PneumaCult™: An Integrated Culture Medium System for *in vitro* Human Airway Modeling

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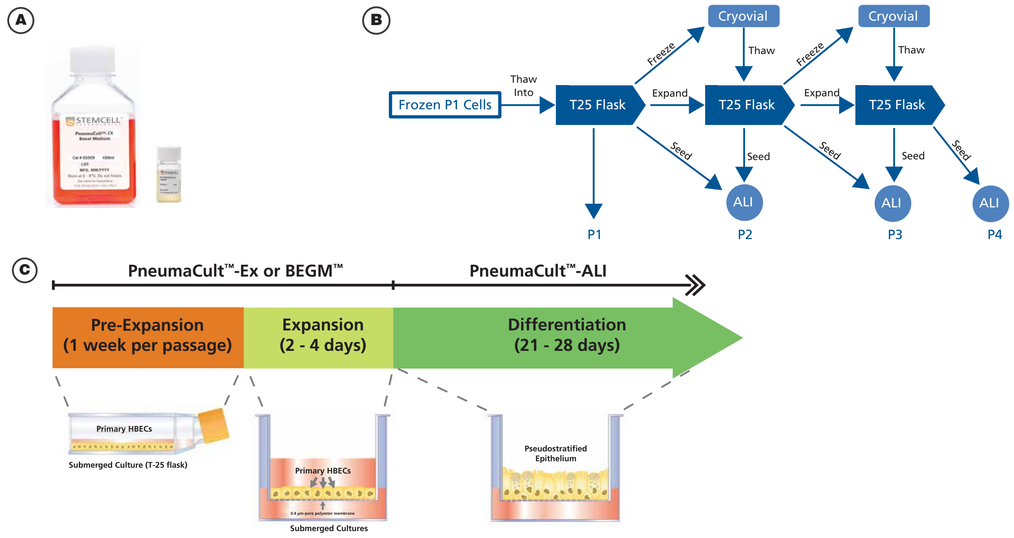
Introduction

Modeling of the human airway *in vitro* can be useful for a number of basic and applied research applications including the studies of viral infection, drug transport and toxicity, wound repair and disease modeling. To effectively recapitulate the human airway *in vitro*, primary human airway epithelial cells can be cultured at the air-liquid interface (ALI) using specialized media, resulting in differentiated cultures that exhibit morphological and functional characteristics that mimic the *in vivo* airway. In addition, primary airway epithelial cells can be expanded in submerged culture for several passages whilst retaining the ability to differentiate. We have recently launched PneumaCult™-ALI, a serum- and Bovine Pituitary Extract (BPE)- free medium to support efficient mucociliary differentiation of primary human bronchial epithelial cells (HBECs) in ALI culture. Here we describe PneumaCult™-Ex, a serum- and BPE-free medium that supports rapid expansion of HBECs. Primary HBECs cultured in PneumaCult™-Ex can successfully undergo mucociliary differentiation when cultured in PneumaCult™-ALI medium, thus providing a fully-integrated serum- and BPE-free workflow for human airway modelling.

Materials and Methods

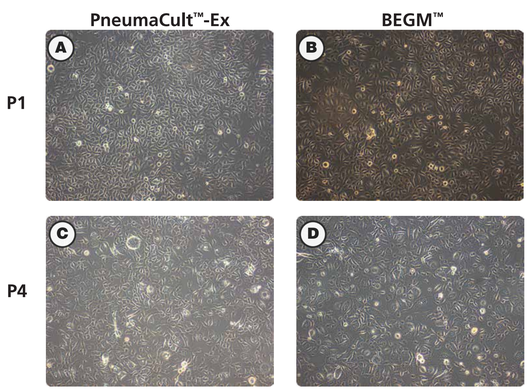
FIGURE 1: PneumaCult™-Ex and schematic of culture methods



A) PneumaCult™-Ex consists of one basal medium (490 mL) and one 50X supplement (10 mL). **B)** Schematic for serial passaging and assessment of differentiation potential. Commercially available primary normal human airway epithelial cells such as Lonza's HBECs [Passage (P)1; Catalog #CC-2540s] were thawed and seeded directly into T-25 cm^2 culture flasks containing PneumaCult™-Ex or control medium (BEGM™; Lonza) at a density of 3.5 x 10^3 cells/cm^2. Culture media were fully replenished every other day and cultures were passaged once cells reached approximately 80% confluence. At each passage, cells were enzymatically dissociated using Trypsin-EDTA (0.05%) and then re-plated at a density of 1 x 10^4 cells/cm^2 in PneumaCult™-Ex or BEGM™. **C)** Protocol for ALI differentiation: ALI cultures were prepared using aliquots of HBECs that had been expanding using either PneumaCult™-Ex or BEGM™, and differentiation was performed using PneumaCult™-ALI. Fold expansion was measured over 4 passages and the differentiation potential was assessed during each of the first 3 passages after which HBECs typically lose the ability to efficiently differentiate at the ALI.

