Effect of culture conditions on fluid and mucus secretion by airway epithelial cells

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Introduction

The human airway is lined by a thin layer of fluid called airway surface liquid, which is mainly produced by submucosal gland cells. This fluid has a role in keeping the lungs infection-free by trapping microbes and pathogens. The airway surface liquid of Cystic Fibrosis airways is dehydrated and more acidic, although the mechanisms involved are still under investigation.

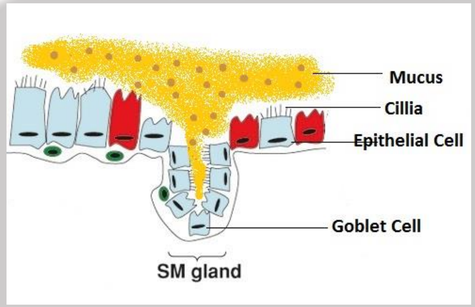


Figure 1. Submucosal gland secreting fluid. Diagram of a submucosal gland secreting fluid into the airway to make up the airway surface liquid.

Adapted from: https://commons.wikimedia.org/wiki/File:Diagram_representing_putative_lung_epithelial_progenitors_and_their_locations_in_the_adult_respirato.jpg

Aims

The main aims of this research project were to find the culture conditions that produce submucosal gland cells that best represent those found in the body (in vivo).

Calu-3 cells are a human airway epithelial cell line. We aimed to investigate the fluid and mucus secretions of Calu-3 cells under two different conditions; submerged and air-liquid interface (ALI) to see how these culture conditions affect the expression of the Cystic Fibrosis Transmembrane Regulator (CFTR) and the impact this has on cell secretions.

Methods

The Calu-3 cells were cultured in two different conditions: submerged and ALI. Submerged cells were grown with media underneath and on top of the membrane, whereas ALI cells were grown only with media underneath.

Fluid Secretion Assay

The Calu-3 cells were treated different pharmaceuticals, Forskolin and CFTR inhibitors (CFTRi-172 and GlyH-101) and were compared to a control. Forskolin stimulates HCO_3^- secretion, therefore was expected to cause the pH of the secreted fluid to increase. The use of CFTR inhibitors were expected to reduce fluid secretion in both conditions.

Methods

PAS Assay

The fluid secretion assay samples were collected and analysed using a spectrophotometer to measure the concentration of glycoprotein in the sample. This was compared to a standard curve using known concentrations of purified pig mucin.

Results - Fluid Secretion Assay

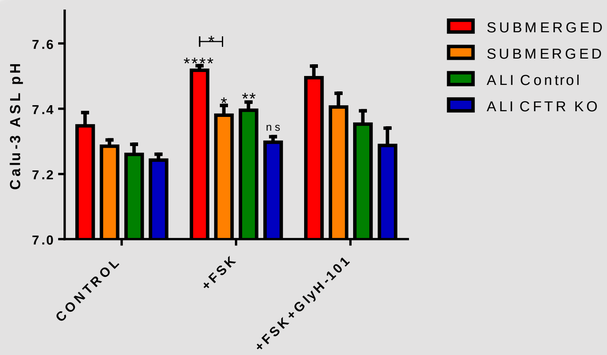


Figure 2. Comparison of pH of fluid secreted between Wild Type Calu-3 cells and CFTR Knock Down in response to pharmaceuticals. Comparison of changes in fluid pH betweensubmerged and ALI cells exposed to Forskolin (FSK) and CFTR inhibitor GlyH-101, showing ALI cells result in a higher volume than ALI. Data are Mean ± SEM

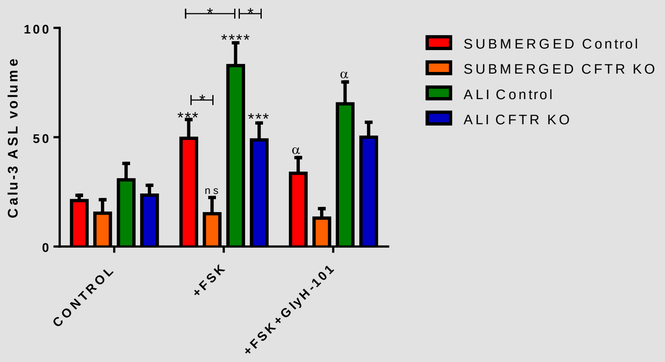


Figure 3. Comparison of volume of fluid secreted between Wild Type and CFTR Knock Out Calu-3 cells in response to pharmaceuticals. Comparison of changes in volume secreted between submerged and ALI cells exposed to Forskolin (FSK) and CFTR inhibitor 172, showing ALI cells secrete increased volume than submerged. Data are Mean ±SEM

Results – PAS Assay

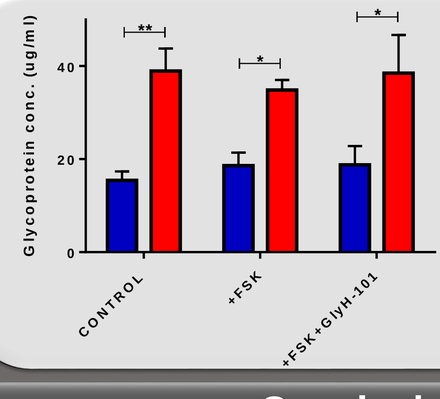
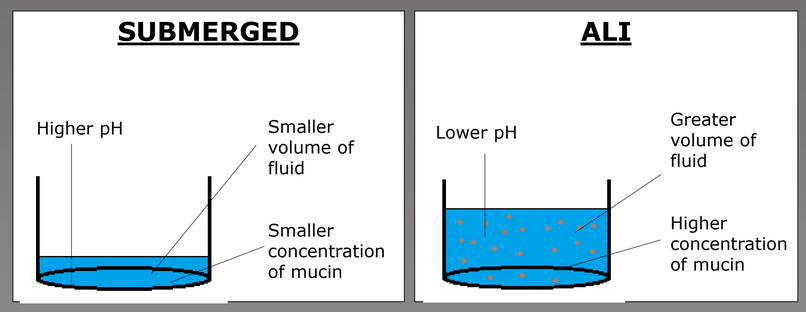


Figure 4. Glycoprotein concentration in fluid samples from Calu-3 fluid secretion assays. Concentration of glycoprotein (ug/ml) in samples of fluid secreted from Calu-3 cells. Data are Mean ± SEM.

Conclusion



- ALI grown Calu-3 cells secreted a greater volume of fluid than submerged cells
- ALI secreted fluid contained a higher concentration of mucin and a lower pH.
- Forskolin increased volume of secreted fluid and pH in both ALI and submerged cultures
- In CFTR KO cells, volume of secreted fluid increased in ALI cultures, but not submerged when exposed to Forskolin
- When Forskolin was applied, the increase in fluid secretion which was due to CFTR was greater in submerged cells than ALI grown cells.
- **ALI-grown Calu-3 cells may express a CFTR-independent ion conductance that drives fluid secretion in the presence of Forskolin, whereas fluid secretion from submerged grown cells is predominantly mediated by CFTR.**

Acknowledgements

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