Recapitulating the human airway *in vitro* using PneumaCult™-ALI, a novel medium formulation for the differentiation of primary human bronchial epithelial cells at the air-liquid interface

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Introduction

The epithelial lining of the tracheo-bronchial region of the human respiratory tract is classified as a pseudostratified, ciliated, columnar epithelium. The predominant cell types in the bronchi include ciliated cells, secretory cells (primarily mucus-secreting goblet cells), and basal cells. The tracheo-bronchial epithelium acts as a protective barrier, defending against a variety of inhaled insults such as toxins, pollutants, and pathogens. Tight junction proteins maintain the integrity of the bronchial epithelium and are critical to its function as a physical barrier. In addition, particulate matter and pathogens can be trapped by secreted mucus and transported out of the airway by the coordinated action of ciliated cells in what is known as the mucociliary elevator. Researchers use a variety of *in vitro* models to study the human airway. Although the use of immortalized cell lines and primary cells from animals is common, data generated using these model systems is not directly applicable to the human system. Culture of primary human bronchial epithelial cells (HBECs) is possible in standard submerged culture; however, these cells fail to undergo mucociliary differentiation. Culturing HBECs at the air-liquid interface (ALI) in the presence of specialized media drives differentiation towards a mucociliary phenotype. This *in vitro* model recapitulates many of the characteristic properties of the *in vivo* human airway, including mucus secretion, cilia motility, and formation of cellular tight junctions, thus providing a physiologically relevant model of the human airway. PneumaCult™-ALI provides a standardized method for mucociliary differentiation of HBECs when cultured at ALI.

