Lung Tissue Biopsy Dissociation

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Abstract

Our client conducts asthma research by obtaining lung tissue samples before and after an asthmatic response. Individual cells are then dissociated away from the tissue and studied with flow cytometry. The current device being used for this process is the Miltenyi GentleMACS™ Dissociator, which does not yield viable cells for flow cytometry testing. A small tissue biopsy, 12 mm^3 or less, is desired to minimize patient pain and reduce recovery time. This design team was tasked with creating a dissociation device that can successfully dissociate small tissue samples to yield viable cells that can be analyzed by flow cytometry. This device must yield at least 10,000 cells from a single biopsy. To accomplish this task, a microfluidic device that utilizes shear force was 3D printed. Testing has shown that this device dissociates cells at about 17,000 cells/mm^3 while testing on the GentleMACS™ yields about 36,000 cells/mm^3. From these values we can conclude that our device is able to dissociate lung tissue of approximately 13 mm^3, but not as successfully as the GentleMACS™. Future testing should look at using smaller, fresh tissue samples.

Background

Asthma:

- Affects 8% of population in U.S. leading to 10.5 million primary care visits per year.[1]
- Unknown etiology cause sensitization to harmless antigens which triggers inflammation and constriction of the airways.[2]
- Eosinophils are a major cell type associated with asthmatic inflammation
  - Current source of EOS in blood not ideal
  - Biopsies allow a more accurate study of EOS[3]

Client Information:

- Dr. Sameer Mathur is the director of Allergy and Immunology Clinics and the Chief of Allergy at the VA Hospital.
- Performs research on asthma, comparing lung biopsies before and after an asthmatic reaction.
- **Requires a device that dissociates more cells than the Miltenyi GentleMACS™ to study the lung asthmatic response using flow cytometry.**

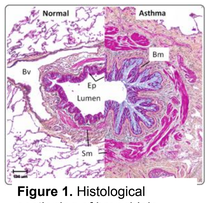
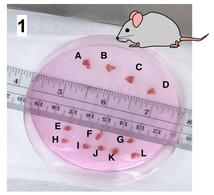


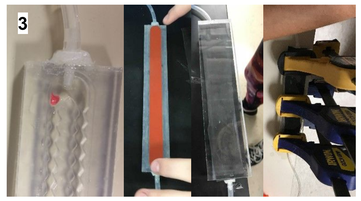
Figure 1. Histological sectioning of bronchiole illustrating an asthmatic reaction. Normal tissue on left. Inflamed tissue on right.[4]

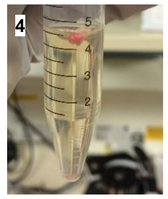
Design Criteria

- Dissociate 12 mm^3 tissue samples
- Obtain more than 10,000 cells per sample
  - Ideally white blood cells
- Device must cost ≤ $10 per use
- Device must be sterilizable
- Device must not lyse cells or alter cell surface markers

Testing and Results







1. Murine lung tissue biopsies taken and imaged in DMEM
2. Samples placed in collagenase solution and incubated at 37°C for 45 min
3. Sample inserted into the microfluidic device and device assembled
4. Peristaltic pump used to push 5 mL of liquid over the tissue 2X, then centrifuged
5. Particle counter used to count cells obtained from the tissue

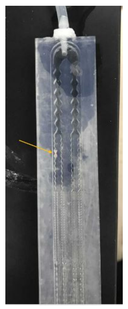


Figure 2. Final 3D printed design with tissue sample in small channel (arrow).

Fabricated using Viper SLA 3D printer
Material: Accura 60

Calculations performed at 14.4 mL/min on SolidWorks

Flow Rate Tests:
Old Pump Highest Setting: 99.6
Measured Flow Rate: 15 mL/min

New Pump Highest Setting: 48
Measured Flow Rate: 30 mL/min

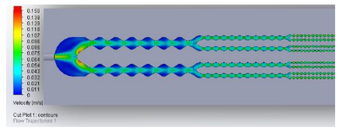
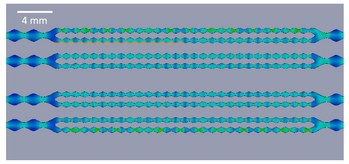


Figure 3. (Top) Velocity profile in device, velocity ranged from about 10 mm/s (blue) to 100 mm/s (red). **(Bottom)** Shear stress profile through middle and smallest channels of the device. Shear stress ranged from ~0 (blue) to ~1.28 Pa (green). This stress is similar to what is present in an arteriole[5].



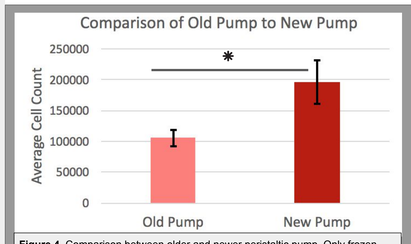


Figure 4. Comparison between older and newer peristaltic pump. Only frozen tissue was used for this comparison. The older pump yielded a maximum flow rate of 15 mL/min compared to 30 mL/min for the newer pump. Error bars depict SEM. Old pump AVE=1.05E+05, SEM=1.30E+04, n=3. New pump AVE=1.96E+05, SEM=3.5E+04, n=12. T-test p-value = 0.015.

