

Computational Modelling of Lung Mucosal and Humoral Clearance of Pathogenic Bacteria Including *Mycobacterium Tuberculosis*

Abstract

Mycobacterium tuberculosis (Mtb) infects human hosts primarily through the respiratory tract and resides intracellularly in human lung macrophages, after obtaining access to the lung epithelial layer. As a critical part of human immunity, the mucosal layer covering the epithelial structure of the lung provides protection by blocking Mtb access to the epithelial layer through decreased diffusivity, directing flow to expel bacteria from the human lung, and supporting antibody diffusion and antigen-antibody neutralization reactions. This project aims to model bacterial sterilization in the bronchial mucus layer to determine whether human mucosal immunity can readily protect the host from Mtb. This project simulates prolonged exposure up to 100 seconds to Mtb inoculum and includes both inhalation and exhalation movements in the human lung. Both antibody and Mtb were simulated as dilute species transported by diffusion and flow solutions in the airway, the mucus layer, and the periciliary layer of the bronchiole. The model demonstrates that human mucosal immunity can readily sterilize and control bacterial infection even at a high dose concentration of 10 mol/m^3 . The project concludes mucosal immunity is indeed a great avenue for the development of novel therapeutics to combat Mtb infections.

Introduction

Mycobacterium tuberculosis (Mtb) causes tuberculosis (TB), leading to more than one million deaths worldwide annually¹. Mtb aerosolizes and invades human airways, and increasing research evidence has shown that the human immune system, upon sensing Mtb insult to the body, does not achieve clearance of Mtb infection². Existing methods of treating the disease are often limited by poor targeting of the lungs and require long-term, invasive

medication³. The high rate of deaths caused by disease urges bioengineers to find a solution by improving certain aspects of human immunity. Recently, human mucus emerged as a new avenue to engineer. Engineered mucus allows the initial blockage of Mtb before resulting in system insult after accessing the epithelium, and the therapeutic method is usually non-invasive - minimizing side effects⁴. Engineering human mucus to be more dynamic and respond to natural or external stimuli to change its structure or protein concentration can strengthen the mucus' ability to fight infection. However, it is not yet clear to what extent our existing human mucus can clear Mtb. With this knowledge, we will be able to learn which mucosal protection characteristics (i.e., parameters), if any, need to be improved with therapeutics. This model seeks to simulate a bacterial invasion inside the lung with multiple cycles of respiration, without native mucus. The goal of this work is to probe whether natural mucosal conditions can effectively defend against the bacteria.

In order to explore mucosal protection and clearing in the human lung, a model airway was constructed *in silico* to demonstrate how mucociliary clearance works in the lungs by observing particle transport through the mucus layer, periciliary layer, and lung epithelial tissue in COMSOL. This work builds upon a previous COMSOL model created by Bartlett et al. that also stimulated the lung airway mucosal layers. However, Bartlett's model focused on the dosage distribution of differently-sized particles and its effect on mucosal penetration⁴. While the paper briefly looked at the antibody response in the lung, the model did not include a time-dependent simulation modeling inhalation and exhalation. Bartlett et al.'s model also did not consider antibody diffusion in the mucociliary layers and its impact on neutralizing pathogenic particles. From there, this model focuses on how the lung's antibody response neutralizes the pathogenic particles over time, with the aid of the mucociliary layer's decreased diffusivity and bacteria-expelling flow.

In order to address the consideration of antibody reaction and time-dependent respiration, time-dependent stimulation involving non-constant airflow with an added reaction to the periciliary layer

and the mucus layer was used to mimic the mucosal antibody response.

Methods

The bronchial epithelium in the lung consists of three main layers: the mucosal layer, the periciliary layer (PCL), and the epithelial layer. The mucosal layer is responsible for creating mucus which selectively controls the transport of molecules such as bacteria before they reach the epithelial layer. The periciliary layer consists of cilia that beat the mucus along one's mucus layer and the epithelial layers consist of epithelial cells that provide barrier protection, clearance of particulate, initiation of immune responses, and repair following injury⁹. Specifically, the epithelial cells produce antibodies that diffuse through the periciliary and mucosal layers. These three layers form the lung's immune response to pathogenic bacteria.

