



Tissue Dissociation

Prepared by: Samuel Moss, Patrick Kasl, Hailey Frye, Saketh Challa, Elizabeth
Gunderson, Jesse Fan

Client: Dr. Sameer Mathur

Advisor: Dr. Wan Ju-Li

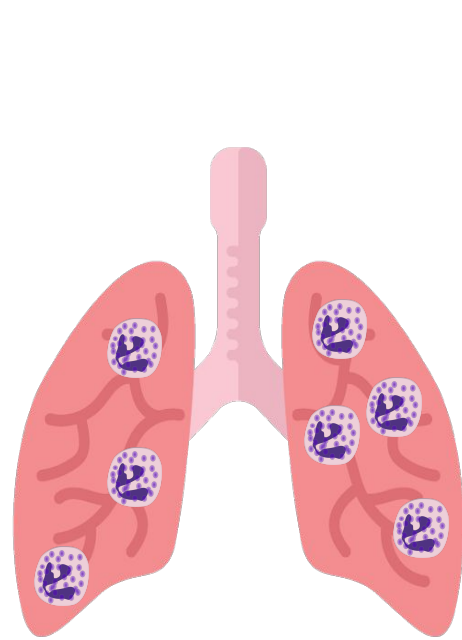



Problem Statement

Dr. Mathur's research group receives tissue samples from lung biopsies that are too small and/or time sensitive to allow for use of common tissue dissociation methods. Our goal is to develop a tissue dissociation method that will allow for interrogation of gene expression as well as cell surface markers. Specifically, Dr. Mathur is interested in the effect of tissue resident eosinophils on asthma.

