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An integrated mathematical epithelial cell model for airway surface liquid regulation by mechanical forces

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ABSTRACT

A robust method based on reverse engineering was utilized to construct the ion-channel conductance functions for airway epithelial sodium channels (ENaC), the cystic fibrosis transmembrane conductance regulator (CFTR), and calcium-activated chloride channels (CaCC). The ion-channel conductance models for both normal (NL) and cystic fibrosis (CF) airway epithelia were developed and then coupled to an adenosine triphosphate (ATP) metabolism model and a fluid transport model (collectively called the integrated cell model) to investigate airway surface liquid (ASL) volume regulation and hence mucus concentration, by mechanical forces in NL and CF human airways. The epithelial cell models for NL and CF required differences in Cl^- secretion (decreased in CF) and Na^+ absorption (raised in CF) to reproduce behaviors similar to *in vitro* epithelial cells exposed to mechanical forces (cyclic shear stress, cyclic compressive pressure and cilial strain) and selected modulators of ion channels and ATP release. The epithelial cell models were then used to investigate the effects of mechanical forces and evaporative flux on ASL and mucus homeostasis in both NL and CF airway epithelia. Because of reduced CF ASL volumes, CF mucus concentrations increased and produced a greater dependence of ASL volume regulation on cilia-mucus-ATP release interactions in CF than NL epithelial nodules. Similarly, the CF model was less tolerant to evaporation induced ASL volume reduction at all ATP release rates than the NL model. Consequently, this reverse engineered model appears to provide a robust tool for investigating CF pathophysiology and novel therapies.

1. Introduction

ASL height (volume) regulation is critical to hydrate mucus sufficiently to maintain mucus transport and host defense. Cystic fibrosis (CF) patients are vulnerable to airways mucus hyperconcentration, mucus obstruction, and pulmonary infections. This vulnerability reflects the fact that CF subjects exhibit a dysfunction of cystic fibrosis transmembrane conductance regulator (CFTR) mediated ASL volume regulation. In series with submucosal gland secretion (Ballard and Spadafora, 2007; Widdicombe and Wine, 2015), ASL height is primarily governed by osmotic gradients generated by ion channels embedded in the apical membrane of superficial airway epithelia (Boucher, 2007). Multiple experiments have demonstrated that these channels are, in part, regulated by external and internal mechanical forces through adenosine triphosphate (ATP) release into the luminal compartment (Button et al., 2013; Button et al., 2007; Tarran et al., 2005). Three different types of physical forces can trigger ATP release: cyclic shear stress, cyclic compressive stress, and cilial strain. Three distinct ion channels respond to the ATP release: epithelial sodium channels (ENaC), CFTR, and calcium-activated chloride channels (CaCC). However, ASL height homeostasis in the *in vivo* environment, reflecting the interactions between epithelia cells, mechanical forces, and thermodynamics, is not as easily characterized as ASL homeostasis in *in vitro* experiments. Thus, a numerical study of ASL height regulation may be useful to provide insights on ASL volume regulation *in vivo* in normal (NL) and CF lungs.

A high-fidelity computational fluid dynamics (CFD) method can accurately predict mechanical forces and water evaporative fluxes in the human airways and, consequently, could be coupled with a biophysical cell model of ion and water transepithelial transport to predict ASL height. Also available is an extracellular ATP

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metabolism model for airway epithelia (Zuo et al., 2008). However, this ATP model has not been coupled to ion transport models (Zuo, 2007). Herschlag et al. (2013) developed a cell model that predicted ASL height based on mechanical forces, but the model lumped all the interactions into three variables, *i.e.*, ATP concentration ([ATP]), ion concentration, and ASL height. Without distinct variables and solving for individual regulatory pathways, the utility of the model was limited. Warren et al. (2009) developed a cell model to predict ASL height by osmosis. Compared to reported ATP/ion/water transport models, their model solved the biophysical mechanisms for Na^+, Cl^- and K^+ transport with independent descriptions of the major ion channels, ion cotransporters, and ion pumps on both apical and basolateral membranes. They also included an ATP-trigged intracellular calcium component to model the activation of CaCC. However, they did not include other pathways required in ASL height regulation, e.g., extracellular ATP or its metabolites and their activation/inhibition of CFTR and ENaC. Garcia et al. (2013) also developed a biophysical model for water and ion transport. Compared to Warren et al. (2009)'s model that used conductance-voltage relations to simulate ion flux with conductances fitted to experimental data, Garcia et al. used the Goldman-Hodgkin-Katz equation to simulate ion flux with permeabilities fitted to experimental data. Both methods showed good agreement with the experimental data.