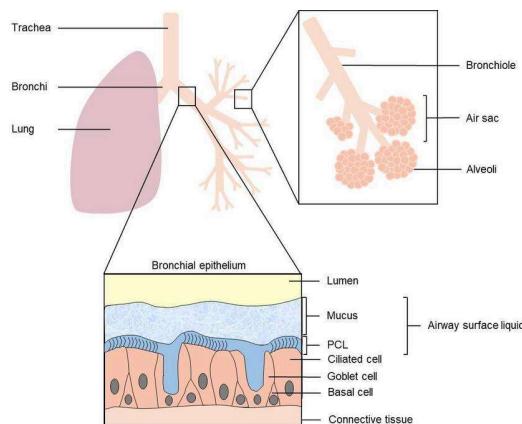


Figure 1. Anatomy of the bronchial epithelium.
Illustration adapted from reference 10.

The model simulates the bacteria clearance and antibody binding of bacteria inside the bronchiole of the lung in several respirations. As Figure 1 shows, a bronchiole is an air tube in a cylindrical shape. Therefore, our group used the 2D Axisymmetric model in COMSOL Multiphysics 6.2 to model the bronchiole. The model's dimensions are as modeled in Figure 2. Besides the lumen which is the air tube in the middle, the model contains three layers: the mucus layer, the periciliary layer, and the epithelial layer.

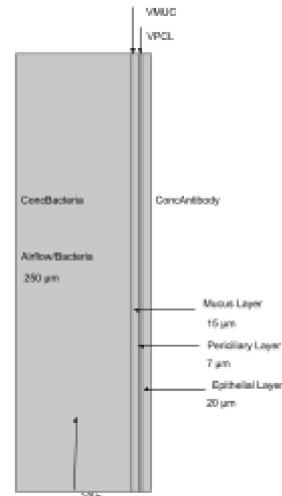


Figure 2. Schematic diagram of the bronchial epithelium. Length specified in radius direction. Z direction (vertical) in total 1000 μm . Leftmost boundary is the symmetry axis.

The bacteria is assigned to be a diluted species in all physics. It flows in the z-direction of the cylinder where the air flows from one cross-section of the lumen to the other. Due to the physiology of the human lung, the mucus layer, and PCL each have a smaller velocity in the opposite direction of the bacteria flow within the layers¹¹. Moreover, the bacteria diffuses through the mucus and periciliary layer into the epithelial layer through diffusion. In this model, convection takes place when the air breathed in the lung causes air transport of both diffusion and flow with bacteria to occur as modeled by the following equation:

$$\frac{\partial c_i}{\partial t} + \nabla \cdot (-D_i \nabla c_i) + u \cdot \nabla c_i = R_i$$

An oscillatory function: $vAIR * \sin((\pi/3)*t)$ [m/s] was set for the velocity to change signs to simulate the inhale and exhale of a respiration cycle. The oscillatory function was formed by considering the average breath time of 6 seconds (including the inhale and exhale) where the sine function demonstrates that maximum velocity occurs at the end of the inhale and exhale), and was directly assigned to be the solution for the bacteria dilute species for the flow solution in the airway. As the velocity depends on the number of sub-branches and

the radius of the bronchioles, it was also assumed that the maximum velocity in the air tube/lumen is 2 m/s.

At the same time, the bacteria flows into the lumen, the epithelial layer produces antibodies that diffuse into the PCL and mucus layer to cause antibody binding and kill the bacteria. Antibody is assigned to be existing only in the mucus layer and PCL layer as it exists extracellularly and does not aerosolize. For simplicity, we did not include antibody production by plasma B cells, instead, we assumed an inlet for antibodies at the intersection between the epithelial layer and the PCL, with it diffusing out to establish a concentration gradient across the mucus and PCL layers.

While we simply assigned bacteria in the airway with an oscillatory flow solution as specified, we simulated laminar flow with COMSOL default Navier-Stokes governing equations in mucus and PCL layers with parameters specified in Table 1. All transported species in either layer were assigned the flow profiles corresponding to the matching physics.

