Air Liquid Interface Models:

A Collection of Tools to Support Powerful Studies that Mimic *In Vivo* Models

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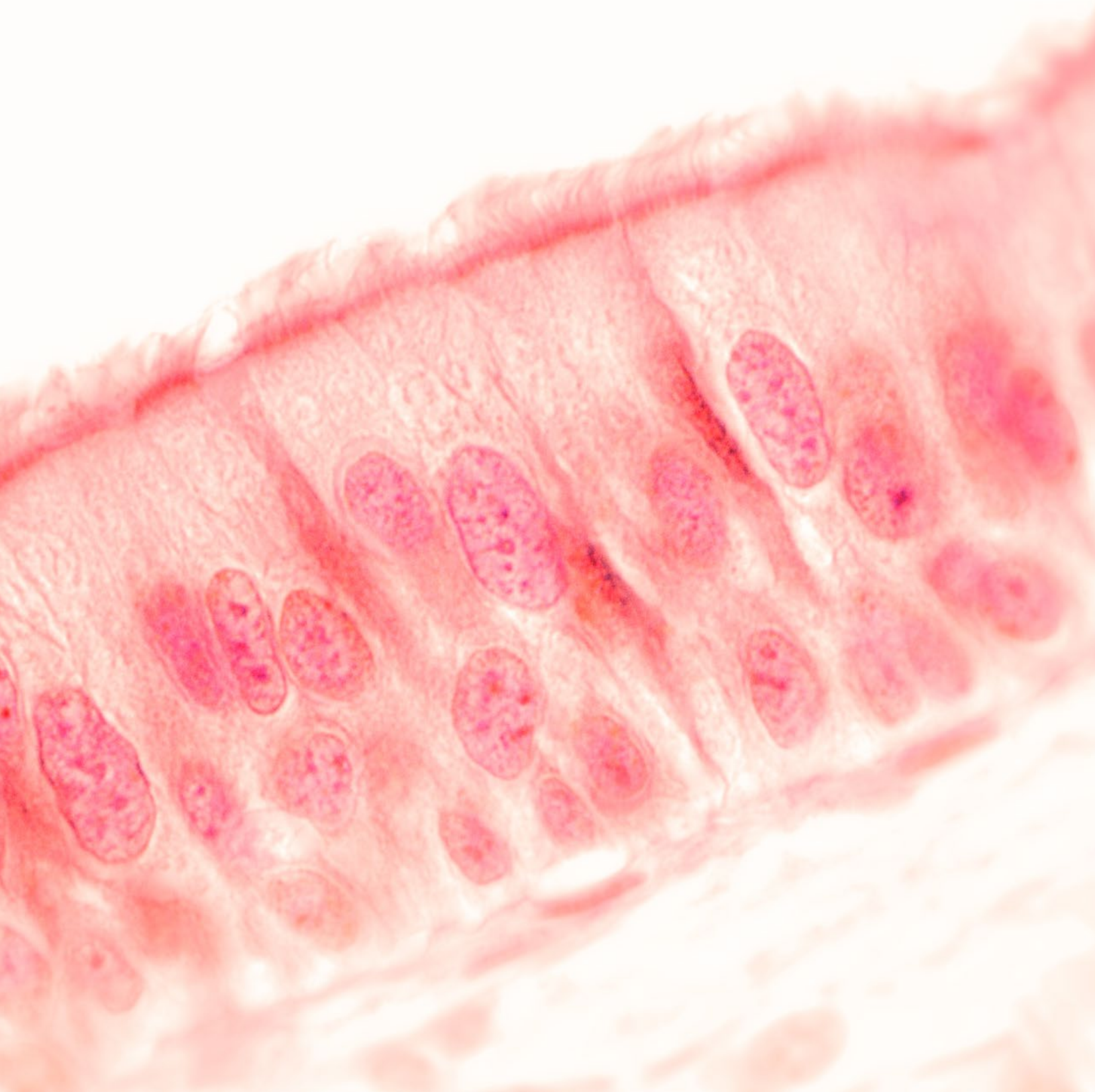
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Mimic the disease.
Advance the science.

Corning 3D cell culture solutions give you the power to generate and study physiologically relevant models that closely mimic *in vivo* behaviors in human tissues, tumors, and organs. In fact, lung organoids generated using Corning® Matrigel® matrix and air-liquid interface models created using Transwell® and Falcon® permeable supports are being used to study a variety of diseases, including infectious diseases such as COVID-19. Complex 3D models help you understand how infectivity works, how a virus attacks the body, and how it spreads from one cell to the next. So you can move one step closer to uncovering the best ways to treat and prevent disease. **To learn more, visit www.corning.com/3D.**

A Powerful Tool for the Study of Airway Biology: Human Tracheobronchial Epithelial Model Grown at the Air-liquid Interface

Application Note

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Introduction

Normal human tracheobronchial epithelium protects the airways by providing a barrier against injury from external insults. Intact cellular organization and function of the epithelial barrier provides a sterile and stable inner environment for living organisms. Ciliated and nonciliated columnar cells, secretory cells, and basal cells are arranged in a pseudostratified pattern to form complex epithelium structure and function. Two distinct compartments, apical and basolateral are developed by all cells creating tight junctions and contacting basement membrane, while some cells will reach the luminal surface[1]. When exposed to particles such as pollutants, bacteria, or virus, these particles will be cleared by the co-operation of ciliated cells and secretory cells[2].
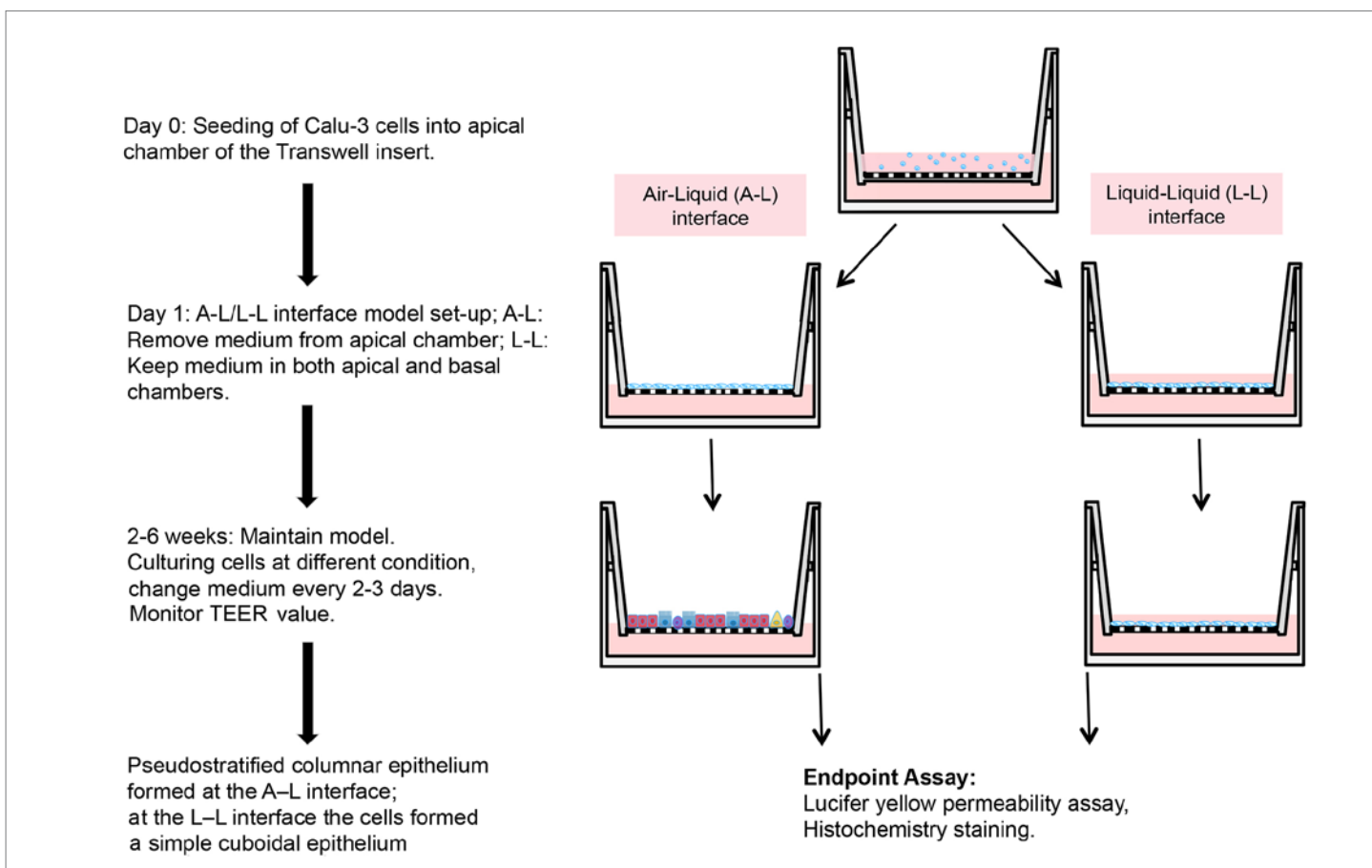
Human tracheobronchial epithelial cells grown at the air-liquid interface (ALI) culture have emerged as a powerful tool for the study of airway biology. Air-liquid interface culture for 3 to 6 weeks can result in the formation of well-differentiated, polarized human tracheobronchial epithelial cultures that resemble *in vivo* pseudostratified mucociliary epithelium. *In vitro* models using primary cells and cell lines are essential for understanding the function of the epithelium. Studies with cell lines are convenient, reproducible, and inexpensive compared to experiments involving primary cells. Calu-3, an epithelial cell line derived from human lung adenocarcinoma, has been widely used in studying bronchial epithelium, its closest resemblance to this epithelium *in vivo*[3]. ALI models of culturing epithelial cells has shown its application in response to stimulations including wounding, anion secretion, and viral infection[4,5,6].

The Transwell® permeable support insert from Corning Life Sciences is a useful tool that mimics an *in vivo* environment, which is why it has been widely used in a variety of studies, including drug transport/absorption, cell migration and differentiation, and co-culture applications. In addition, several *in vitro* models have also been constructed to better explore the natural physiology of cellular mechanism.

Reagents and Materials

Calu-3 cell line (ATCC Cat. No. HTB-55)

MEM medium (Corning Cat. No. 10-010-CV)

Fetal bovine serum (FBS, Corning Cat. No. 35-076-CV)

Transwell permeable support insert (Corning Cat. No. 3470, 0.4 µm PET membrane)

24-well plate (Corning Cat. No. 3524)

96-well assay plate (Corning Cat. No. 3915)

Lucifer yellow (Sigma Cat. No. L0144)

HBSS (Corning Cat. No. 21-020-CV)

HEPES (Corning Cat. No. 25-060-Cl)

Voltohmmeter (MilliporeSigma)

SpectraMax® M4 plate reader (Molecular Devices)

Workflow

Day 0: Seeding of Calu-3 cells into apical chamber of the Transwell insert.

Day 1: A-L/L-L interface model set-up; A-L: Remove medium from apical chamber; L-L: Keep medium in both apical and basal chambers.

2-6 weeks: Maintain model. Culturing cells at different condition, change medium every 2-3 days. Monitor TEER value.

Pseudostratified columnar epithelium formed at the A–L interface; at the L–L interface the cells formed a simple cuboidal epithelium

Air-Liquid (A-L) interface

Liquid-Liquid (L-L) interface

Endpoint Assay:
Lucifer yellow permeability assay, Histochemistry staining.

Procedure

1. Model Generation

Cells were seeded into Transwell® inserts on day 0. Prior to seeding, Transwell inserts were pre-incubated at 37°C with culture medium for at least 30 minutes. Calu-3 cells were seeded into the apical chamber of the Transwell inserts at densities of 0.25 x 10^5, 0.5 x 10^5, 1 x 10^5, 2 x 10^5, and 4 x 10^5 cells/well with 150 µL of culture medium. 800 µL of medium was added to the basal chamber. The plate was placed in a humidified 37°C/5% CO_2 incubator and cultured overnight. On day 1 for the A-L interface, the culture
medium in apical chamber was removed after seeding for 24 hours, and cells were cultured with 500 µL culture medium in the basal chamber. For L-L interface, the medium in both apical and basal chambers were kept. Culture medium was changed every 2 to 3 days, and cultured for 3 weeks.

