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

Kevin Tyo, Fang Tian
Cell Biology R&D, ATCC, Gaithersburg, MD 20877

ATCC®
Credible leads to Incredible

Poster # P1601

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 promises 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 cost 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 plate inserts and cultured under air-liquid interface (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 from 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 transepithelial/transendothelial electrical resistivity measurements. Additionally, H&E and alcian blue imaging and MUC5AC and α-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

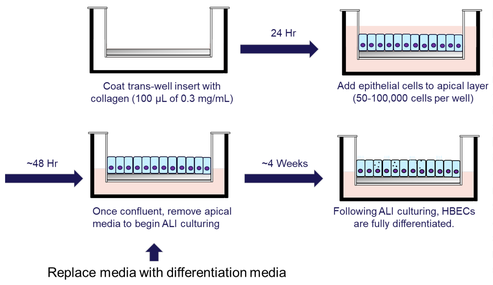


Figure 1. Schematic of airway model fabrication process. Trans-well inserts with PET membranes are added to either every well within a 24-well plate or to interior wells only. Inserts are apically coated with 0.3 mg/mL collagen solution and incubated overnight at 4˚C. Membrane inserts are twice rinsed using PBS prior to cell seeding. For plates with interior inserts only, 2 mL of PBS is added to the outer wells. Primary human bronchial epithelial cells are then apically seeded on the trans-well inserts at 10^5 cells per well and incubated for 48-72 hours to ensure full confluency. Afterwards, apical media is removed, and basal media replaced with selected differentiation media. Cells are cultured under ALI for at least 4 weeks to ensure epithelial differentiation. After this incubation period, the airway model is fully mature, containing basal, goblet, and ciliated cells.

Results

Microscopy images of bronchial epithelial cell differentiation

A

Media Differentiation Test	
Primary Lot #	Differentiation Media
1	100% Bronchial Growth Media 80:20: Bronchial:Fibroblast Growth Lifeline Cell Technology Media STEMCELL Technologies Media
2	
3	
4	
NuLi-1 Control	

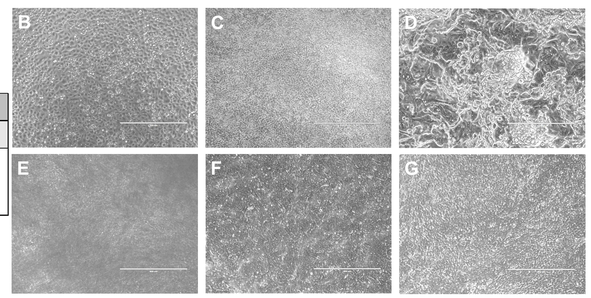


Figure 2. Morphology of airway models is dependent upon ALI media. (A) Layout of first set of studies comparing different media during ALI incubation. Four different primary cell lots were tested as well as NuLi-1 hTERT-immortalized primary cells. Microscopy images representative of HBECs (B) prior to ALI culturing, (C) all models after one week of ALI, as well as 5-week model incubation using (D) complete bronchial growth media, (E) 80:20 bronchial: fibroblast growth media, (F) Lifeline Cell Technology media or (G) STEMCELL Technology media. Little to no morphology differences were shown between different primary HBECs lots incubated in the same differentiation media (data not shown). Scale bar represents 400 µm.

Comparing resistivity measurements between airway models

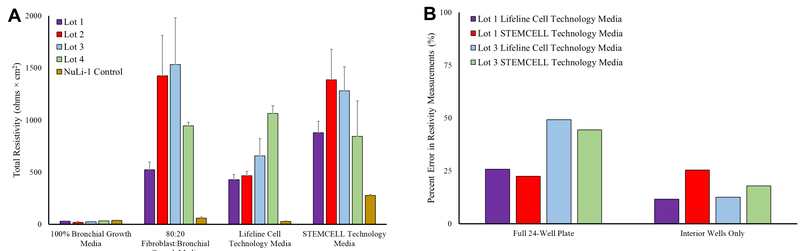


Figure 3. Comparing TEER differences in airway models grown in different differentiation media as well as assessing edge effect. (A) Total resistivity measurements from airway models incubated using different differentiation media. (B) Comparisons of percent error between airway models using all wells within a 24-well plate and models incubated in the interior wells only.

Comparing airway model histology

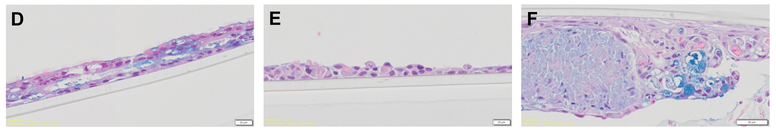


Figure 4. Representative alcian-blue stained histological images of airway models. Models comprised of primary HBECs incubated with (A) complete bronchial growth media, (B) 80:20 bronchial: fibroblast growth media, (C) Lifeline Cell Technology media, or (D) STEMCELL Technology media. Images of airway models comprised of hTERT-immortalized cell NuLi-1 controls cultured in either (E) Lifeline Cell Technology media or (F) STEMCELL Technology media. Scale bars represent 20 µm.

