TISSUE DISSOCIATION MICROFLUIDIC DEVICE

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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 focuses 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 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 conducted to determine the effectiveness of this device.

Background

Asthma Background

- Triggered by airborne allergens
- T cells lead inflammatory response in bronchial tubes [1]
- Eosinophil counts are high with asthmatic reaction. Eosinophils are a type of white blood cell, 12-17 um in diameter, making up 5% of white blood cells [2]

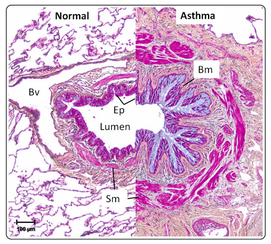

Figure 1: Asthmatic Bronchial Tissue. This visual depicts the cellular level reaction of asthma in a bronchial tube cross section. In comparison to normal tissue, inflammation is present.

Analyzing Asthma Tissue

- Transbronchial biopsy
- 1-2 mm tissue sample [3]
- Dissociation is the process of liberating individual cells from tissue aggregate and ECM. Mechanical forces and enzymatic digestion can be applied.
- Current method requires 4 mm tissue sample size
- Flow cytometry: uses lasers to focus on individual, stained cells, and measures light scattered and fluorescence emitted [4]

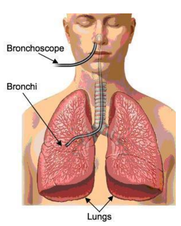

Figure 2. Bronchoscopy method used to obtain tissue samples

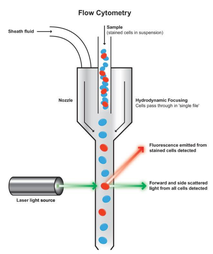

Figure 3. Flow cytometry diagram. This is the method used to analyze individual cells

Problem and Aims

Problem:

- Lab receives biopsy sized tissue samples for research
- GentleMacs device is not successful at dissociating 1-2 square mm tissue samples mechanical dissociation

Project Goal:

- Develop a device to dissociate 1-2mm lung biopsy samples into individual cells with intentions of analyzing cell markers via flow cytometry.
- Test to determine if viable cells can be recovered via the microfluidic device.

Design Specifications

- 50% of cells dissociated with a +/- 10% margin of error
- Minimal to no disruption of cell surface characteristics especially eosinophils
- The device does not lyse cells during dissociation
- Material is biocompatible and resistant to cell adhesion
- Can open to allow retrieval of potentially non dissociated tissue
- Allows for easy collection of dissociated tissue
- < $10 per use
- Ergonomic as a lab bench device
- Transparent lid for viewing during dissociation

Final Design

Figure 4: Design progress left to right. **Left:** Comparison of original design in blue to clear design and white design. The only difference between the clear and white design is that the white design has a rubber gasket around it to prevent leaving from the top. **Middle:** White design with putty on the lid to present leakage is the final design. **Right:** Final design in preliminary set up with makeshift clamps used for testing.

Flow Analysis: Navier-Stokes, Macroscopic balances and FEA

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

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

Equations 1 and 2: Reduction of Navier-Stokes equations of motion for arbitrary flow in the xy plane. This equation is extremely complicated to solve.

Macroscopic balance:

$$\frac{\Delta p}{\rho}+\sum\frac{1}{2}v^2(.45(1-\beta))+\sum\frac{1}{2}v^2\left(\frac{1}{\beta}-1\right)^2=0$$

Equation 3: Reduction of the macroscopic mechanical energy to get pressure differential from friction factors, assuming the channel can be approximated by a series of sudden contractions (first term) and sudden expansions (second term), where beta is (small)/(large) cross-sectional area.

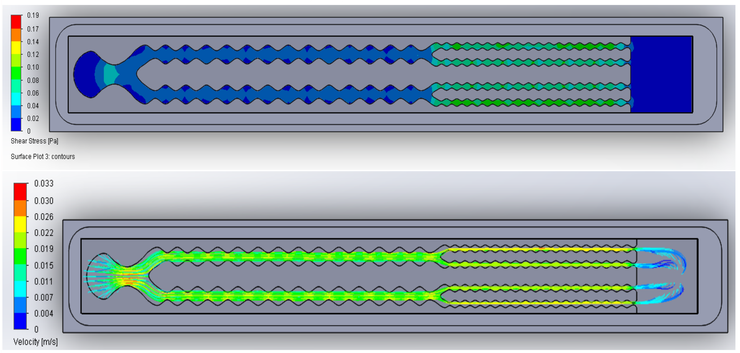

Figure 5: Results of FEA flow simulation showing shear stress (top) and velocity (bottom) profiles for a volume flow rate of 1mL/s. Velocity ranges from about .007m/s to a max of .024m/s in the smallest channel, similarly, shear stress ranges from a minimum of approximately 0Pa to about .1 Pa in the smallest channel.