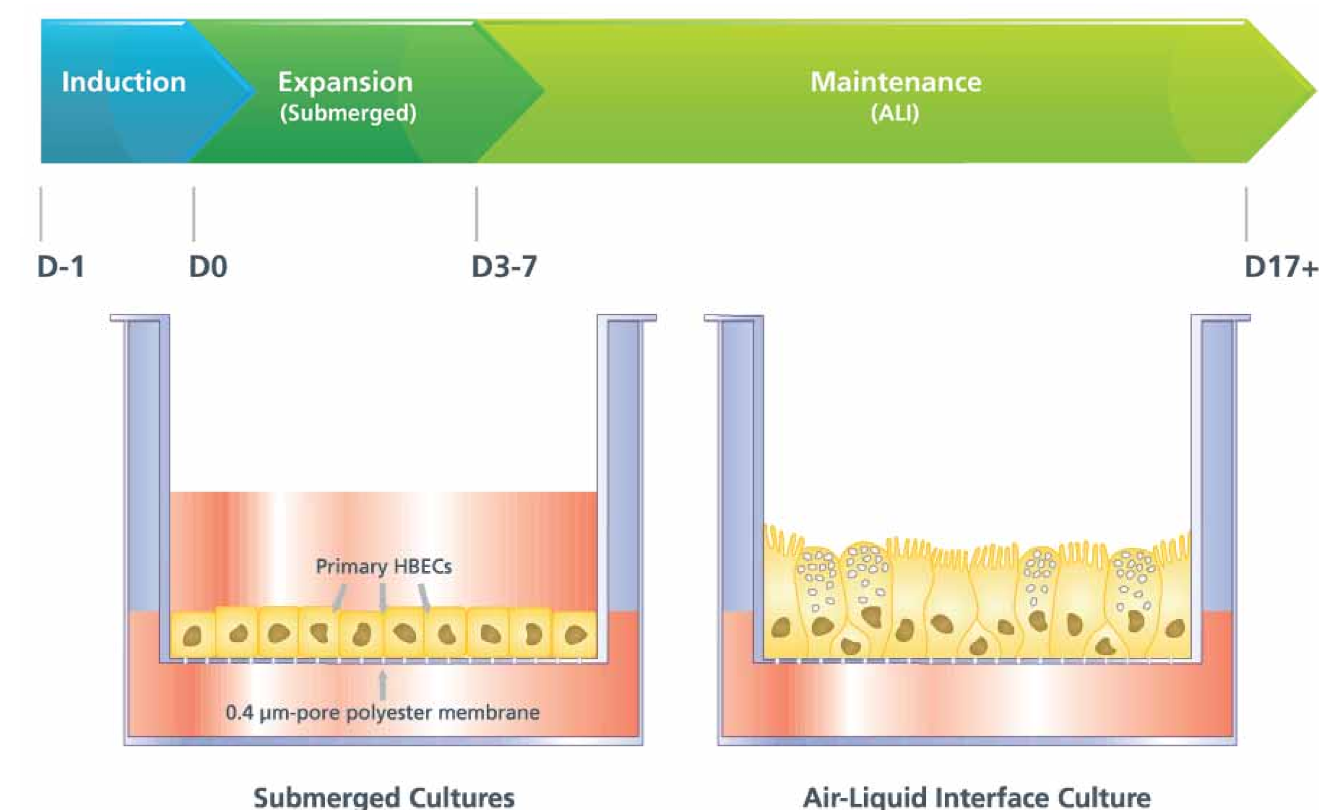
PneumaCult™-ALI

FIGURE 1: PneumaCult™-ALI consists of a basal medium and 4 supplements

- 10X supplement
- 100X Induction Supplement
- 100X Expansion Supplement
- 100X Maintenance Supplement

1 kit is sufficient to establish and maintain ALI culture in >2 x 12-well plates for over 28 days post air-lift.

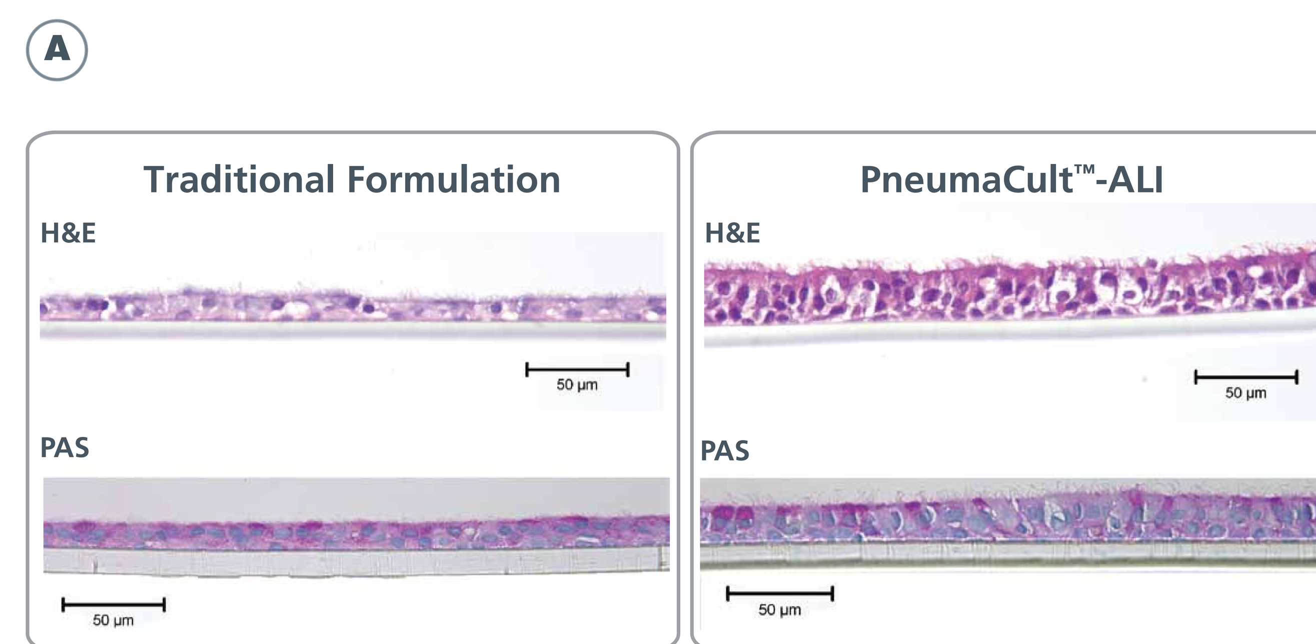
FIGURE 2: Schematic of the protocol for differentiating HBECs at the ALI using PneumaCult™-ALI

