A modelling framework for airway epithelial fluid and ion transport with multiple cell types: implications for success or failure in gene therapies for cystic fibrosis

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Introduction:

- We have developed a multicellular modelling framework for airway epithelial ion transport.
- The model allows us to explore the impacts of different cell types and gene therapy when CFTR correction is imperfect.

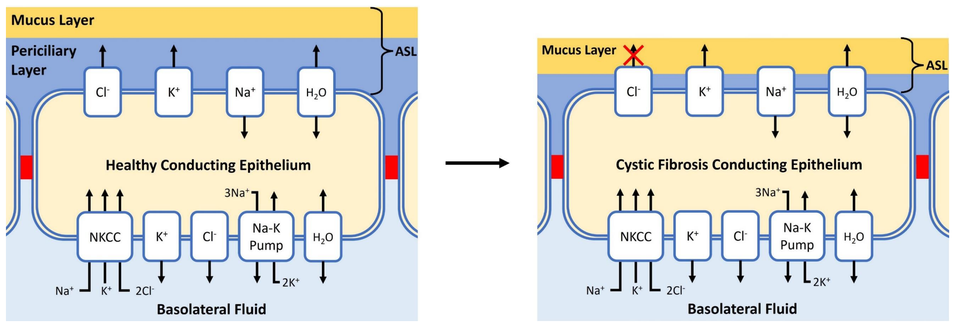

Fig. 1: Schematic of the ion transport pathways included in typical airway models, controlling hydration of the airway surface liquid (ASL) which is reduced in CF. K^+, Cl^- and Na^+ represent ion channels, H_2O represents aquaporins and NKCC represents the $Na^+/K^+/2Cl^-$ co-transporter.

The airway epithelium is not homogenous:

- A range of epithelial cell types are expressed in the upper airways (**Fig. 2**).
- Transport properties vary considerably across these cell types.
- Ionocytes account for up to 50% of CFTR expression, despite making up only 1-2% of epithelial cells.[1][2]

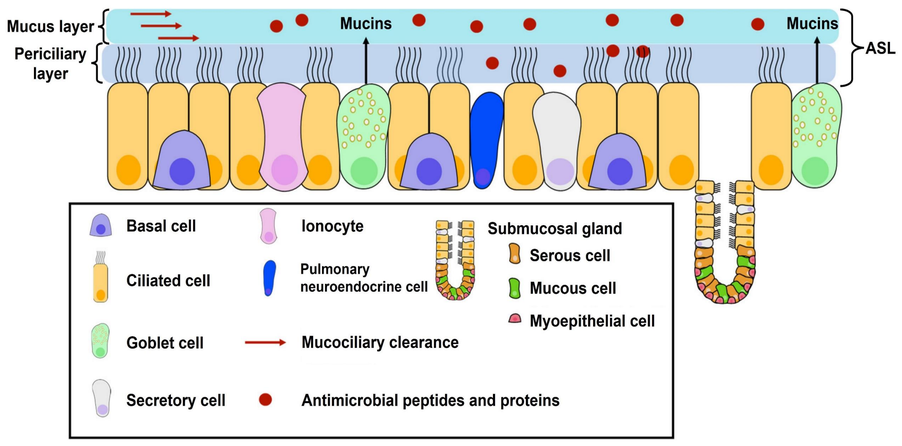

Fig. 2: Representation of cell types in the upper airway epithelium. Figure modified from Zajac et al.[3]

A cell-type specific role for CFTR in anion secretion and absorption:

- Simple model with epithelium split into two distinct cell types, as shown in **Fig. 5**.
- **Secretory cell:** contains $Na^+/K^+/2Cl^-$ co-transporters (NKCC) but no basolateral Cl^- channels (ClC_{ba}), resulting in Cl^- secretion from the cell via CFTR.
- **Absorptive cell:** contains ClC_{ba} but no NKCC, resulting in Cl^- absorption into the cell via CFTR.