In this study, we developed an ion-channel conductance model that coupled the previously developed fluid secretion model (Warren et al., 2009) with an ATP metabolism model (Zuo et al., 2008) to not only reproduce known steady state cellular responses reported experimentally, but to investigate the effects of mechanical forces and evaporative flux on the ASL volume homeostasis for NL and CF airway epithelia. The integrated model takes advantage of the two existing airway epithelial cell models that contain the fully described ATP metabolism and the ion-channel conductance/water transport descriptions of the known regulatory pathways for ASL volume homeostasis. The ultimate goal was to combine this integrated model with a thermo-fluid CFD lung mechanics model (Wu et al., 2015; Wu et al., 2014) that predicts *in vivo* local mechanical forces and water loss rates. The approach enables the prediction of ASL volume by accounting for CFD-predicted local evaporative fluxes and cellular responses to CFD-predicted local mechanical forces in a disease specific manner.

2. Method

2.1. An overview of the model

Fig. 1 shows the schematic view of both normal (NL) and CF epithelial cells. The cell model elements reacting to mechanical forces are summarized into eight basic processes marked in Fig. 1(a) (Zuo, 2007):

1. Mechanical forces trigger luminal ATP release from airway epithelial cells.
2. Extracellular ATP is metabolized into adenosine diphosphate (ADP), adenosine monophosphate (AMP), adenosine (ADO) and inosine (INO).
3. ATP binds to purinoceptor 2 receptor (P2Y2-R) and triggers phosphatidylinositol 4,5-bisphosphate (PIP_2) metabolism into inositol trisphosphate (IP_3) and diglyceride (DAG). IP_3 activates intracellular calcium release. Intracellular Ca^{2+} activates CaCC channel to increase Cl^- secretion.
4. ADO binds to the adenosine A_{2b} receptor and increases cyclic adenosine monophosphate (cAMP), which subsequently activates CFTR channel via protein kinase A to increase Cl^- secretion.
5. An increase of DAG concentration activates enzyme protein kinase C (PKC), which also activates the CFTR channel.
6. CFTR channel inhibits ENaC channels, decreasing Na^+ absorption (Stutts et al., 1995). However, there exists contradictory evidence that CFTR does not inhibit ENaC (Nagel et al., 2005).
7. P2Y2-R-mediated depletion of PIP_2 inhibits ENaC channel, decreasing Na^+ absorption.
8. As ion channels modulate extracellular ion concentrations, osmotic gradients are created that drive water flux through the epithelial cell membranes. For example, an increase of Cl^- secretion increases ASL height, whereas an increase of Na^+ absorption decreases ASL height.

Thus, we present three models to predict the processes illustrated in Fig. 1:(a) an ATP model (processes 1 and 2); (b) an ion-channel conductance model (processes 3 to 7); and (c) a fluid transport model (FTM) (process 8). Note that these models do not account for: 1) the elements that regulate the extracellular pH (Coakley et al., 2003; Falkenberg & Jakobsson, 2010); 2) direct mechanical effects on CFTR (Zhang et al., 2010) or ENaC function (Althaus et al., 2007); or 3) the mass of mucins in the mucus layer. The differences between NL and CF epithelia cells are that steps 4, 5 and 6 in Fig. 1(b) involving CFTR channels are dysfunctional in CF cells due to mutations in the CFTR gene.

2.2. ATP model and fluid transport model

The ATP model was developed and validated by Zuo et al. (2008) (see the reproduced result in Fig. 2(a), which shows the hydrolysis of 100 μM ATP to its metabolites). The FTM was developed, extensively tested, and validated by Warren et al. (2009). As shown in Fig. 1, this model includes three apical membrane channels: CaCC, ENaC and CFTR, and two basolateral membrane channels; *i.e.*, a basolateral chloride channel (BCC) and calcium-activated potassium channel (CaKC). It also includes a Na-K-2Cl cotransporter and an active sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase) on the basolateral surface. Fig. 2(b) shows the FTM prediction of 33% hypotonic challenge (reproduced as in Warren et al. (2009)) compared to data from Okada et al. (2006). After hypotonic solution administration, water was driven by osmotic pressure and ions were driven by the electrochemical gradients across the membrane until equilibrium was again reached. The cell swelling and deswelling dynamics were modeled accurately by the FTM. More details of the ATP and FTM model are provided in the Supplementary Material.

2.3. ATP release by mechanical forces

Extracellular ATP is released by cyclic shear stress, compressive stress, and cilial strain, which are the types of forces airway naturally experiencing during tidal breathing or coughing. Each type of stress was modeled as follows.

First, Tarran et al. (2005) found that ATP was released by cyclic shear stress (CSS) as given by J_{ATP_CSS} in Fig. 3(a). As no single function described the relationship between [ATP] and cyclic shear stress, interpolation of measurement data was used. The ATP release range by CSS was approximately between [0, 130] nM.

Second, Button et al. (2007) found that ATP was released by cyclic compressive pressure (CCP), as denoted by J_{ATP_CCP} in Fig. 3(b). The release of ATP could be well described by the function $J_{ATP\_CCS} = P_{max}\frac{\Delta P}{K_p+\Delta P}$, where ΔP is the average change in CCP, K_p and P_{max} were fitted to match the data. The ATP release range by CCP was approximately between [0, 60] nM.

Third, Button et al. (2013) reported that cilial strain (CS) stimulated by mucus viscosity triggered the ATP release J_{ATP_CS}. They showed that the ATP release increases with mucus concentration