# Effect of MUC5AC and MUC5B mucins on airway clearance by simulated cough

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## Introduction

The airways are lined with epithelial cells that produce mucus layers which protect the body from pathogens inhaled from the air every day. This critical layer bind/trap these pathogens and then must be efficiently cleared by the cilia-mediated mucus transport system. *Coughing* is an essential "backup" mechanism used to clear the thicker/adherent mucus associated with viral or bacterial infections, or lung disease. The human lungs can clear mucus with a cough at up to 500 mph. Due to the biophysical properties of mucus, diseased and thicker mucus can affect the effectiveness of a cough clearance and cause buildup of mucus in the lungs.

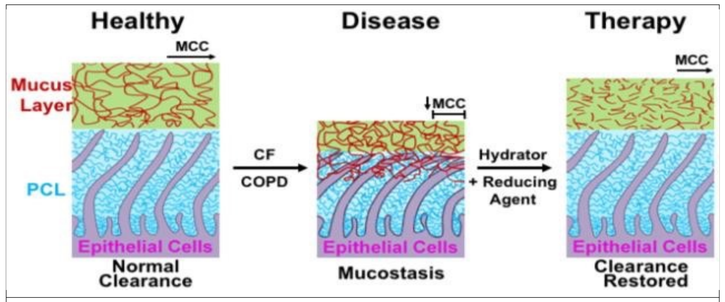


**Figure 1.** Role of mucus dehydration in the disease pathogenesis of CF and COPD. I hypothesize that disease- related increases in mucus concentration lead to 1) a collapse of the PCL, 2) adhesion of the mucus to the cell surface, and 3) subsequent reduction in cilia- and cough- mediated clearance.

Understanding quantitatively how thicker mucus clears differently by cough is critical to helping treat people with upper respiratory disease. The best-known phenotype that correlates with seemingly unrelated airway diseases, such as COPD, Asthma, and cystic fibrosis (CF), is the accumulation of thick, "sticky" mucus in the airways. Previous literature has shown1 that proper hydration of both mucus and periciliary layer (PCL) is required for efficient mucus clearance and that abnormalities in both layers are manifested in lung diseases (**Fig. 1**). Adherent mucus initiates inflammatory responses, airway wall damage and serves as a growth medium for pathogenic microorganisms. Mucus is dominated by two large (up to 100 MDa), heavily glycosylated mucin proteins, MUC5B and MUC5AC which gives mucus is viscoelastic properties. It has been recently hypothesized that different airway diseases, such as chronic bronchitis and asthma, have different ratios of MUC5AC to MUC5B expression. As of now it is not yet confirmed why the body produces higher ratios of MUC5AC to MUC5B in patients with diseases such as COPD and Asthma. It has been hypothesized before that because of the sticker mucus that pathogens are trapped more readily by the sticker mucus and can prevent more infections. This tested study hypothesizes that MUC5AC-dominant mucus clears differently from MUC5B-dominant mucus due to differences in their biophysical properties during a simulated cough.

**Mucus Clearance and Lung Health:** Normal mucus is composed of roughly 98% water and 2% solids components, consisting of mucins (~0.5%), which represent the main "gel-forming" polymers secreted by glands and superficial epithelial cells, globular proteins (~0.6%), and salt (~0.9%). Compared to normal mucus, diseased mucus in patients experiencing an upper respiratory illness can range from 6%-10% solids which is extremely sticky. Patients who have these higher mucus concentrations describe it as mucus getting trapped in their airways but can't cough it up. Often these individuals require medical assistance to clear it (i.e., respiratory physiotherapy)
The continual mucus clearance from the lung represents a key innate defense system that protects the airway surface against exposure to inhaled infectious and noxious particles. Abnormal clearance of mucus is an important contributor to the phenotype of patients with chronic bronchitis (CB) due to: 1) environmental causes such as cigarette smoke; 2) genetic causes, such as cystic fibrosis (CF), or 3) a combination of the two, such as in Asthma. The best-known phenotype that correlates with clearance reduction is the increased production and accumulation of thick, "sticky" mucus in the airways9. The net result is mucus-plugged airways and the eventual clearance failure that leads to airflow obstruction and bacterial infection. Without a proper way to clear the mucus in these situations, there can be dangerous effects on the lungs of the affected individual that can lead to infections or trouble breathing.

