This device would require slightly more for the technician work than the modification device since a few steps would be added to the procedure. 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 able to be adjusted to prevent lysis 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) cannot generate significant enough shear forces at a low rotational speed to dissociate cells without lysing them.

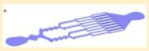
Design Idea:	Modification of current design 	Microfluidic 	Screw Device 
Performance (40)	24 (3/5)	**32 (4/5)**	24 (3/5)
Ease of fabrication (25)	10 (2/5)	**20 (4/5)**	15 (3/5)
Cost/usage (20)	12 (3/5)	**20 (5/5)**	12 (3/5)
Ease of use (15)	**15 (5/5)**	9 (3/5)	12 (4/5)
Total (100)	61	**80**	63

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 is best suited solution for the engineering problem. The team believes this design best meets all the criteria addressed in the preliminary design specifications and will work the best to suit the client's needs.

V. Fabrication

Before beginning fabrication, parameters such as channel width dimensions and enzyme use will be determined. A SolidWorks model will also be developed before fabrication begins. The team will use 3D printing as a way to test out our initial design and then will make the appropriate adjustments for the fabrication of the final design. Once the solidworks file is updated with the exact parameters found to be most effective, it can then be converted to file compatible with SprutCAM. This automated system will be used to micromill our final design into polyethylene plastic. Two team members have access to a micromill in Professor Beebe's lab. Another potential way to fabricate our design is to use laser cutting. Polyethylene plastic is compatible with both of these fabrication methods, however, the team is leaning towards the use of micromilling.

VI. Testing and Future Results

There are several factors that have been determined to control the number of cells with viable surface markers that are collected after dissociation. As a part of our future testing we will run single variable testing on the following variables in the following devices.

Enzymes play a large role in breaking up the extracellular matrix (ECM) of lung tissue samples. If the enzyme is too concentrated, it can disturb the surface markers of the cell. If it is not concentrated enough it will not break apart the ECM efficiently and dissociation will not be effective. As a part of our testing, we will determine the most efficient concentration for breaking apart the ECM and keeping the surface markers of the cell intact. Currently, our client uses Collagenase, and has found through his own research that this enzyme has the best results for breaking apart the ECM while maintaining the cell surface markers. We will continue to use Collagenase, but we will experiment with soaking the tissue in different concentrations of Collagenase solution and for varying amounts of time before sending it through the microfluidic device.

As a part of the microfluidic device, we will change the width of the constrictions in the channels keeping the total width of the channels at each stage constant. By changing the constriction channel sizes, we can increase or decrease the shear forces experienced by the tissue. We will alter the air pressure imposed on the tissue sample. Higher air pressures will increase the affect the forces have on the tissue while also decreasing the time required for dissociation. By finding the right balance of constriction channel width and air pressure, we can maximize dissociation and frequency.

We will try various fluid mediums for the tissue sample and accommodate for the differences in viscosity. This will include a trial of running a low concentration of Collagenase solution through the microfluidic device. If this shows better results than a control solution, we will also experiment here with different concentrations of Collagenase.

After each variable is tested the change of viable cell recovery will be measured via flow cytometry. Flow cytometry will give us an effective overview of the success of our alterations by determining the presence of cells and if those cells contain surface markers useful for interrogation.

VII. Discussion of Future Work

With the chosen final design, future work involves creating a SolidWorks model, fabrication, and then numerous tests designed to determine the best procedure. Based on the results, these steps may be iterated through to find the best mix of procedure and design for producing the highest percent of viable cells. The team will also be trying to increase speed and efficiency, while minimizing cost and cell-site disruption.

The first task will be to create a SolidWorks assembly of our device. This will include making many modifications to the current microfluidic device. The design found through the team's research was originally designed to be used on tumor tissues. The team will therefore need to do additional research on lung tissues to optimize the microfluidic device flow to have the exact shear forces necessary to pull apart the lung tissue. We can change the forces the tissue experiences in the device by changing the channel width. The number of channels and channel length will also be changed to adjust the time the tissue will be under these forces.

When our SolidWorks assembly is complete we will move on to fabrication. In order to visually examine our initial design, we will create a prototype which will be 3D printed. This will be a quick and cheap way to see our first design so we can make any initial adjustments. Once we are satisfied, we will need to have a more accurate prototype before we begin testing. For this, we will either use laser cutting or micromilling. Our material is polyethylene plastic, which is compatible with both of these fabrication methods and has the advantage of being low-cost. This will allow us to do numerous tests with modified designs.

Our longest lasting step will be testing which will involve a lot of data collection. We will be trying to optimise the quantity of viable cells that we get as output, and we will be experimenting with different procedures, slightly different channel designs, different amounts or types of enzymes, and other factors mentioned in our testing section. Based on the data we collect from our testing and experiments, we will make changes to our device and procedure to optimise the output. This combination of device and procedure will be our final result from our project.

VIII. 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 to dissociate tissues of 1-2 square millimeters instead of the standard 3-2 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 diminishing channels as well as pressurized air to force tissue samples that are in solution through channels with smooth constrictions. The team believes that this device will allow the client to achieve his 50% viable cell count based on results seen in a similar study. The team will move forward with the microfluidic design and begin material selection and fabrication of the design in order to start testing and optimizing the process to meet the client's specifications.

IX. 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].

X. Appendix

Appendix A: Product Design Specifications

Microscale Tissue Biopsy Device
Product Design Specifications
2017/10/11

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% 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 because the samples are human tissue. The device should also have completely sealed electronics if they are necessary. 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. Accuracy and reliability will be later refined based on client needs.

