Modelling Epithelial Barrier Function And Mucociliary Clearance Using Small Airway Epithelium Cells

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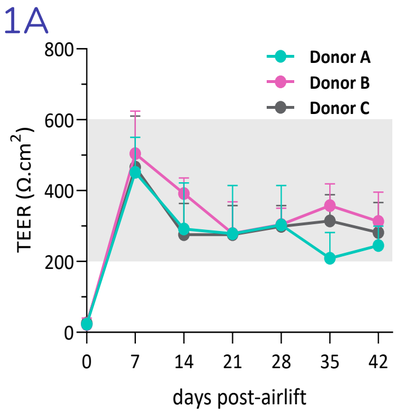
Small Airways in Health and Disease

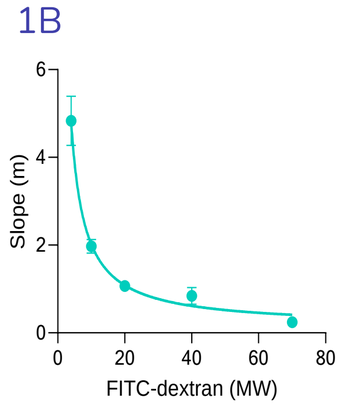
- The small airways form a first line defence mechanism against inhaled particles and facilitate gas exchange by distributing air to the alveoli. The small airways play a crucial role in disease and are often the initial site of inflammation and obstruction in conditions such as bronchiolitis obliterans, asthma, and chronic obstructive pulmonary disease.
- Our ability to study small airway physiology *in vivo* is limited due to their small size and location deep within the lungs, making them difficult to visualize and access using non-invasive imaging techniques. At Newcells Biotech, we have developed a complex, multicellular system to enable the study of small airway physiology and disease *in vitro*.
- Primary human basal cells are expanded in culture flasks prior to seeding onto semi-permeable inserts under submerged conditions. On reaching confluency, the cells are 'air-lifted', i.e. apical medium is removed, to allow air-liquid interface (ALI) culture conditions. Promoting epithelial polarisation and cell differentiation to form a heterogeneous population of epithelial cells representative of the small airways.

Epithelial Barrier Integrity

1A



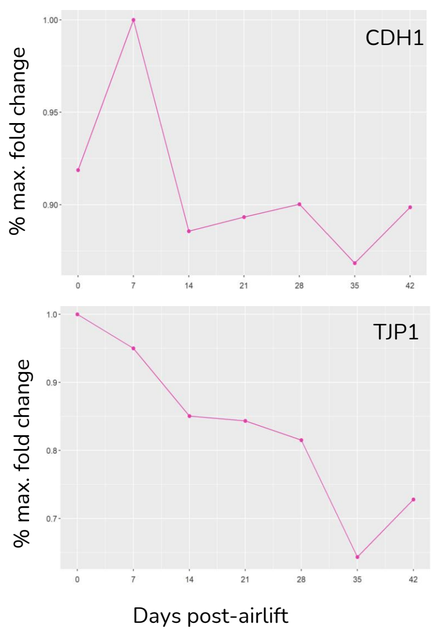
1B



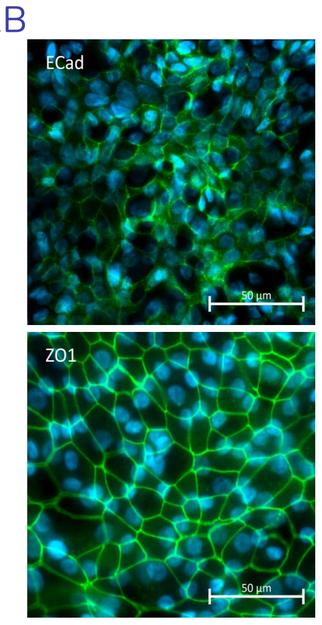
(1A) Transepithelial electrical resistance (TEER), an indicator of epithelial barrier integrity, is increased 7-days post-airlift, confirming epithelial polarisation and establishment of epithelial junctions. TEER is reduced from day 7 to day 14, in line with increased cellular ion transport. TEER is maintained from day 14 through to day 42.