2. TEER Value Monitor

The TEER (transepithelial electrical resistance) value was measured every 2 to 3 days using a Voltohmmeter (MilliporeSigma) to confirm the development of tight junctions. Both the basolateral and apical chamber were filled with fresh pre-equilibrated medium and TEER was read after allowing the cell culture to get

stable potential for approximately 15 to 30 min. to avoid any temperature fluctuation-induced TEER changes, because TEER measurement is sensitive to temperature[7]. Medium was removed from the apical side of the insert immediately after TEER readings to re-establish the ALI condition.

3. Lucifer Yellow Permeability Assay

On day 21, the Lucifer yellow permeability assay was performed. To prepare the donor solution, 100 mM stock solution of Lucifer yellow was diluted with HBSS/10 mM HEPES buffer to 300 µM. The receiver solution used was HBSS/HEPES buffer with a DMSO content matched to that in the donor solution. The Transwell inserts were washed once with HBSS/HEPES buffer, transferred into a new 24-well receiver plate with receiver solution, and then 50 µL of the donor solution was added to the apical chamber. The plate was placed at 37°C with orbital shaking for 60 min. Then 100 µL of the solution in the receiver plate was transferred to a black assay plate and the fluorescence was read at 480 nm/ 530 nm using a SpectraMax® M4 plate reader. In the meantime, a series of Lucifer yellow solution with different concentrations were diluted and the standard curve was plotted. The concentration of Lucifer yellow in the receiver plate was calculated from the standard curve.

4. Cell Staining

After culturing in the Transwell® inserts, Calu-3 cells, together with the Transwell membrane, were cut from the insert and fixed in 10% formalin overnight at 4°C. After dehydration and paraffin embedment, 5 µm sections were prepared and stained with hematoxylin and eosin (H&E), periodic acid–Schiff (PAS) stain for neutral proteoglycans, or Alcian blue for acid proteoglycans, and viewed with a lighted microscope.

To demonstrate mucus cell differentiation, confluent layers of cells grown at ALI model were subjected to PAS staining to visualize neutral and acidic glycoproteins (likely mucins).

5. Calculations

Transepithelial Electrical Resistance (TEER):

TEER of insert and medium alone was subtracted from measured TEER and calculated multiplying by the insert area.

$$TEER = (R_m - R_b) \times A$$

Where:

$TEER$ is the transepithelial electrical resistance

R_m is the resistance reading obtained for the cell monolayer

R_b is the resistance reading obtained for the blank insert (without cells)

A is the surface area of insert filter membrane [cm²]

Apparent Permeability (P_{app}):

$$P_{app}[cm/s] = \frac{Flux \times V_d}{t \times A} = \frac{dQ}{dt} \times \frac{1}{A \cdot C_0}$$

Where:

Flux is the fraction of the donated amount recovered in the receiver chamber

V_d is the volume in the donor chamber [cm³]

C_0 is the initial concentration in the donor solution [mM]

A is the surface area of insert filter membrane [cm²]

t is the incubation time [sec.]

dQ/dt is the amount of drug transported within a given time period [pmol/sec.]

Results

1. Microscopic Observation

In the L-L interface model, Calu-3 cells became confluent and lacked clear structure after 3 weeks of culturing in the Transwell inserts. In the A-L model, protuberances were observed after 3 weeks cultivation (Figure 1).

2. Evaluation of Cell Monolayer Integrity

TEER is a widely accepted quantitative technique to measure the integrity of tight junction dynamics in cell culture models of epithelial monolayers. TEER values are strong indicators of the integrity of the cellular barriers before they are evaluated for transport of drugs or chemicals. TEER measurements can be performed in real-time without cell damage and generally are based on measuring ohmic resistance or measuring impedance across a wide spectrum of frequencies.
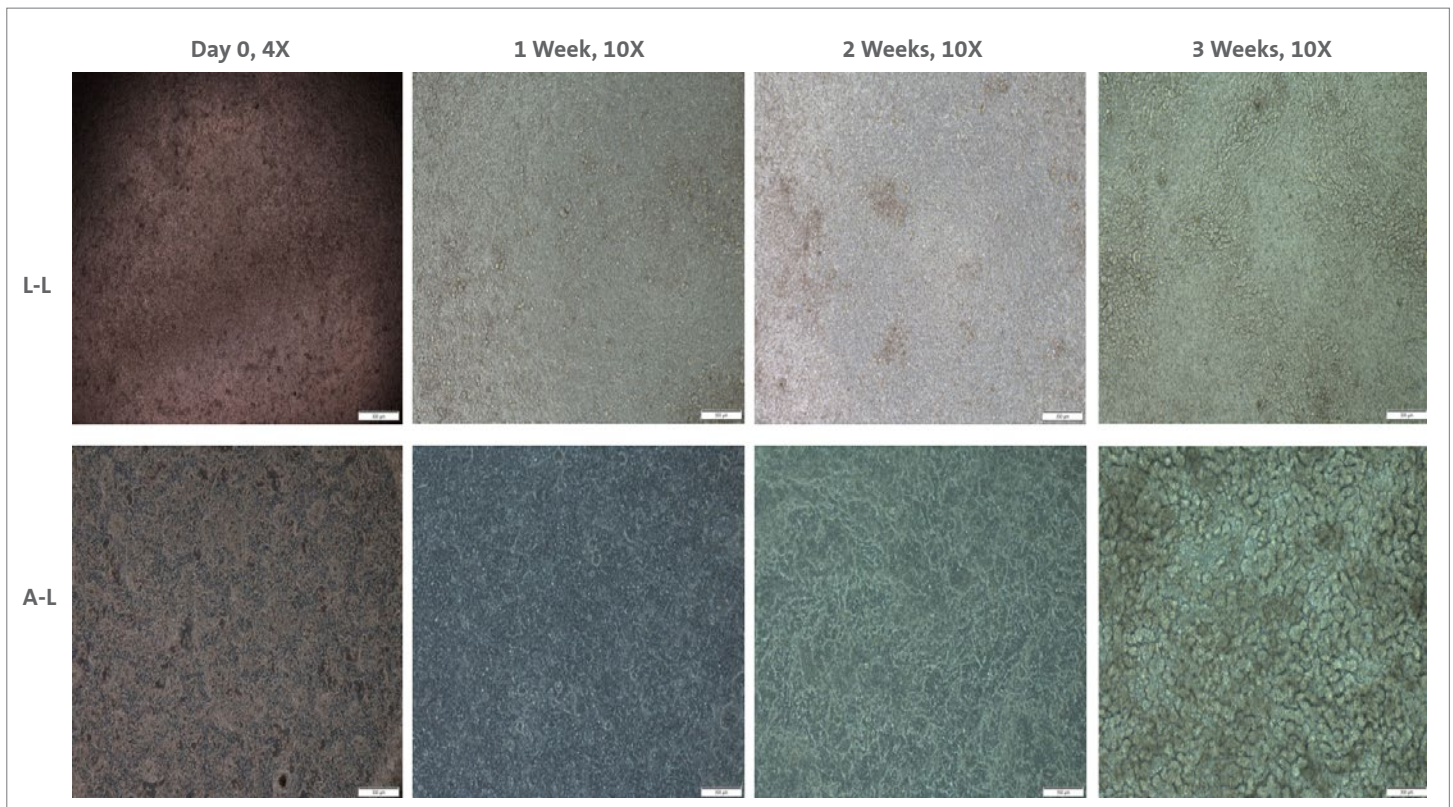

Figure 1. Calu-3 bronchial epithelial model. Cultivation of Calu-3 cell at L-L model (upper) and A-L model (below). Microscopy images photos were taken at day 0, 1 week, 2 weeks, and 3 weeks under microscope.
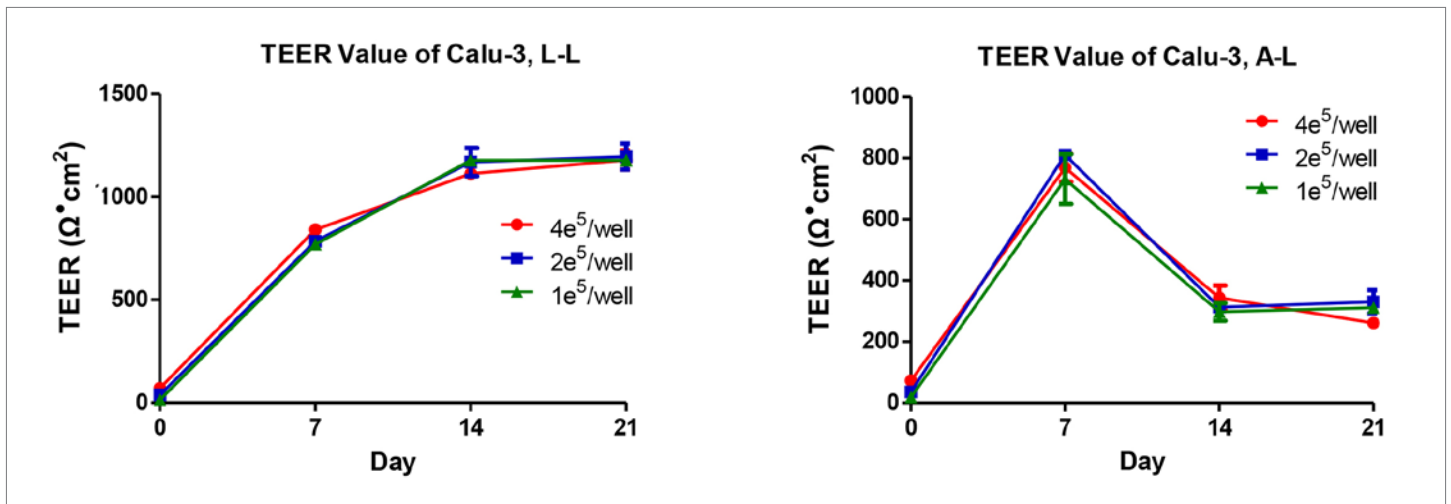
Figure 2. TEER measurements of Calu-3 in L-L and A-L models. Time-course of TEER development for the L-L model (left) and A-L model (right) at day 7, 14, and 21. Seeding densities of Calu-3 cells: 4e5 (red squares), 2e5 (blue dots), and 1e5 (green triangles) per insert.

In the L-L interface model, the TEER values of Calu-3 cells reached more than 1,000 $\Omega \cdot cm^2$ at day 14 and remained stable until day 21. In the A-L interface model, high TEER values were reached in one week, then decreased and stabilized at approximately 300 $\Omega \cdot cm^2$ (Figure 2).

Lucifer yellow was also used as a paracellular marker for monolayer integrity to confirm the results of TEER measurements. Lucifer yellow travels across the cell monolayer only through passive paracellular diffusion and has low permeability. It is not able to pass across cell monolayers when tight junctions between cells are maintained. Figure 3 shows the permeability (P_{app}) of Lucifer yellow for Calu-3 epithelial L-L and A-L models. P_{app} was much lower than 1 x 10^{-6} cm/s for both L-L and A-L models. Tight junctions were formed after 3 weeks of cultivation in the A-L model.

3. Haematoxylin-eosin (HE) Staining

The results of HE staining indicated that pseudostratified columnar epithelium was formed in 3 weeks in the A-L model (Figure 4).

Cells exhibited poor morphological quality in the L-L model. They appeared dedifferentiated, flat, or dwarf, with reduced numbers of cilia and appearance of secretory protrusions in the L-L model; While in the A-L model, cells were highly polarized, confluent, and retained a mix of ciliated and secretory cells.