We included a reaction that antibodies neutralize Mtb in both the mucus layer and the PCL. The reaction rate constant Rck in which the antibody neutralizes Mtb is $10^{16} [M^{-20}s^{-1}]^{12}$. We assume that this reaction rate constant applies to antibody-antigen reactions throughout both the mucus layer and PCL as Borrebaeck et al. claimed that the *in vitro* reaction rate constant should be generally applicable to all conditions *in vivo* if no other catalytic event occurs, as they obtained the parameter in *in vivo* resembling settings. Our model further assumes that it takes roughly 20 antibodies to neutralize one bacteria, and the reaction occurs only if 20 antibodies counter an Mtb bacterium, i.e.:

$$\text{Reaction Rate} = Rck[\text{Bacteria}][\text{Antibody}]^{20}$$

This assumption is reasonable as Mtb is known to have high dissociation factor K_D with most antibody isotypes¹³. Also note that this assumption does not account for the size of bacteria, as the varying sizes of bacteria might result in a different Rck , and a different amount of antibodies needed to neutralize one bacteria. Additionally, this reaction rate is linked

to the concentration of bacteria and antibodies. The more concentrated the antibody or bacteria are, the quicker the depleting reaction of both is.

The model was formed based on the following initial conditions. The first initial condition is that the epithelial layer contains a significant amount of antibody concentration compared to the mucus layer, PCL, and lumen with little initial antibody concentration, as the immune system won't be primed until bacteria enter. Thus, the amount of antibodies in the lumen is negligible. In addition, there is a constant velocity for both the PCL and mucus layer in the opposite direction of the bacteria flow. The antibody reaction starts at first inhale and assumes a much larger concentration in the epithelial layer than in the PCL and mucus layer. For the diffusivity, the diffusivity of air was assumed to be way larger than the diffusivity in the mucus layer which was assumed to be 100 times larger than the diffusivity in the mucus layer since air can be more easily diffused than the sticky mucus layer. The PCL is more diffusible compared to the mucus layer which was assumed to be 10 times larger than the diffusivity in the mucus layer. It was also assumed the bacteria can almost not diffuse into the cell which leads to a diffusivity of the epithelial layer close to zero.

Variable Name	Value [unit]	Description	Source
conc	20 [mol/m^3]	Initial concentration of antibody in the mucus layer.	Reference 4
conc2	1000 [mol/m^3]	Initial concentration of antibody in the epithelial layer.	Reference 4
muAIR	$18.95 \times 10^{-6} [\text{Pa}^*s]$	Viscosity of air at 310.15 Kelvin	Reference 6
muMUC	$302 [\text{Pa}^*s]$	Viscosity of the mucus layer.	Reference 4
muPCL	$200 \times 6.922 \times 10^{-4} [\text{Pa}^*s]$	Viscosity of the PCL	Reference 4
vMUC	5 [mm/min]	Velocity of mucus layer flow	Reference 4
mucDiff	$1.26 \times 10^{-11} [\text{m}^2/\text{s}]$	Diffusivity of the mucus layer	Reference 4
airDiff	$\text{mucDiff} \times 100$	Diffusivity of	Assumption

		air/bacteria flow.	
Rck	-10^6 [M ⁻²⁰ s ⁻¹]	Reaction rate at which IgG neutralizes bacteria	Reference 12
Rc	$Rck \cdot c^{20} \cdot c^2$	Concentration-dependent reaction rate at which IgG neutralizes bacteria	Assumption
vPCL	2.4 [mm/min]	Velocity of PCL	Reference 4
pclDiff	mucDiff*10	Diffusivity of the PCL layer	Assumption
epiDiff	mucDiff*10 ⁻¹⁰	Diffusivity of the epithelial layer	Assumption

Table 1. Table of Parameters and Assumptions. Refer to Appendix I for the full table.

After setting up the parameters and initial conditions as demonstrated in Table 1, a time-dependent study was run. In the time-dependent study, the airflow with bacteria velocity was assumed to follow the oscillatory function and the rate at which IgG neutralizes bacteria was set to a variable that is dependent on the concentration of the antibody in the lumen and epithelial layer.

