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NEW ANALYSIS OF THE MECHANISMS CONTROLLING THE BRONCHIAL MUCUS BALANCE

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ABSTRACT

Lining the bronchi in the lung, the mucus constitutes a protective layer from harmful pathogens and particles. Its displacement due to the coordinated beating of the bronchial epithelium cilia contributes to its clearance from the lung. As this mucociliary clearance is impaired in numerous pathologies, a comprehensive understanding of this process is critical. A simple mass balance over the mucus layer in an airway suggests that the cilia beating, on its own, is not sufficient enough for controlling the bronchial mucus balance in the entire lung. In this article, we show through a mathematical modeling approach that the evaporation of the water contained in the mucus layer could act as a control mechanism of the mucus balance. Furthermore, we suggest that other control mechanisms act in parallel to evaporation to maintain the mucus balance. Our model and results could be useful in the study and management of several mucus-related airway diseases, such as cystic fibrosis or exercise-induced asthma.

Keywords: mucus, evaporation, epithelium, cilia, respiratory physiology

INTRODUCTION

In mammals, the lung is the organ responsible for the respiratory gases exchange between the body and the environment. As the gas exchange occurs through the alveolar membrane, it can be viewed as a surface process. To maximize the gas exchange surface while containing the lung in a small volume, the evolution optimized the organ shape to distribute the air to the alveoli in a compact manner. In human, the trachea branches in a dichotomous manner, each level of branching being called a generation. Morphometric data estimate that the average lung is made of 24 generations, the first 17 forming the bronchial tree, while the last 7 ones are regrouped in grape-like structures called acini, where the alveoli are budding from. To protect the large surface of the lungs (50-100 square meters in human), most of the bronchial epithelium is covered with a watery protective layer, called the Airway Surface Liquid (ASL). The ASL is made of two sublayers. On top, the mucus layer, which traps inhaled pathogens and particles. Beneath is the Periciliary Layer (PCL), which seems to act like a lubricating layer for mucus displacement. This displacement is induced by the metachronal beating of small cilia covering the bronchial epithelium, which are bathed in the PCL (see Figure 1). Trapped in the mucus layer, pathogens are expelled from the bronchi through the cilia beating. This mucociliary clearance constitutes the major defense mechanism against pathogens in the bronchi.

The literature seems to confirm that this mucociliary clearance is impaired in numerous respiratory pathologies. Thus, a correct description of the bronchial mucus dynamics is critical in the understanding of these diseases, and in the development of new treatments. Therefore, the objective is to provide an accurate description of the bronchial mucus balance and of its possible control mechanisms.

MUCUS BALANCE AND CONTROL MECHANISMS

First, simple calculation based on a mass balance over the mucus layer in a bronchus tends to indicate a potential disequilibrium of the mucus dynamics. In steady state, in healthy patients, the quantity of mucus entering a bronchus should be equal to the quantity of mucus leaving this airway at the same

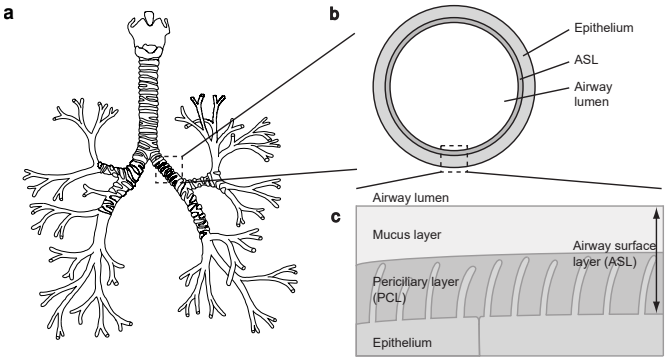

Figure 1: **a)** Picture of a lung cast: bronchial region. **b)** Schematic transverse view of an airway, with the different layers surrounding the lumen of the airway. **c)** The cilia penetrate in the ASL and generate the displacement of the mucus.

time, due to cilia beating. As the airways are branching in a dichotomous manner, the mucus entering an airway is coming from the two daughter bronchi down the tree:

$$2\pi(R_{E_{i+1}}^2 - R_{\mu_{i+1}}^2)v_{\mu_{i+1}} = \pi(R_{E_i}^2 - R_{\mu_i}^2)v_{\mu_i}$$

where R_E and R_μ are the radii from the center of the airway to the mucus and to the epithelium, respectively, v_μ the mucus displacement velocity and i and $i+1$ subscripts indicate the generations indices, numbered from the trachea.