Background Material

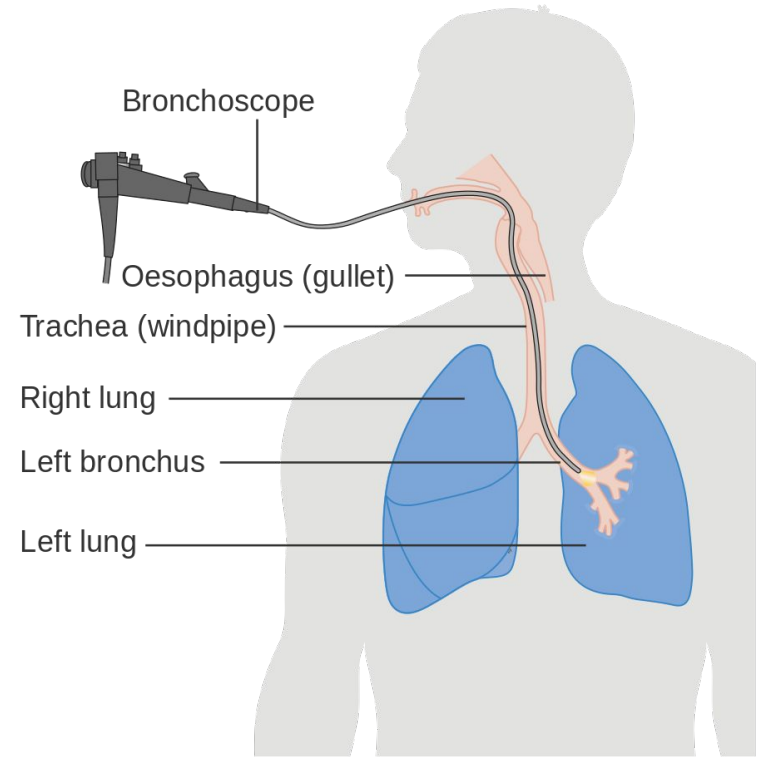
Asthma and the Eosinophil





Background Material

Lung Biopsies



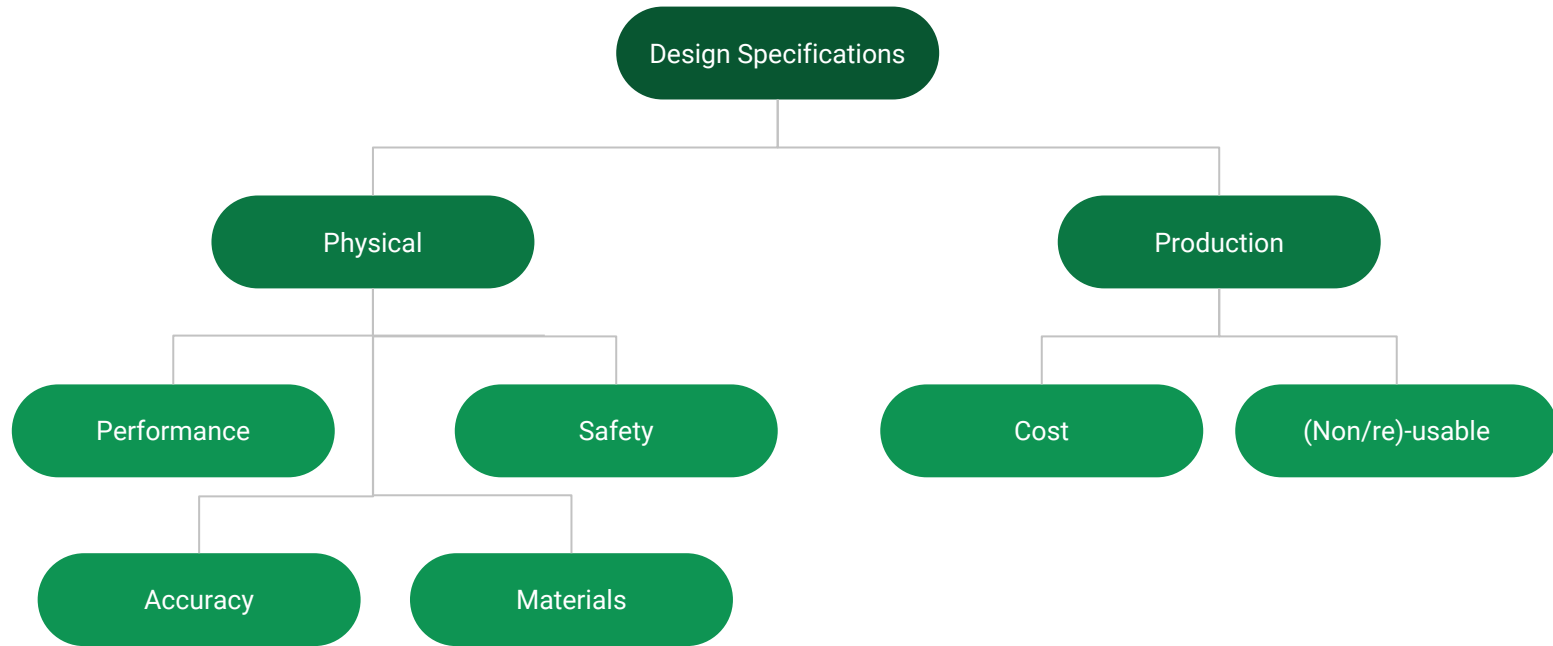
Background Material

Current Methods

- Literature standard: enzymatic
 - Long incubation
 - Surface markers damage/change
- gentleMACs
 - Larger sample required