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 our goal of the cost per run being $5-$10. The material should be able to be micro-milled and will need to not induce any inflammatory reaction with the cells. The current material consideration is a polyethylene plastic.

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 we select. 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 if the device was compatible with the current dissociation device (gentleMACS) used in the lab.

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 B: Calculations of Shear Forces in Current Design

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.

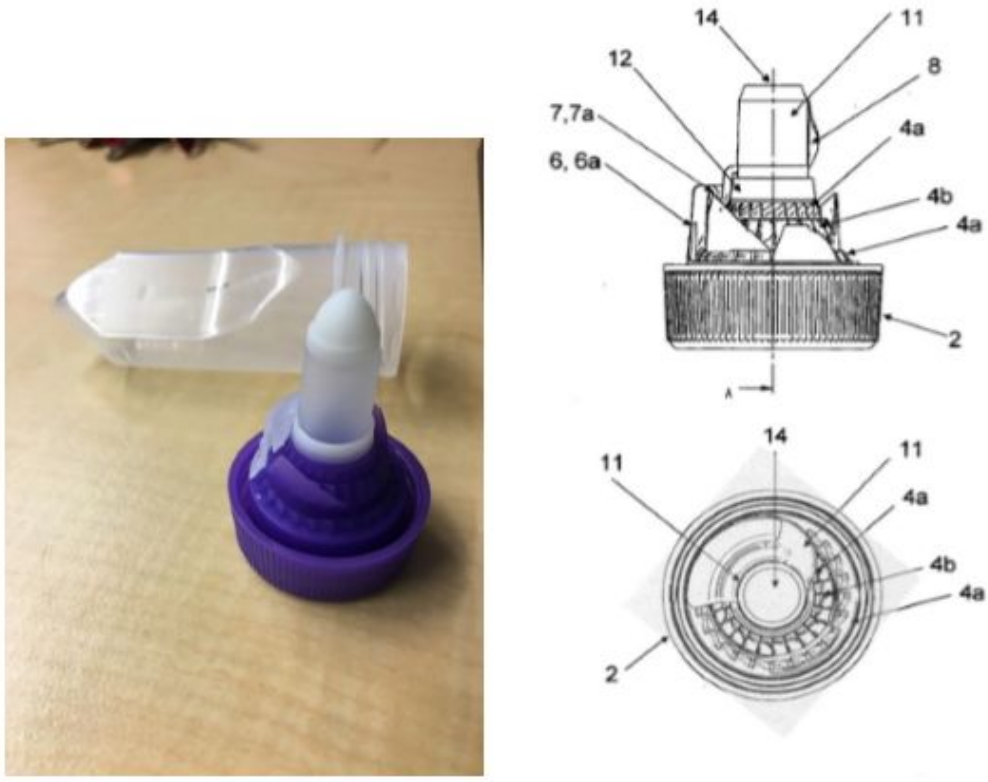


Figure 1: Device and its schematic: **Left**: Image of device. **Right**: Schematic of device. Rotor can be seen as white plastic on the image and 11 on the schematic. Stater is the purple plastic and consists of 2,4ab, 6, 6a, 7 and 7a.

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.

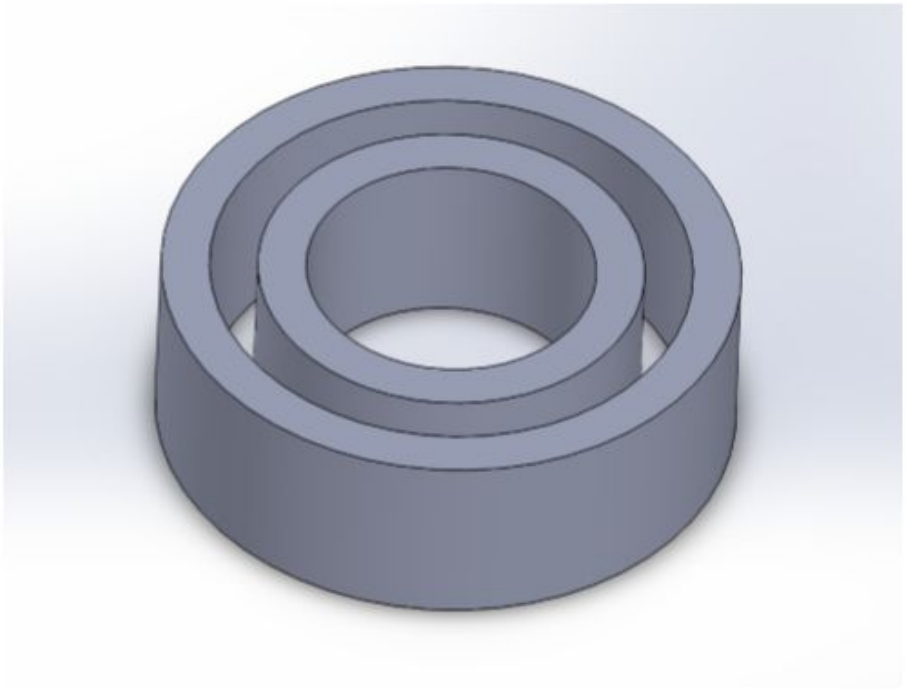


Figure 2: Simplification of device that makes it a simple rotor and stator with one stationary cylinder and one cylinder rotating with a given angular velocity.

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.