(1B) Size selectivity of the epithelial barrier is confirmed by decreased flux of FITC-dextran molecules of increasing molecular weight.

2A



2B

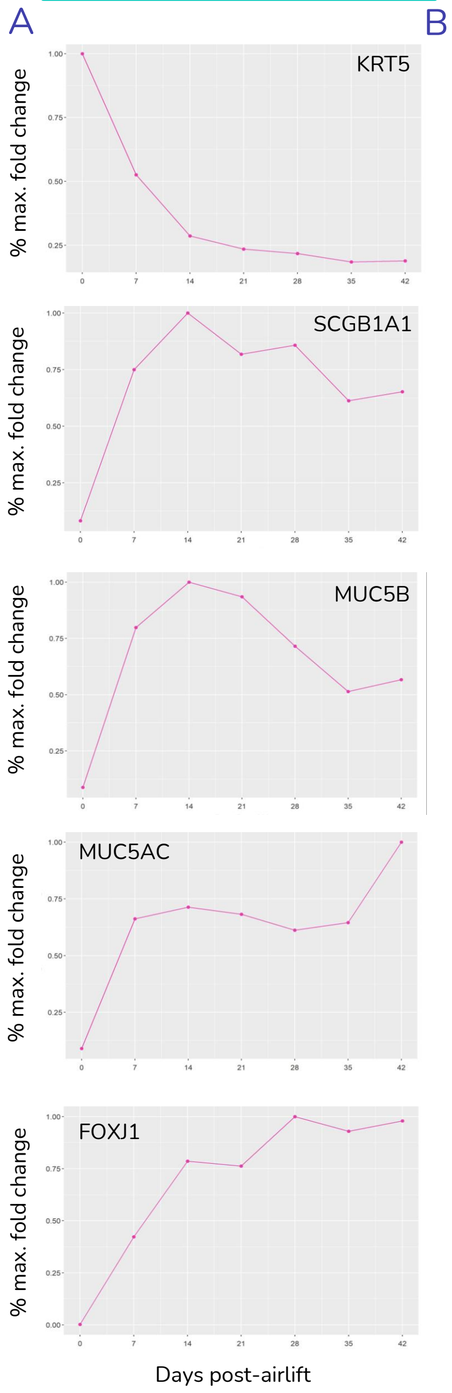


(2A) Gene expression (relative to maximal expression) of key junctional genes CDH1 (E-Cadherin) and TJP1 (ZO-1), throughout the differentiation period, from day 0 (day of air-lift) to day 42, indicating presence of epithelial junctions, in-line with literature data.

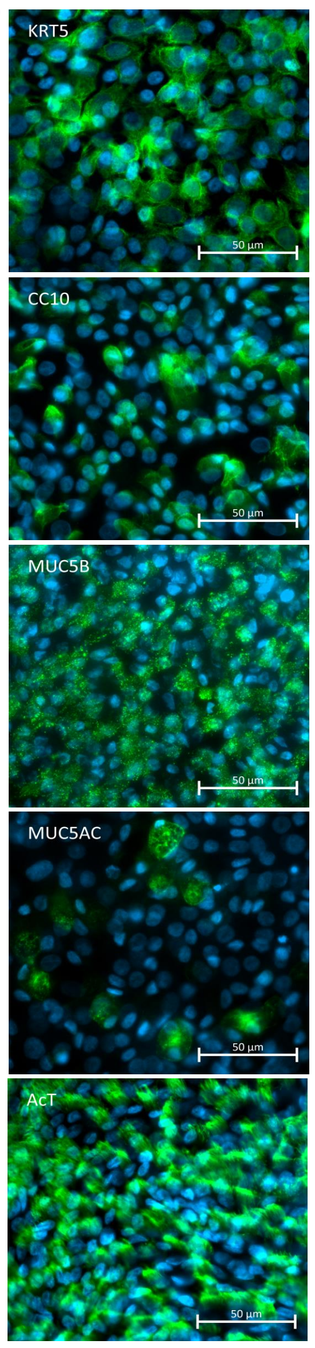
(2B) Representative images showing expression, and localisation of ZO1 and E-Cadherin proteins at epithelial cell: cell junctions.