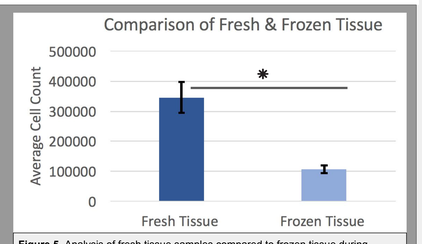


Figure 5. Analysis of fresh tissue samples compared to frozen tissue during dissociation with our device. All samples were filtered and dissociated using the old peristaltic pump. Error bars depict SEM. Fresh tissue AVE=3.46E+05, SEM=5.08E+04, n=2. Frozen tissue AVE=5.08E+04, SEM=1.30E+04, n=3. T-test p-value = 0.0004.

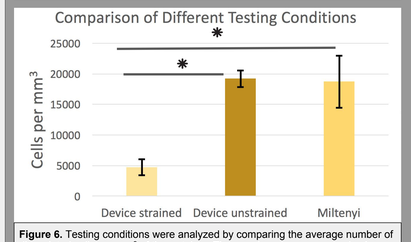


Figure 6. Testing conditions were analyzed by comparing the average number of dissociated cells per mm^3 of tissue taken. Tissue volume was measured using ImageJ. All samples were frozen and used with the newer peristaltic pump. Error bars depict SEM. Device strained AVE=4.76E+03, SEM=1.3E+03, n=5. Device unstrained AVE=1.91E+04, SEM=1.3E+03, n=2. Miltenyi AVE=1.87E+04, SEM=4.24E+03, n=5. ANOVA yielded p-value = 0.01 between groups. T-test between device strained and device unstrained p-value = 0.0015. T-test between device strained and Miltenyi p-value = 0.013. T-test between device strained and Miltenyi p-value = 0.95.

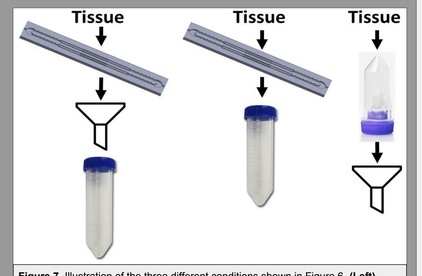


Figure 7. Illustration of the three different conditions shown in Figure 6. **(Left)** Tissue is run through microfluidic device, filtered through 50 µm filter, and centrifuged. **(Middle)** Tissue is run through the device and centrifuged. **(Right)** Tissue is run through Miltenyi GentleMACS™, filtered through 50 µm filter, then centrifuged.

Final Design

- Microfluidic device with series of channels with decreasing width
  - Widest channel = 1.6 mm; Narrowest channel = 0.3 mm; Channel height = 2 mm
- Reusable, can be re-designed quickly and cheaply
  - Cost per use: $2 or less

Successful Design Features:

- Reusable, sterilizable, low cost
- Repeatable dissociation protocol
- Consistent dissociation of tissue into >10,000 cells/mm^3
- Successfully solved lingering leaking problems
- Undissociated tissue can be retrieved from the device

Design Features to Improve:

- Experiment with programmable peristaltic pump for oscillating flow
- Use sealing method other than clamps and rubber gasket
- Idealize channel dimensions by combining computer simulation with experimentation
- Experiment with number of times solution is run through device

Conclusions

Interpretation of Statistical Results:

- The new pump (higher flow rate) dissociated tissue more effectively
- Dissociation of fresh tissue yielded more cells than frozen tissue
- The Miltenyi and unstrained device conditions were more successful at dissociating tissue than the strained device condition
- **When tissue samples were unstrained prior to centrifugation, the microfluidic device dissociated as effectively as the Miltenyi GentleMACS™**

Limitations:

- Use of frozen tissue limits discovery of viable cells
- Fresh tissue is more difficult to obtain
- No access to human samples
- No access to samples exhibiting asthmatic response

Future Work

- Investigate mirrored design with oscillating flow
- Determine type and integrity of dissociated cells
- Assess accuracy of predicted shear forces in the device
- Use cytotoxicity tests to determine cell viability after dissociation
  - Will fresh tissue yield more viable cells than frozen tissue?
- Use different tissue types in the device to assess if it can be marketed to a wider range of research groups

Acknowledgments

Dr. Sameer Mathur, Dr. Krishanu Saha, Paul Fichtinger, Robert Swader, Elizabeth McKernan, TEAM Lab, Morgridge Fabrication Lab

References

[1] Centers for Disease Control. "Most Recent Asthma Data". Internet: www.cdc.gov/asthma/most_recent_data.htm, June 2017 [Apr. 18].
[2] M. Kudo, Y. Ishigatsubo, I Aoki, "Pathology of asthma". *Frontiers in Microbiology*, vol. 4, no. 263, 2013.
[3] E. Kelly, et al. "Mepolizumab Attenuates Airway Eosinophil Numbers, but Not Their Functional Phenotype, in Asthma" Am J Respir Crit Care Med Vol 196, Iss 11, pp 1385–1395
[4] S. Holgate, S. Wenzel, D. Postma, S. Weiss, H. Renz and P. Sly, "Asthma", Nature Reviews Disease Primers, p. 15025, 2015.
[5]. A. G. Koutsiaris *et al.*, "Volume flow and wall shear stress quantification in the human conjunctival capillaries and post-capillary venules in vivo.," *Biorheology*, vol. 44, no. 5–6, pp. 375–86, 2007.