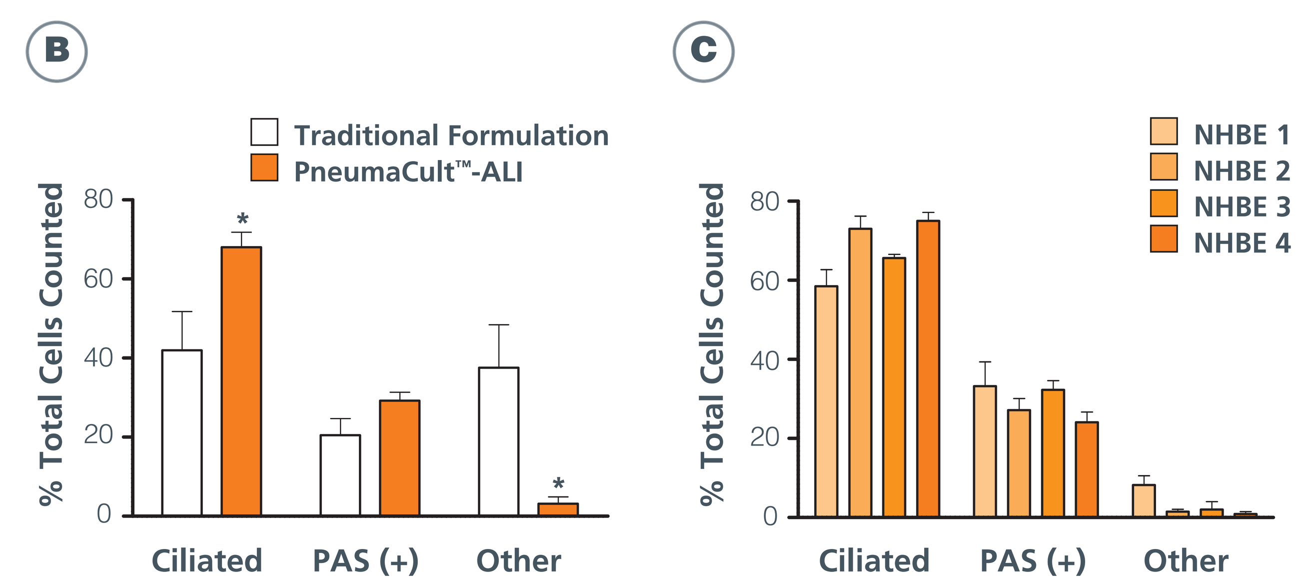


Primary human HBECs initially expanded in bronchial epithelial growth medium (eg. BEGM, Lonza) are cultured in PneumaCult™-ALI Complete Basal Medium (PneumaCult™-ALI Basal Medium + 10X Supplement) supplemented with 10 µl/mL PneumaCult™-ALI Induction Supplement overnight in submerged culture prior to seeding cells onto collagen-1-coated transwell inserts. Cells are then enzymatically dissociated and seeded into transwell inserts and grown to confluence in PneumaCult™-ALI Complete Basal Medium supplemented with 10 µl/mL PneumaCult™-ALI Expansion Supplement. Cells typically reach confluence within 3 to 7 days. Confluent cultures are then raised to ALI by aspirating medium from apical and basal chambers and replacing with PneumaCult™-ALI Complete Basal Medium supplemented with 10 µl/mL Maintenance Supplement in the basal chamber only. Mucociliary differentiation occurs over the course of 14 to 21 days, and can be maintained for over 14 weeks.