As described by Weibel *et al.* [1], a homothetic ratio is linking the radii of airways in two successive generations with, approximately, $R_{E_{i+1}} = 2^{-\frac{1}{3}} R_{E_i}$. With this relation, and assuming that $R_\mu - R_E \ll R_E$:

$$\delta_{\mu_{i+1}} v_{\mu_{i+1}} = 2^{\frac{2}{3}} \delta_{\mu_i} v_{\mu_i} \approx 1.6\, \delta_{\mu_i} v_{\mu_i}$$

This equation shows that the product of the thickness of the mucus layer (δ_μ) and its velocity of displacement due to cilia beating (v_μ) must be multiplied by a factor 1.6 at each generation to ensure the mucus balance in each generation. Along the bronchial tree, considering 16 generations covered by the mucus layer, this relation implies that the product $\delta_{\mu_i} v_{\mu_i}$ should be multiplied by a factor $2^{\frac{2\times 15}{3}} \approx 1000$ to ensure the mucus balance in the bronchial region. According to previous experimental and simulation results, this increase factor seems overestimated compared to the actual increase factor taking place over the whole bronchial tree (see details in [2]).

This result leads to think that the cilia beating, although the main mechanism of displacement of the bronchial mucus, cannot be the only mechanism able to control the mucus balance in the bronchi.

In parallel, it is interesting to recall previous experimental and theoretical studies that show that the bronchi, at least the proximal ones, tend to be a site of heat and water vapor exchanges [3, 4]. However, the main paradigm is still that the air entering the bronchi is close to body temperature and saturated in water vapor, for having been conditioned in the upper airways [5]. This point of view tends to minimize the magnitude of the heat and water transfers in the bronchi.

Actually, the cited studies show that it takes several generations in the bronchial tree to have the air saturated with water and at body temperature. It means that, during each respiratory cycle, heat and water are lost from the body to the air, mainly through a cycle of evaporation during inspiration and partial condensation during expiration [3]. These transfers obviously occur in the most external layer of the epithelium facing the bronchial lumen, i.e., the ASL, which has to be considered as a zone of transfers and phase change.

As mentioned, the bronchial evaporation mainly takes place in the proximal airways. Obviously, it will influence the ASL, by modifying the volume of mucus in the first bronchi through evaporation or condensation of its water content. Thus, depending on its magnitude, evaporation could act as a control mechanism of the bronchial mucus balance, otherwise only controlled by the non-realistic value of the $\delta_\mu v_\mu$ product.

To test this hypothesis, we have developed a mathematical model, described in the next section, that is able to calculate the heat and mass transfers in the bronchi. This model brings out new features, which have been validated by comparison with previous experimental and modeling results. This consolidates its use as a good computational model of the physicochemical processes occurring in the bronchial mucus layer.

MODELING THE HEAT AND WATER TRANSFERS IN THE BRONCHI

The mathematical model is based on the description of the heat and water vapor transfers in the lumen of the bronchi and, in relation, on the the transport of heat in the tissues surrounding the lumen, i.e., the ASL, epithelium, muscle layers and connective tissue. A detailed description of the model can be found in Karamaoun *et al.*, 2018 [2].

In the lumen of an airway, unsteady transport of heat and water vapor are considered. The temperature and concentration fields are assumed to be axisymmetric. Radial and axial diffusive transports of heat and mass are assumed. The velocity field presented in Tawhai *et al.* is used, with $v(r) = V_{\text{av}} \frac{\alpha+2}{\alpha} \left(1 - (r/R_\mu)^\alpha\right)$, where α is a function of the Reynolds number of the flow in the airway and V_{av} is the average velocity of the flow in the airway [4]. The balance equations for heat and mass transport in the lumen are:

$$V_{\text{av}} \frac{\alpha+2}{\alpha} \left(1 - \left(\frac{r}{R_\mu}\right)^\alpha\right) \frac{\partial C}{\partial z} + \frac{\partial C}{\partial t} = D \frac{1}{r} \frac{\partial}{\partial r}\left(r \frac{\partial C}{\partial r}\right) + D \frac{\partial^2 C}{\partial z^2} \quad (1)$$

$$V_{\text{av}} \frac{\alpha+2}{\alpha} \left(1 - \left(\frac{r}{R_\mu}\right)^\alpha\right) \frac{\partial T}{\partial z} + \frac{\partial T}{\partial t} = \kappa_a \frac{1}{r} \frac{\partial}{\partial r}\left(r \frac{\partial C}{\partial r}\right) + \kappa_a \frac{\partial^2 C}{\partial z^2} \quad (2)$$

where D is the diffusion coefficient of water in air and κ_a is the thermal diffusivity of air. At the end of the airway ($z = L$), the axial diffusive fluxes are assumed to be equal to zero.

