Development of an Air-Liquid Interface Model using Primary Human Bronchial Epithelial Cells and HTS Transwell®-24 Permeable Supports from Corning

Application Note

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Introduction

Historically, two-dimensional (2D) monolayer cultures with numerous primary and immortalized cell lines have been used for drug screening and disease modeling. However, cell culture models of the mammalian airway have been unsuccessful in 2D due to the inability of airway epithelial cells to differentiate well in submerged culture[1]. On the other hand, primary airway epithelial cells cultured at the air-liquid interface (ALI) on permeable membranes polarize and form a pseudostratified epithelium containing mucus-secreting goblet cells and ciliated cells with beating cilia, both characteristic cell types of intact airways.[1-5] HTS Transwell-24 permeable supports from Corning are 24-well cell culture systems containing permeable culture inserts that sit in 24-well receiver plates or reservoirs. The membrane inserts are connected by a rigid, automation-friendly tray that enables all 24 Transwell inserts to be handled as a single unit. In this study, primary human bronchial epithelial cells from a healthy donor (NHBE) and from a donor with cystic fibrosis (D-HBE-CF) were cultured on HTS Transwell-24 polyethylene terephthalate (PET) 0.4 µm inserts coated with Collagen I. After a confluent monolayer was formed, cells were air-lifted and cultured at the ALI for 28 to 30 days to form a pseudostratified epithelial structure containing ciliated cells, goblet cells, and basal stem cells. The differentiated ALI culture yielded high TEER values consistent with what has been reported in the literature for primary bronchial cells[3] and displayed positive staining for tight junction protein ZO-1. Because the ALI model developed exhibits the cell types and functions necessary for a primary bronchial epithelium model and is amenable to both healthy and diseased cells, it can easily be employed for throughput assays for therapy research and drug development.

Materials and Methods

Monolayer Formation

Human bronchial/tracheal epithelial cells (passage 3) from a healthy donor (NHBE; Lonza Cat. No. CC-2541) and from a donor with cystic fibrosis (D-HBE-CF; Lonza Cat. No. 00196979) were thawed in PneumaCult™-Ex Plus culture medium (STEMCELL Technologies Cat. No. 05040) supplemented with 0.096 µg/mL hydrocortisone (STEMCELL Technologies Cat. No. 07925) and cultured in a humidified 5% CO_2 37°C incubator for 3 to 4 days prior to seeding on Transwell permeable supports. HTS Transwell-24 PET 0.4 µm inserts (Corning Cat. No. 3378) were coated with 100 µL of 10 µg/cm^2 rat tail Collagen I (Corning Cat. No. 354236) diluted in 0.02 N acetic acid (MilliporeSigma Cat. No. AX0073-6), incubated for 1 hour at room temperature, and rinsed with 150 µL of Dulbecco's Phosphate-Buffered Saline (DPBS; Corning Cat. No. 21-031-CM). Coated Transwell permeable supports were used immediately or stored at 4°C until cell seeding (up to 72 hours). Cells were harvested with Accutase® Cell Detachment Solution (Corning Cat. No. 25-058-CI) and were seeded in 100 µL culture medium onto previously-coated Transwell-24 PET inserts. NHBE were seeded at 1.5 x 10^5 cells/cm^2 and D-HBE-CF were seeded at 2 x 10^5 cells/cm^2. Transwell receiver wells were filled with 0.7 mL culture medium, and cells from both donors were cultured for 6 days until confluent monolayers were formed. Medium was exchanged 2 times prior to air-lift.

Air Lift and Culture at ALI

To initiate air-lift, culture medium was removed from the Transwell inserts and wells. Only the medium from the receiver wells was replaced with 0.7 mL PneumaCult-ALI Medium for differentiation (STEMCELL Technologies Cat. No. 05001) supplemented with 0.48 µg/mL hydrocortisone and 0.0004% heparin (STEMCELL Technologies Cat. No. 07980). Cells were cultured at the ALI for 28 to 30 days with media changes 3 times per week. Inserts were rinsed once a week with 150 µL of DPBS to remove mucus. After 28 to 30 days post-air-lift, transepithelial electrical resistance (TEER) values were determined using a World Precision voltage meter (EVOM). Prior to TEER measurements, enough culture medium was added to submerge the probes (200 µL/insert and 300 µL/well) and cultures were equilibrated to room temperature. For all staining and imaging applications, cells were then fixed with cold 4% paraformaldehyde (Boston Bioproducts Cat. No. BM-155) for 15 minutes and rinsed with DPBS.