## Methodology

**Methods/ Procedures:**
**Cell Culture-** First, to generate MUC5AC and MUC5B, mucus for these studies, cells must be grown that specifically to secrete either MUC5AC or MUC5B mucus. This was done in work by using A549 cancer cells that either had the gene coded for MUC5B or MUC5AC expression to be knocked out using CRISPR/Cas9 so that only one type of mucus is secreted by the cells. (Note, under normal conditions, these cells produce 1:1 ratio of MUC5AC:MUC5B). These cells are then grown on permeable support (Transwell™ Clear; Corning) on top of A549 media for 2-3 weeks in an incubator that simulates human body conditions (i.e., 37°C, 5% $CO_2$, 95% humidity). After 2-3 weeks, a thick layer of mucus accumulated on top of the cells. This mucus was harvested using PBS solution and humification inside an incubator. After harvesting the mucus using this technique, it was spin-concentrated to specific concentrations to simulate healthy (2% solids), mild lung disease (6%), and severe lung disease (10%). This was done by determining the percent solids of the harvested mucus and then using a centrifuge to concentrate the mucus down to a volume that would give one the desired mucus concentration.

**Preparation of cell cultures for cough simulation-** After the mucus of the desired genotype was concentrated to the desired concentration, fluorescent beads were added so that the cough clearance can be tracked by fluorescence microscopy. This was done by first making a mucus solution containing a 1:10000 concentration of 1-micron beads in PBS solution mixed with roughly 80-100 microliters of the desired mucus concentration.
**Inverted cell culture preparation**
Next, one must acquire an inverted cell culture that contains human airway cells and prepare to add this mucus to the inverted cell culture. The purpose of an inverted cell culture is to grow cells in a way that can be inserted into the cough clearance stage as well as they are grown this way so that the cultures are exposed without walls. This allows us to blow across them to observe the cells as if they were in the respiratory system.
First, the inverted cell culture was washed multiple times with PBS to ensure that there is no residual mucus or other molecules could affect the clearance of the desired type of mucus during the cough study. After washing, the mucus sample on placed on top of the inverted cell culture. Next, a Kimwipe™ mesh that helps create a mucus "island", created using a laser cutter, was carefully added to the luminal surface of the culture. After this step, roughly 100 microliters of Perfluorocarbon was placed on top of the mesh so that the mucus sample was not dehydrated. Finally, the culture with island and mucus was incubated in an incubator for 30 minutes. This process was repeated of samples for MUC5AC and MUC5B mucus at concentrations of 2%,6%, and 10% solids.

**Cough simulation-** In this work, to simulate a cough, a vacuum was used to pull air across the surface of the cell culture in one direction. This was an effective way to simulate a cough because pulling in one direction is the same as pushing in the other direction. Another reason that a pulling motion was used was because it resulted in less cell damage than pushing air on the cells likely due to distention of the membrane. The vacuum pump that was used in these studies was capable of generating an airflow velocity of ~32 m/s, which is consistent with the human cough associated with the distal airways. Importantly the inverted culture needs a specific stage that contains an eyelet to place the inverted culture into. A iron cast mold was used to create this eyelet for the inverted culture out of silicon that is sturdy enough to transport the force of the vacuum pump. After the inverted culture is in place the cough is then pulsed using LabVIEW software in order to control the length of the simulated cough and at what speed using a stepper that acts to adjust flow speed from the vacuum.

For the tracking of the mucus (fluorescent beads), the cell culture was positioned into a custom-designed "cough" chamber on a fluorescence microscope (Eclipse Ti2; Nikon) that can focus on the beads and track/record their movement. This was done by taking a series of images (at 100 ftp) of the cough during the airflow where the beads are pushed, and during the elastic recoil of the beads moving backward. The length of the cough lasted for 200 milliseconds and a recoil length of 800 milliseconds in order to record the recovery of the mucus post-cough.