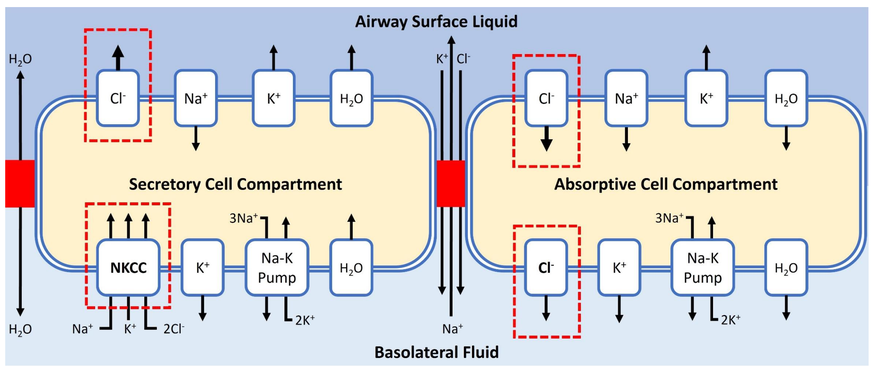

Fig. 5: Schematic of a two-cell secretory-absorptive model of airway epithelial fluid/ion transport. Apical Cl^- channels, basolateral Cl^- channels and NKCC are highlighted as different in each cell.

Modelling airway epithelial fluid/ion transport:

- To understand how different cell types contribute to ASL regulation and how this could inform therapeutic strategies for CF, we developed, and solved, **a multicellular computational modelling framework for fluid/ion flux in airway epithelia** (**Fig. 3**).

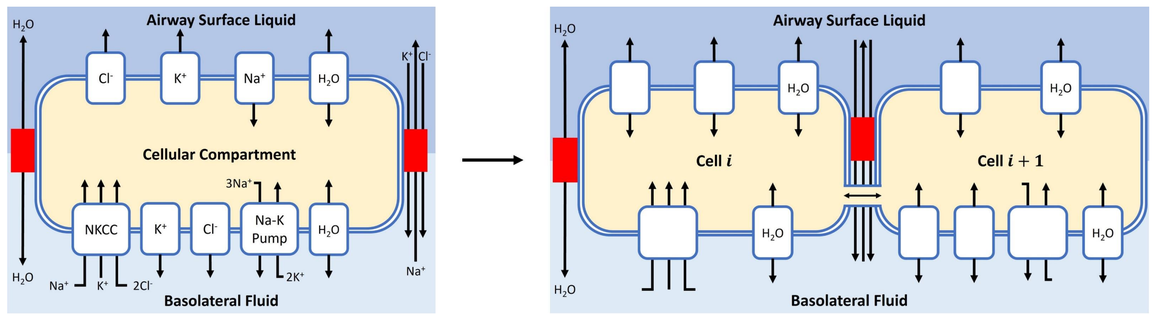

Fig. 3: Schematic of typical single-cell models (left) and a multicellular model (right) of fluid and ion transport in airway epithelium. White boxes show channel-mediated diffusion, osmosis or active/secondary-active transport processes and red boxes show paracellular flux. Cell-cell lateral diffusion is included in the mathematical framework.

- The model is governed by a series of differential equations defining the rate of change of model variables and equations defining the flux of fluid and ions

Channel-mediated electro-diffusion:

$$I_{ion} = P_{ion} {z_{ion}}^2 \frac{V_m F^2}{RT} \frac{a_i - a_o e^{(-z_{ion} F V_m / RT)}}{1 - e^{(-z_{ion} F V_m / RT)}}$$

Fluid flux:

$$OSM_j = [Na^+]_j + [Cl^-]_j + [K^+]_j + [\psi]_j$$

$$J_{H_2O} = P_{H_2O} v_{H_2O} \left(OSM_j - OSM_{j+1}\right)$$

NKCC steady-state turnover rate:

$$v_{NKCC} = \frac{k_f^{full} k_f^{empty} [Na^+]_s [K^+]_s [Cl^-]_s^2 - k_b^{full} k_b^{empty} [Na^+]_i [K^+]_i [Cl^-]_i^2}{\sum_{n=1}^{16} Z_{NKCC}^n}$$

Gene therapy in a secretory-absorptive model of the CF airways:

- Mis-localisation of CFTR to the basolateral membrane has been observed when attempting to overexpress CFTR.[5] The model confirms that this would result in reduced outward apical Cl^- currents, hence reducing hydration of the ASL.
- Our secretory-absorptive model (**Fig. 5**) goes beyond previous single-cell models, allowing further predictions to be made about the extent of success for gene therapy, which depends on the ability to target appropriate cells (**Table 1**).

