

Human Airway Epithelial Cell Culture and COVID-19 Research

Application Note

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Human Airway Epithelial Cells and the Respiratory Tract

Human airway epithelial (HAE) cells are commonly used models for studying respiratory tract biology, disease, and therapy¹. Airway epithelial cells include tracheal, bronchial, small airway, and alveolar cells. These can be cultured as primary cells isolated from lung tissue e.g., primary HAE cells, differentiated pluripotent stem cells, or as immortalized or tumor cell lines such as Calu-3, a well-characterized human lung cancer cell line commonly used in models of human respiratory function, structure, and inflammatory responses^{1,2}.

In addition to its central air conducting role, the airway epithelium acts as a frontline defense against inhaled pathogens, including respiratory viruses³. Mechanisms of defense include the formation of a complex physicochemical cellular barrier, efficient maintenance of mucociliary clearance by differentiated luminal cells (secretory goblet and ciliated cells), and through immunological functions³. Therefore, human airway cell cultures which can be efficiently infected can also be used to model various mechanisms of viral pathogenesis during infection and human disease.

3D Cell Culture of Human Airway Epithelial Cells

HAE cells are traditionally cultured as 2D submerged cultures on plastic typically coated with extracellular matrix proteins such as collagens¹ (Figure 1). Such submerged conditions can however result in the loss of the differentiated luminal cells with cells mainly demonstrating a basal cell phenotype¹.

Various 3D cell culture systems have since been developed including air-liquid interface (ALI) and organoid cultures to study the airway epithelium. Based on a dual compartment model separated by a microporous membrane, permeable support systems are an established technique for ALI culture. Organoids are a newer technology which are gaining popularity in the study of lung epithelial cell function⁴. These are 3D structures that originate from stem/progenitor cells typically embedded in hydrogel (e.g., Corning® Matrigel® matrix) culture, which self-organize into airway-like tissue structures¹. Both ALI and organoid models provide greater physiological relevance versus conventional cell culture to further elucidate mechanisms of viral pathogenesis in the *in vivo* airway.

Air-Liquid Interface (ALI) Culture and Coronavirus Research

Tracheobronchial cells are one of the first targets of human respiratory viruses such as coronaviruses⁵. These cells can be cultured in ALI on permeable supports for 21 to 28 days where the apical side of the cell layer is exposed to air while the basolateral side is submerged in medium⁵. The cells differentiate and form a pseudostratified epithelial layer containing many different functional cell types such as basal, ciliated, and mucus-secreting goblet cells⁵. This 3D-like system effectively models the architecture and cellular complexity of the human upper conducting airway⁶. Another advantage of using permeable supports is the ability to generate multiple ALI cultures in an automation-friendly format for throughput cell-based assays of both healthy and diseased airway epithelium⁷ (Figure 2).

Key benefits of primary human airway epithelial ALI culture in modeling virus pathogenesis are the efficiency of infection by human and animal-transmitted coronaviruses (e.g., SARS- and MERS-CoV), the comparability of gene expression patterns and architectural functionality to the *in vivo* epithelium, and the ability to study virus infection, replication and host interactions in natural target cells⁵. Primary bronchial ALI cultures have been successfully used to study most human coronaviruses, a subset of which (e.g., MERS-CoV, SARS-CoV, HCoV-HKU1), having also been investigated with established primary alveolar ALI cultures (as reviewed in Reference 5).

The emerging literature on SARS-CoV-2 infection is primarily based on ALI cultures of primary bronchial cells inoculated with the virus to study infectious particle isolation, propagation, cytopathic effects, and anti-viral drug efficacy using a variety of techniques e.g., plaque assays, light microscopy, transmission electron microscopy, RT-PCR, and genome sequencing and analysis (Table 1). SARS-CoV-2 effectively infects and replicates in human airway epithelial ALI culture and has been shown to be directionally released on the apical side of the cell layer⁸. Furthermore, treatment with type I and III interferons significantly decreased virus replication in these ALI cultures demonstrating the therapeutic potential of IFNs to treat COVID-19⁸. A more recent study examines viral tropism along the human respiratory tract with higher levels of SARS-CoV-2 infectivity evident in proximal (high) versus distal (low) pulmonary epithelial ALI cultures⁹. While establishing human airway ALI cultures may be perceived as labor intensive, they are a valuable research tool for analysis of human respiratory pathogens such as SARS-CoV-2¹⁰.

Human Airway Organoids (AOs) and Coronavirus Research

Several approaches have been explored to generate mammalian airway organoids (AOs) as reviewed in Barkauskas, et al⁴. AOs have been derived from a variety of origin cell types including trachea or large airway basal cells⁴, alveolar cells¹¹, human iPSC¹², and embryonic lung¹³. AOs have the same ability to differentiate into polarized structures consisting of ciliated, goblet, and basal cells without the need for a permeable support system¹⁴.

