Lung Tissue Biopsy Dissociation

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ABSTRACT

- Patients with asthma present eosinophils in the lumen of their blood, airways, and inflamed lung tissue
- Eosinophils have different surface markers depending on location in lung
- Project goal: successfully dissociate eosinophils to analyze their surface markers after an asthmatic reaction
- The design should minimize cell damage while dissociating enough cells to run flow cytometry (20,000 cells)

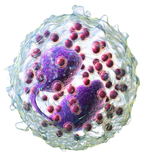

Figure 1: *Eosinophil*[1]

BACKGROUND

Motivation

- Eosinophil research can lead to advancements in drug delivery mechanisms and further knowledge of asthmatic physiology

Research

- gentleMACS has been used for over a decade on various rodent lungs, from Guinea pigs to mice[2]
- The gentleMACS tube dissociates tissue using various ridges, rotors, and spacers[2]
- Why not a microfluidic device?

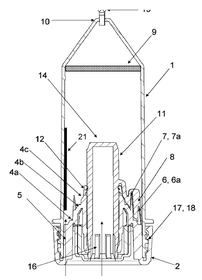

Figure 2: *GentleMACS conical tube*[3]

$$Reynolds\ Number = \frac{\rho v L}{\mu}$$

$$If\ L = 0.0001m$$

Re>3000[4] to achieve turbulence

$$If\ \rho = 1000\ \frac{kg}{m^3}\ and\ \mu = 0.00089\ Pa{*}sec$$

$$v(velocity)\ = 26.7\ \frac{m}{sec}\ \text{or}\ 60\ \text{mph}$$

Cells would die instantly upon wall collision

DESIGN CRITERIA

Client Requirements

- Dissociated cells must be viable and have and unchanged surface markers
- Duration of dissociation must be less than 4 hours
- Cost of single use device must be less than $10 and sterilizable

Hydrogel Requirements

- Stiffness, swellability, and size adjustability
- Cell should not attach or interact chemically with the hydrogel material
- Beads should not decompose in enzyme solution
- Biocompatible

FINAL DESIGN

- 3 mm^3 spherical sodium alginate hydrogels were synthesized by dropping 3.5% sodium alginate from pipette into a 200mM $CaCl_2$ solution stirred at 200rpm
- 1-2 mm^3 tissue samples were soaked in .15% collagenase and 5 mM Ca for 20 minutes at 37℃
- 4.4 g of hydrogels, 2.4 ml enzymes, and 4 pieces of tissue biopsies were added to gentleMACS conical tube
- To dissociate the tissue, the lung tissue setting on the gentleMACS machine was used three times

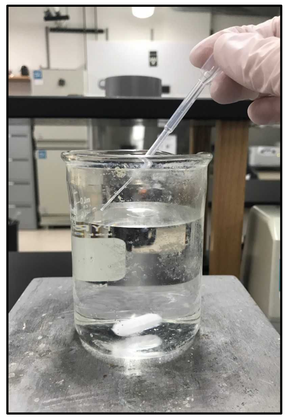

Figure 3: *Hydrogel Synthesis with beaker of $CaCl_2$, stir bar, and pipette of Alginate*

TESTING AND RESULTS

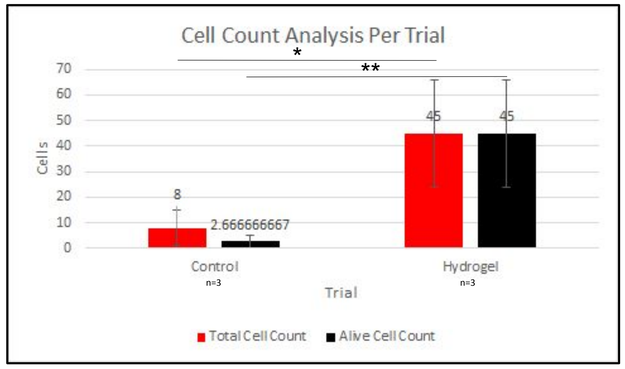

Figure 4: *Number cells alive (black) and total cells (red) in both control and experimental (hydrogel) conditions as counted in the hemocytometer with Trypan Blue*

