Evaluating Airway ALI Model Fabrication Methods and Comparing Differentiation Potential of Primary and hTERT-immortalized Epithelial Cells

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Abstract

Human respiratory research encompasses a variety of fields including drug development, disease modeling, and toxicology testing. Despite the availability of traditional in vitro airway models, there is a persistent concern with their lack of physiological relevance to the human lung. Within the past decade, several advanced in vitro airway models have been constructed, which promise to provide more relevant applications in human respiratory research. However, the numerous variables associated in the generation of these advanced models can cause incomplete or inconsistent differentiation, resulting in research delays or costly overruns. In these studies, we showcase an optimal method of fabricating airway models consisting of human bronchial tracheal epithelial cells (HBECs) grown in collagen-coated 24-well plates and cultured under air-liquid interfaces (ALI) for 5 weeks. Model generation using different lots of primary HBECs as well as hTERT-immortalized HBECs were compared. In addition, various commercial media designed to promote epithelial differentiation were evaluated. Next, primary HBECs ATCC and other commercial companies were evaluated and compared on epithelial differentiation and model morphology using optimized processes validated during the first phase of the study. All airway models were evaluated via weekly microscopy and transpithelial/transendothelial electrical resistance measurements. Additionally, H&E and aziain blue imaging and MUC5AC and α1-tubulin immunohistochemical analysis from histological samples of mature airway models were generated. These studies elucidate techniques and procedures to reliably generate 3D airway models with consistent full epithelial differentiation across replicates using both ATCC and other commercial primary HBECs.

Background

Microscopy images of bronchial epithelial cell differentiation

Results

Comparing resistivity measurements between airway models

Comparing resistivity measurements from different commercial lots

Comparing airway model histology

Comparing commercial cells

Comparing commercial media

Conclusion

- Fully differentiated mature airway models using cell lines from either ATCC or commercial vendors were successfully generated.
- Lot variation was observed between models; however, media choice plays a much larger role in model variability and maturation.
- Both commercial differentiation media provided the best levels of epithelial differentiation.
- Variability between models was minimized by incubating models in the interior wells only as well as using PBS in the outer wells.
- Despite the utility of hTERT cells, NuLi-1 cells lines were shown to be unable to form airway models with appropriate epithelial morphology and are not an appropriate substitute for primary HBECs in airway model fabrication.
- These studies demonstrated that ATCC primary HBECs are an effective tool to generate airway models with appropriate epithelial differentiation, model morphology, and function variability.

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