4. Periodic Acid Schiff (PAS) and Alcian Blue Staining of Proteoglycans/Mucus

Neutral proteoglycans were stained with PAS (red) and nuclei were stained with nuclear fast red (Figure 5, left panel). Acid proteoglycans were stained with Alcian blue (blue; Figure 5, right panel). The results showed that secretory products and vesicles were visible in cells cultured at the A–L interface for 3 weeks.
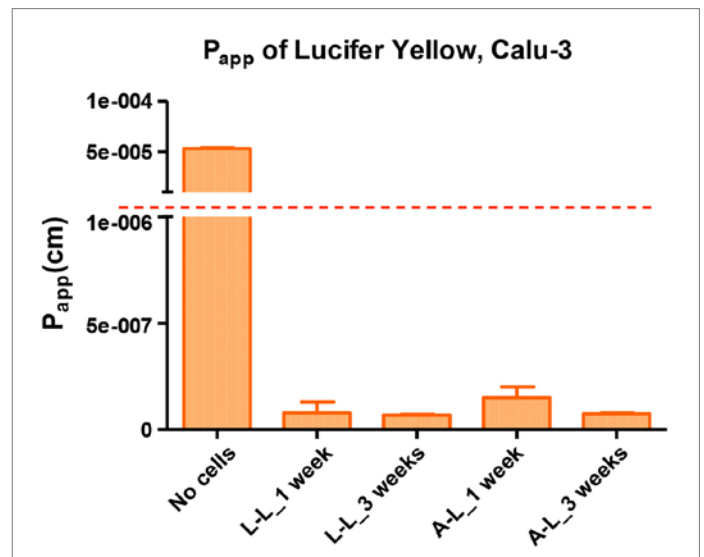

Figure 3. Lucifer permeability of the Calu-3 epithelial models after 1 week and 3 weeks.
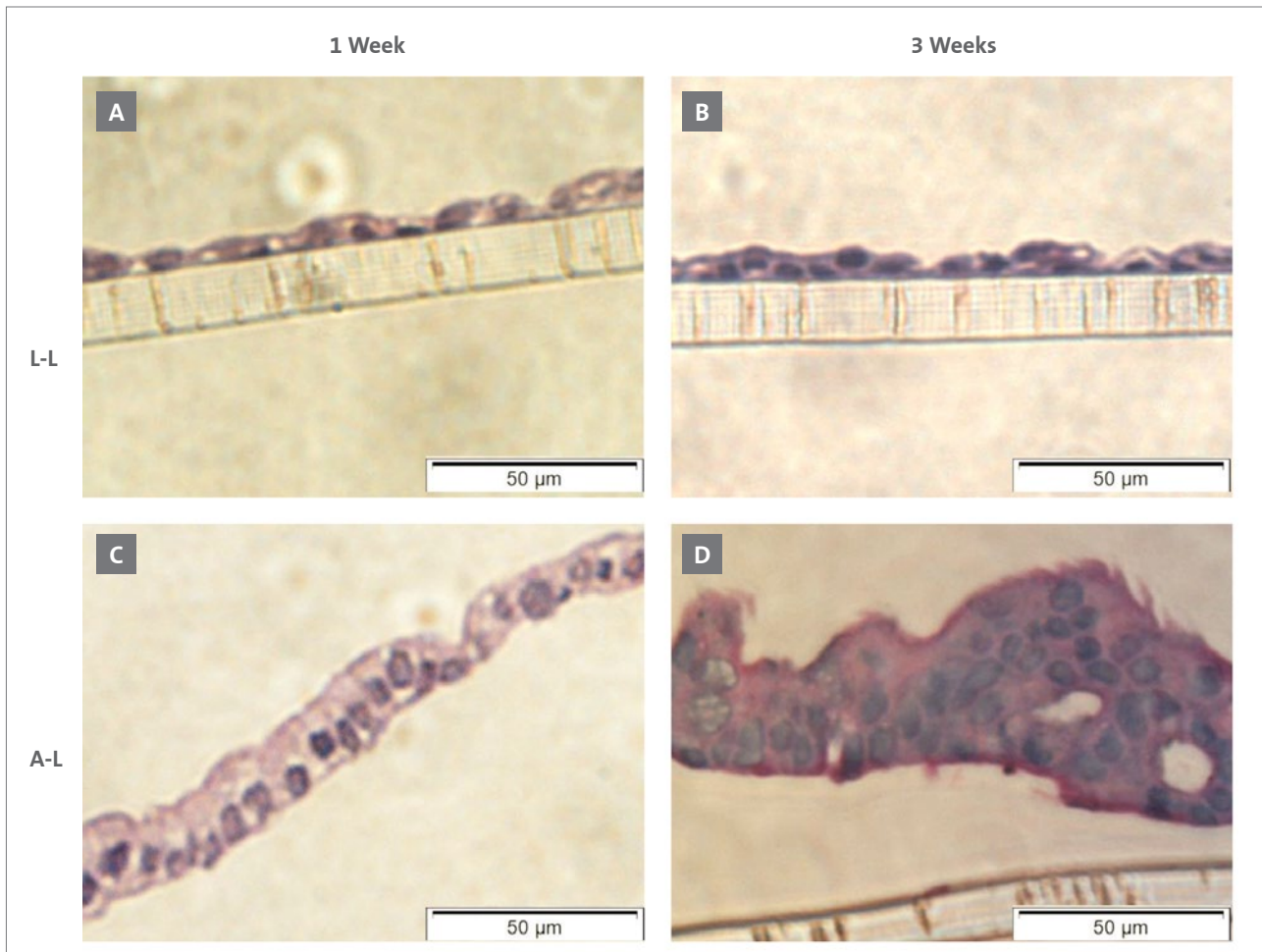
Figure 4. Haematoxylin-eosin stain of Calu-3 cells at the L-L and A-L interfaces after 1 week and 3 weeks in culture. Haematoxylin-eosin stain at the L-L interface revealed a flat cell monolayer (A, B). At the A-L interface, pseudostratified mucociliary epithelium morphology was formed (C, D).
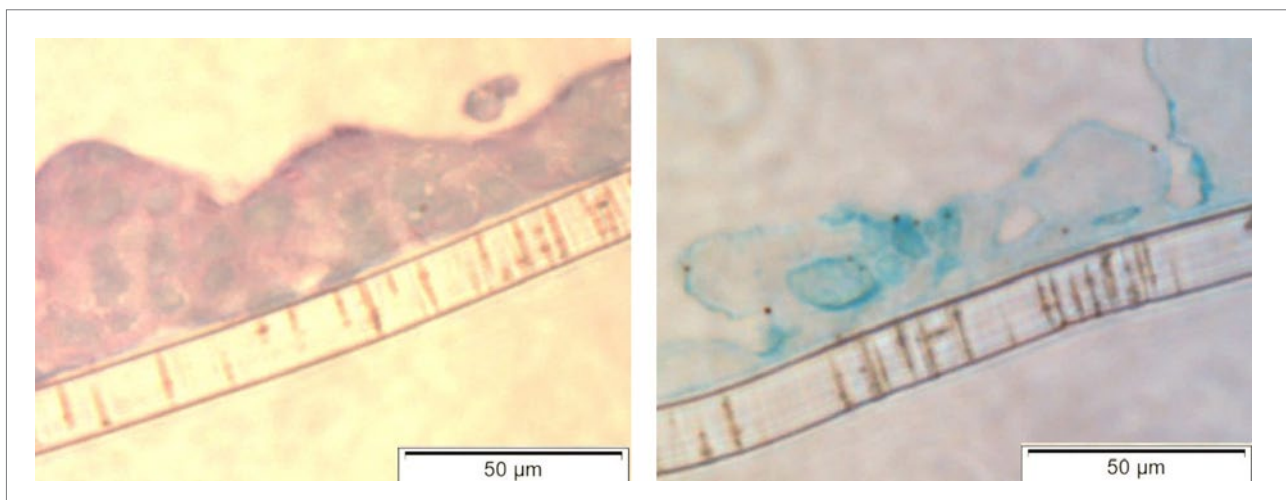

Figure 5. PAS (left) and Alcian blue stain (right) for Calu-3 cells at the A-L interfaces at 3 weeks in culture.

Conclusion

The study of airway biology can be achieved by cultivation of human tracheobronchial epithelial cells, primary cells, and cell lines at the air-liquid interface. In the present study, a successful bronchial epithelial model had been set up by using Calu-3 cells cultivated at the air-liquid interface for 3 weeks. Several methods were performed to verify the integrity of this model, including measurement of TEER value for tight junctions between cell monolayers and Lucifer yellow permeability. TEER reflects the ionic conductance of the paracellular pathway in the epithelial monolayer. This method is non-invasive and can be applied to monitor liver cells during their various stages of growth and differentiation. However, various factors have been shown to affect TEER values, including temperature, medium formulation, passage number of cells, and usage of electrodes[7]. Histochemical staining confirmed the pseudostratified mucociliary epithelium morphology.

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Studying
COVID-19
With Lung Organoids

With airway organoids, researchers use *in vitro* versions of human lung material to mimic a SARS-CoV-2 infection. These innovative studies can help reveal better ways to prevent and treat diseases, such as COVID-19.

How a Lung Organoid is Formed

LUNG ORGANOID

1 **BASAL CELLS:**
This outer layer of basal cells surrounds the lumen.

2 **CLUB AND GOBLET CELLS:**
These secretory cells generate mucins that trap microbes and other particles.

3 **CILIATED CELLS:**
These ciliated cells move trapped substances to the beat of a coordinated rhythm.
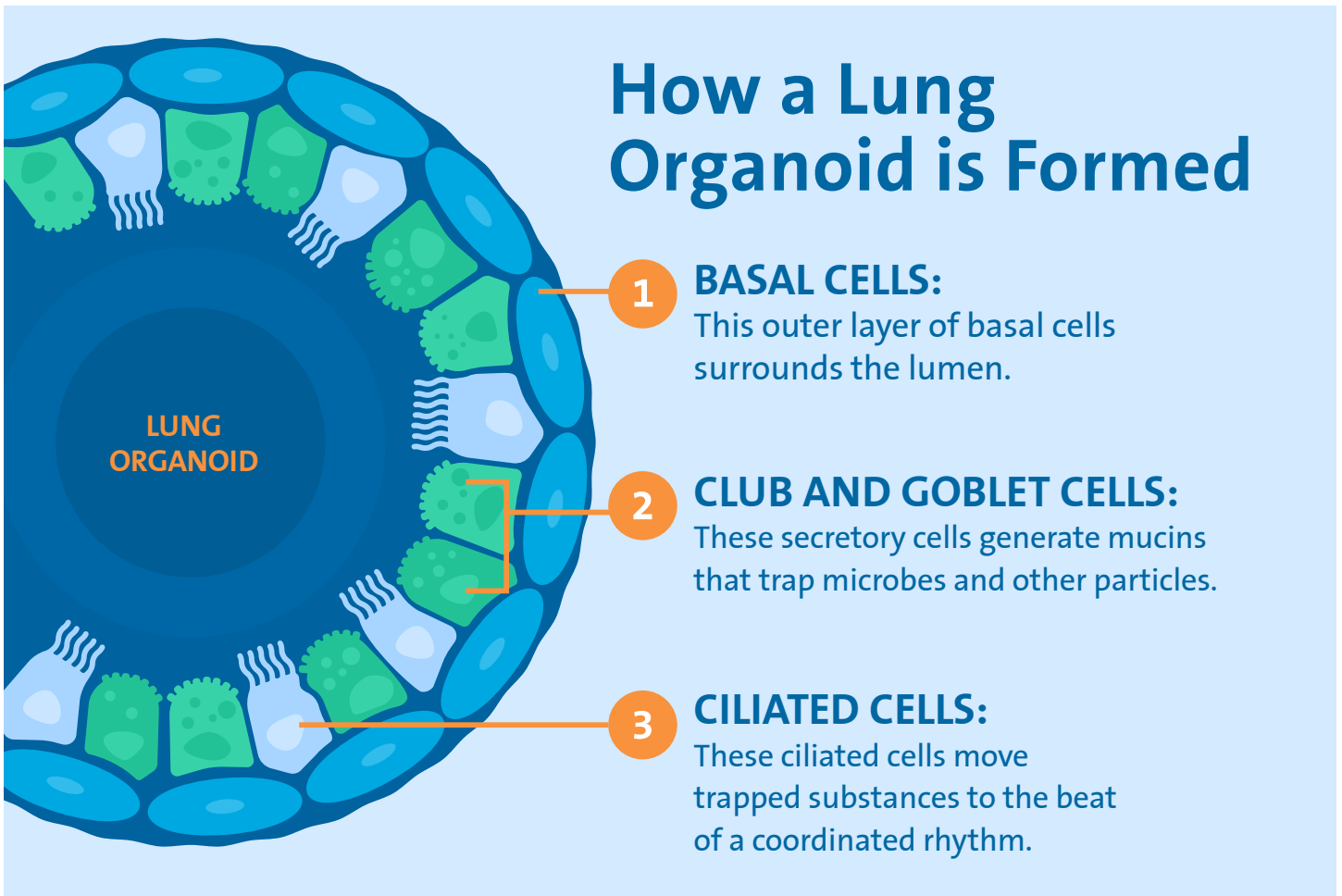
Matrigel® Matrix

Hydrogels, such as Corning® Matrigel® matrix for organoid culture, are a popular scaffold choice to generate organoid models.