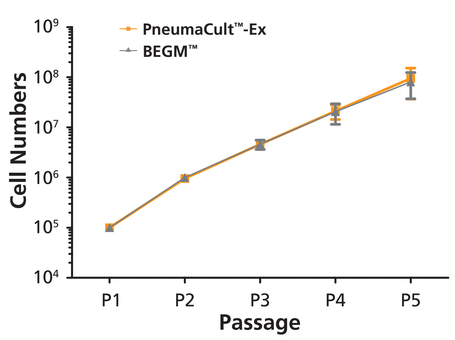
Results

FIGURE 2: HBECs cultured in PneumaCult™-Ex maintain a typical cobblestone morphology



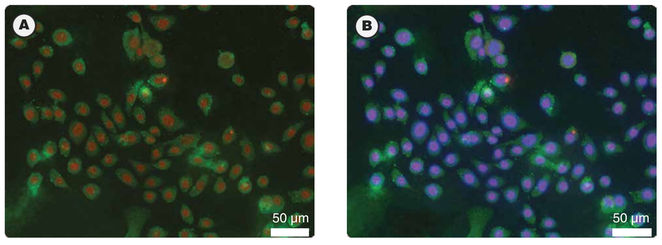
A,B) Cryopreserved P1 HBECs post-thaw, expanded in PneumaCult™-Ex, retaining a similar cobblestone morphology to cells cultured in BEGM™. Confluent cultures are shown at 5 days post seeding. **C,D)** The HBECs retain their normal cobblestone morphology and continue to expand at later passage (P4). Confluent cultures are shown at 7 days post seeding. All pictures taken through a 10X objective.

FIGURE 3: HBECs cultured in PneumaCult™-Ex (BPE-free) and BEGM™ (containing BPE) show comparable expansion



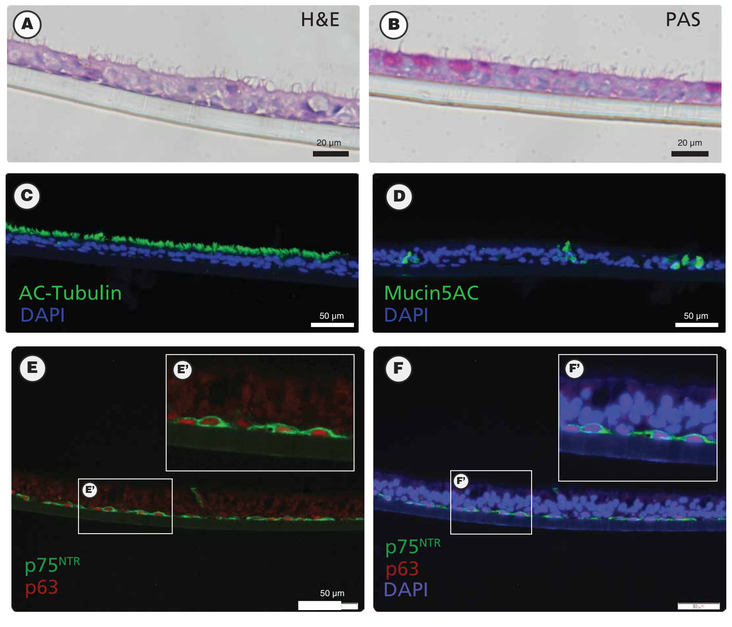
In 7 independent donor samples, the average fold expansion over 4 passages was not significantly different between cells cultured in PneumaCult™-Ex and cells cultured in BPE-containing BEGM™ medium (7.1 ± 1.4 vs. 7.2 ± 1.9; mean ± SD, n = 7, p = 0.9 in paired t-test).