Model Validation

A



B



(A) Gene expression data shows relative expression of epithelial specific genes, KRT5, SCGB1A1, MUC5B/MUC5AC, and FOXJ1 throughout the 28-day differentiation period, with changes in expression reflecting differentiation of basal, club, goblet and ciliated cells, respectively.

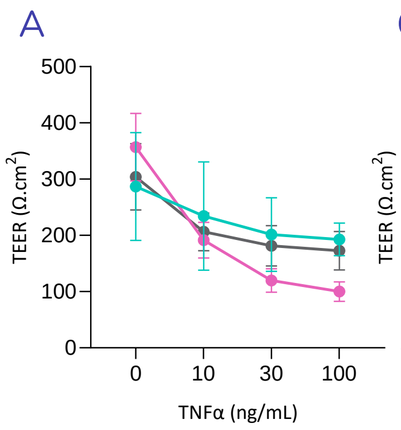
(B) Presence of key epithelial cell types, basal, club, goblet and ciliated cells, at 28-days post-airlift is confirmed by immunocytochemical detection of KRT5, CC10, MUC5B/MUC5AC and acetylated-Tubulin (AcT) respectively.

Cytokine Induced Damage

Increased permeability of the epithelial layer due to loss of barrier integrity is a hallmark of lung disease.

Tumour Necrosis Factor-α (TNFα) is reported to be increased in lung diseases. We stimulated cells basolaterally with TNFα at specified concentrations for 72 hours and observed a dose-dependent epithelial breakdown response.

A



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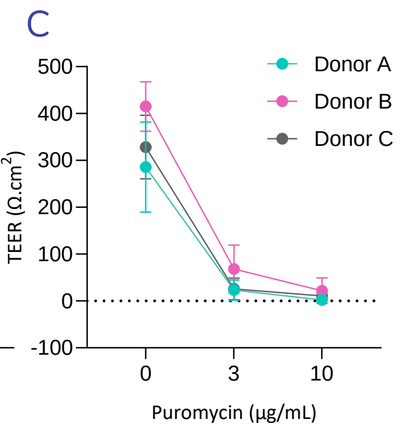
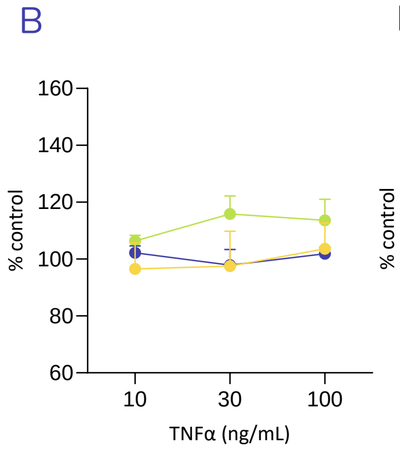


B



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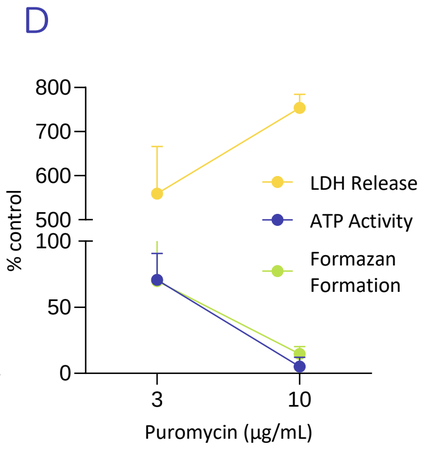


(A) Exposure of **Small Airway Epithelial Cells (SAEC's)** to increasing concentration of the TNFα reduces TEER, in-line with cytokine induced breakdown of the epithelial barrier.