In the ASL, the epithelium and the connective tissue of the airway, the characteristic time of the radial diffusion of heat is way smaller than the inspiration time and than the characteristic time of the longitudinal diffusion of heat. Therefore, it can

be assumed that the transport of heat in the ASL, the epithelium and the muscles layer of the airway is purely radial and at quasi-steady state, which writes $\frac{\kappa_l}{r}\frac{\partial}{\partial r}\left(r\frac{\partial T_l}{\partial r}\right) = 0$, where κ_l is the thermal diffusivity of liquid water and T_l is the temperature in the airway wall.

In the connective tissue surrounding the airway wall (subscript c), the energy balance is:

$$\frac{dT_c}{dt} = \frac{P}{\rho_l c_{p,l}} - \frac{\kappa_l}{\delta_c}\frac{\partial T_l}{\partial r} \tag{3}$$

where P is the amount of heat produced by the blood flow in the connective tissue, ρ_l and $c_{p,l}$ are the density and the heat capacity of liquid water. δ_c, the thickness of the connective tissue involved in the heat transfer, is expressed as: $\delta_c = \sqrt{\kappa_l t^{\text{in}}}$, where t^{in} is the inspiration time. The second term of the right-hand side member of this equation characterizes the heat transferred from the connective tissue to the mucus–lumen interface. The first term of its right-hand side member characterizes the heat production in the connective tissue. The heat production term, due to the renewal of the blood in the connective tissue, is expressed as $P = \beta(T_{\text{body}} - T_c)$, with β a coefficient related to the blood flow in the connective tissue capillaries. For $t^{\text{in}} = 2$ s, $\beta \approx 2 \times 10^5$ W m^{-3} K^{-1} [2]. This equation constitutes one of the originality of the model, in a way that it does not evaluate *a priori* the temperature in the tissues, except at its interface with blood vessels, where it is assumed that body temperature is reached.

At the air-mucus layer interface ($r = R_\mu$), $T = T_l$ and $C = C_{\text{sat}}(T)$, which expresses that the air in contact with the mucus layer is saturated with water. Furthermore, the heat balance at the interface is written:

$$-\lambda_a\frac{\partial T}{\partial r} + \lambda_l\frac{\partial T_l}{\partial r} = \mathscr{L}D\frac{\partial C}{\partial r}$$

where λ_a is the thermal conductivity of air, λ_l is the thermal conductivity of liquid water, and $\mathscr{L}$ is the latent heat of vaporization of water.

In the trachea, $T(r, z = 0, t) = T^{\text{in}}$ and $C(r, z = 0, t) = \text{HR}^{\text{in}}C_{\text{sat}}(T^{\text{in}})$, where T^{in} and HR^{in} are the temperature and relative humidity of the inspired air at the inlet of the trachea. At the inlet of an airway belonging to generation i, $T(r, z = 0, t) = T^{\text{out}}_{i-1}(t)$ and $C(r, z = 0, t) = C^{\text{out}}_{i-1}(t)$, where $T^{\text{out}}_{i-1}(t)$ and $C^{\text{out}}_{i-1}(t)$ are the averaged temperature and water concentration at the outlet of an airway belonging to generation $i - 1$. This homogenization of the concentration and temperature profiles at the inlet of an airway is written as a way to take into account the recirculations of the flow induced by the bifurcations.

At the beginning of the first inspiration phase, the air in the bronchial region of the lungs is assumed to be saturated with water and at body temperature. Then, to simulate the inspiration phase, the above equations are solved successively for each generation, starting with the trachea. To simulate an expiration phase, the heat and mass transports equations for expiration are also solved successively, but starting with the last bronchial generation. Several cycles can be simulated consecutively, until the temperature profile of the connective tissue obtained at the end of a cycle is almost equal to that obtained at the end of the previous cycle.

RESULTS & DISCUSSIONS

Figure 2a presents the calculated average temperature of the air at inspiration (○) and expiration (□). These results are in very good agreement with the experimental results from McFadden *et al.* [3], and from other previous modeling works. They show an interesting dynamics of the air temperature in the bronchi. At inspiration, the air is warmed up as it moves towards the distal bronchi, up to a generation where it is close to saturation and body temperature. At expiration, one would expect that the expired warm air would stay at body temperature as it is expelled. On the contrary, the air temperature decreases as it progresses towards the proximal airways, which is in good agreement with experimental measurements.

These results confirm previous observations regarding the thermal inertia of the tissue layers surrounding the bronchi, and validate our original approach. During inspiration, in the first generations, the blood flow in the connective tissue is not able to offset significantly the loss of energy due to air heating and water evaporation; it results in a significant decrease of the tissue temperature. Subsequently, energy is transferred back from the saturated air coming from the alveoli to the connective tissue during expiration, leading to condensation of water. This condensation has a significant influence on the magnitude of the evaporation flux over a respiratory cycle.