Results and Discussion

Mtb z-direction velocity corresponds to an oscillatory function oscillating between 1.7 m/s and -1.7 m/s

We ought to first check if our simulated bacteria flow in the airway matches what we expect to see - an oscillatory function cycling with 6 seconds intervals.

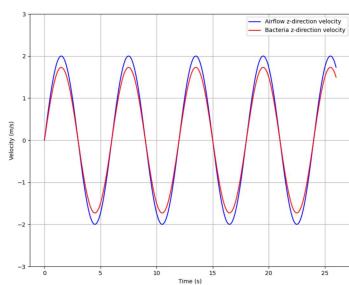


Figure 3. Assigned airflow z-direction velocity (blue) and simulated bacteria z-direction velocity (red).

Deriving the z-direction velocity from COMSOL to generate Figure 3, we see that the bacteria dilute species indeed obeys an oscillating function with 6-second intervals (in red), corresponding to the intervals of the assigned airflow solution (in blue). However, we discovered that the bacteria got peaking velocities at 1.7 m/s and -1.7 m/s, instead of the 2 m/s and -2 m/s velocities of the actual airflow assigned. We think this is due to the interaction of the bacteria with the surrounding mucus layer's viscous properties and the bacteria species' diffusion.

Antibody develops a concentration gradient in the mucus layer and PCL with the concentration profile oscillating between inhalation and exhalation phases

At the initial start point, where $t = 0$ second, shown by the left panel of Figure 3, the antibody is at a high concentration (1000 mol/m³) at the inlet, the intersection between the mucus layer and PCL, and the antibodies very quickly occupy up the entirety of both layers as the simulation starts. At the inhalation phase versus the exhalation phase, the antibody concentration shows a different profile, as showcased by a comparison between $t = 15$ seconds (inhalation phase) and $t = 18$ seconds (exhalation phase) respectively in the middle and the right panel of Figure 4. Here exhalation has a lower concentration profile probably because of the reaction depletion of antibodies when reacting with bacteria.

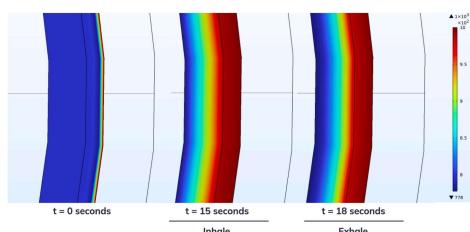


Figure 4. Antibody concentration in mucus and PCL layers since the start of simulation. Unit: mol/m³. In each panel, the black line amidst the gradient is the intersection between the mucus layer and PCL. The PCL here is the outer layer.

To better understand how such concentration gradient changes between the inhalation and exhalation phases, we plotted the average concentration of antibodies in the mucus layer with respect to time.

As noted in Figure 5, the average antibody concentration in the mucus layer oscillates between inhalation and exhalation phases after a quick development of the concentration gradient in roughly 10 seconds. The black arrow notes the inhalation phase, at $t = 15$ seconds after the start of the simulation, corresponding to the Figure 4 middle panel. The inhalation phase here is formally defined as the scenario of the physics after a full inhalation motion, at which point bacteria concentration is fully developed in the airway. The black arrow notes the exhalation phase, at $t = 18$ seconds after the start of the simulation, corresponding to the Figure 4 right panel. The exhalation phase here is formally defined as the scenario of the physics after a full exhalation motion, at which point bacteria concentration is removed in the airway.

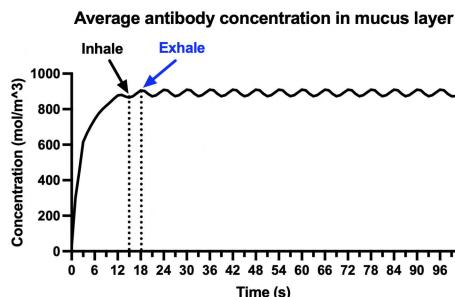


Figure 5. The average antibody concentration in the mucus layer since the start of the simulation and up to 100 seconds into the simulation.

