Highly Expandable Human Alveolar Organoids to Study Respiratory Diseases

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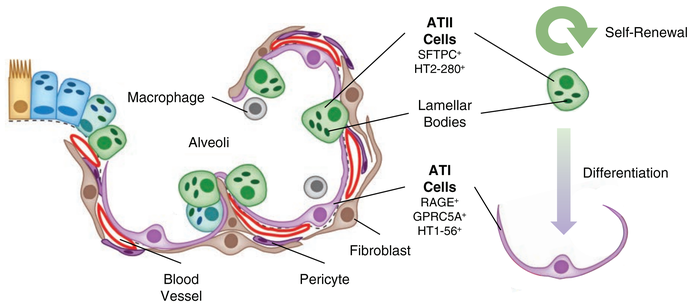
INTRODUCTION

In vitro organoid models for culturing human pulmonary epithelial cells have emerged as powerful tools for studying adult stem cells and lung biology. In addition, the COVID-19 pandemic has highlighted the dire need of physiologically relevant alveolar infection models to improve our understanding of respiratory diseases and develop novel drugs.

Alveoli are located in the most distal portion of the airway and the breathing units of our lungs. The alveolar sacs are composed of epithelial cells, stromal cells, endothelial cells, immune cells, and extracellular matrix molecules (Figure 1). Alveolar epithelial type 1 (ATI) cells are large, flat, terminally differentiated cells responsible for gas exchange. Cuboidal alveolar epithelial type 2 (ATII) cells are the stem cells of the alveoli capable of self-renewal and differentiation. They replenish both ATII and ATI cell populations during homeostatic turnover or after an injury. Importantly, traditional two-dimensional (2D) culture techniques have proven insufficient for the long-term culture of ATII cells.



Maintaining ATII cell phenotype and their capability to self-renew without support from stromal or feeder cells has been challenging due to the inability to properly mimic the complicated alveolar stem cell niche in vitro. To address the lack of optimized culture conditions, we have developed PneumaCult™ Alveolar Organoid Expansion (AvOE) and Alveolar Organoid Differentiation (AvOD) Media for the highly efficient expansion of isolated primary human ATII cells and subsequent differentiation into ATI cells.

FIGURE 1: Schematic of the Structure and Cell Composition of Alveoli
Figure was adapted from Rock JR et al.[1]

METHODS

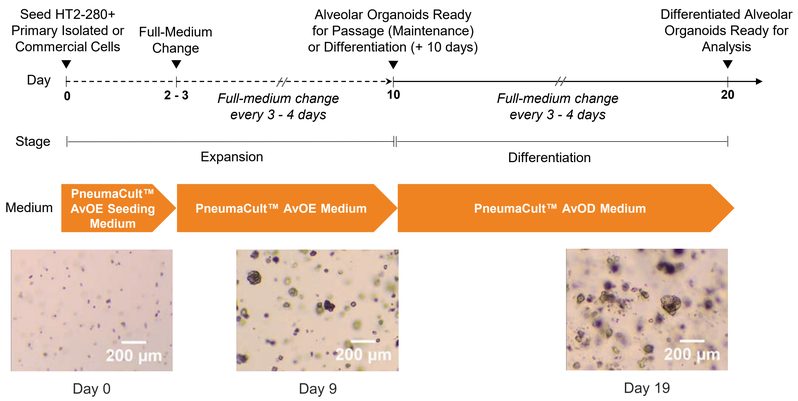


FIGURE 2. Generation of Alveolar Organoids Using PneumaCult™ Alveolar Organoid Media

During the expansion stage, primary isolated or cryopreserved human ATII single cells are seeded in Corning® Matrigel® domes with serum-free PneumaCult™ AvOE Seeding Medium. On days 2 - 3, after a full-medium change, cultures are expanded using PneumaCult™ AvOE Medium to obtain mature ATII organoids. After 10 - 14 days of culture, organoids are either passaged and subcultured in PneumaCult™ AvOE Medium, or differentiated into ATI cell-containing organoids by simply switching to PneumaCult™ AvOD Medium for additional 10 days. Alternatively, organoids in the expansion phase can be cryopreserved for biobanking. Representative bright field images at different stages of the workflow are shown at the bottom.