Staining and Imaging

Histological staining. Fixed cells were paraffin-embedded and sectioned following the protocol in the "Preparation of Transwell® Inserts for Histology Guidelines for Use" (Corning Lit. Code CLS-AN-335) prior to staining with hematoxylin and eosin (H&E) and Periodic Acid-Schiff-Alcian Blue (PAS-AB). Brightfield images were taken using a Keyence BZ-X710 digital microscope with a 40X/0.75 NA plan fluor lens.

Immunofluorescence (IF) staining. Fixed cells were sectioned using a cryostat. Sections were stained with antibodies for markers of differentiated cell types: p63 antibody (Genetex Cat. No. GTX102425) with CF®488A-conjugated secondary antibody (Biotium Cat. No. 20015), Alexa Fluor® 555-conjugated beta-IV tubulin antibody (Abcam Cat. No. ab204034), and Alexa Fluor 647 conjugated Muc5AC antibody (Abcam Cat. No. ab218714). Confocal imaging was conducted on a Leica TCS SP5 laser scanning confocal microscope with a 40X/1.3 NA plan apo oil immersion

lens. Additionally, fixed cells were permeabilized and blocked for 1 hour in 0.2% Triton™ X-100 (Integra Cat. No. T756.30.30), 1% BSA (MilliporeSigma Cat. No. A9576), and 5% FBS (Corning Cat. No. 35-010-CV) in DPBS. Cells were stained with Alexa Fluor 488 conjugated ZO-1 antibody (Thermo Fisher Cat. No. 339188) or with isotype control (Thermo Fisher Cat. No. MA5-18167) overnight at 4°C. Cells were counterstained with 10 µg/mL Hoechst 33342 (Thermo Fisher Cat. No. H3570) for 15 minutes prior to imaging with a Thermo Fisher CellInsight® CX7 HCS platform in confocal mode with a 20X objective.

Results and Discussion

In this study, HTS Transwell®-24 PET 0.4 µm inserts from Corning were utilized to generate assay-ready models of both healthy and diseased human airway epithelia. Specifically, healthy (NHBE) and cystic fibrosis-affected (D-HBE-CF) primary bronchial epithelial cells were cultured on Transwell inserts at the ALI for 28 to 30 days to form a pseudostratified epithelium modeling native bronchial tissue.

H&E staining confirmed the formation of a pseudostratified epithelium with a ciliary brush border with both NHBE and D-HBE-CF cultured at the ALI (Figure 1). Beating cilia were observed within 10 days for NHBE and within 12 days for D-HBE-CF, which is consistent with previously reported results for primary epithelial cells. Typically, ciliated cells appear after 11 to 14 days in culture at the ALI, with higher passage cells taking longer than lower passage cells.[4] The presence of ciliated cells in the HTS Transwell-24 cultures was further verified by immunofluorescence staining (Figure 3) for beta-IV tubulin.[3] Likewise, histological staining and immunofluorescence clearly demonstrated the presence of functional mucus-producing goblet cells via PAS-AB[5] and Muc5AC[3,4] staining for mucins present in and on goblet cells (Figures 2 and 3, respectively). Last, the third differentiated cell type – basal stem cells – was identified in the cultures by positive p63[4] staining (Figure 3). It is important to note that not only were all 3 differentiated cell types present in the ALI cultures for both healthy and cystic fibrosis-affected cells, but the cells were organized similarly to an intact airway epithelium with ciliated cells appearing on the apical (air) side and basal stem cells appearing on the basolateral (liquid) side (Figure 3).