Comparing model resistivity from different commercial lots

A

Primary Cell Comparison		
Cell Type	Study Designation	Differentiation Media Used
ATCC 1˚ Bronchial Tracheal Epithelial Cells (3 Lots)	ATCC Lot 1	STEMCELL Media
	ATCC Lot 2	Lifeline Media
	ATCC Lot 3	
1st Commerical Option (2 Lots)	1st Commerical (Lot 1)	STEMCELL Media
	1st Commerical (Lot 2)	1st Commerical Media
2nd Commerical Option (2 Lots)	2nd Commerical (Lot 1)	STEMCELL Media
	2nd Commerical (Lot 2)	2nd Commerical Media
3rd Commerical Option (1 Lot)	3rd Commerical (Lot 1)	STEMCELL Media 3rd Commerical Media

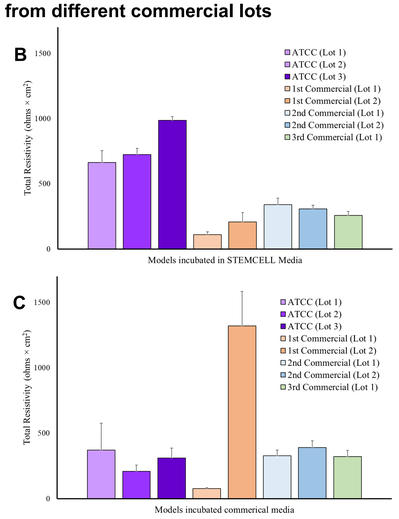


Figure 5. Comparing commercial cells. (A) Layout of second set of studies comparing differentiation potential of primary cells from both ATCC and commercial vendors. TEER values from airway models comprised of primary HBECs from either ATCC or other commercial vendors incubated in either (B) STEMCELL Technology media or (C) respective commercial ALI differentiation media.

Functional testing of models

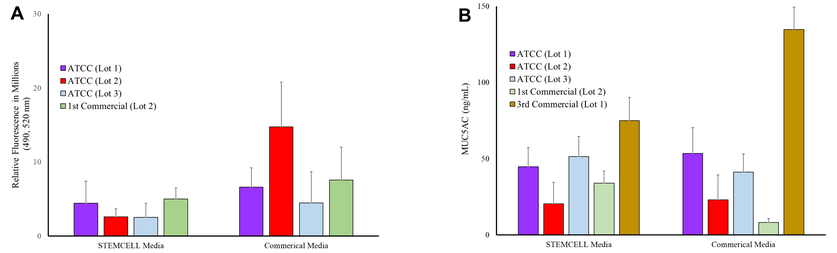
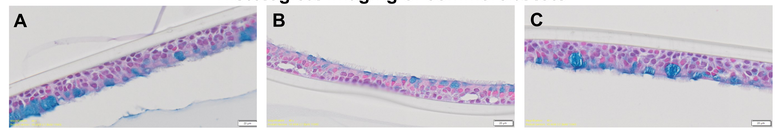


Figure 6. Functional studies on selected airway models. (A) Tight junctions were evaluating by comparing the rate of transmembrane diffusion of FITC-Dextran in selected airway models. (B) Mucin production (MUC5AC), an indicator of epithelial differentiation, was evaluated in selected airway models via ELISA.

Histological imaging of commercial lots



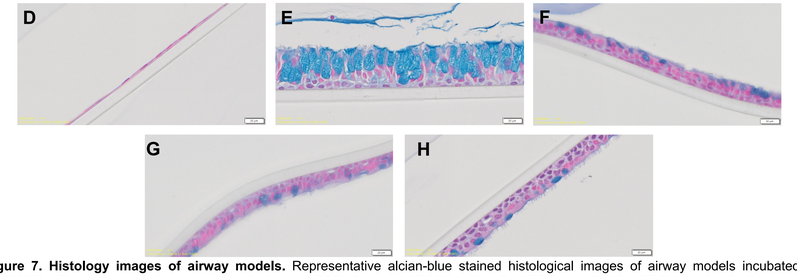


Figure 7. Histology images of airway models. Representative alcian-blue stained histological images of airway models incubated in STEMCELL Technology media comprised of primary HBECs from (A) ATCC lot # 1, (B) ATCC lot # 2, (C) ATCC lot # 3, (D) Commercial Vendor 1 (Lot 1), (E) Commercial Vendor 1 (Lot 2), (F) Commercial Vendor 2 (Lot 1), (G) Commercial Vendor 2 (Lot 2), or (H) Commercial Vendor 3 (Lot 1). Scale bars represent 20 µm.

Immunohistochemistry microscopy imaging

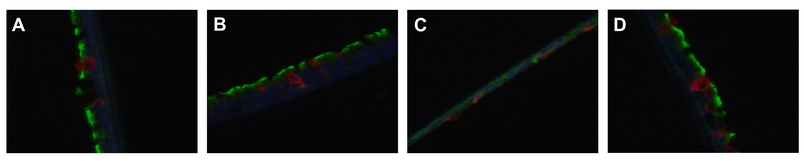


Figure 8. IHC microscopy confirms differentiated functionality in airway models. Representative images of airway models from (A) ATCC lot# 1, (B) ATCC lot# 2, (C) ATCC lot# 3, and (D) Commercial vendor 3 (Lot 1). Red fluorescence in images is from MUC5AC, green fluorescence stems from α-tubulin, with DAPI control as blue.

Conclusion

- Fully differentiated mature airway models using cell lines from either ATCC or commercial vendors were successfully generated.
- Lot-to-lot variation was observed between models; however, media choice plays a much larger role in model variability and maturation.
- Both commercial differentiation medias 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 model morphology and are not an appropriate substitute for primary HBECs in airway model fabrication.
- These results demonstrate that ATCC primary HBECs are an effective tool to generate airway models with appropriate epithelial differentiation, model morphology, and mature functionality.

ATCC 10801 University Boulevard, Manassas, Virginia 20110-2209 **Phone:** 800.638.6597 **Email:** SalesRep@atcc.org **Web:** www.atcc.org