RESULTS

FIGURE 3. PneumaCult™ AvOE Medium Supports High Organoid-Forming Efficiency

Organoid-forming efficiency (OFE) serves as a functional measure of the health of an organoid culture and the performance of the medium. **(A)** The entire dome of initially 160 single-seeded cells expanded in PneumaCult™ AvOE Medium was captured on day 9; each ATII organoid formed was counted and indicated (total 44; OFE = 27.5%). **(B)** Bar graph showing OFE of ATII organoid cultures derived from three different donors (means ± SD). OFE = (organoids formed/cells seeded) x 100%.

FIGURE 4. PneumaCult™ AvOE Medium Supports Long-Term Expansion and Cryopreservation For Biobanking

(A) Cumulative doublings at each passage (n = 4 independent donors) for organoids expanded in PneumaCult™ AvOE Medium (orange) and published formulation (gray). Long-term passaging of alveolar organoids and a >10,000-fold expansion was achieved by both PneumaCult™ AvOE Medium and published formulation. **(B)** Population doublings (mean ± SD) of organoids generated from cells previously cryopreserved at early and late passages, and expanded in PneumaCult™ AvOE Medium. The cultured organoids retained their high expansion potential after thawing.

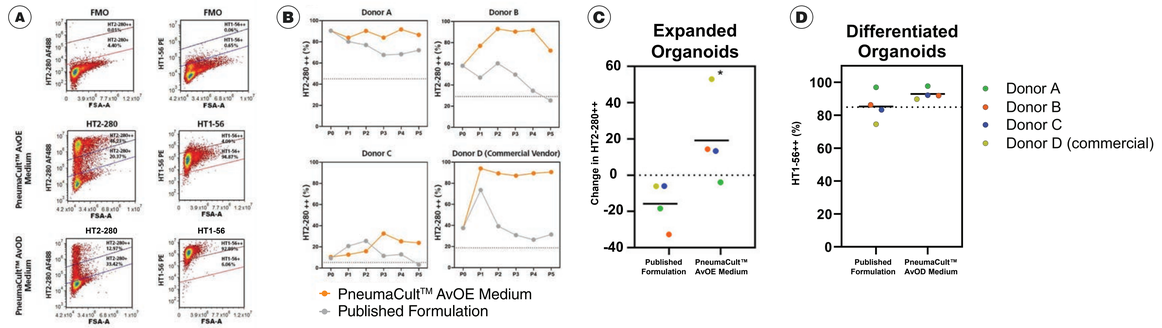


FIGURE 5. PneumaCult™ Alveolar Organoid Media Maintain High Levels of ATII and ATI Cell Marker Expression

(A) Flow cytometry gating of Fluorescence Minus One (FMO) controls for HT2-280 and HT1-56 markers (top), stained cells from organoids expanded in PneumaCult™ AvOE Medium (middle), and subsequently differentiated in PneumaCult™ AvOD Medium (bottom). PneumaCult™ AvOE Medium maintained both ATII marker expression (HT2-280++) and lineage potential to differentiate into ATI cells (HT1-56++) in PneumaCult™ AvOD Medium. **(B)** Organoids derived from 4 independent donors and expressing high levels of HT2-280 were cultured in PneumaCult™ AvOE Medium or published formulation, and compared at each passage. Dashed line depicts 50% of starting HT2-280++%. **(C)** Scatter plot illustrating the percent change in HT2-280 expression between P0 and P5 from the same donors. Organoids cultured in PneumaCult™ AvOE Medium maintained higher levels of HT2-280 marker expression compared to those cultured using published formulation. Mean is displayed as a solid line. * = $p < 0.05$ in a one-tailed paired T-test (n = 4). **(D)** Organoids cultured in PneumaCult™ AvOD Medium very efficiently differentiated into ATI cells, as shown by the average expression of HT1-56 in 92.9% ± 3.4% of the cells across multiple donors (n = 4).

