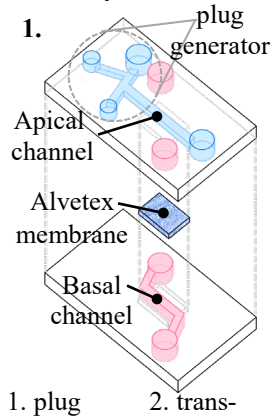
Pulmonary "inflammation-on-a-chip" to discover novel mechanisms of airway inflammation

Foreword: Because I am a BioMAT trainee with two years of NIH support, my adviser, Dr. Shuichi Takayama, allowed me to build my own project. Motivated by my interest in immunopathology, **I independently created this project** after becoming familiar with the work of our established airway disease collaborator, Dr. Rabindra Tirouvanziam at Emory University. My adviser plans to expand my idea into a full grant proposal that I will write with him.

Motivation: Inflammatory airway diseases (IADs), including COPD[a], CF[b], and asthma, are the fifth leading cause of mortality globally.[1] Chronic pulmonary inflammation can lead to fibrosis, recurrent infection, and loss of lung function that requires transplant. Neutrophils (PMNs[c]) are the perpetrators of this excessive inflammation, making them a target of therapeutics and motivating the study of mechanisms driving their pro-inflammatory conditioning.



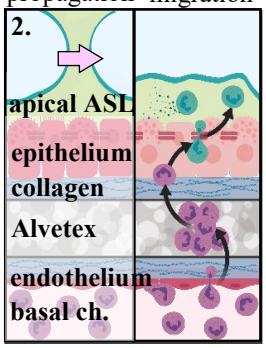


Fig. 1*: Device design.*
Fig. 2*: Cross-section.*

In vivo models are insufficient due to significant differences in the function of inflammatory mediators and disease phenotype. They also do not offer the ability to systematically eliminate confounding variables, making *in vitro* models attractive for mechanistic studies. In typical *in vitro* studies, investigators expose either blood PMNs or epithelial cell lines to a disease-related stimulus (e.g. smoke, bacteria) in a static system and measure cells' responses. Despite these efforts, no therapeutics have yet halted the cycle of damage inflicted by PMNs, motivating more sophisticated mechanistic studies to inform therapeutic strategies.

In vitro models neglect **fluid mechanical stress (FMS**, caused by thickened lung mucus) and **PMN transmigration into the lumen** despite evidence that severe, FMS-induced crackling sounds are one of the top three clinical predictors of poor prognosis in CF and COPD,[4,5] and PMNs that transmigrate into diseased lung fluids acquire a pro-inflammatory phenotype.[2,3] These processes may be connected: thickened mucus causes FMS-induced epithelial inflammation that recruits PMNs and makes them pro-inflammatory. Stressors are known to induce sterile inflammation in many cell types.[7] Bronchial epithelial cells produce exosomes, cytokines and miRNA in response to compressive mechanical stress, and hypoxia-stressed epithelial cells release exosomes that activate proinflammatory signaling in macrophages.[8,9] **I hypothesize** that fluid mechanical stress induces sterile inflammation of the epithelium resulting in epithelial exosomes, miRNA and cytokines that contribute to the inflammatory phenotype of PMNs. **The objective of my research is to establish a novel microfluidic model of pulmonary inflammation,** incorporating FMS and PMN transmigration, to discover pro-inflammatory pathways that are inaccessible with current models.

Aim 1: Design, develop and optimize the "lung inflammation-on-chip" microfluidic device.

I am currently modifying the lab's established lung device,[2] which already includes FMS, to incorporate PMNs by adding a porous Alvetex™ membrane that our Emory collaborators use to model PMN transmigration.[3] To generate confluent, primary epithelial and endothelial cells on the membrane, I will adapt established Transwell coculture methods: I will continuously flow media on both sides until confluence, which I will evaluate with trans-bilayer electrical resistance and staining for tight junctions.[10] To differentiate the epithelial cells, I will remove media from the apical channel and flow 5% CO_2 air for 14 days with media flowing in the basal channel. Liquid plugs will be generated in the absence of neutrophil flow with PBS + 1.2 mg/mL of the surfactant