The biophysical properties of the mucosal immunity and the reaction from humoral response provide robust protection against the bacteria inoculum

We then want to visualize the bacteria concentration gradient in the physics simulated, and see if the combination of decreased diffusivity in mucus, the expelling flow of mucus layer and PCL, and the antibody in both layers are able to readily neutralize all bacteria. To this end, we visualized the bacteria concentration in a 2D slice plot, Figure 6.

In Figure 6, we are able to directly see that not even the PCL (therefore, definitely not the epithelium) gets extensively penetrated by bacteria at the end of

inhalation or exhalation - the bacteria do not seem to reach that deep in physics. However, what is particularly interesting is that while the bacteria concentration can be largely removed at the end of exhalation phases in later time points, such as at $t = 96$ seconds in Figure 6, at an earlier time point $t = 6$ seconds into the simulation, we see lingering bacteria concentration in the mucus layer. We think the reason that there is not a full clearance of the bacterium in the mucus layer at the end of the exhalation phase at $t = 6$ seconds is primarily because of the yet-to-be-developed antibody concentration at the interface between the airway and the mucus layer.

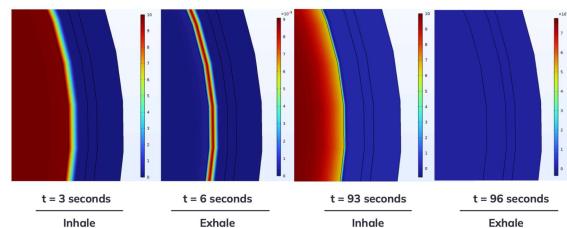


Figure 6. Bacteria concentration is effectively cleared out by biophysical properties of mucosal and humoral immunity at later time points. Unit: mol/m³. Here from the most inward layer to the most outward one, delineated with black lines between each layer, is the airway, the mucus layer, PCL, and the epithelial layer.

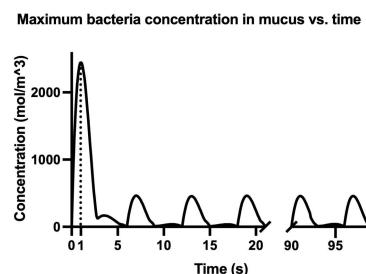


Figure 7. Maximum bacteria concentration in the mucus layer with respect to time. The peaking concentration at $t = 1$ second is noted in the figure.

In Figure 7 we are able to see that, through the time simulated, we are able to see that the maximum bacteria concentration in mucus peaks at $t = 1$ second into the simulation and immediately drops to follow an oscillating pattern corresponding to the assigned inhalation and exhalation phases, with 6-second intervals beyond $t = 5$ seconds. This matches our

hypothesis that at early time points, the bacteria more readily invades into the anatomical physics with our antibody concentration gradient not yet developed. We also wanted to visualize how many bacteria got access to the epithelial layer. In Figure 8, we compared the integrated bacterial content in the epithelium with and without the bacteria-antibody neutralization reaction. We are able to see that with reaction, the integrated epithelial bacteria content remains controlled at a low level of around $1.6E-11$ mole. Interestingly, even with the reaction disabled, the integrated bacteria content still got controlled at a low amount, plateauing at around $1.5E-9$, by just the mucus protection, while bacteria occupied the mucus layer and PCL at large as soon as $t = 20$ seconds into simulation. The hypothesis here about the plateauing after $t = 30$ seconds is that our way of modeling the epithelial layer is too arbitrary that not that many bacteria could flow in or out with an extremely small diffusion coefficient assigned to the epithelial layer.

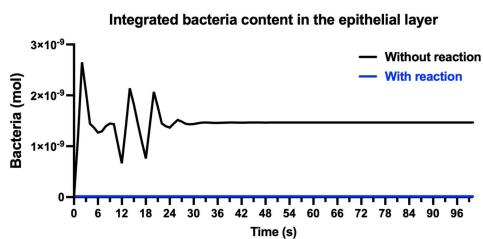


Figure 8. Bacteria obtained access to epithelium with and without reaction enabled. Unit: mol.