Figure 2b presents the evaluation of the magnitude of the various mechanisms controlling the bronchial mucus balance. The mucociliary transport contribution, noted $\Delta M_i/S_i$ (■), is calculated as the difference between the amount of mucus entering and leaving each generation by time unit. The calculation is based on profiles of v_μ and δ_μ that follow a geometric progression along the bronchial tree [2]. The calculated contribution of evaporation is noted E_i (⧫). The other control mechanisms are grouped in the term $B_i = E_i - \Delta M_i$ (•), that ensures the global mucus balance when evaporation and mucus displacement do not have the same order of magnitude. In the upper bronchial tree, the magnitude of the evaporation by surface unit, noted E_i/S_i (⧫), clearly dominates the magnitude of the mucociliary transport by surface unit, $\Delta M_i/S_i$ (■). As the air is heated and saturated down the bronchial tree, the trend is inverted (around generation 9 for these inspiration conditions) and the mucus displacement term dominates the evaporation as control mechanism of the mucus balance.

These results show that the bronchial mucus balance is controlled by several mechanisms. On the one hand, the main mechanism driving the mucus dynamics is its displacement due to cilia beating. We show by a simple mass balance that the mucus, while pushed upward the bronchial tree, has less bronchial surface to spread on, which can potentially disrupt its balance in healthy lungs. On the other hand, evaporation appears to be largely non-negligible in the upper part of the bronchial tree, at a point where it seems reasonable to suggest that other mechanisms (•), such as water replenishment from the epithelium, are critical to prevent mucus dehydration. In-

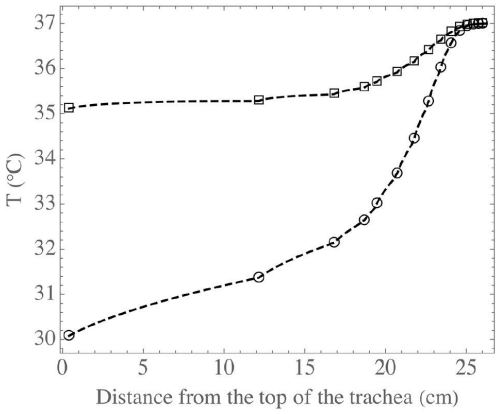

Fig 2a Average temperature of the air at the end of inspiration (○) and expiration (□) on a flow cross-section as a function of the distance from the top of the trachea, at rest, with $T^{\text{in}} = 30°\text{C}$ and $\text{RH}^{\text{in}} = 0.8$.

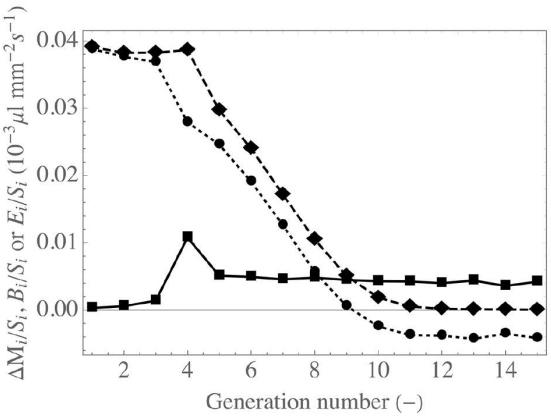

Fig 2b Magnitude of the mechanisms controlling the bronchial mucus balance, by unit surface S_i, with $T^{\text{in}} = 34°\text{C}$ and $\text{RH}^{\text{in}} = 0.95$. (■): mucus displacement contribution; (♦): evaporation contribution; (•): other control mechanisms contribution.

terestingly, the evaporation flux in the first generations, at rest, is of the same order of magnitude than some mucus replenishment values obtained in *in vitro* epithelial cell cultures [6]. Down the tree, the balance disruption due to mucus displacement is still to be considered, while evaporation has become negligible. Under these conditions, it is reasonable to make the hypothesis that one or several control mechanisms play a role (•), one being a possible excess water absorption from the mucus by the epithelial cells, which is coherent with observations from other authors [7].

CONCLUSION

Altogether, this study brings out interesting new insights in the dynamics of the bronchial mucus. The magnitude of the heat and water transfers in the bronchi suggests that these phenomena play an important role in the control of the bronchial mucus balance, and in the dynamics of the bronchial system in general. The management of several related pathologies, such as cystic fibrosis, COPD or exercise-induced asthma, could benefit from recommendations based on the type of approach presented in this article. As an example, the occurrence of exercise-induced asthma in athletes has been related to inspired air conditions and integrity of the bronchial epithelial membrane [8]. Our results allow interpreting these events as correlated: the intense evaporation occurring in the bronchi while inspiring cold air at high inspiration flow possibly leads to a degradation of the bronchial epithelium integrity. As an example, one recommendation would be a pre-conditioning of the inspired air (in temperature and relative humidity) during high-intensity training in athletes.

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