Survanta; the target speed of the liquid plug is 2 mm/s at an air pressure of 1 kPa to model physiological conditions of sublethal stress.[11] Liquid plug speed and pressure drop will be measured with established methods from our lab.[2] Viscosity of the plug-generating fluid will be measured with rheometry. Computer-controlled electromechanical actuators will create the air flow switching that generates liquid plugs. To validate transmigration, neutrophils will flow on the basal side of undamaged epithelium and the apical side will be incubated with either a) RPMI control or, to induce transmigration, b) RPMI+100 nM LTB4 or c) patient airway surface liquid (ASL). PMN analysis described in [3], including flow cytometry and measurements of metabolism and bacterial killing, will validate that the model produces proinflammatory PMNs.[3]

Aim 2: Model stress-induced inflammation and infer novel inflammatory networks

Epithelial cells will be exposed to 0, 6, 12 or 24 hours of liquid plugging at a constant rate and pressure drop (5 plugs/min and 1 kPa), and then PMNs will transmigrate through the stressed epithelium (see Figs. 1-2 for diagrams and Fig. 3 for workflow). Exosomes will be isolated with an ExoQuick kit and lysed with 5% Triton X. 40 cytokines (including IL-8, CXCL1, IL-1β, IL-6, and IL-10) will be measured with Luminex assays, and neutrophil elastase (NE) will be measured with ELISA for intra- and extra-exosomal groups (extracellular NE activity is a predictor of lung function in CF adults[3]). miRNA will be isolated from supernatant and exosomal lysate with the miRNeasy Mini Kit. miRNA microarrays will identify frequently occurring miRNAs in the supernatant and exosomal lysate. Quantitative RT-PCR will validate the microarray data.[12] I will compare the ASL and PMNs from models with stressed and unstressed epithelium, and I will include no-PMN devices as controls. I will also compare transmigrated and non-transmigrated PMNs from the same device. These comparisons will be made at all 4 timepoints so I can evaluate how the system evolves from healthy to diseased over extended stress exposure.

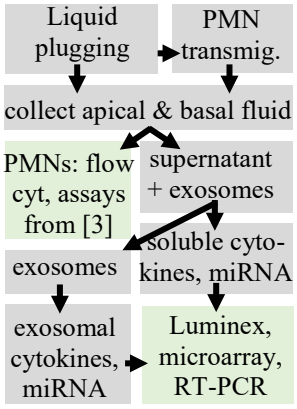


Fig. 3*. Aim 2 workflow.*

To interpret my data, **I initiated a collaboration** with Dr. Kelly Arnold at Univ. of Michigan, whose lab specializes in analyzing COPD patient sputum and blood using data-driven computational approaches to infer cytokine networks driving cell behavior, tissue phenotype and disease progression. The Arnold lab will use systems biology computational methods to identify inflammatory PMN markers from my flow cytometry data and infer proinflammatory cytokine networks between epithelium and PMNs from the 40-plex Luminex assays. **My hypothesis is correct if** a) PMNs that transmigrate through stressed epithelium are pro-inflammatory (based on assays from [3] or cell surface marker expression) and b) proinflammatory cytokines or miRNA are produced by the epithelium in response to FMS that stimulate PMN inflammation.

Broader Impacts: I am uniting leaders in microfluidics, immunology and systems-level data analysis to engineer a novel IAD pathology model and discover immunological mechanisms that will **inform therapeutic design**, ultimately **reducing lung transplants and extending lifespans**. This work is applicable to all IADs and the device can also be used to model immunopathology of pneumonia, lung cancer, or idiopathic pulmonary fibrosis. I will disseminate my results in publications, conferences and seminars with incarcerated people, and I will mentor undergraduates and minority high school students through ENGAGES. ● [a]chronic obstructive pulmonary disease. [b]cystic fibrosis. [c]polymorphonuclear leukocytes.

Ref: [1]"(COPD)." *WHO*, 2017. [2]Tavana *et. al* (2011) *Biomed. Microdev.* [3]Forrest *et. al* (2017) *J Leukoc Bio.* 1-11. [4]Konstan *et. al* (2007) *J Peadiatr* 151:134-9. [5]Jacome *et. al* (2017) *Clin Resp J* 612-620. [6]Unpub., Tirouvanziam Lab [7]Fleshner *et. al* (2017) *Trends in Immuno* 38(10):768-76. [8]Park *et. al* (2012) *Mech. of Allergy* 130:1375-83. [9]Moon *et. al* (2015) *Cell Dth* 6. [10]Hermanns *et. al* (2004) *Lab Invest.* 84:736-52. [11]Yalcin *et. al* (2007) *J App Physio* 103(5):1796:807. [12]Ohshima *et. al* (2010) *PloS ONE* 5:e13247.