Conclusion

This project seeks to better understand what mucus conditions protect human hosts from Mtb infection by adding the complexities wrought by airflow and antibody-mediated neutralization of bacteria to a COMSOL model of a simulated bronchiole under Mtb infection. The results demonstrate that the antibody diffuses thoroughly into the PCL, but not completely in the mucus layer. Under the conditions in the model, Mtb can be cleared efficiently enough to prevent epithelial cell layer infection.

However, in reality, one million people die from TB annually, so clearly these conditions do not accurately

depict representative parameters of mucus. Assuming the correctness of the model, one possible explanation of how mucosal and humoral immunity fails is that Mtb's infection dose can be as low as 1 bacterium. Even though our simulation showed that the Mtb content that got access to the epithelium is as low as $1.6E-11$ mole, this means that few bacteria still can possibly evade the modeled immunity and cause the disease.

Flaws also exist in the model, making it deviate from reality. One obvious flaw includes the assumption that antibody production begins immediately upon inhalation as the immune system must be triggered by bacterium detection to begin antibody production. This would significantly impact our findings and likely lead to less effective clearance of Mtb (before it gets access to epithelium). Thus, future iterations of our model should include these vagaries to better understand how mucus can be engineered to prevent TB.

Additionally, gathering biophysical data on the ranges in mucosal parameters amongst individuals would provide a more representative description of the current capacities of human mucus to fend off infection and provide finer granularity into which parameters can be engineered to aid in TB protection. Future works should be focused on experimentation-based validation of key parameters. Experimental lab data of the diffusivity, viscosity, velocity, and concentration of the concentration-dependent antibody can increase the accuracy of the model.

Regardless, this model fairly robustly indicates the importance and efficacy of mucosal immunity and potential related therapeutic usages. Our model may best instead be seen as one potential ideal in mucosal conditions that can hopefully be recapitulated with the use of therapeutics. Potentially a better Mtb therapeutic could lie along the lines of engineering a better mucus layer with enhanced biophysical protections. Our model proves the assumption that the mucus layer will be able to react to bacteria inside the mucus layer of the bronchiole.

Works Cited

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Appendix I: Full Table 1 and Meeting Agenda

Table 1. Full Table of Parameters and Assumptions used in the Model

Variable Name	Value [unit]	Description	Source
conc	20 [mol/m ³]	Initial concentration of antibody in the mucus layer.	Reference 4
conc2	1000 [mol/m ³]	Initial concentration of antibody in the epithelial layer.	Reference 4
T	310.15 [K]	Average human body temperature.	Reference 4
muAIR	18.95*10 ⁻⁶ [Pa*s]	Viscosity of air at 310.15 Kelvin	Reference 6
muMUC	302 [Pa*s]	Viscosity of the mucus layer.	Reference 4
muPCL	200*6.922*10 ⁻⁴ [Pa*s]	Viscosity of the PCL	Reference 4
radiusAI R	250 [um]	Radius of bronchioles varies between 0.25mm to 0.5mm	Reference 7
radiusM UC	15 [um]	Radius of the mucus layer/the thickness of the mucus layer varies between 0.1-50um	Reference 4
rhoMUC	1000 [kg/m ³]	Density of the mucus layer	Reference 4
rhoAIR	1.29 [kg/m ³]	Density of air/bacteria flow	Reference 8
rhoPCL	1000 [kg/m ³]	Density of the PCL	Reference 4
vAIR	2 [m/s]	Velocity of air/bacteria flow	Assumption
vMUC	5 [mm/min]	Velocity of mucus layer flow	Reference 4
mucDiff	1.26*10 ⁻¹¹ [m ² /s]	Diffusivity of the mucus layer	Reference 4
airDiff	mucDiff*100	Diffusivity of air/bacteria flow.	Assumption
Rck	-10 ⁶ [M ⁻²⁰ s ⁻¹]	Reaction rate at which IgG neutralizes bacteria	Reference 12
Rc	Rck*c ²⁰ c ²	Concentration-dependent reaction rate at which IgG neutralizes bacteria	Assumption
vPCL	2.4 [mm/min]	Velocity of PCL	Reference 4
pclDiff	mucDiff*10	Diffusivity of the PCL layer	Assumption
epiDiff	mucDiff*10 ⁻¹⁰	Diffusivity of the epithelial layer	Assumption