FIGURE 4: HBECs cultured in PneumaCult™-Ex maintain widespread basal cell marker expression



A) Passage 3 HBECs cultured in PneumaCult™-Ex demonstrated extensive co-labeling of basal cell markers p63 (red) and $p75^{NTR}$ (green). **B)** Merged image for p63, $p75^{NTR}$ and DAPI.

FIGURE 5: HBECs cultured in PneumaCult™-Ex successfully differentiated in PneumaCult™-ALI



Early-passage (P1 - 3) HBECs cultured in PneumaCult™-Ex successfully differentiated in PneumaCult™-ALI to a pseudostratified mucociliary epithelium, as indicated by the qualitative observation of beating cilia under brightfield microscopy (data not shown) and **A)** H&E staining, as well as by **B)** the presence of goblet cells as indicated by positive Periodic acid-Schiff (PAS) staining at 28 days post air-lift. **C, D)** The presence of cilia and goblet cells was also demonstrated by immunofluorescence staining of the cilia marker acetylated (AC)-tubulin (green) in C and goblet cell marker Mucin5AC (green) in D. **E,F)** Appropriate positioning of basal cells along the transwell insert is visualized by immunofluorescence staining of basal cell markers $p75^{NTR}$ (green) and p63 (red) in cryosectioned ALI cultures differentiated from P2 HBECs cultured in PneumaCult™-Ex.

FIGURE 6: The time course of trans epithelial electrical resistance (TEER) for the HBECs cultured in PneumaCult™-ALI

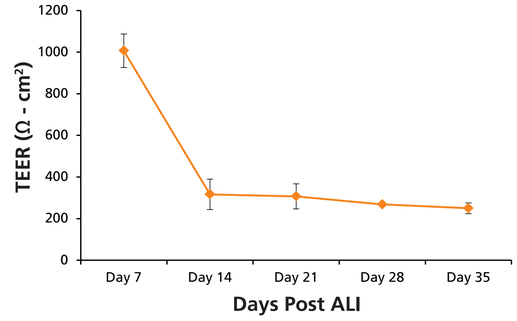
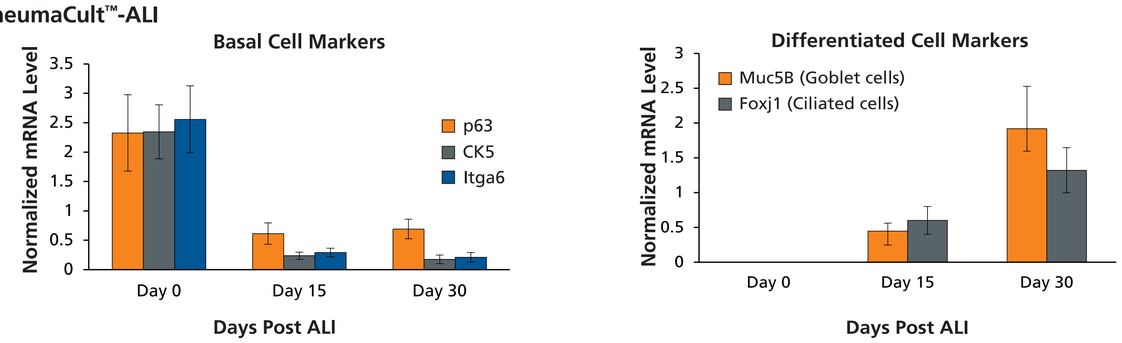


FIGURE 7: Dynamic changes of marker gene expression suggest successful differentiation of HBECs cultured in PneumaCult™-ALI



Gene expression profile for basal cells (p63, $p75^{NTR}$ and Itga6), goblet cells (Muc5B) and ciliated cells (Foxj1) determined by qPCR analysis. Expression data were normalized to the housekeeping gene TBP. Data presented are the mean of three independent experiments with triplicate wells for two different donors. Error bars indicate SD.

Conclusions

- PneumaCult™-Ex is a serum- and BPE-free medium that supports the expansion of primary human bronchial epithelial cells (HBECs).
- By supporting efficient expansion of primary HBECs in monolayer culture, PneumaCult™-Ex enables generation of large numbers of cells for use in respiratory research, toxicity testing and drug development.
- HBECs cultured in PneumaCult™-Ex maintain an epithelial cell morphology and expression of key basal cell markers, p63 and $p75^{NTR}$.
- PneumaCult™-Ex, together with PneumaCult™-ALI, creates an optimized BPE-free culture system for differentiating HBECs into a pseudostratified mucociliary epithelium resembling the human airway.