PneumaCult™-Ex Plus, a Novel Defined and Feeder-Free Medium, Supports the Improved Expansion of Primary Human Airway Epithelial Cells and Maintenance of their Mucociliary Differentiation Potential in Later Passages

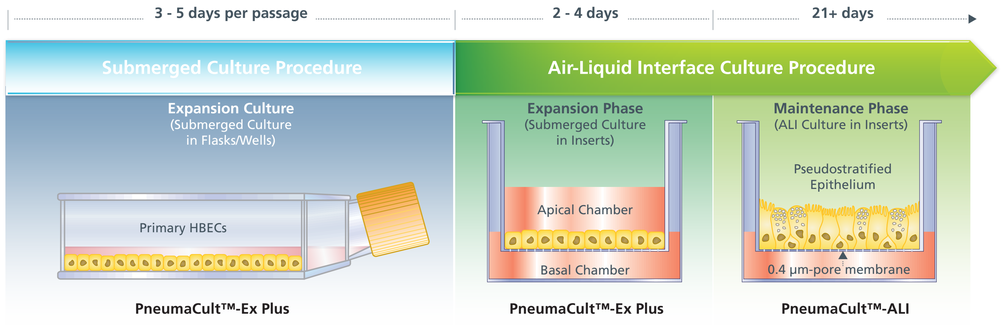
Juan Hou[1], Tyler Brown[1], Arisa Yoshikawa[1], Michael J. Riedel[1], John Stingl[1], Terry E. Thomas[1], Allen C. Eaves[1,2], and Sharon A. Louis[1]
[1] STEMCELL Technologies Inc., Vancouver, Canada [2] Terry Fox Laboratory, BC Cancer Agency, Vancouver, B.C., Canada

Introduction

Traditional feeder-free and Bovine Pituitary Extract (BPE)-containing media formulations for the expansion of primary human bronchial epithelial cells (HBECs) typically support the maintenance of their mucociliary differentiation potential for a limited number of passages in vitro. A novel culture system comprising an inactivated mouse embryonic fibroblast feeder layer and a specialized medium has been recently reported to improve the expansion of HBECs while maintaining their differentiation potential, even after extended passaging[1,2]. However this feeder-dependent method is cumbersome and undefined, thus limiting its utility. We have developed a novel feeder- and BPE-free culture medium, PneumaCult™-Ex Plus, that promotes extended passaging of HBECs without the loss of their differentiation potential at later passages. PneumaCult™-Ex Plus allows for the rapid expansion of HBECs, while maintaining their ability to form a pseudostratified mucociliary epithelium at air-liquid interface (ALI) for more passages, compared to other commercial HBEC expansion media.

Materials and Methods

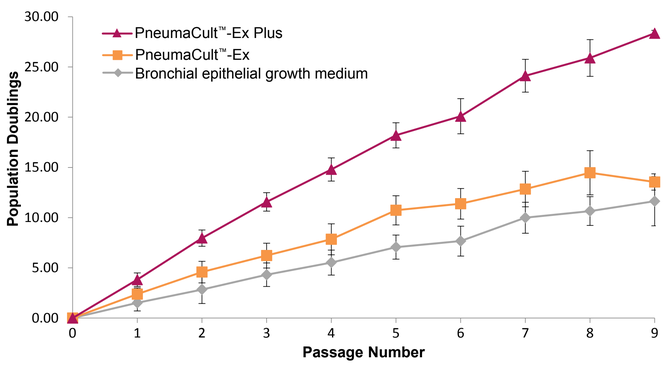
FIGURE 1: PneumaCult™ culture system



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 either PneumaCult™-Ex Plus, PneumaCult™-Ex or commercial bronchial epithelial growth medium 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, the cells were enzymatically dissociated using Trypsin-EDTA (0.05%) and then re-plated at a density of 5 x 10^3 cells/cm^2 in the diffrerent medium. Fold expansion was measured over 8 passages and the differentiation potential was assessed at each passage.