How Does SARS-CoV-2 Infect Organoids?

1 Spike proteins on the virus hook to the organoid's ACE2 receptors.

2 Once bound, the organoid's ACE2 receptors allow viral entry for SARS-CoV-2.

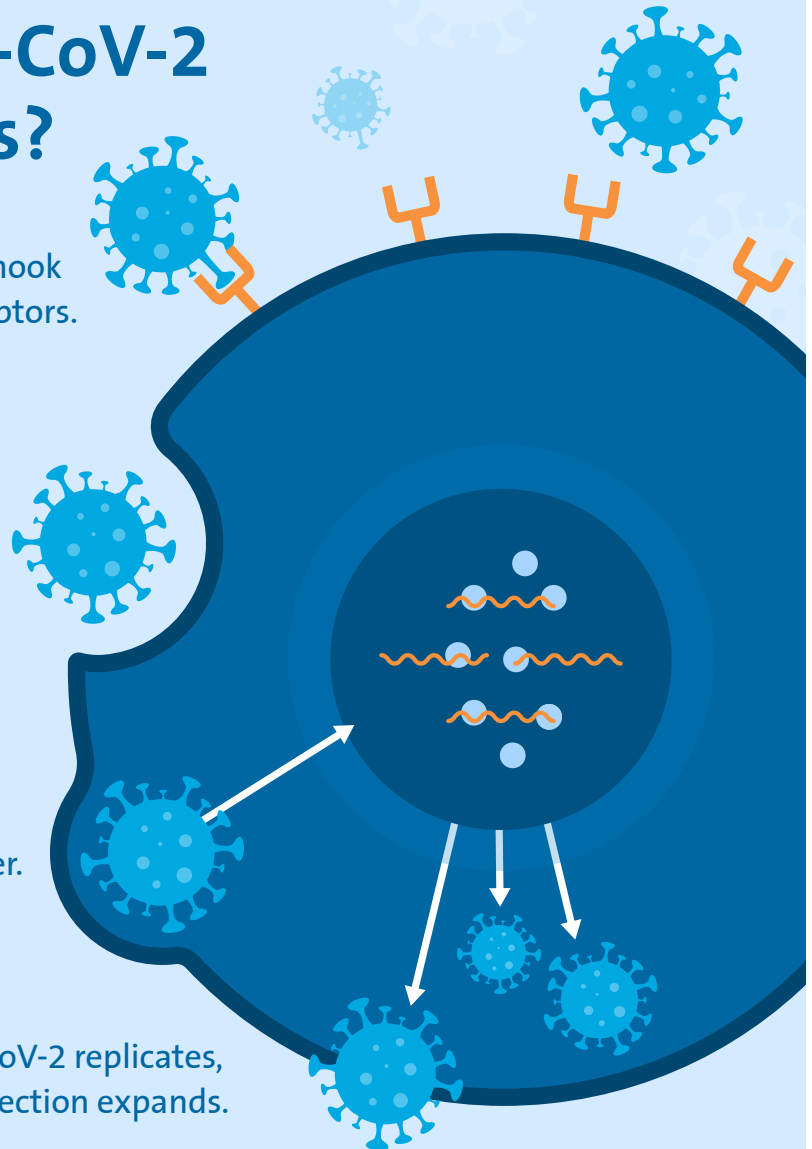**3** The viral and organoid membranes fuse together.

4 SARS-CoV-2 replicates, and infection expands.

Corning solutions, such as Matrigel matrix, support research to understand diseases like SARS-CoV-2 more completely, as well as the development of more effective therapies and treatments.

Mimic the Disease. Advance the Science.

Organoid models can help mimic *in vivo* conditions so that you can advance the understanding of complex diseases. To order supplies or access technical resources and protocols, visit:

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2. https://www.embopress.org/doi/full/10.15252/embj.2019101526
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Development of an Air-Liquid Interface Model using Primary Human Bronchial Epithelial Cells and HTS Transswell®-24 Permeable Supports from Corning

Application Note

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Introduction

Historically, two-dimensional (2D) monolayer cultures with numerous primary and immortalized cell lines have been used for drug screening and disease modeling. However, cell culture models of the mammalian airway have been unsuccessful in 2D due to the inability of airway epithelial cells to differentiate well in submerged culture[1]. On the other hand, primary airway epithelial cells cultured at the air-liquid interface (ALI) on permeable membranes polarize and form a pseudostratified epithelium containing mucus-secreting goblet cells and ciliated cells with beating cilia, both characteristic cell types of intact airways.[1-5] HTS Transswell-24 permeable supports from Corning are 24-well cell culture systems containing permeable culture inserts that sit in 24-well receiver plates or reservoirs. The membrane inserts are connected by a rigid, automation-friendly tray that enables all 24 Transswell inserts to be handled as a single unit. In this study, primary human bronchial epithelial cells from a healthy donor (NHBE) and from a donor with cystic fibrosis (D-HBE-CF) were cultured on HTS Transswell-24 polyethylene terephthalate (PET) 0.4 µm inserts coated with Collagen I. After a confluent monolayer was formed, cells were air-lifted and cultured at the ALI for 28 to 30 days to form a pseudostratified epithelial structure containing ciliated cells, goblet cells, and basal stem cells. The differentiated ALI culture yielded high TEER values consistent with what has been reported in the literature for primary bronchial cells[3] and displayed positive staining for tight junction protein ZO-1. Because the ALI model developed exhibits the cell types and functions necessary for a primary bronchial epithelium model and is amenable to both healthy and diseased cells, it can easily be employed for throughput assays for therapy research and drug development.

Materials and Methods

Monolayer Formation

Human bronchial/tracheal epithelial cells (passage 3) from a healthy donor (NHBE; Lonza Cat. No. CC-2541) and from a donor with cystic fibrosis (D-HBE-CF; Lonza Cat. No. 00196979) were thawed in PneumaCult™-Ex Plus culture medium (STEMCELL Technologies Cat. No. 05040) supplemented with 0.096 µg/mL hydrocortisone (STEMCELL Technologies Cat. No. 07925) and cultured in a humidified 5% CO_2 37°C incubator for 3 to 4 days prior to seeding on Transswell permeable supports. HTS Transswell-24 PET 0.4 µm inserts (Corning Cat. No. 3378) were coated with 100 µL of 10 µg/cm² rat tail Collagen I (Corning Cat. No. 354236) diluted in 0.02 N acetic acid (MilliporeSigma Cat. No. AX0073-6), incubated for 1 hour at room temperature, and rinsed with 150 µL of Dulbecco's Phosphate-Buffered Saline (DPBS; Corning Cat. No. 21-031-CM). Coated Transswell permeable supports were used immediately or stored at 4°C until cell seeding (up to 72 hours). Cells were harvested with Accutase® Cell Detachment Solution (Corning Cat. No. 25-058-CI) and were seeded in 100 µL culture medium onto previously-coated Transswell-24 PET inserts. NHBE were seeded at 1.5 x 10⁵ cells/cm² and D-HBE-CF were seeded at 2 x 10⁵ cells/cm². Transswell receiver wells were filled with 0.7 mL culture medium, and cells from both donors were cultured for 6 days until confluent monolayers were formed. Medium was exchanged 2 times prior to air-lift.

Air Lift and Culture at ALI

To initiate air-lift, culture medium was removed from the Transswell inserts and wells. Only the medium from the receiver wells was replaced with 0.7 mL PneumaCult-ALI Medium for differentiation (STEMCELL Technologies Cat. No. 05001) supplemented with 0.48 µg/mL hydrocortisone and 0.0004% heparin (STEMCELL Technologies Cat. No. 07980). Cells were cultured at the ALI for 28 to 30 days with media changes 3 times per week. Inserts were rinsed once a week with 150 µL of DPBS to remove mucus. After 28 to 30 days post-air-lift, transepithelial electrical resistance (TEER) values were determined using a World Precision voltage meter (EVOM). Prior to TEER measurements, enough culture medium was added to submerge the probes (200 µL/insert and 300 µL/well) and cultures were equilibrated to room temperature. For all staining and imaging applications, cells were then fixed with cold 4% paraformaldehyde (Boston Bioproducts Cat. No. BM-155) for 15 minutes and rinsed with DPBS.

Staining and Imaging

Histological staining. Fixed cells were paraffin-embedded and sectioned following the protocol in the "Preparation of Transswell® Inserts for Histology Guidelines for Use" (Corning Lit. Code CLS-AN-335) prior to staining with hematoxylin and eosin (H&E) and Periodic Acid-Schiff-Alcian Blue (PAS-AB). Brightfield images were taken using a Keyence BZ-X710 digital microscope with a 40X/0.75 NA plan fluor lens.

Immunofluorescence (IF) staining. Fixed cells were sectioned using a cryostat. Sections were stained with antibodies for markers of differentiated cell types: p63 antibody (Genetex Cat. No. GTX102425) with CF®488A-conjugated secondary antibody (Biotium Cat. No. 20015), Alexa Fluor® 555-conjugated beta-IV tubulin antibody (Abcam Cat. No. ab204034), and Alexa Fluor 647 conjugated Muc5AC antibody (Abcam Cat. No. ab218714). Confocal imaging was conducted on a Leica TCS SP5 laser scanning confocal microscope with a 40X/1.3 NA plan apo oil immersion

lens. Additionally, fixed cells were permeabilized and blocked for 1 hour in 0.2% Triton™ X-100 (Integra Cat. No. T756.30.30), 1% BSA (MilliporeSigma Cat. No. A9576), and 5% FBS (Corning Cat. No. 35-010-CV) in DPBS. Cells were stained with Alexa Fluor 488 conjugated ZO-1 antibody (Thermo Fisher Cat. No. 339188) or with isotype control (Thermo Fisher Cat. No. MA5-18167) overnight at 4°C. Cells were counterstained with 10 µg/mL Hoechst 33342 (Thermo Fisher Cat. No. H3570) for 15 minutes prior to imaging with a Thermo Fisher CellInsight® CX7 HCS platform in confocal mode with a 20X objective.

Results and Discussion

In this study, HTS Transwell®-24 PET 0.4 µm inserts from Corning were utilized to generate assay-ready models of both healthy and diseased human airway epithelia. Specifically, healthy (NHBE) and cystic fibrosis-affected (D-HBE-CF) primary bronchial epithelial cells were cultured on Transwell inserts at the ALI for 28 to 30 days to form a pseudostratified epithelium modeling native bronchial tissue.