Recent advances enabling long-term (i.e., >1 year) expansion of human AOs from biopsies or bronchoalveolar lavage fluid has improved the reproducibility and ease of availability of these organoid models¹⁵. Once established, differentiated AOs can be expanded indefinitely, display phenotypic and genotype stability, are amenable to modification by lentivirus and CRISPR technology and are therefore ideal tools for disease modeling^{15,16}. AOs have allowed analysis of cystic fibrosis¹⁵ and rapid assessment of the infectivity of emerging respiratory viruses e.g., influenza, in humans¹⁷. An essential aspect of studying respiratory disorders is comparing gene expression of healthy versus diseased tissue for disease model characterization and screening. High throughput gene expression analysis of AOs using the nCounter® PlexSet™ assay has been performed for healthy and asthmatic primary bronchial cells¹⁸ (Figure 3).

Organoids are suitable as new disease models to study SARS-CoV-2 biology and to screen for therapeutics using human disease-relevant tissues^{19,20}. COVID-19 patients typically present with respiratory symptoms however, almost 25% of patients also exhibit gastrointestinal indications²⁰ and liver damage is likewise a common feature in severe COVID-19 patients²¹. In emerging COVID-19 literature, lung²², small intestinal^{23,24}, colonic^{20,25}, brain²⁶, and liver ductal²¹ organoids have been derived from primary or stem cells. This has facilitated the isolation and propagation of SARS-CoV-2 virus for downstream PCR, genome sequencing, *in vivo* transplantation and high throughput screening analysis (Table 1). An hPSC-derived lung organoid platform containing alveolar type II cells expressing ACE2 has been described which demonstrates SARS-CoV-2 infectivity, a robust physiological immunomodulatory response and amenability to high throughput anti-viral drug screening²². Lamers, et al. report that organoid-derived human airway epithelium cultured on a Collagen I-coated Transwell® permeable support (Corning) are productively infected by SARS-CoV and SARS-CoV-2 viruses which specifically target ciliated cells²³. The data generated thus far strongly support that human organoids are effective *in vitro* models to study the systemic biology, pathogenesis, and potential treatment of coronaviruses²³. The continued development of the human airway organoid model, in particular, will be of valuable importance to further study SARS-CoV-2 infectivity, replication kinetics, host-virus interactions, and immunomodulatory responses, and as a tool for antiviral drug discovery and development to help fight the current pandemic²⁷.

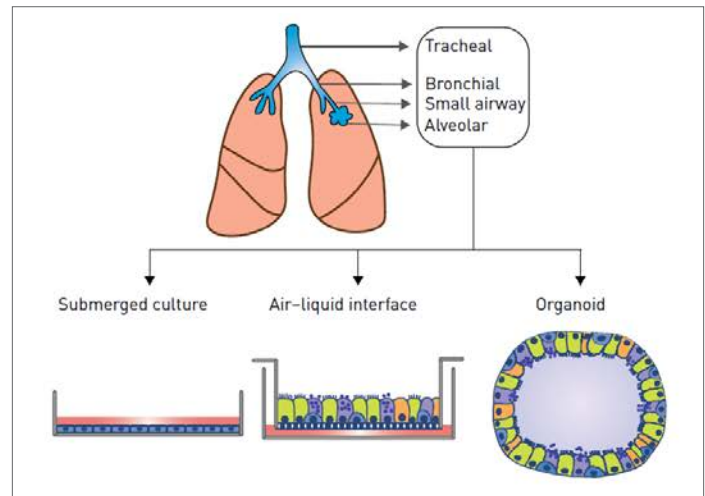


Figure 1. Various culture systems used for culture of airway epithelial cells. Cells can be grown as a simple submerged culture on plastic typically coated with extracellular matrix (ECM)-derived proteins, at air-liquid interface (ALI) using a permeable support system, or in 3D organoid culture within a biological matrix. Image adapted from Reference 1.

