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A spatial model of fluid recycling in the airways of the lung

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ABSTRACT

The genetic disease cystic fibrosis (CF) is a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, and results in viscous mucus and impaired mucociliary clearance leading to chronic recurring pulmonary infections. Although extensive experimental research has been conducted over the last few decades, CF lung pathophysiology remains controversial. There are two competing explanations for the observed depletion of periciliary liquid (PCL) in CF lungs. The low volume hypothesis assumes fluid hyperabsorption through surface epithelia due to an over-active epithelial Na^+ channel (ENaC), and the low secretion hypothesis assumes inspissated mucins secreted from glands due to lack of serous fluid secreted from gland acini.

We present a spatial mathematical model that reflects *in vivo* fluid recycling via submucosal gland (SMG) secretion, and absorption through surface epithelia. We then test the model in CF conditions by increasing ENaC open probability and decreasing SMG flux while simultaneously reducing CFTR open probability. Increasing ENaC activity only results in increased fluid absorption across surface epithelia, as seen in *in vitro* experiments. However, combining potential CF mechanisms results in markedly *less* fluid absorbed while providing the largest reduction in PCL volume, suggesting that a compromise in gland fluid secretion dominates over increased ENaC activity to decrease the amount of fluid transported transcellularly in CF lungs *in vivo*. Model results also indicate that a spatial model is necessary for an accurate calculation of total fluid transport, as the effects of spatial gradients can be severe, particularly in close proximity to the SMGs.

1. Introduction

The genetic disease cystic fibrosis (CF) results in an accumulation of mucus in many major organs, with more than 90% of mortality arising from lung complications (Quinton, 1990). A monolayer of predominantly ciliated epithelia line the inside of the lungs from the trachea to bronchioles, and are located underneath the airway surface liquid (ASL); two mostly distinct layers consisting of serosal fluid from the cell surface to the tips of the cilia (the periciliary liquid or PCL) and above it, a layer of varying thickness consisting of a gel of mucins. The ASL has antimicrobial and antibacterial properties (Smith et al., 1996; Bals et al., 1998), and is maintained at an optimal volume through a balance of ion/water transport to maintain healthy hydration of the airways. Lung surface epithelia allow passive transport of fluid through leaky membranes and osmotic gradients, and active/passive ion transport through channels, exchangers and transporters located on cell membranes.

The mucus layer is a tangled macromolecular mesh comprising 90% gel-forming mucins such as MUC5AC and MUC5B secreted by goblet surface epithelia and cells from the gland mucous tubule (Button et al., 2012). The other 10% of the mesh consists primarily of tethered mucins MUC1, MUC4 and MUC16 that are attached to surface cells, and therefore densely pack the negatively charged PCL fluid (Sheehan et al., 2007).

CF is a mutation in the gene for the protein cystic fibrosis transmembrane conductance regulator (CFTR). In healthy lungs, the CFTR transports Cl^- through cell membranes and is associated with water secretion that replenishes the ASL. CFTR dysfunction results in compromised mucociliary clearance which causes mucus accumulation and disrupts the natural innate defense molecules inherent in healthy serosal fluid. The mechanisms which increase mucus from a healthy volume are still unclear, and two main hypotheses attempt to explain this phenomenon.

In the *low volume hypothesis*, it is assumed that normal CFTR function inhibits the ENaC tonically, whereby the malfunction results in the hyper-absorption of PCL Na^+ and water by the cell layer, thus depleting PCL fluid (Tarran et al., 2001, 2006; Matsui et al., 2000, 1998). Depleted or inspissated PCL causes cilia to become stuck, disrupting healthy mucociliary clearance mechanisms. The *low secretion hypothesis* assumes CFTR function is highest in serous acini located at the distal end of submucosal glands (SMG), where a defective CFTR results in reduced secretion of fluid (Shen et al., 1994; Wu et al., 2007; Salinas et al., 2005). This is due to compromised

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Cl^- secretion from the acini where Na^+ generally follows via electroneutrality and subsequently fluid is secreted via osmosis.

The PCL is predominantly hydrated by fluid secreted from SMGs (Wu et al., 2007), which are found approximately once per 1 mm^2 (10^6 μm^2) of tracheal surface (Tos, 1970). Therefore, we have constructed a model to analyse how this spatial arrangement of fluid-secreting glands affects water fluxes and cellular/PCL ion concentrations, and to test the validity of the two hypotheses for CF conditions. As the amount of fluid above the cell distribution is paramount to maintaining healthy lung hydration, we have also constructed the model to predict PCL volume. We contend that *fluid recycling* is the important factor in considering CF lung epithelia, where fluid is secreted from the glands and reabsorbed across the cell layer. This assumption is derived from the facts that surface epithelia are primarily absorptive in basal conditions due to large amounts of fluid secreted from the glands into the ASL (≈ 1.5 L/d) (Kilburn, 1968; Widdicombe et al., 1997) and PCL evaporation rates are comparatively low (4.34×10^{-8} L/m^2 s, estimated by Novotny and Jakobsson, 1996a, b). Essentially, we demonstrate that the balance between the secretion and absorption of ions and water effectively controls airway hydration, and use this to predict cellular behaviour *in vivo*.

Previous mathematical models have thus far focused on time-dependent solutions and transepithelial water flux through singular, primarily water-secreting cells, all identical in nature. No spatial model has been constructed to our knowledge, and no model contains transport of the PCL as a whole. Although Warren et al. (2010) published a spatio-temporal model focused on the balance between fluid secretion from epithelia and evaporation from the airways, the epithelia in their model are secretory and model predictions indicate a dehydrated PCL without a significant contribution of fluid from either the glands or from some other source.