Previous Work

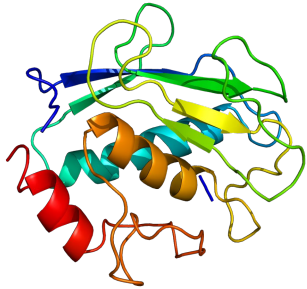




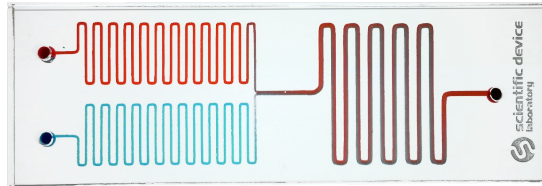


Alternative Designs Considered

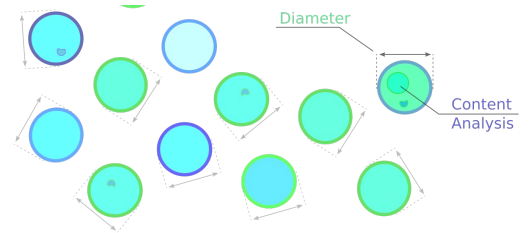
Enzyme with Gentle Agitation



Enzyme and Microfluidic Device



Hydrogels with gentleMACs dissociator





Design Matrix

	Weight	Enzyme and Gentle Agitation		Enzyme and Microfluidic Device		Hydrogel Matrix with the gentleMACS Dissociator	
Cost	10	5/5	10	3/5	6	4/5	8
Ease of Fabrication	15	5/5	15	3/5	9	4/5	12
Duration of Dissociation Process	20	3/5	12	4/5	16	5/5	20
Cell Viability	20	2/5	8	4/5	16	5/5	20
Effect on Cell Surface Markers	25	4/5	20	3/5	15	5/5	25
Ease of Use	10	5/5	10	3/5	6	4/5	8
TOTAL	100		75		68		93



gentleMACs dissociation + Hydrogel

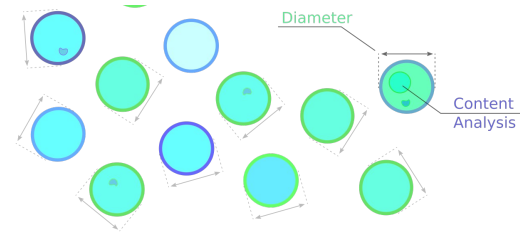
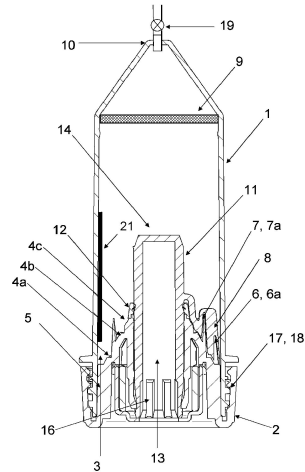


Fig. 1



gentleMACs Dissociator

Established but limited

Tube enclosure - functional design

Our patented enclosure design directs the sample flow towards the stator to ensure thorough dissociation and homogenization.

Pierceable septum - maximal safety

A pierceable septum allows for the removal of dissociated or homogenized tissue sample without the need to open the tube. This keeps hazardous material safely away from the operator.

Stator - exact control

At the fixed stator site, the sample is processed through mechanical shearing that occurs within the narrow gaps defined by the distance between the stator teeth and the rotor.

Sealing gasket - no loss of material

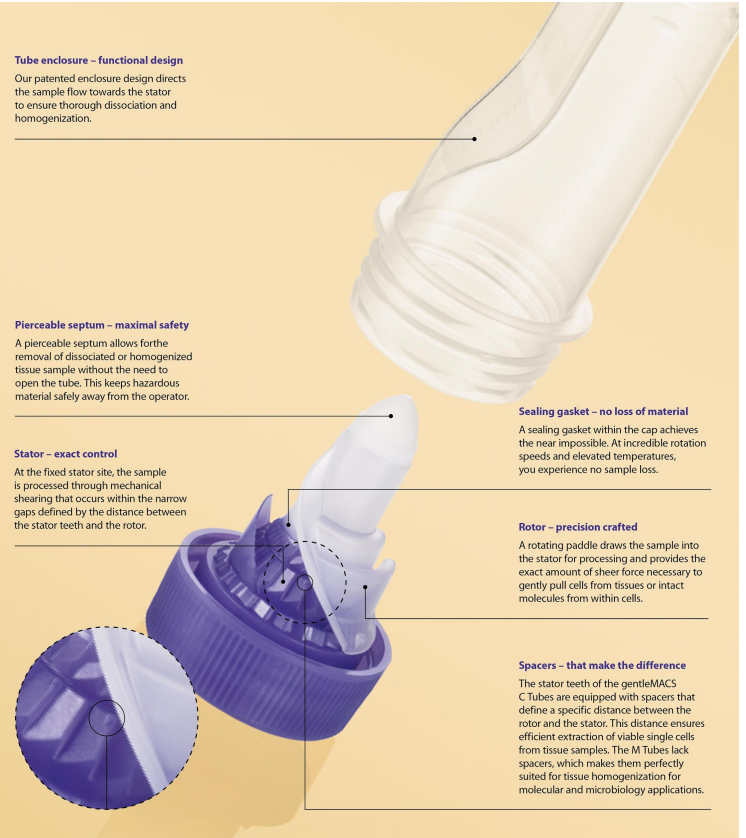
A sealing gasket within the cap achieves the near impossible. At incredible rotation speeds and elevated temperatures, you experience no sample loss.