Stratification and differentiation into the appropriate cell types enabled the formation of a tight barrier in the ALI cultures. The formation of a tight epithelium was demonstrated by the presence of tight junction protein ZO-1 and high TEER values, per previously published studies.[2,3] The pattern of ZO-1 fluorescence encircling the cells in NHBE and D-HBE-CF ALI cultures confirms tight junction formation (Figure 4). As a result of the epithelial barrier, both NHBE and D-HBE-CF ALI cultures displayed high TEER values (Figure 5) that are consistent with literature for pseudostratified epithelium formed by primary human bronchial epithelial cells (200 to 600 ohm*cm^2).[3]

Reports in the primary literature show that airway epithelial cells cultured on permeable membranes at the ALI for 21 to 28 days can form a fully differentiated pseudostratified epithelium with functional ciliated cells, mucus-secreting goblet cells, and basal

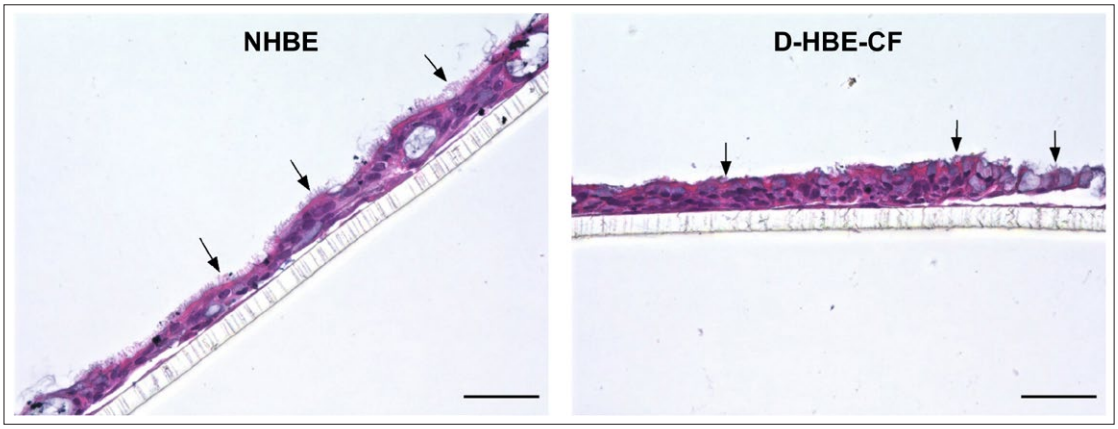

Figure 1. NHBE and D-HBE-CF primary bronchial epithelial cells cultured at the ALI form pseudostratified epithelia. H&E staining of cells from both healthy and cystic fibrosis-affected donors formed a pseudostratified epithelial structure when cultured on HTS Transwell-24 permeable supports from Corning at the ALI for 28 to 30 days. Cilia were present on the apical side of the culture (black arrows). Scale bar = 10 µm.

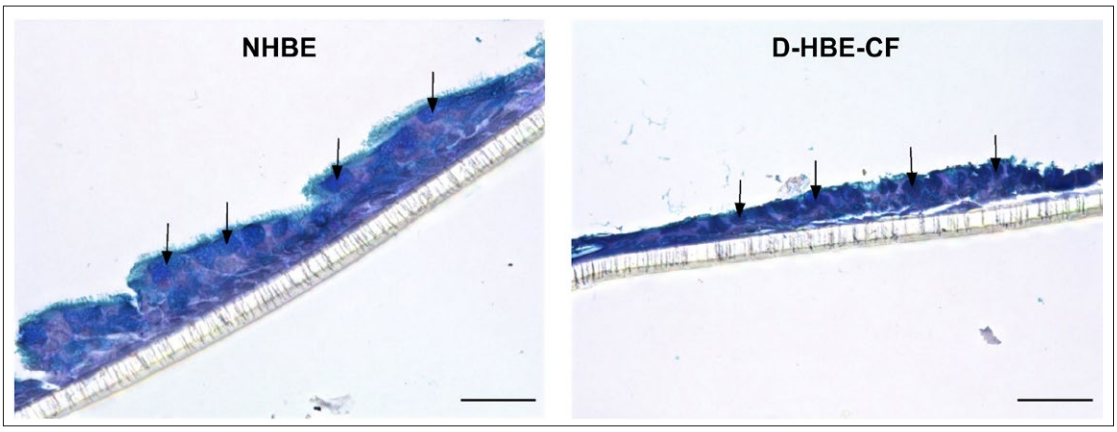

Figure 2. Pseudostratified epithelia of ALI cultures contain mucus-producing goblet cells. PAS-AB staining indicates mucus-producing goblet cells (black arrows) in ALI cultures generated on HTS Transwell-24 permeable supports from Corning with both NHBE and D-HBE-CF cells following 28- to 30-day culture. Scale bar = 10 µm.