2. Model construction

We consider a continuous distribution of absorptive epithelial cells between neighbouring submucosal glands in a one dimensional, three compartmental model. In order to model the effects of fluid secretion from the glands, we have included a term for lateral water movement in the PCL layer, W_p, as a volumetric flux. If we assume that the area is square, we have a domain of 1000×1000 μm^2, indicating a linear boundary of $x \in [0, 1000]$. Water is secreted from the glands into the PCL, where it is translated into PCL water flux at the boundaries $x = 0, 1000$ μm, and renders boundary conditions for W_p. Fig. 1 shows water secreted from the glands entering the domain at the two boundaries, as indicated by the arrows. We assume that secretions from the glands will push water inwards, forcing the flux to be zero in the middle of the domain due to symmetry. This effectively cuts our domain in half, creating a new boundary condition $W_p(500\ \mu m) = 0\ \mu m/s$, and therefore it is only necessary to solve the system numerically to $x \in [0, 500]$.

The three compartments represent the PCL, cell and underlying serous bath (plasma) which are denoted by subscripts p, i and s, respectively. Water is driven across both apical and basolateral membranes due to osmotic gradients, which gives rise to changes in cell volume. The plasma located underneath the cell layer is considered to be an infinite bath, preserving constant ionic concentrations. Cellular and PCL compartments are assumed well stirred in the y–z direction.

3. Model equations

3.1. Compartmental volume

As we assume that PCL water and cell volume are homogenous in the y–z direction, we therefore describe the PCL and cell 'volume' as one-dimensional heights. We assume that H_p is constant over the spatial domain and although it may not be a good approximation, we do so for simplicity. If we assume otherwise, the time-dependent fluid layer could be modelled only with the equations of fluid mechanics (as well as constitutive equations for the flow of the viscous fluid in response to gradients in height), which is a model that is orders of magnitude more difficult and complex than the one presented here. Differential equations describing PCL height (H_p) and cell height (H_i) are derived using conservation of water mass and incompressibility:

$$\frac{\partial H_p}{\partial t} = -H_p \frac{\partial W_p}{\partial x} - J_a^w, \tag{1}$$

$$\frac{\partial H_i}{\partial t} = J_a^w - J_b^w, \tag{2}$$

where W_p is the PCL water flux (in units of μm/s) and measures the volume of water transported laterally above the epithelia, as shown in Fig. 1. Volumetric fluxes of water through the apical and basolateral cell membranes are represented by J_a^w and J_b^w (in units of μm mM/s), respectively. The partial differential equation governing H_i represents the change in cellular height over the spatial distribution.

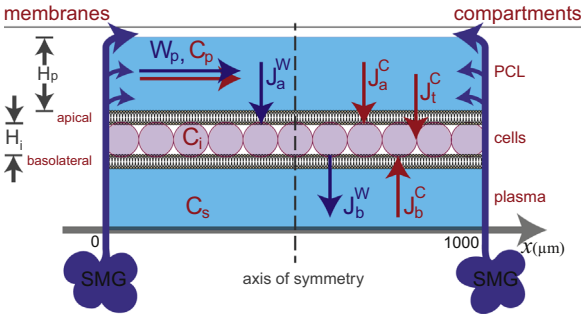

Fig. 1. Schematic of full compartmental model with ionic and water fluxes over a distribution of 'continuous' cells in between secretions from submucosal glands (SMGs) at $x = 0, 1000$ μm. The glands secrete fluid at the boundaries of the domain and push the PCL inwards, which is then transported transcellularly. Ions flow into the cell via the apical or basolateral membrane and from the PCL directly to plasma via the tight junctions. W_p – PCL water flux, c_p – PCL ion concentration, c_i – cellular ion concentration, c_s – plasma ion concentration, H_p – PCL height, H_i – cellular height, J_a^w – water flux through apical membrane, J_b^w – water flux through basolateral membrane, J_a^d – ionic flux through apical membrane, J_b^d – ionic flux through basolateral membrane, J_t^d – ionic flux through tight junction. Arrows denote positive flux direction, and we assume the negligible thickness of cellular membrane.

3.2. Ions

The change in ionic species concentration in PCL (c_p^j) or cellular compartment (c_i^j) is derived from conserving the number of ions, and in generic form is given by

$$\frac{\partial}{\partial t}(H_p c_p^j) = H_p \mathcal{D}_{c_j} \frac{\partial^2 c_p^j}{\partial x^2} - H_p \frac{\partial}{\partial x}(c_p^j W_p) - J_a^{c_j} - J_t^{c_j}, \tag{3}$$

$$\frac{\partial}{\partial t}(H_i c_i^j) = J_a^{c_j} + J_b^{c_j}, \tag{4}$$

where we have assumed that the lateral velocity of ions in the PCL is equivalent to that of the water. The species included in this model are the ions Na^+, K^+, Cl^-, HCO_3^-, H^+, and the molecule CO_2. Ionic fluxes through the apical, basolateral, and tight junctional membranes are represented by $J_a^{c_j}$, $J_b^{c_j}$ and $J_t^{c_j}$ for ion species c_j. The diffusion coefficient is $\mathcal{D}_{c_j}$ for each ionic species. We assume that intercellular diffusion and advection of ions or water between