(B) No significant increase in LDH release or decrease in ATP- or MTT- activity (formazan formation), was observed following exposure of SAEC's to TNFα, suggesting TNFα induced breakdown of the epithelial barrier was not observed as a result of cellular toxicity.

(C) Puromycin, an antibiotic with known toxic side effects in the lung, was used as a positive control. A decrease in TEER is triggered with increasing concentrations of Puromycin, indicating a loss in barrier integrity.

(D) High increase in LDH release as well as a decrease in ATP- and MTT- activity was observed following exposure of SAEC's to Puromycin, indicating drug induced cellular toxicity.

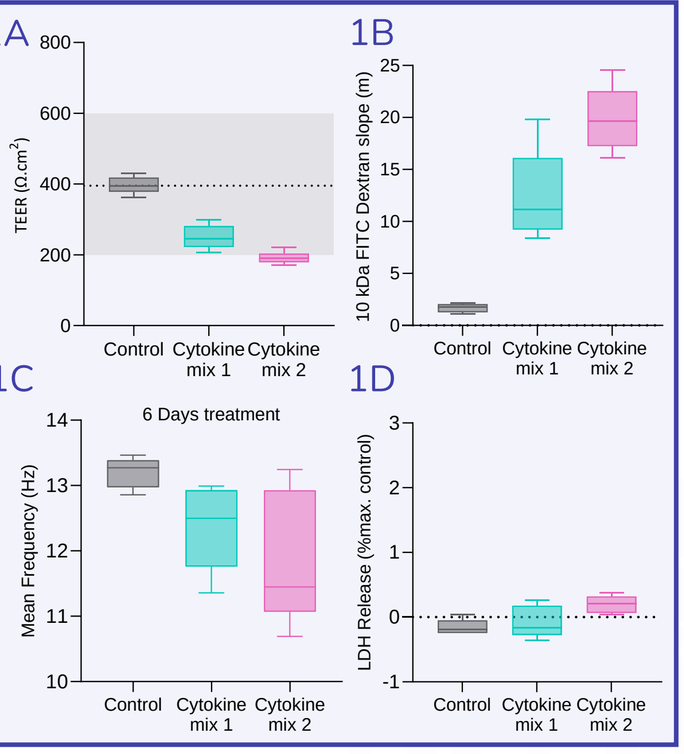
In Development

1. Modelling Idiopathic Pulmonary Fibrosis (IPF) in Small Airway Epithelial Cells (SAEC's)

On treating differentiating SAECs with variations of cytokine mixes for 12 days, an increase in epithelial damage was observed as seen through a reduction in **(1A)** TEER and increase in **(1B)** FITC dextran permeability. The cytokine induced damage to the epithelial barrier was not caused due to cytotoxicity as **(1D)** LDH release was not increased.

2. Ciliary Beat Frequency

On treating differentiating SAECs with variations of cytokine mixes for 10 days **(1C)**, a decrease in ciliary beat frequency was observed compared to the control.



Newcells Biotech's SAEC model

Model Features:

- 3 validated primary basal cell donors
- Fully differentiated and multicellular
- Optimised under Air-Liquid Interface conditions

Available Analytical Techniques and Data Outputs:

- ✓ Epithelial barrier integrity: TEER and FITC-dextran flux measurements.
- ✓ Epithelial damage studies: cytokine-induced barrier breakdown.
- ✓ Gene and protein expression: qPCR and immunocytochemistry.
- ✓ Biomarker analysis: ELISA based detection of secreted factors.
- ✓ Cellular toxicity assessment: LDH release and cellular metabolism.

Lung eBook available online or contact us at enquiries@newcellsbiotech.co.uk

Also available: Fibroblast-to-Myofibroblast Transition (FMT) assay.

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