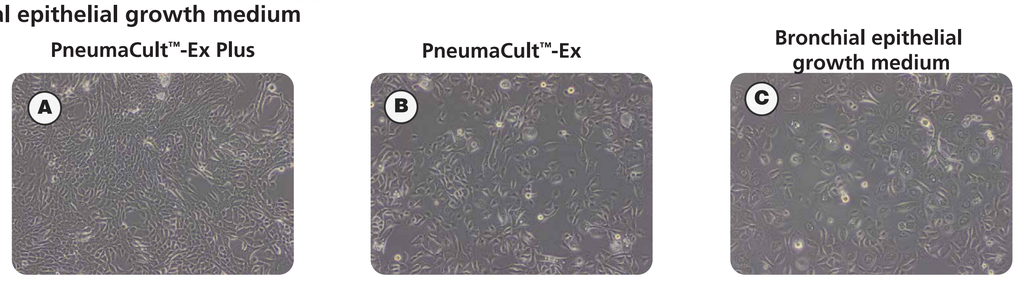
Results

FIGURE 2: HBECs cultured in PneumaCult™-Ex Plus showed more rapid expansion compared to those cultured in PneumaCult™-Ex and commercial bronchial epithelial growth medium



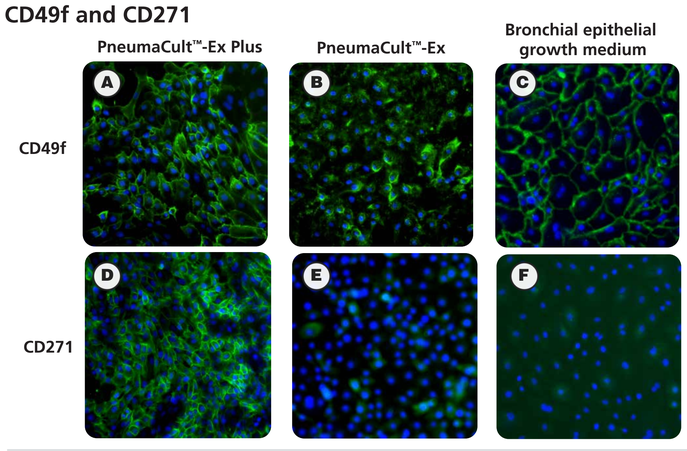
Commercially available, cryopreserved, passage 1 (P1) HBECs were seeded into PneumaCult™-Ex Plus, PneumaCult™-Ex or commercial bronchial epithelial growth medium. Cells cultured in PneumaCult™-Ex Plus have a significantly higher proliferation rate over 9 passages compared to those maintained in either PneumaCult™-Ex or commercial bronchial epithelial growth medium (n=6).

FIGURE 3: Representative morphology of HBECs cultured in PneumaCult™-Ex Plus, PneumaCult™-Ex, or Bronchial epithelial growth medium



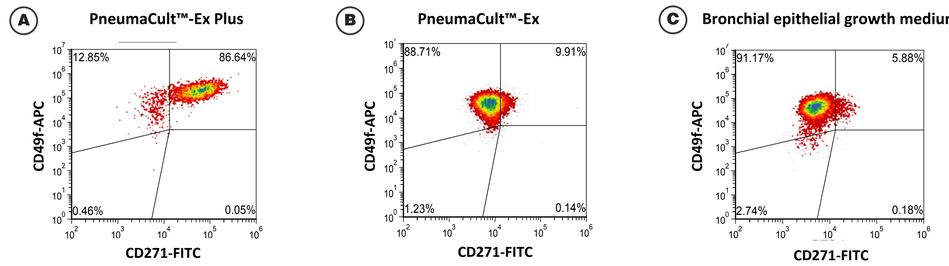
Representative live culture images for P4 HBECs cultured in PneumaCult™-Ex plus or control media (PneumaCult™-Ex or commercial bronchial epithelial growth medium). Compared to the cells cultured in PneumaCult™-Ex **(B)** and commercial bronchial epithelial growth medium **(C)**, HBECs cultured in PneumaCult™-Ex Plus **(A)** are smaller and more tightly packed. All images were taken using 10X objective.