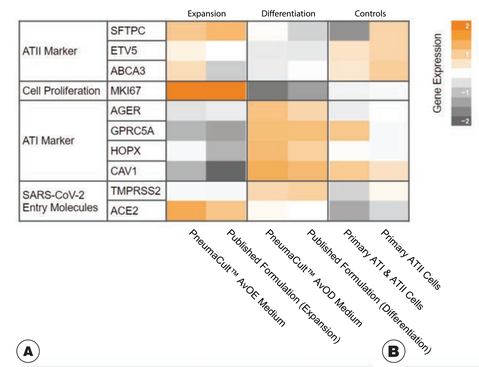


FIGURE 6. Gene Expression Analysis of Organoids Cultured in PneumaCult™ Alveolar Organoid Media Shows Presence of Alveolar Markers

Normalized gene expression of organoids expanded in PneumaCult™ AvOE Medium and differentiated in PneumaCult™ AvOD Medium compared to those cultured using a published formulation. The presence of canonical ATII and ATI genes as well as SARS-CoV-2 key entry markers, ACE2 and TMPRSS2, is shown. Published RNA-Seq datasets of primary alveolar epithelial cells and primary ATII cells were used as controls.

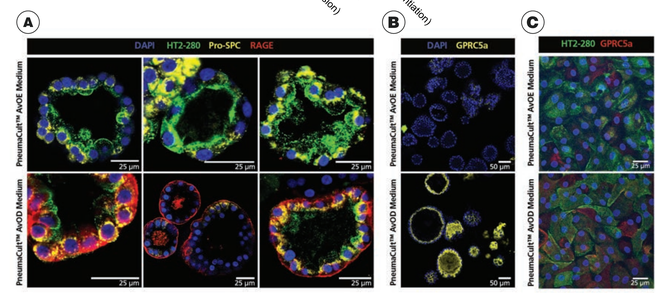


FIGURE 7. Immunohistochemistry Confirms Expression of ATII and ATI Cell Markers in Alveolar Organoids and Alveolar Epithelial Monolayers

(A) Organoids from 3 donors expanded in PneumaCult™ AvOE Medium (top) expressed the ATII markers HT2-280 (green) and prosurfactant protein C (pro-SPC, yellow). When further differentiated in PneumaCult™ AvOD Medium (bottom), ATII marker expression was downregulated, while ATI marker RAGE (red) was upregulated. **(B)** Differentiated organoids also expressed high levels of ATI marker GPRC5a (yellow). **(C)** Organoids seeded into Transwell® inserts and cultured in PneumaCult™ AvOE Medium formed a confluent monolayer, and could be cultured at the air-liquid interface. These cells expressed higher levels of HT2-280 and lower levels of GPRC5a compared to alveolar monolayers differentiated in PneumaCult™ AvOD Medium.

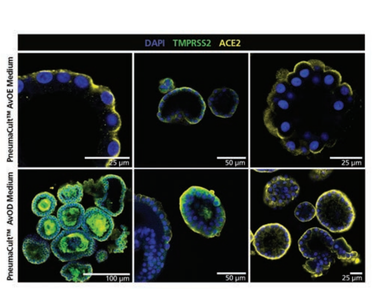


FIGURE 8. Organoids Cultured in PneumaCult™ Alveolar Organoid Media Express TMPRSS2 and ACE2, Key Markers for SARS-CoV-2 Entry

Organoids from 3 donors expanded in PneumaCult™ AvOE Medium (top) and differentiated in PneumaCult™ AvOD Medium (bottom) expressed proteins associated with SARS-CoV-2 entry, TMPRSS2 and ACE2.

Summary

- Organoids generated using PneumaCult™ Alveolar Organoid Expansion and Differentiation Media recapitulate alveolar physiology and key features of ATII and ATI cells in vitro that make them a suitable tool for disease modeling, drug discovery, and toxicity testing.
- PneumaCult™ AvOE Medium promotes efficient and reproducible long-term expansion of ATII organoids and offers a complete workflow to subsequently differentiate ATII cells into ATI cells using PneumaCult™ AvOD Medium.
- Alveolar organoids cultured in PneumaCult™ AvOE Medium can be cryopreserved for biobanking.

References
1. Rock JR & Hogan BLM Annual Review Cell & Developmental Biology 2011

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