Characterization of Differentiated Cultures

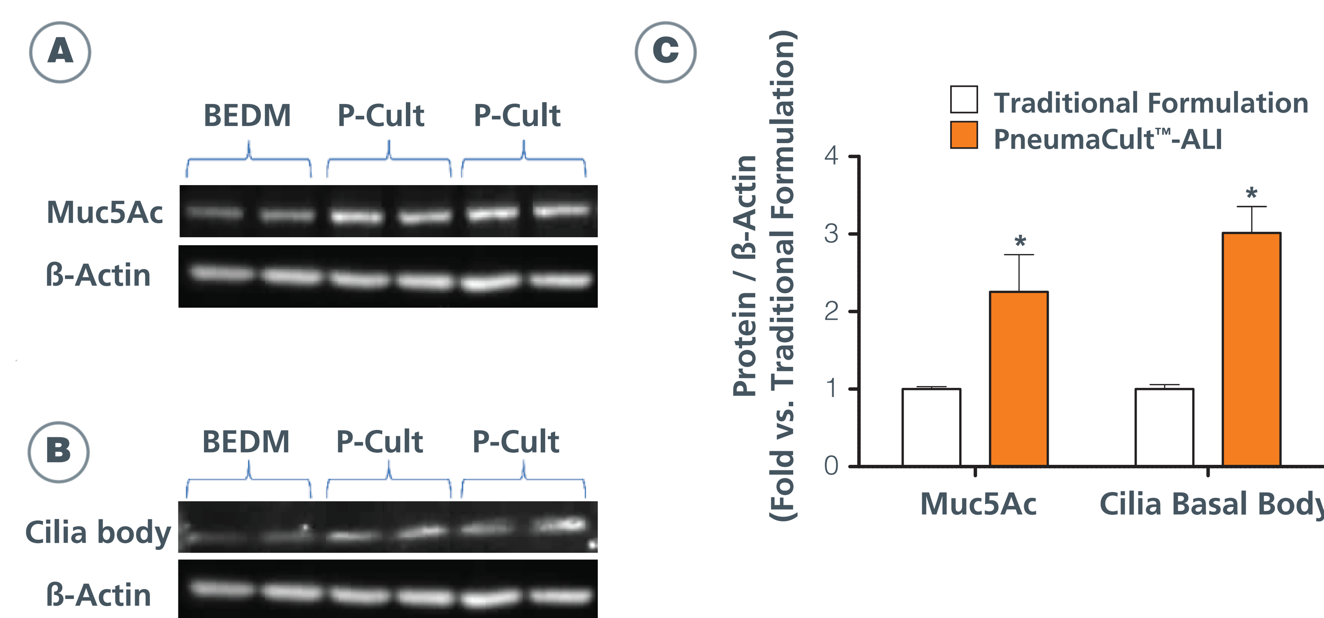
FIGURE 3: HBECs differentiated in PneumaCult™-ALI show consistent formation of a pseudostratified, mucociliated epitheilum