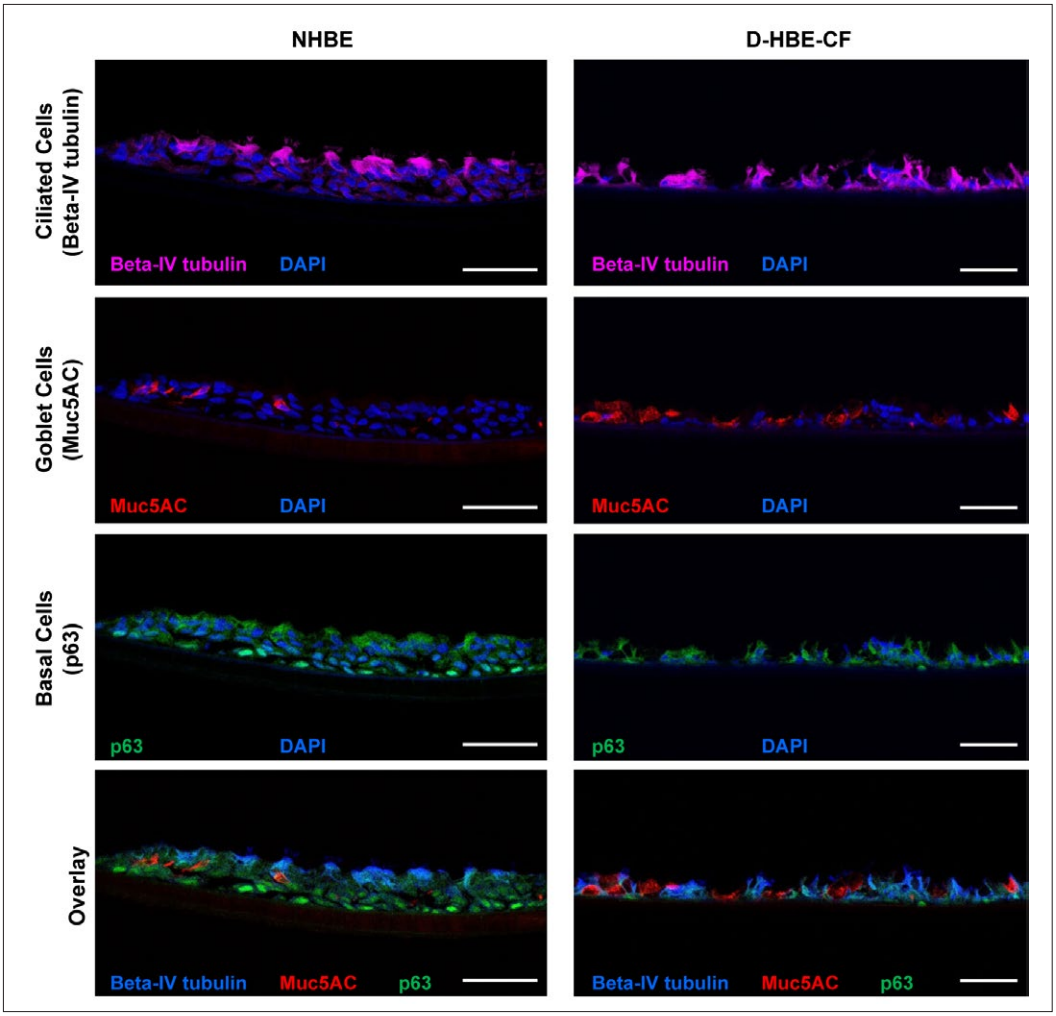

Figure 3. NHBE and D-HBE-CF primary bronchial epithelial cells cultured at the ALI form pseudostratified epithelia containing 3 differentiated cell types. IF staining indicates that ALI cultures from both donors contained ciliated cells (Beta-IV tubulin), mucus-producing goblet cells (Muc5AC), and basal cells (p63) with the ciliated cells appearing on the apical (top) side and the basal cells appearing on the basolateral (bottom) side. Scale bar = 10 µm.