H&E staining confirmed the formation of a pseudostratified epithelium with a ciliary brush border with both NHBE and D-HBE-CF cultured at the ALI (Figure 1). Beating cilia were observed within 10 days for NHBE and within 12 days for D-HBE-CF, which is consistent with previously reported results for primary epithelial cells. Typically, ciliated cells appear after 11 to 14 days in culture at the ALI, with higher passage cells taking longer than lower passage cells.[4] The presence of ciliated cells in the HTS Transwell-24 cultures was further verified by immunofluorescence

staining (Figure 3) for beta-IV tubulin.[3] Likewise, histological staining and immunofluorescence clearly demonstrated the presence of functional mucus-producing goblet cells via PAS-AB[5] and Muc5AC[3,4] staining for mucins present in and on goblet cells (Figures 2 and 3, respectively). Last, the third differentiated cell type – basal stem cells – was identified in the cultures by positive p63[4] staining (Figure 3). It is important to note that not only were all 3 differentiated cell types present in the ALI cultures for both healthy and cystic fibrosis-affected cells, but the cells were organized similarly to an intact airway epithelium with ciliated cells appearing on the apical (air) side and basal stem cells appearing on the basolateral (liquid) side (Figure 3).

Stratification and differentiation into the appropriate cell types enabled the formation of a tight barrier in the ALI cultures. The formation of a tight epithelium was demonstrated by the presence of tight junction protein ZO-1 and high TEER values, per previously published studies.[2,3] The pattern of ZO-1 fluorescence encircling the cells in NHBE and D-HBE-CF ALI cultures confirms tight junction formation (Figure 4). As a result of the epithelial barrier, both NHBE and D-HBE-CF ALI cultures displayed high TEER values (Figure 5) that are consistent with literature for pseudostratified epithelium formed by primary human bronchial epithelial cells (200 to 600 ohm*cm^2).[3]

Reports in the primary literature show that airway epithelial cells cultured on permeable membranes at the ALI for 21 to 28 days can form a fully differentiated pseudostratified epithelium with functional ciliated cells, mucus-secreting goblet cells, and basal
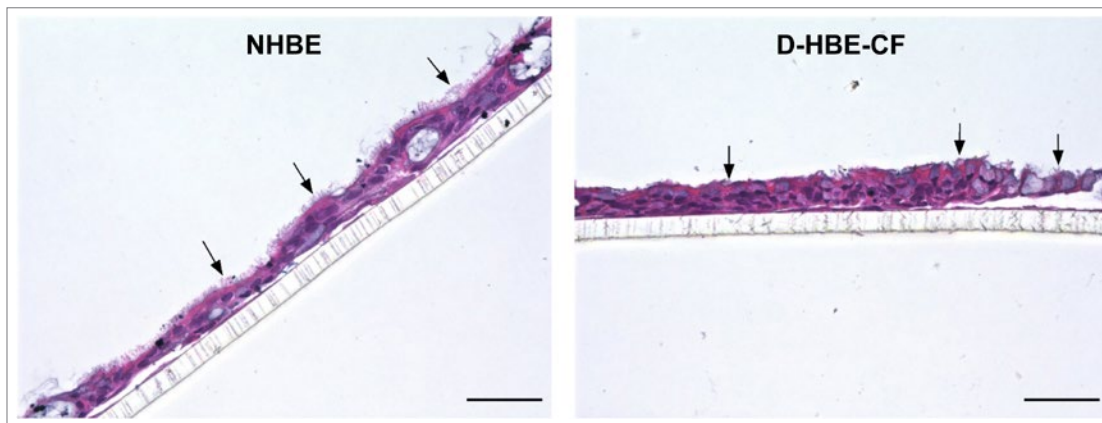

Figure 1. NHBE and D-HBE-CF primary bronchial epithelial cells cultured at the ALI form pseudostratified epithelia. H&E staining of cells from both healthy and cystic fibrosis-affected donors formed a pseudostratified epithelial structure when cultured on HTS Transwell-24 permeable supports from Corning at the ALI for 28 to 30 days. Cilia were present on the apical side of the culture (black arrows). Scale bar = 10 µm.
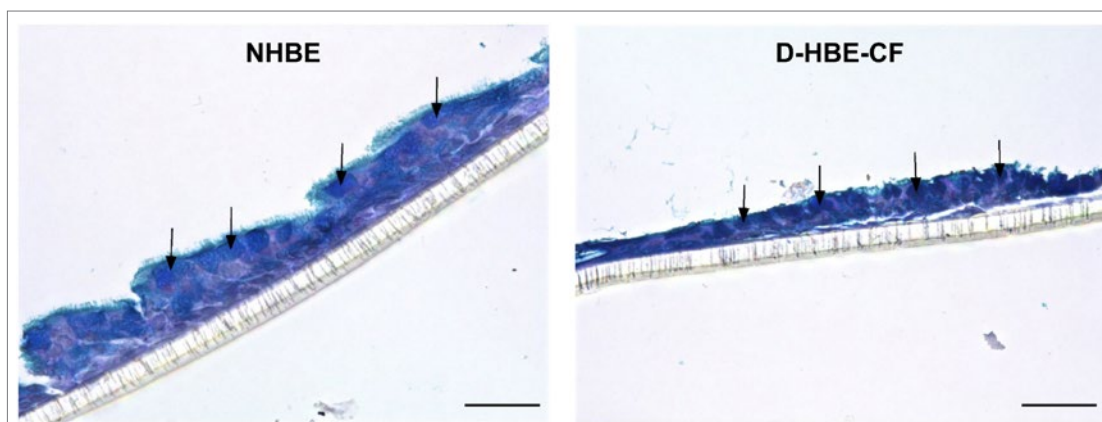

Figure 2. Pseudostratified epithelia of ALI cultures contain mucus-producing goblet cells. PAS-AB staining indicates mucus-producing goblet cells (black arrows) in ALI cultures generated on HTS Transwell-24 permeable supports from Corning with both NHBE and D-HBE-CF cells following 28- to 30-day culture. Scale bar = 10 µm.
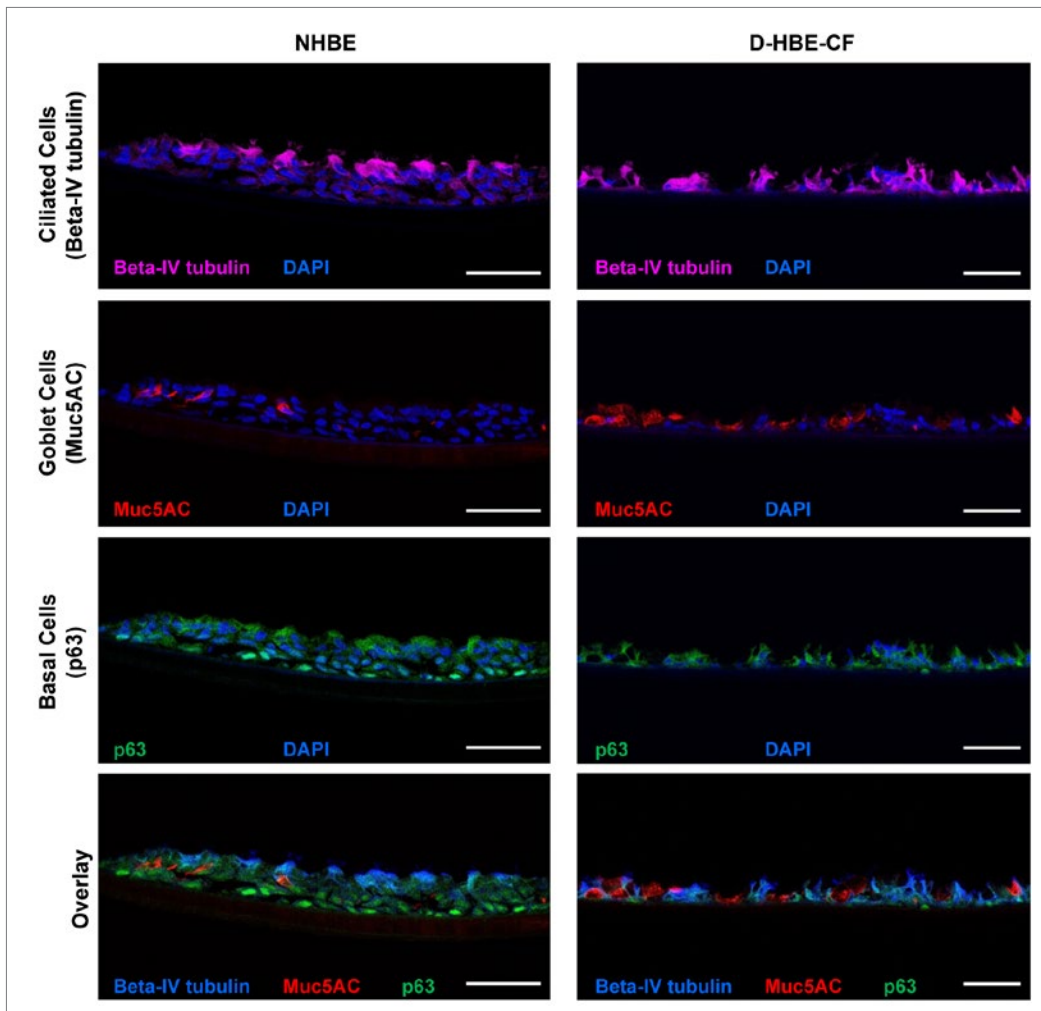
Figure 3. NHBE and D-HBE-CF primary bronchial epithelial cells cultured at the ALI form pseudostratified epithelia containing 3 differentiated cell types. IF staining indicates that ALI cultures from both donors contained ciliated cells (Beta-IV tubulin), mucus-producing goblet cells (Muc5AC), and basal cells (p63) with the ciliated cells appearing on the apical (top) side and the basal cells appearing on the basolateral (bottom) side. Scale bar = 10 μm.
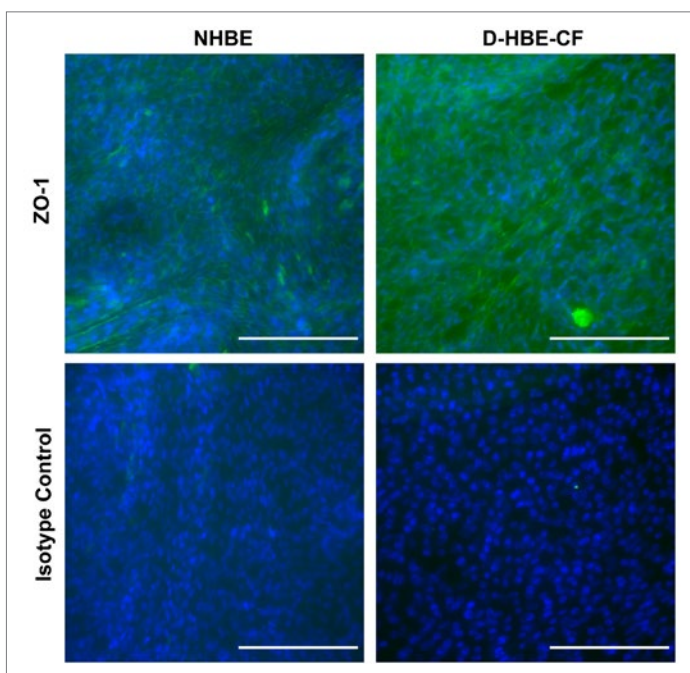
Figure 4. ALI cultures generated on HTS Transwell®-24 permeable supports express tight junction protein ZO-1. After 28 to 30 days of culture at the ALI, NHBE and D-HBE-CF cultures displayed staining for tight junction protein ZO-1 (green) encircling cells. Nuclei (blue) were counterstained with 10 μg/mL Hoechst 33342. Scale bar = 100 μm.