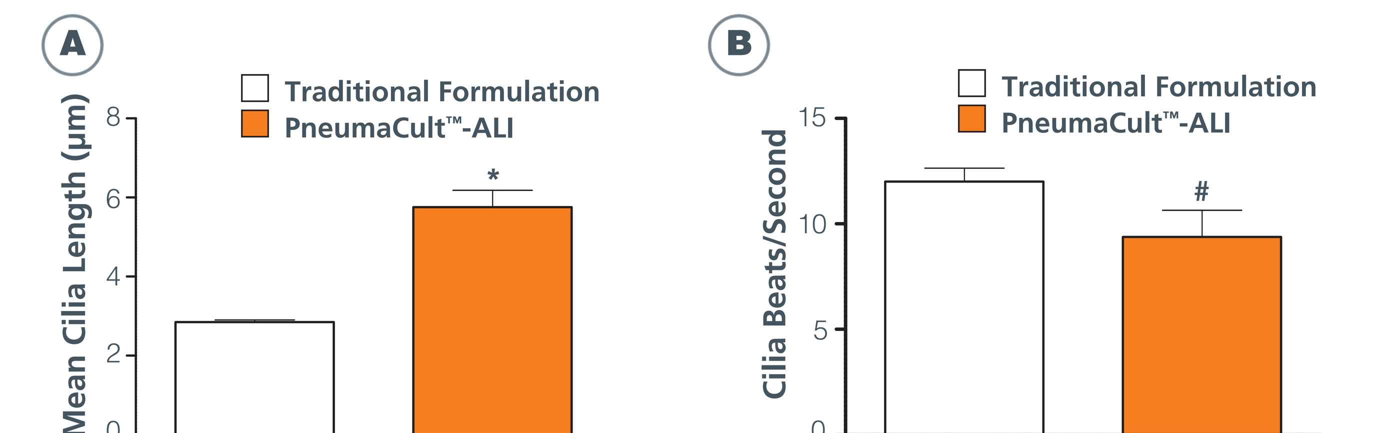
Ⓐ Hematoxylin and Eosin (H&E) or Periodic Acid-Schiff (PAS) staining of cultures differentiated using PneumaCult™-ALI shows a pseudostratified epithelium with extensive ciliagenesis and the presence of mucus-producing goblet cells. Ⓑ Pooled data indicating that the frequency of ciliated cells is significantly increased in cells cultured using PneumaCult™-ALI compared to a traditional formulation, thus reducing the number of cells that are not ciliated or mucus-producing (n=4; *P<0.05). Ⓒ Data from individual donors is shown to illustrate the reproducibility of mucociliary differentiation when using PneumaCult™-ALI. Each bar represents the mean ± SEM of 3 to 8 wells.

FIGURE 4: Expression of mucociliary proteins are increased in HBECs differentiated using PneumaCult™-ALI compared to a traditional formulation



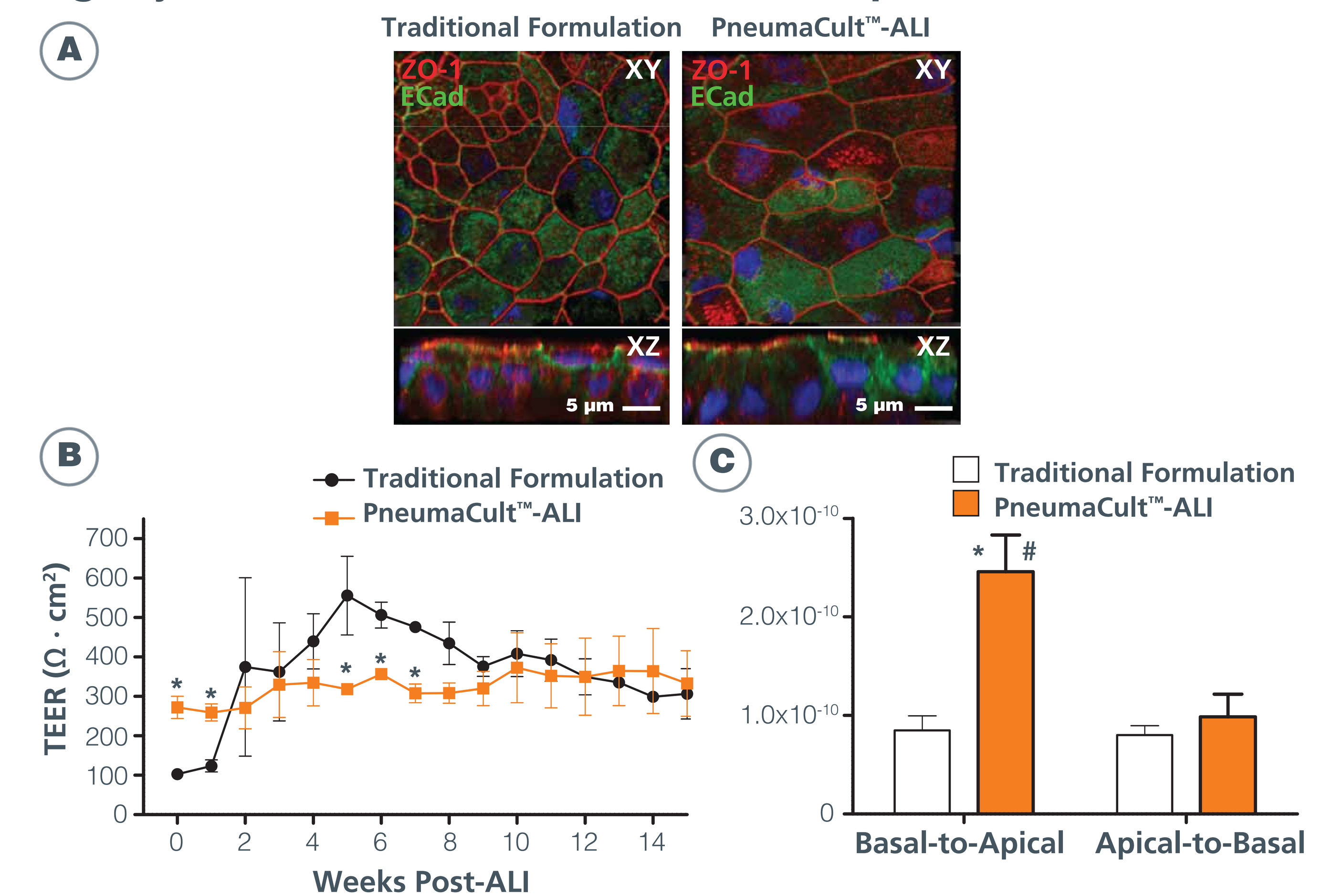
Ⓐ, Ⓑ Representative Western blots for Muc5Ac and Cilia Basal Body protein. Ⓒ Quantification of relative protein levels in cells cultured using PneumaCult™-ALI compared to a traditional formulation (n=10 - 12; *P<0.05).