FIGURE 4: HBECs cultured in PneumaCult™-Ex Plus maintain widespread expression of the basal cell markers CD49f and CD271



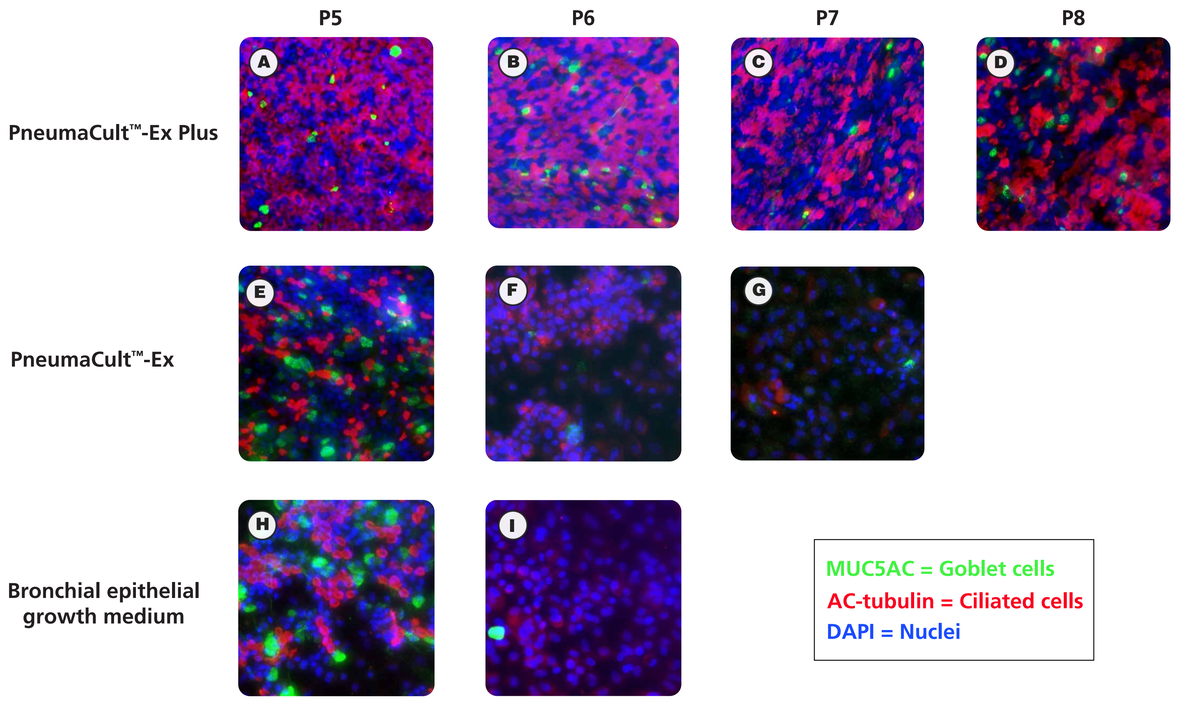
Immunocytochemistry detection of basal cell markers CD49f **(A, B, and C)** and CD271 **(D, E, and F)** for P4 HBECs cultured in PneumaCult™-Ex Plus **(A and D)**, PneumaCult™-Ex **(B and E)**, and commercial bronchial epithelial growth medium **(C and F)**. All images were taken using 10X objective. Nuclei are counterstained with DAPI.

FIGURE 5: HBECs cultured in PneumaCult™-Ex Plus maintain a larger population of $CD271^+CD49f^+$ basal cells