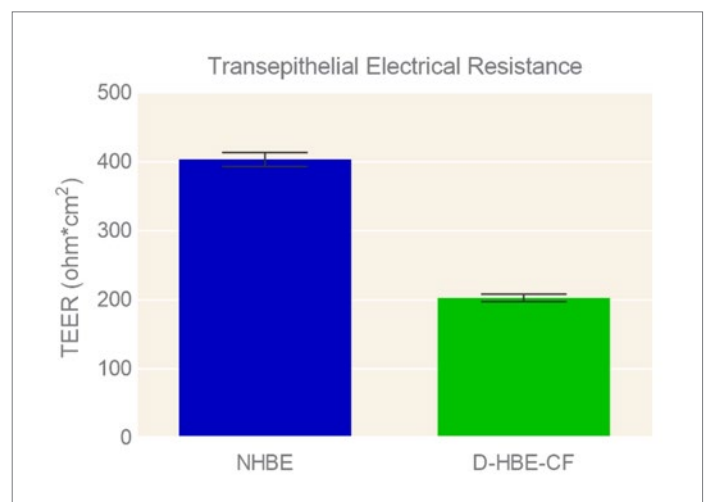**Figure 5. ALI cultures generated on HTS Transwell-24 permeable supports demonstrate high TEER values.** After 28 to 30 days of culture at the ALI, NHBE and D-HBE-CF cultures displayed high TEER values (mean ± SEM) that were >400 ohm*cm^2 for NHBE and >200 ohm*cm^2 for D-HBE-CF.
For each donor, TEER was measured from 24 inserts 3 independent times. N = 72.

stem cells.[2-5] Traditionally, these models are generated in a low-throughput manner with individual permeable supports. The use of the HTS Transwell®-24 PET inserts in this study simplifies the generation of multiple ALI cultures in an automation-friendly format. The ALI models developed here exhibit the cell types and functions necessary of a primary bronchial epithelium model, are amenable to both healthy and diseased cells, and can easily be employed for throughput assays for therapy research and development.

Conclusions

Transswell-24 permeable supports from Corning can be used to culture primary human bronchial epithelial cells at the ALI to form pseudostratified epithelium to model the human airway.

HTS Transwell-24 permeable supports simplify the generation of multiple ALI cultures in an automation-friendly format for throughput cell-based assay of both healthy and diseased airway epithelium.

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COVID-19

Using Air-Liquid Interface Models to Mimic Infection

Air liquid interface (ALI) cultures are a gold standard for studying disease models such as cystic fibrosis and asthma, investigating essential dermal processes such as wound healing, and advancing understanding of respiratory epithelium infections including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). With one side exposed to liquid media and the other to air, ALI models may be used to replicate *in vivo* conditions for cell-to-cell signaling and disease modeling. Here's how they work — and why it matters for COVID-19 research.
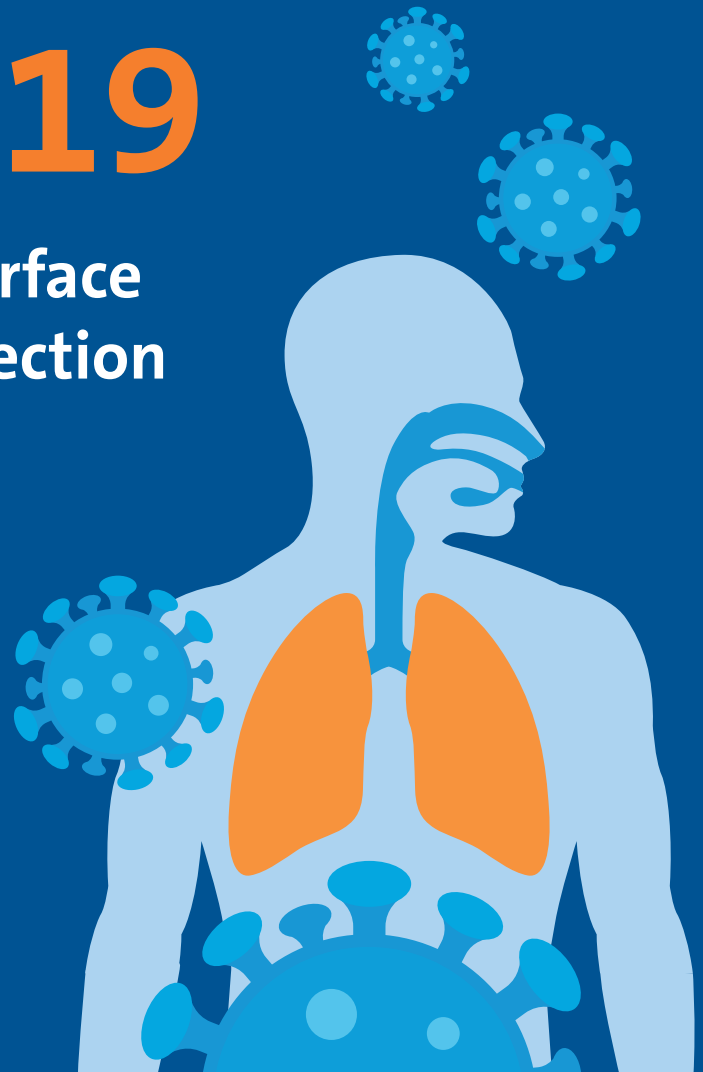
How ALI Cultures are Generated

ALI models may recapitulate key traits of the *in vivo* airway:
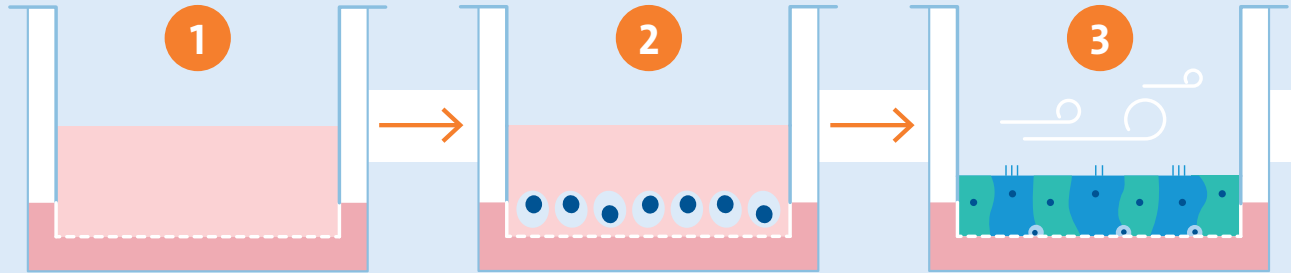
1

A porous filter — such as a Transwell®, Falcon®, or Corning® BioCoat® permeable support, typically precoated with collagen — creates apical and basolateral compartments.

2

After being cultured on the apical surface of a permeable support membrane, confluent cells are subjected to "air-lift," where the medium is supplied only to the basal chamber.

3

Because the liquid remains on the basal side, apical cells become surrounded by air while still getting fed and humidified by the media through a microporous membrane.

4

Cells differentiate. Now, they're ready to be infected.

Within three to six weeks

ALI Models in Action to Study COVID-19

Because ALI cultures mimic native cells, infection models can be generated that replicate *in vivo* viral behaviors.

Viruses infect the differentiated cells based on specific viral infection pathways. SARS-CoV-2, for example, infects by attaching to angiotensin-converting enzyme 2 (ACE2) receptors within ciliated cells.[1]
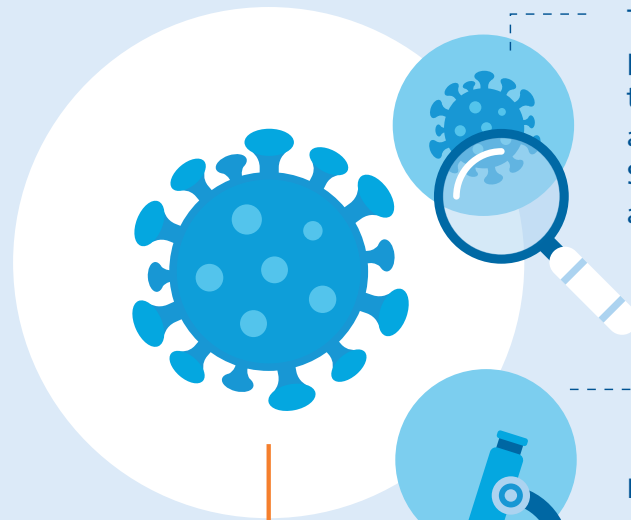
This makes ALI models a physiologically relevant tool to study ACE2 expression as a target for exploring SARS-CoV-2 disease pathology and antiviral treatments.

In fact, researchers used ALI models in the earliest studies of the novel coronavirus, before SARS-CoV-2 even had a name.[2]

ACE2
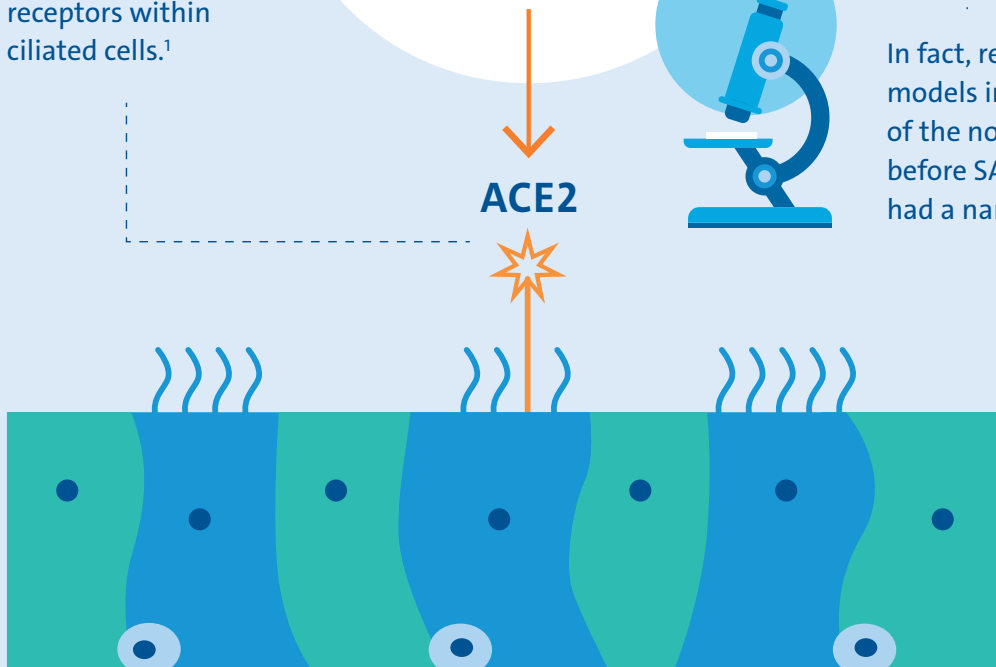
Ready to Get Started?

Air liquid interface models are inexpensive, relatively fast, and allow researchers to more accurately mimic *in vivo* conditions compared to using conventional cell culture models. When you're ready to get started, visit www.corning.com/diseasemodels to learn more about Transwell, Falcon, and Corning BioCoat permeable supports.

Mimic the disease.
Advance the science.

To learn more, visit:

www.corning.com/diseasemodels

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CORNING

Human Airway Epithelial Cell Culture and COVID-19 Research

Application Note

CORNING

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Corning Incorporated, Life Sciences

Human Airway Epithelial Cells and the Respiratory Tract

Human airway epithelial (HAE) cells are commonly used models for studying respiratory tract biology, disease, and therapy[1]. 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 viruses[3]. 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 functions[3]. 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 collagens[1] (Figure 1). Such submerged conditions can however result in the loss of the differentiated luminal cells with cells mainly demonstrating a basal cell phenotype[1].

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 function[4]. 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 structures[1]. 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 coronaviruses[5]. 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 medium[5]. The cells differentiate and form a pseudostratified epithelial layer containing many different functional cell types such as basal, ciliated, and mucus-secreting goblet cells[5]. This 3D-like system effectively models the architecture and cellular complexity of the human upper conducting airway[6]. 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 epithelium[7] (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 cells[5]. 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 layer[8]. 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-19[8]. 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 cultures[9]. 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-2[10].