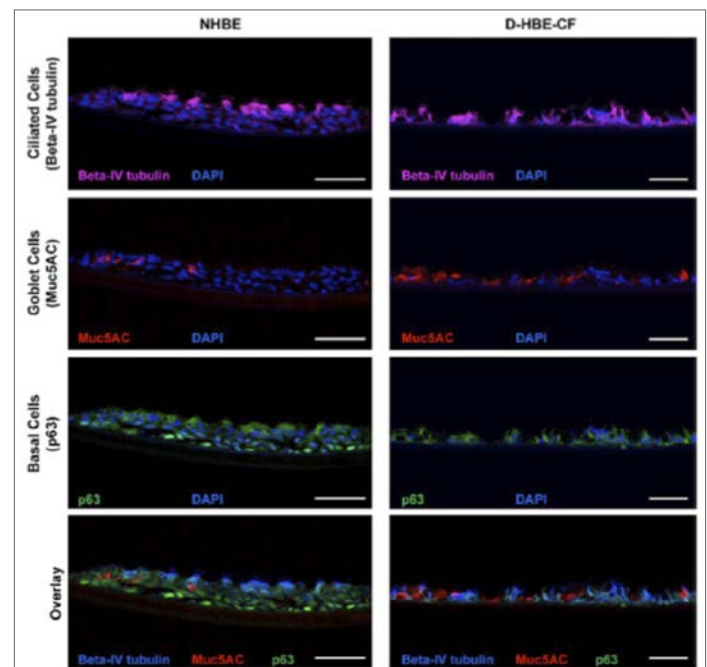


Figure 2. Human bronchial/tracheal epithelial cells from a healthy donor (NHBE; Lonza CC-2541) and from a donor with cystic fibrosis (D-HBE-CF; Lonza 00196979) cultured at the ALI form pseudostratified epithelia containing 3 different cell types⁷.

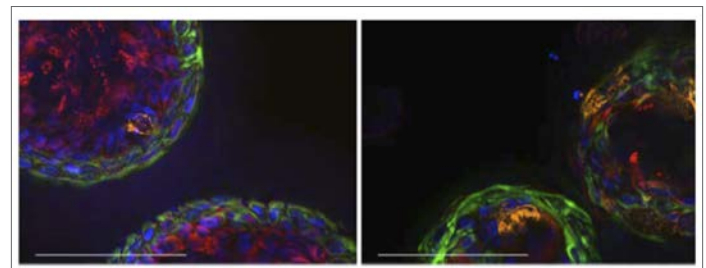


Figure 3. Normal (left) versus asthmatic (right) airway organoids cultured in Corning Matrigel matrix. Multi-color fluorescent labels indicate specific cell types: basal cells (green), ciliated cells (red), mucus production from goblet cells (orange), nuclei (blue)¹⁸.

Table 1. Emerging Articles Using 3D Cell Culture Models to Study COVID-19

Application	3D Model	Culture System	Reference No.
Modeling of SARS-CoV-2 infection, replication, cytokine response profiling and sensitivity to interferons	ALI culture of human airway epithelial cells	Transwell® permeable supports	8
Exploration of SARS-CoV-2 infection susceptibility in nasal, airway and alveolar regions	ALI culture of primary human nasal epithelial (HNE), bronchial large epithelial (LAE), and type II alveolar cells	Transwell permeable supports	9
Isolation of SARS-CoV-2 virus from patient samples for downstream RT-PCR and viral genome sequencing	ALI culture of human airway epithelial cells	Transwell permeable supports	10
Investigation of the effect of broad-spectrum antiviral drug NHC against SARS-CoV-2, MERS-CoV and SARS-CoV	ALI culture of human airway epithelial cells	Transwell permeable supports	28
Investigation of human small intestinal organoids as a model for SARS-CoV-2 infection and biology	(1) Human small intestinal organoids (hSIOs) derived from primary gastric tumor samples (2) Human airway organoids derived from adult human lung stem cells (3) ALI culture of dissociated human airway organoids	Corning® Matrigel® matrix, Transwell permeable supports	23
Infection of bat and human intestinal organoids by SARS-CoV-2	Human small and large intestinal organoids from donor samples Bat intestinal organoids from euthanized specimens	Corning Matrigel matrix	24
Study of SARS-CoV-2 infection and high throughput anti-viral drug screening using human pluripotent stem cell-derived colonic organoids	(1) Human pluripotent stem cell-derived colonic organoids (hPSC-COs) (2) <i>In vivo</i> transplantation of hPSC-COs in humanized mice	Corning Matrigel matrix	20
Investigation of the SARS-CoV-2 lifecycle in human intestinal epithelial cells	Human primary intestinal organoids derived from colonic resection tissue	Corning Matrigel matrix	25
Development of an hPSC-derived lung organoid platform to model COVID-19 and high throughput screening to identify drug therapeutics	Human pluripotent stem cell (hPSC)-derived lung organoids	Corning Matrigel matrix	22
Investigation of the infectivity and liver tissue damage of SARS-CoV-2 in human liver ductal organoids	Primary liver ductal organoids derived from human liver biopsies	Corning Matrigel matrix	21
Modeling of infection and CNS pathologies of SAR-CoV-2 in human brain organoids	hiPSC-derived human brain organoids	Corning Matrigel matrix	26

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