FIGURE 5: Cells differentiated using PneumaCult™-ALI form cilia that are morphologically and functionally similar to those found in the human airway



Ⓐ The average cilia length in cells differentiated using PneumaCult™-ALI is significantly longer than those formed using a traditional formulation and similar to that found in the *in vivo* human airway (Leopold *et al.*, PLoS One; 4(12):e8157, 2009). n=3 donors in triplicate; *P<0.05. Ⓑ Cilia beat frequency is significantly lower in cells cultured in PneumaCult™-ALI but both are within the normal range found in the *in vivo* human airway (Chilvers & O'Callaghan, Thorax; 55(4):314, 2000 and Katz *et al.*, Chest; 92(3):491, 1987). n=2 donors in triplicate; #P<0.05.

FIGURE 6: Cells differentiated using PneumaCult™-ALI form tight junctions and a stable, direction-specific barrier



Ⓐ Representative immunofluorescent confocal images of ZO-1 (red) and E-Cadherin (ECad; green) in cells cultured in PneumaCult™-ALI or a traditional formulation. Ⓑ Trans-epithelial electrical resistance (TEER) measurements over 15 weeks of ALI culture. Ⓒ Measurement of directional passive diffusion of 4KDa FITC-labeled Dextran. Cells cultured in PneumaCult™-ALI exhibit greater basal-to-apical diffusion. N=18; *P<0.05 compared to traditional formulation; #P<0.05 compared to apical-to-basal diffusion.

FIGURE 7: HBECs differentiated in PneumaCult™-ALI exhibit mucus cell hyperplasia in response to IL-13 treatment

Representative PAS stained sections showing hyperplasia of goblet cells following 14 days treatment with 10 ng/mL IL-13.

Summary

- PneumaCult™-ALI is a defined, serum and Bovine Pituitary Extract (BPE)-free medium formulation that allows for the reproducible differentiation of primary human bronchial epithelial cells when cultured at the air-liquid interface.
- Cells differentiated in PneumaCult™-ALI exhibit several phenotypic characteristics of the human bronchial epithelium, including a pseudostratified, mucociliated phenotype.
- PneumaCult™-ALI can be used to establish air-liquid interface cultures for detailed study of airway epithelium development, function, and repair.

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