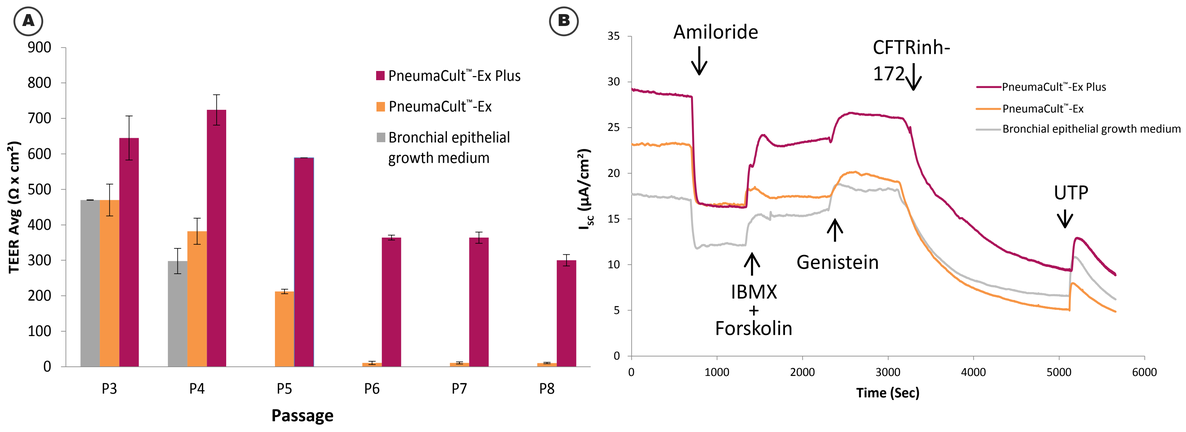
P4 HBECs cultured in PneumaCult™-Ex Plus **(A)**, PneumaCult™-Ex **(B)**, and commercial bronchial epithelial growth medium **(C)** were characterized by flow cytometry for expression of the basal cell markers CD49f and CD271. HBECs cultured in PneumaCult™-Ex Plus **(A)** have a higher percentage of cells co-expressing CD49f and CD271 compared to those cultured in PneumaCult™-Ex **(B)** and commercial bronchial epithelial growth medium **(C)**.

FIGURE 6: HBECs cultured in PneumaCult™-Ex Plus differentiate into a pseudostratified mucociliary epithelium at later passages in PneumaCult™-ALI



P4 HBECs were seeded and passaged in either PneumaCult™-Ex Plus, PneumaCult™-Ex, or commercial bronchial epithelial growth medium, followed by ALI differentiation at each passage (P5 - 8) with PneumaCult™-ALI Medium. The ALI cultures at 28 days post air-lift were fixed and stained with antibodies for cilia marker acetylated-tubulin (red) and the goblet cell marker MUC5AC (green). The nuclei are counterstained with DAPI (blue). All images were taken using 20X objective.

FIGURE 7: Electrophysiological characterization of differentiated HBECs previously expanded in PneumaCult™-Ex Plus, PneumaCult™-Ex, and commercial bronchial epithelial growth medium



(A) Transepithelial electrical resistance (TEER) for ALI cultures at 28 days post air-lift using HBECs expanded in either PneumaCult™-Ex Plus, PneumaCult™-Ex, or commercial bronchial epithelial growth medium. **(B)** Representative characterization of the ion channel activities for the ALI cultures at 28 days post air-lift using HBECs expanded in PneumaCult™-Ex Plus, PneumaCult™-Ex, or commercial bronchial epithelial grown medium. Amiloride: Epithelial Sodium Channel (ENaC) inhibitor. IBMX and Forskolin: Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) activators. Genistein: CFTR potentiator. CFTRinh-172: CFTR inhibitor. UTP: Calcium-activated Chloride channels (CaCCs) activator. I_{SC}: Short Circuit Current. All ALI differentiation cultures were performed using PneumaCult™-ALI Medium.

Conclusions

- PneumaCult™-Ex Plus is a serum- and BPE- free medium that supports higher expansion of HBECs compared to a commercial expansion media
- HBECs expanded in PneumaCult™-Ex Plus maintain better mucociliary differentiation potentials
- PneumaCult™-Ex, together with PneumaCult™-ALI, creates an optimized BPE-free culture system for expanding and differentiating HBECs into a pseudostratified mucociliary epithelium, resembling the human airway both morphologically and electrophysiologically

References

1. Liu X, et al. (2012) Am J Pathol 180(2): 599-607
2. Suprynowicz F, et al. (2012) PNAS (109): 20035-20040