Rotor - precision crafted

A rotating paddle draws the sample into the stator for processing and provides the exact amount of sheer force necessary to gently pull cells from tissues or intact molecules from within cells.

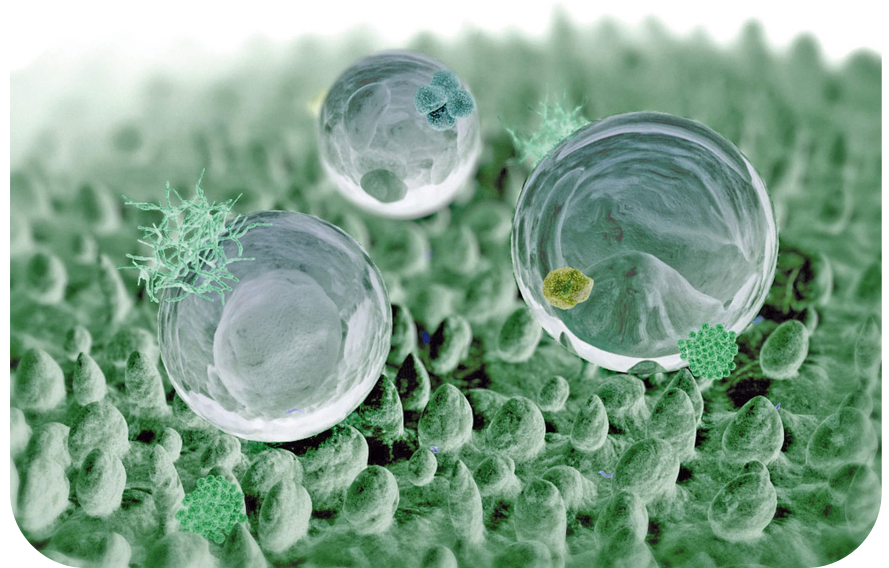
Spacers - that make the difference

The stator teeth of the gentleMACS C Tubes are equipped with spacers that define a specific distance between the rotor and the stator. This distance ensures efficient extraction of viable single cells from tissue samples. The M Tubes lack spacers, which makes them perfectly suited for tissue homogenization for molecular and microbiology applications.



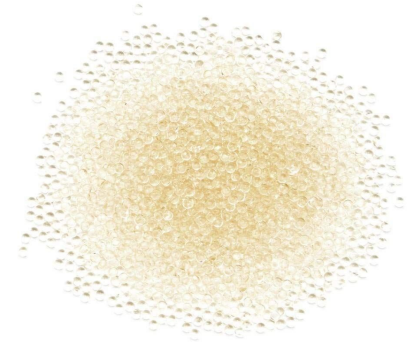
Hydrogel Selection Criteria

- Hydrogel composition
 - Material
 - Natural: Alginate, Chitosan, Agarose
 - Synthetic: PEG+Monomers, PHEMA
 - Cross linking
 - Stiffness
 - Swellability
- Size of beads
 - Inertial force application
 - Contact level



Future Work

- Additional Research
 - Determination of ideal hydrogel
 - Enzyme selection
 - gentleMACs settings
- Create or purchase hydrogels
 - Miracle-Gro/ “Water Beads”
 - Polyacrylate
- Prototype hydrogel system





References and Acknowledgments

Ahmed, E. (2018). *Hydrogel: Preparation, characterization, and applications: A review*. Science Direct.

Qiu, X., De Jesus, J., Pennell, M., Troiani, M. and Haun, J. (2018). *Microfluidic device for mechanical dissociation of cancer cell aggregates into single cells*. [online] Royal Society of Chemistry.

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Questions / Comments?