Human Airway Organoids (AOs) and Coronavirus Research

Several approaches have been explored to generate mammalian airway organoids (AOs) as reviewed in Barkauskas, et al[4]. AOs have been derived from a variety of origin cell types including trachea or large airway basal cells[4], alveolar cells[11], human iPSC[12], and embryonic lung[13]. AOs have the same ability to differentiate into polarized structures consisting of ciliated, goblet, and basal cells without the need for a permeable support system[14].

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 models[15]. 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 fibrosis[15] and rapid assessment of the infectivity of emerging respiratory viruses e.g., influenza, in humans[17]. 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 cells[18] (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 indications[20] and liver damage is likewise a common feature in severe COVID-19 patients[21]. In emerging COVID-19 literature, lung[22], small intestinal[23,24], colonic[20,25], brain[26], and liver ductal[21] 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 screening[22]. 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 cells[23]. 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 coronaviruses[23]. 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 pandemic[27].
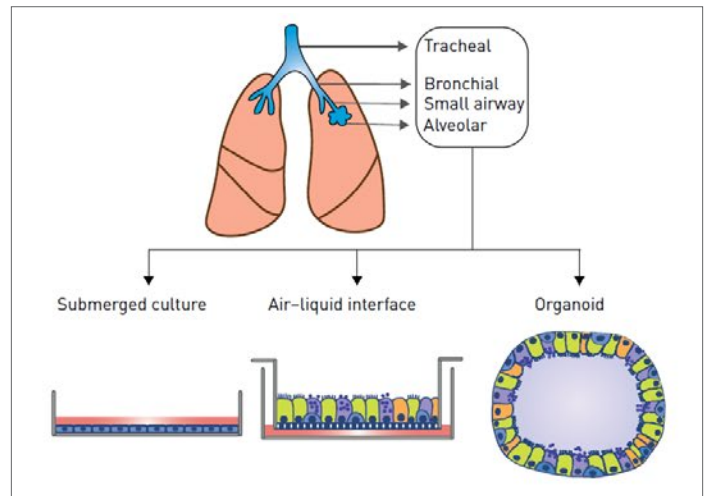
Figure 1. Various culture systems used for culture of airway epithelial cells. Cells can be grown as a simple submerged culture on plastic typically coated with extracellular matrix (ECM)-derived proteins, at air-liquid interface (ALI) using a permeable support system, or in 3D organoid culture within a biological matrix. Image adapted from Reference 1.
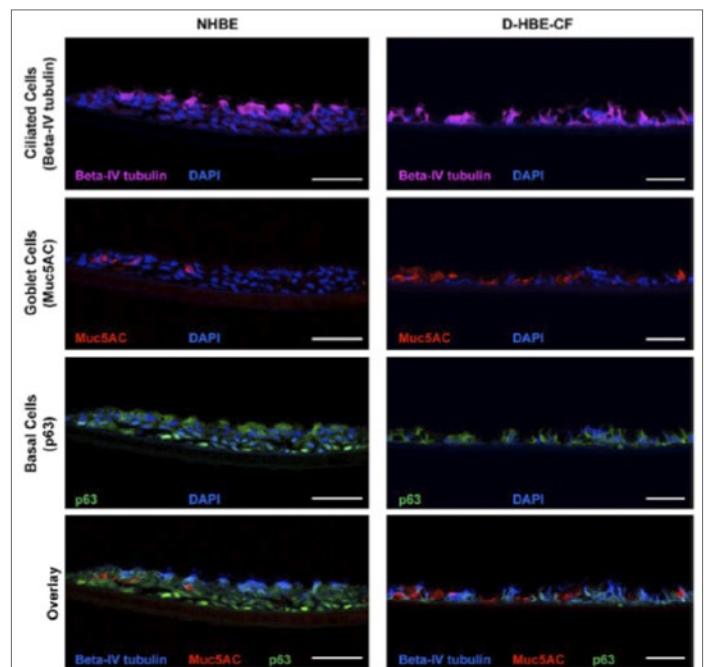
Figure 2. Human bronchial/tracheal epithelial cells from a healthy donor (NHBE; Lonza CC-2541) and from a donor with cystic fibrosis (D-HBE-CF; Lonza 00196979) cultured at the ALI form pseudostratified epithelia containing 3 different cell types[7].

Figure 3. Normal (left) versus asthmatic (right) airway organoids cultured in Corning Matrigel matrix. Multi-color fluorescent labels indicate specific cells 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

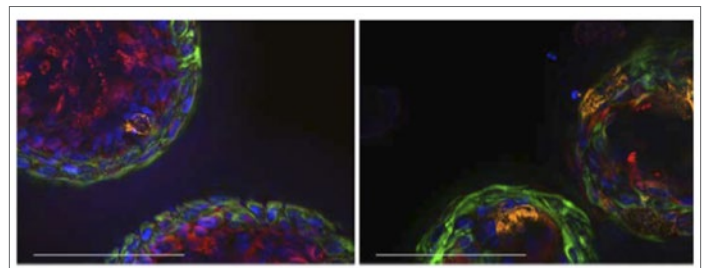| Application	3D Model	Culture System	Reference No.
Modeling of SARS-CoV-2 infection, replication, cytokine response profiling and sensitivity to interferons	ALI culture of human airway epithelial cells	Transwell® permeable supports	8
Exploration of SARS-CoV-2 infection susceptibility in nasal, airway 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) *In vivo* 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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Citations Summary for Transswell® Permeable Supports from Corning

Studying COVID-19 at the Air-Liquid Interface

CORNING

With the emergence of COVID-19 (SARS-CoV-2) at the end of 2019 and its rapid spread in 2020, several studies are focused on understanding the virus biology and rapidly assessing the potential of existing drugs and the development of new active compounds.

Human airway air-liquid interface (ALI) cultures have been commonly used to model various mechanisms of coronavirus pathogenesis where the cells are efficiently infected by human or animal-transmitted CoV, including SARS-CoV, SARS-CoV-2, and MERS-CoV. The citations below highlight some of the recent publications in which Transswell permeable supports from Corning were used for ALI as an *ex vivo* model. These studies demonstrate Transswell permeable supports as an effective tool to study coronavirus infection and for the development of targeted therapies.

1. **A Novel Coronavirus from Patients with Pneumonia in China**
   Zhu N, et al. N Engl J Med. (2020), 382(8): 727-733.

   This study is one of the first publications reported after the COVID pandemic. Human airway epithelial cell cultures generated on an air-liquid interface on Transswell-COL permeable supports formed well-differentiated, polarized cultures resembling *in vivo* pseudostratified mucociliary epithelium. Data supports that differentiated cells on ALI culture used for virus infection from patient samples and propagation of human respiratory secretions onto human airway epithelial cell cultures. This was followed by whole genome sequencing of culture supernatant which allowed for successful detection of a new human coronavirus named 2019-nCoV.

2. **An Orally Bioavailable Broad-Spectrum Antiviral Inhibits SARS-CoV-2 in Human Airway Epithelial Cell Cultures and Multiple Coronaviruses in Mice**
   Sheahan TP, et al. Sci Transl Med. (2020), 12(541).

   Using ALI culture of primary human airway epithelial cells on Transswell-COL permeable supports, this study demonstrates the potency of a drug compound on COVID-19 strains including COVID-19. Viral infection of differentiated airway epithelial cultures and dose response study using nucleoside analog NHC (EIDD-1931) demonstrated that NHC was potently antiviral against SARS-CoV-2, MERS-CoV, and SARS-CoV in primary human epithelial cell cultures without cytotoxicity. Overall, the data support ALI culture of human airway epithelial cells as a tool to study and understand antiviral activities of the nucleoside prodrug, EIDD-2801, and opportunities for the development of targeted therapies for recent and emerging coronavirus infections.

3. **Type I and Type III IFN Restrict SARS CoV-2 Infection of Human Airway Epithelial Cultures**
   Vanderheiden A, et al. J Virol. (2020) Jul 22;JVI.00985-20. doi: 10.1128/JVI.00985-20

   In this study, researchers modeled SARS-CoV-2 infection on primary human airway epithelial (pHAE) cultures using Transswell permeable supports when maintained at ALI to create a polarized, pseudostratified epithelial layer. This culture system re-capitulates the unique features of the human respiratory tract, including mucus production and coordinated cilia movement. The data supports that SARS-CoV-2 effectively infects and replicates in pHAE cultures and is directionally released on the apical side of ALI culture. Furthermore, results demonstrate SARS-CoV-2 sensitivity to interferon treatment as a possible therapy.

4. **SARS-CoV-2 Productively Infects Human Gut Enterocytes**
   Lamers MM, et al. Science. (2020), 369:50-54.

   Using Collagen I-coated Transswell inserts this study demonstrates that ALI culture of dissociated human airway organoids were productively infected by SARS-CoV and SARS-CoV-2 viruses (from patient samples) which specifically target ciliated cells. Transcriptomic analysis of SARS-CoV-2 infected differentiated organoids identified enriched genes upon SARS-CoV-2 infection in differentiated intestinal organoids. The data generated from subsequent studies strongly suggest that human organoids are effective *in vitro* models to study the biology, pathogenesis, and potential treatment of coronaviruses.

5. **Replication of SARS-CoV-2 in Human Respiratory Epithelium**
   Milewska A, et al. J. Virol. (2020), doi: 10.1128/JVI.00957-20

   This work describes fully differentiated human airway epithelium on an ALI culture using Transswell permeable supports as a model to study novel human coronavirus (SARS-CoV-2). Researchers demonstrate that the SARS-CoV-2 effectively replicates in the HAE cultures. The infection was polarized as the release occurred at the apical side of the epithelium. Furthermore, SARS-CoV-2 replication *in vitro* and *ex vivo* was effectively blocked by serum obtained from patients who recovered from COVID-19. Overall, results support that this *ex vivo* model constitutes a convenient tool to study the viral infection.

6. **Characterization and Treatment of SARS-CoV-2 in Nasal and Bronchial Human Airway Epithelia**
Pizzorno A, et al. (2020), doi: https://doi.org/10.1101/2020.03.31.017889

Researchers highlight new insights on SARS-CoV-2 biology and drug combination therapies against COVID-19. To characterize viral infections induced by SARS-CoV-2, human reconstituted airway epithelia were cultured at the ALI on Transwell® inserts. Data supports antiviral efficacy of remdesivir and the therapeutic potential of the remdesivir-diltiazem combination as a rapidly available option to respond to the current unmet medical need imposed by COVID-19. Their results indicate the relevance of this model for the preclinical evaluation of antiviral candidates.

7. **Human Intestinal Tract Serves as an Alternative Infection Route for Middle East Respiratory Syndrome Coronavirus**
Zhou J, et al. Sci Adv. (2017), 3(11): eaao4966

This study demonstrates that human primary intestinal epithelial cells, small intestine explants, and intestinal organoids were highly susceptible to MERS-CoV and can sustain robust viral replication. In polarized Caco-2 cells cultured on Transwell inserts, apical MERS-CoV inoculation was more effective in establishing infection than basolateral inoculation. Researchers hypothesize that the human gastrointestinal tract could serve as an alternative route to acquire MERS-CoV infection.

8. **Species-Specific Colocalization of Middle East Respiratory Syndrome Coronavirus Attachment and Entry Receptors**
Widagdo W, et al. J Virol. (2019), 93(16): e00107-19.

Primary normal human bronchial epithelial cells (NHBE) cells were cultured on Transwell permeable supports and differentiated at the ALI to mimic the human airway environment. Results demonstrate that Neuraminidase treatment prior to MERS-CoV infection of these cells significantly reduced the number of infected cells. These data support the importance of MERS-CoV-recognized glycotopes as an attachment factor during infection of human airway epithelial cells.

9. **Conditionally Reprogrammed Human Normal Airway Epithelial Cells at ALI: A Physiological Model for Emerging Viruses**
Liu X, et al. Virol Sin (2020) 35:280-289.