Table 1: Secretory-absorptive model outputs after simulating the target effects of gene therapy across eight different scenarios. Scenarios highlighted in bold (1 and 5) are predicted to most likely to result in adequate airway surface liquid (ASL) rehydration. ClC_{ba} represents basolateral Cl^- permeability.

Scenario		Cells affected	Localisation	ASL Depth (μm)
1	Gene therapy returns apical Cl^- and Na^+ permeability to normal level…	**in every cell**	**-**	[illegible]
2		in every cell	but increases ClC_{ba} in every cell (1:1 apical to basolateral)	[illegible]
3		in every cell	but increases ClC_{ba} in every cell (2:1 apical to basolateral)	[illegible]
4		in every cell	but increases ClC_{ba} in every cell (4:1 apical to basolateral)	[illegible]
5		**in secretory cell ONLY**	**-**	[illegible]
6		in absorptive cell ONLY	-	[illegible]
7		in secretory cell ONLY	but increases ClC_{ba} in secretory cell (1:1 apical to basolateral)	[illegible]
8		in absorptive cell ONLY	but increases ClC_{ba} in absorptive cell (1:1 apical to basolateral)	[illegible]

Secretory-absorptive regulation of fluid/ion transport:

- Typical airway fluid/ion regulatory models are bidirectional, with cells capable of alternating between secretory and absorptive states. However, classical epithelial transport is unidirectional (**Fig. 4**).

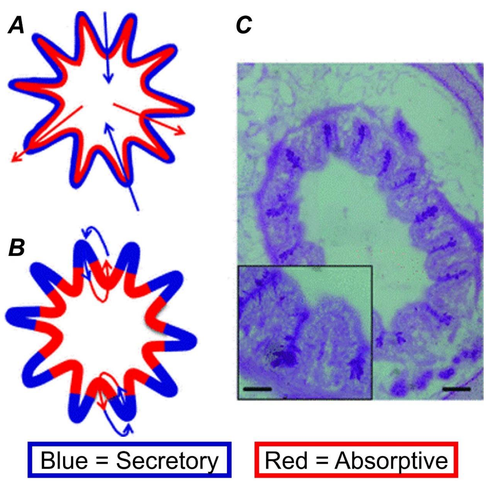

Fig. 4: Alternative models of ion transport control. Secretory-absorptive model of small airway epithelial fluid/ion transport.

A) Bidirectional model: All cells capable of alternating between secretory and absorptive states.

B) Unidirectional model: Cells in the pleats (blue) secrete fluid while cells in the folds (red) absorb secreted fluid.

C) Cross-section of small airway epithelium shows arrangement into pleats and folds.

Figure modified from Shamsuddin and Quinton.[4]

Conclusion:

Multicellular modelling has the potential to further elucidate the roles and importance of different cell types in ASL regulation and inform the design and development of successful therapies for CF.

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1. Plasschaert, L.W., et al., A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. Nature, 2018. 560(7718): p. 377-381.
2. Lei, L., et al., CFTR-rich ionocytes mediate chloride absorption across airway epithelia. The Journal of Clinical Investigation, 2023. 133(20).
3. Zajac, M., et al., Airway Surface Liquid pH Regulation in Airway Epithelium Current Understandings and Gaps in Knowledge. Int J Mol Sci, 2021. 22(7).
4. Shamsuddin, A.K. and P.M. Quinton, Surface fluid absorption and secretion in small airways. J Physiol, 2012. 590(15): p. 3561-74.
5. Farmen, S.L., et al., Gene transfer of CFTR to airway epithelia: low levels of expression are sufficient to correct Cl− transport and overexpression can generate basolateral CFTR. American Journal of Physiology-Lung Cellular and Molecular Physiology, 2005. 289(6): p. L1123-L1130.