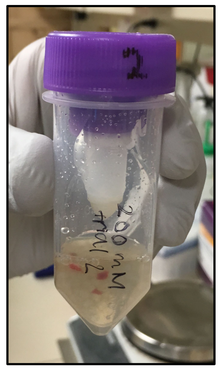

Figure 5: *Tube with hydrogels and tissue samples before dissociation*

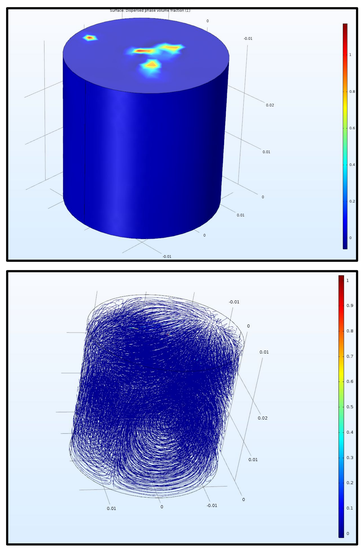

Figure 6: *Comsol simulation with no hydrogel beads. [Top] There is little pressure against the surface of the tube without beads. [Bottom] The flow of particles (water molecules) is more uniform*

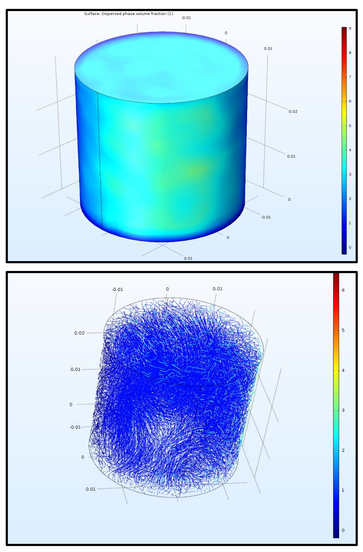

Figure 7: *Comsol simulation with hydrogel beads. [Top] Greater overall pressure applied to all surfaces [Bottom] Flow of particles more chaotic and "turbulent"*

DISCUSSION

- Dissociation with enzymes alone in the gentleMACS machine does not result in appreciable dissociation, samples are too small
- Comsol simulations (Figure 6 and 7) show hydrogel particles aid in mechanical dissociation by increasing the number and force of interactions the tissue has with surroundings
- Homogenization using steel and glass beads is common; these are too stiff and lyse cells; hydrogels were chosen as replacements
- Any biocompatible hydrogel material could be used for dissociation if size and stiffness of beads can be controlled and the hydrogel doesn't degrade in the enzymes
- Size of the beads should be approximately the size of the tissue sample
- The 3.5% sodium alginate was used to create the hydrogel beads; the 2% sodium alginate was not stiff enough and fell apart during the dissociation and a 5% sodium alginate solution was too viscous to make small enough hydrogels.
- Hydrogels used during final testing cross linked with a 200 mM $CaCl_2$ dissociated more cells than 100mM $CaCl_2$
- If Ca^{2+} is required for enzyme activity (i.e. collagenase), alginate is not ideal hydrogel source; alginate expelled into solution is crosslinked into small particles obscuring analysis and culture of cells
- A limitation of this design lies in the fact that it requires the gentleMACS dissociator to agitate the hydrogels and dissociate the tissue

FUTURE WORK

- Synthesize and test different hydrogel material
- Conduct additional tests to determine optimal bead size, swellability, and stiffness for maximum tissue dissociation
- Run final design with tissue sample of inflamed human lung tissue
- Use flow cytometry to determine types of dissociated cells and their surface markers

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[2] Jungblut, M., Oeltze, K., Zehnter, I., Hasselmann, D., and Bosio, A., "Preparation of single-cell suspensions from mouse spleen with the gentleMACS Dissociator," *J Vis Exp.* vol. 11, no. 22, p. 1029, December 2008. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2762924/ [Accessed: 02-Dec-2018].

[3] Miltenyi Biotec, *gentleMACS C Tube.* 2011.

[4] Schlichting, H., and Gersten, K., Boundary-Layer Theory. Springer Verlag, 2017, p. 416-419.