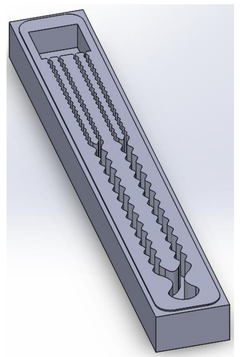

Figure 6: SolidWorks model of final design

Methods and Testing

- A small, 1-2 mm sample was extracted from lung tissue with a biopsy tool to mimic a lung biopsy procedure (Figure 7).
- To aid in cell dissociation, the sample was placed in 50 ml conical tube with either PBS or a Collagenase G solution and then incubated (Figure 8).
- The solution was transferred to the device via a P5000 micropipette and placed in the first well (Figure 9,10). The lid of the device was then clamped to create a tight seal and the peristaltic pump tubing was attached to adaptors on the device. 20 mL PBS was pumped through the device at a flow rate of 1L/5 minutes (1ml/3 seconds) via the peristaltic pump. The solution exited the device through the tubing and was collected in a conical tube.
- The solution was vortexed to separate the cells from the PBS and filtered through a 50 micron filter to remove excess tissue (Figure 11).
- To observe the quantity of dissociated cells, the sample was centrifuged and the pellet was re-suspended in PBS (Figure 12). The solution was loaded into a cytospin device to transfer the cells to a glass slide and stained with HEMA 3. The slides were observed on a 40X Fluorescent microscope (see Results). ImageJ was employed to process the images and calculate the amount of cells obtained in order to compare our device to the current method.

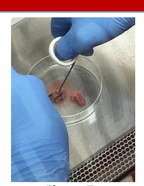

Figure 7

Figure 8

Figure 9

Figure 10

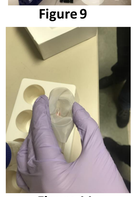

Figure 11

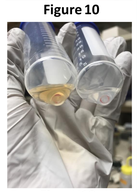

Figure 12

Discussion of Results

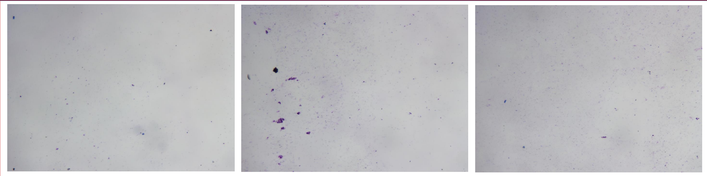

Figure 13: Microscopic images of slides created from cells recovered from the dissociation attempts using the microfluidic device after treatment in collagenase solution

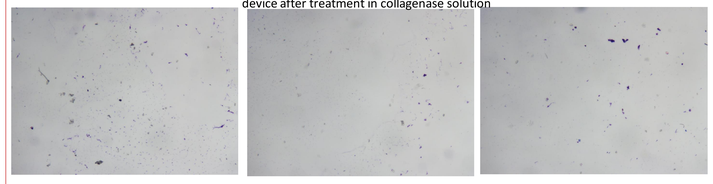

Figure 14: Microscopic images of slides created from cells recovered from the dissociation attempts using the original dissociation method in collagenase solution

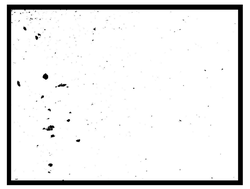

Figure 15: Sample image of analysis of slide images using ImageJ

Cell Count: Collagenase + Microfluidic Device	Cell Count: Collagenase + Original Method
146	410
502	226
395	182

Figure 16: Results of ImageJ cell counting of the two different methods using collagenase

- To analyze the cell count on each slide, 3 photos were taken in 3 different areas
- Photos were analyzed using ImageJ
- Compared the means of the cell count with the device and without the device using the collagenase solution
- p-value: 0.595
- Data is not statistically significant

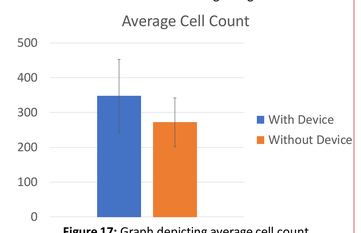

Figure 17: Graph depicting average cell count

Future Work

Modifications to the design of the microfluidic device

- Implement the "groove" for the rubber closer to the channels to improve the leaking issue
- Fabricate a device with even smaller channels
- A higher precision tool will need to be used, such as a laser cutter
- Fabricate transparent cover with more precise port holes

Modifications to the process used to test tissue in the device

- Obtain a pump that maintains a consistent flow rate
- Future groups should conduct multiple rounds of testing to ensure that the results seen are reproducible
- Test dissociation in the absence of an enzyme

Figure 18: SolidWorks model of proposed future design

Acknowledgments

- **Dr. Sameer Mathur,** UW Hospital
- **Dr. Wan Ju Li,** Department of Biomedical Engineering
- **Members of Dr. Mathur's Lab,** UW Hospital
- **Dr. Beebe's Lab,** Beebe Lab in WIMR

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