**Data analysis:** The objective of these analyses was to understand whether the relative ratios of MUC5AC to MUC5B affect mucus cough clearance interactions by quantifying cough mucus clearance speed and displacement. These studies were performed by varying mucus concentration (spanning from normal 2% to CF-like >10% solids) to determine how MUC5AC: MUC5B ratios and mucus concentration combine to affect cough clearance. Here, I tracked the movement of beads using open-source tracking software (ImageJ) and then analyzed the velocity and net displacement using custom-made MATLAB software. After analyzing the tracks, I created a graph that displays how varying mucus concentrations and different ratios of MUC5AC: MUC5B affect cough clearance speeds.

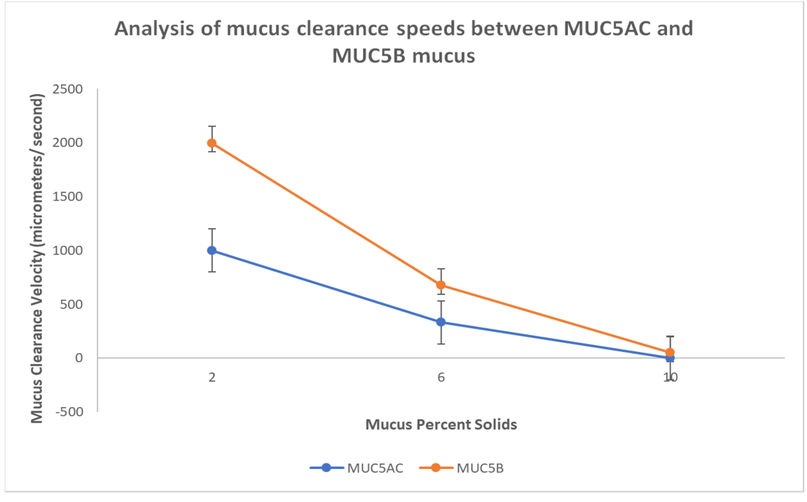


**Figure 2:**
**Cough Clearance of MUC5AC and MUC5B at varying concentrations.** Figure 1 shows the significant decrease in mucus clearance speeds between MUC5AC mucus speeds compared to MUC5B mucus which supports the hypothesis of the study. Figure 1 also shows the effect of mucus concentration (at 2%, 6%, and 10% solids) for both MUC5AC and MUC5B mucus. When mucus concentrations increase for both MUC5AC and MUC5B mucus, the mucus clearance speed decreases insignificant levels. There is a clear linear relationship that as one increases the mucus percent solids, the mucus clearance velocity decreases in both mucus samples.

## Discussion

In this study the important conclusion is that the MUC5AC mucus caused a slower cough clearance speed consistently at different mucus concentrations compared to the MUC5B mucus. Figure 1 showed that at both 2% and 6% mucus that there was a clear trend that MUC5AC had a significantly slower clearance speed than MUC5B. This is consistent with the hypothesis that MUC5AC is a "stickier" mucin that while making it more sticky to inhaled pathogens, result in it being harder to clear mucus from the lungs. In chronic mucus-overproduction diseases, such as asthma, COPD, and CF, the increase in mucus concentration and MUC5AC production, combines to make it extremely difficult for affected individuals to clear their mucus properly.

Another effect that was observed was confirming that increasing mucus concentration led to lower cough clearance speeds. The effects of different types of mucus (MUC5AC and MUC5B) on the clearance of cough were observed to quantify how these different types of mucus affected cough clearance speeds. It was determined that as one increased the mucus concentration on the cell culture, there was a decrease in the speed of the cough clearance, likely due to more friction and adhesion properties of the mucus clinging to the layer of cells underneath it. In these studies, as seen in **Figure 2**, it can be seen that for normal and mucus (2%) and sickly mucus (6%) that MUC5AC is harder to clear because of its significantly lower velocities that were recorded in cough simulations. However, for 10% mucus, the differences between MUC5B and MUC5AC become negligible likely due to increased friction/adhesion dominating any MUC5B/MUC5AC isoform difference.

These interactions will be further researched in the future research in future semesters to see how the cough clearance affects different levels of mucus on the epithelial layer of cells. Further analysis will help to see if when a cough occurs, how the cough affects the layers of mucus that are closer to the cough as well as further from the cough.

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