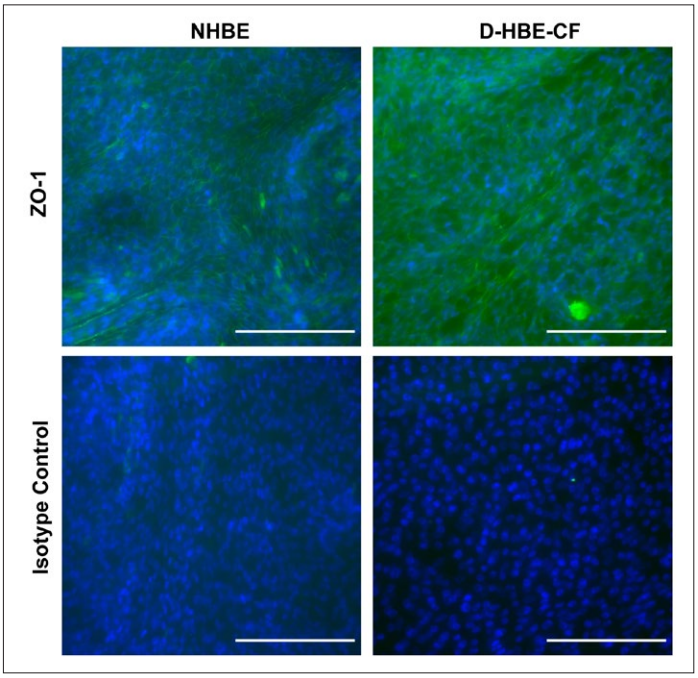

Figure 4. ALI cultures generated on HTS Transwell®-24 permeable supports express tight junction protein ZO-1. After 28 to 30 days of culture at the ALI, NHBE and D-HBE-CF cultures displayed staining for tight junction protein ZO-1 (green) encircling cells. Nuclei (blue) were counterstained with 10 µg/mL Hoechst 33342. Scale bar = 100 µm.

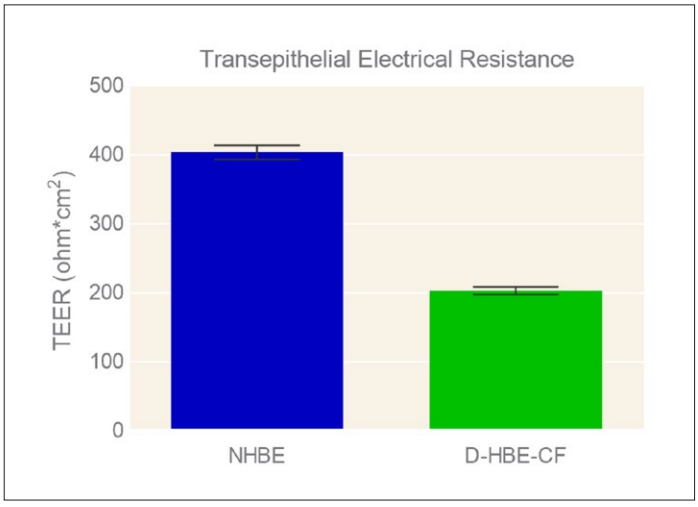

Figure 5. ALI cultures generated on HTS Transwell-24 permeable supports demonstrate high TEER values. After 28 to 30 days of culture at the ALI, NHBE and D-HBE-CF cultures displayed high TEER values (mean ± SEM) that were >400 ohm*cm^2 for NHBE and >200 ohm*cm^2 for D-HBE-CF. For each donor, TEER was measured from 24 inserts 3 independent times. N = 72.

stem cells.[2-5] Traditionally, these models are generated in a low-throughput manner with individual permeable supports. The use of the HTS Transwell®-24 PET inserts in this study simplifies the generation of multiple ALI cultures in an automation-friendly format. The ALI models developed here exhibit the cell types and functions necessary of a primary bronchial epithelium model, are amenable to both healthy and diseased cells, and can easily be employed for throughput assays for therapy research and development.

Conclusions

- Transwell-24 permeable supports from Corning can be used to culture primary human bronchial epithelial cells at the ALI to form pseudostratified epithelium to model the human airway.
- HTS Transwell-24 permeable supports simplify the generation of multiple ALI cultures in an automation-friendly format for throughput cell-based assay of both healthy and diseased airway epithelium.

References

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