This review outlines methods for the establishment of long-term cultures for human normal airway epithelial cells from human nose to lung generated by conditional cell reprogramming (CR) and coupled air-liquid interface (ALI) technologies, and their applications as an *ex vivo* model for studies of emerging viruses. Transwell-COL permeable supports (12-well plates, Corning) for ALI culture has been referenced. Since conditionally reprogrammed cells (CRCs) are stable resources for normal functional airway cells, it has been proposed that CRCs/ALI cultures will facilitate studies on viruses including SARS-CoV-2 infection and the development of novel therapeutics.

10. **Morphogenesis and Cytopathic Effect of SARS-CoV-2 Infection in Human Airway Epithelial Cells**
Zhu N, et al. Nat Comm (2020) 11:3910, https://doi.org/10.1038/s41467-020-17796-z

To better understand the pathogenesis and transmission of SARS-CoV-2, in this study researchers compared the characteristics of the replication dynamics, cell tropism, and morphogenesis of SARS-CoV-2 and human coronavirus NL63 (HCoV-NL63) in human airway epithelial (HAE) cells, which express the shared receptor. Replication dynamics in HAE was confirmed by using fully differentiated HAE cultures derived from three different donors on ALI cultures using Transwell-COL permeable supports (12 mm diameter, Corning) and infected with SARS-CoV-2 or HCoV-NL63. Data support that SARS-CoV-2 is fully adapted to the human airway, which is distinct from other coronaviruses that were reported to have interspecies transmission. Overall, the results open experimental avenues for the understanding of SARS-CoV-2 transmission and pathogenesis.

11. **Type 2 Inflammation Modulates ACE2 and TMPRSS2 in Airway Epithelial Cells**
Kimura H, et al. J Allergy Clin Immunol. (2020) 146:80-88.e8.

Previous studies have demonstrated that for effective host cell entry, SARS-CoV-2 relies on 2 critical proteins, angiotensin-converting enzyme 2 (ACE2) and transmembrane protease, serine 2 (TMPRSS2). To determine whether these 2 key mediators are modulated by IL-13, a cytokine associated with type 2 asthma, primary human airway epithelial cells were cultured and differentiated on collagen-coated Transwell inserts (PET, 12 mm diameter, Corning) at the air-liquid interface and analyzed for the expression of ACE2 and TMPRSS2 using RT-PCR. Results demonstrate that IL-13 suppresses ACE2 expression and increases TMPRSS2 expression in airway epithelial cells from participants with type 2 asthma and atopy. These findings may provide a foundation to elucidate the relative role of these 2 mediators in cell entry and how type 2 cytokines modulate susceptibility to COVID-19.

12. **Single-cell Longitudinal Analysis of SARS-CoV-2 Infection in Human Airway Epithelium**
Ravindra NG, et al. Version 2. bioRxiv. Preprint. (2020) May 7 [revised 2020 Jul 13]. doi: 10.1101/2020.05.06.081695

To reveal insight into viral replication, cell tropism, and host-viral interactions of SARS-CoV-2 researchers performed single-cell RNA sequencing of experimentally infected human bronchial epithelial cells (HBECs) with SARS-CoV-2 at the air-liquid interface cultured on collagen-coated Transwell inserts (0.4 μm, Corning). In-depth analysis of SARS-CoV-2 infection in HBECs and in cells from a pediatric COVID-19 patient identified novel SARS-CoV-2 genes, cell types, and cell state changes associated with infection. Single cell RNA sequence and electron microscopic analyses demonstrate that ciliated cells are the major target cell of SARS-CoV-2 infection in the bronchial epithelium at the onset of infection and that cell tropism expands to basal, club, and BC/club cells over time. Furthermore, SARS-CoV-2 infection elicited intrinsic expression of type I and type III interferons and IL-6 but not IL-1, as well as observed potent induction of the pro-inflammatory cytokine IL-6 and chemokines, which likely contribute to the inflammatory response *in vivo*. Overall studies lead to important future directions including whether other airway and endothelial tissues similarly interact with SARS-CoV-2 and how these interactions vary *in vitro*.

13. **SARS-CoV-2 Infection of Primary Human Lung Epithelium for COVID-19 Modeling and Drug discovery**
   Mulay A, et al. Version 1. bioRxiv. Preprint. (2020) Jun 29. doi: 10.1101/2020.06.29.174623

   In this study researchers established a 3D organoid culture model of the human alveoli to study SARS-CoV-2 infection of the distal lung by utilizing the 3D alveolar organoid and airway ALI culture systems using Corning® collagen-coated (0.4 µm, 24-well) inserts. They studied the effect of a selected panel of drugs which included the known anti-viral cytokine, IFNB1 and investigational drugs for COVID-19 treatment, Remdesivir and Hydroxychloroquine. Validation of the efficacy of various selected candidate COVID-19 drugs confirmed that Remdesivir strongly suppressed viral infection/replication in alveolar organoids. Overall data support that 3D alveolar organoid models and proximal ALI cultures represent a highly relevant preclinical tool to assess SARS-CoV-2 infection and replication and serve as a platform for drug screening and validation.

14. **Conditional Cell Reprogramming for Modeling Host-virus Interactions and Human Viral Diseases**
   Liu X, et al. J Med Virol. (2020) 1-13 Jun 1. doi: 10.1002/jmv.26093. Online ahead of print.

   This review article outlines a comprehensive summary on human physiological cell models for the study of viral infections and discovery of antiviral drugs using long-term cultures for human normal epithelial cells from respiratory, gastrointestinal and genital-urological tracts using conditional cell reprogramming (CR) technology coupled with ALI/LLI culture and propose air-liquid interface cultures (referenced Corning's Transwell) and CRC-coupled ALI/LLI/Organoids (referenced Corning Matrigel® matrix) technologies may serve as an *ex vivo* physiological models for viral infections including SASR-CoV-2 induced injury and drug discovery.

15. **Human iPSC-derived Alveolar and Airway Epithelial Cells can be Cultured at Air-liquid Interface and Express SARS-CoV-2 Host Factors**
   Abo KM, et al. Version 1. https://www.ncbi.nlm.nih.gov/pmc/articlesPMC7302183/ bioRxiv. Preprint. (2020) Jun 4. doi: https://dx.doi.org/10.1101%2F2020.06.03.132639 10.1101/2020.06.03.132639

   In this study researchers establish a novel iPSC-derived alveolar epithelial type 2 cell (iAT2) air-liquid interface (ALI) culture system using Transwell inserts (Corning) to enable modeling of environmental exposures of the human alveolar epithelium, including viral infection. Results support iPSC-derived alveolar and airway epithelial-like cells as a physiologically relevant model system with the potential to model components of SARS-CoV-2 infection such as viral entry, cellular response to pathogen, and viral replication, and may expedite the development of an effective pharmacological intervention for COVID-19.

Uses and Best Practices for Air-Liquid Interface Systems

The following article originally appeared on www.biocompare.com on June 22, 2020.*
Written by Emma Easthope.

Air-liquid interface (ALI) culture is a widely used method to study respiratory tract epithelia and is also known to support differentiation of multi-layered skin models. By seeding cells on to a permeable support suspended in growth media and allowing an initial period of propagation before removing the media from the apical compartment, only the basolateral side of the cell monolayer remains submerged. ALI systems therefore allow researchers to more accurately mimic *in vivo* conditions compared to using conventional cell culture models, making them ideal to perform mechanistic studies of respiratory epithelial cells as drug permeation barriers; to model respiratory diseases like cystic fibrosis and asthma; to investigate essential dermal processes such as wound healing; and to advance understanding of respiratory epithelium infections including Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This article highlights some of these applications and includes tips for using permeable supports to enhance ALI research.

Permeable supports meet a range of requirements

Permeable supports for culturing cells are available in various formats. These include inserts suitable for 6- to 24-well plates, HTS-compatible 24-well permeable supports, and large format inserts that fit into a rectangular tray or cell culture dish. For researchers wishing to reduce the frequency of cell feeding when culturing cells at the ALI for extended periods, a 6-well deep well companion plate designed for use with Falcon® 6-well cell culture inserts holds up to seven times more medium in the basolateral compartment than a regular 6-well plate. ALI studies are typically performed using collagen I or collagen IV-coated inserts with a 0.4 µM pore size, although other pore size inserts may be used depending on the application. Cells can be cultured in ALI on permeable supports for up to 28 days, or longer if handled appropriately.

Wound healing

Primary and immortalized keratinocyte cells are valuable models for investigating the biology of the epidermis. However, for cultured keratinocytes to differentiate into organotypic skin equivalents and display similar architecture to the skin *in vivo*, they require an environment representative of the ALI. This can be produced by casting dermal fibroblasts on to a collagen-coated permeable support that is then submerged in suitable growth media. After seeding keratinocytes on to the fibroblasts and allowing them to establish, the media is removed from the chamber to promote keratinocyte growth under simulated ALI conditions (Figure 1).
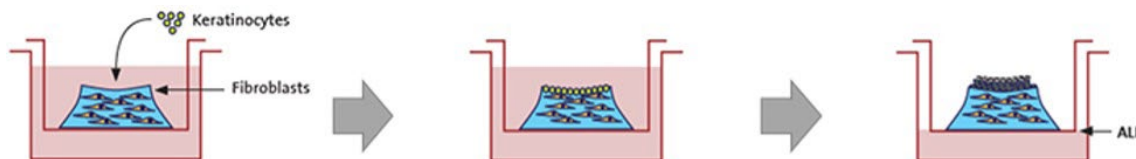

Figure 1. Co-culture of keratinocytes grown on a collagen raft embedded with dermal fibroblasts.

Respiratory diseases

Two-dimensional cell monolayers are of limited use to study the mammalian airway due to the inability of airway epithelial cells to differentiate well in submerged culture. Using permeable supports, researchers have successfully differentiated primary human bronchial epithelial cells (HBECs) into mature airway tissue for improved functional studies and have demonstrated that culturing primary HBECs at the ALI promotes the formation of a pseudostratified epithelium that can be used to model the human airway (Figure 2). This approach has been employed to compare primary airway epithelial cells from healthy human donors to those of donors with cystic fibrosis.
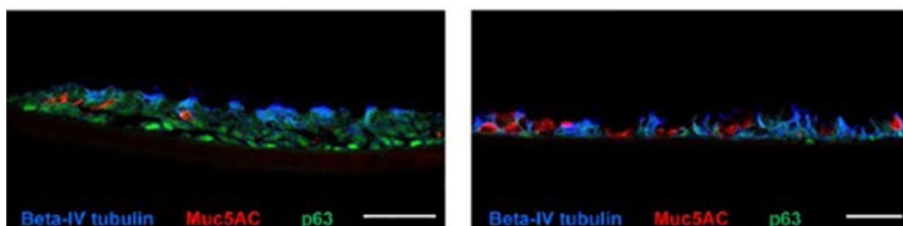

Figure 2. Primary bronchial epithelial cells cultured at the ALI form pseudostratified epithelia comprising ciliated cells (Beta-IV tubulin), mucus-producing goblet cells (Muc5AC), and basal cells (p63), with the ciliated cells appearing on the apical (top) side and the basal cells appearing on the basolateral (bottom) side. Cells from the healthy donor (left) are distinct from those of the donor with cystic fibrosis (right).

SARS-CoV-2

As a result of the COVID-19 pandemic, demand to study cellular behaviors at the ALI has increased dramatically. Recent publications have seen permeable supports used for culturing human airway epithelial cells at the ALI to study the cytopathic effects of the virus and to evaluate the therapeutic potential of a broad-spectrum antiviral drug, while using permeable supports to form differentiated organoid-derived gut cultures has underpinned the finding that SARS-CoV-2 also targets human gut enterocytes.

Tips and tricks for using permeable supports

- Choose a permeable support best suited to your cell type—the type of membrane, pore size, format (e.g., 24-well), and surface (e.g., ECM coating) can all influence results
- Set up the permeable support correctly—first add medium to the multiwell plate, followed by the inserts, then add medium and cells to the inside compartment
- Consider pre-soaking permeable supports in medium prior to seeding to improve cell attachment and spreading
- Follow the recommended medium volumes for optimal cell growth—check the medium level periodically and add fresh medium as required
- Optimize the seeding density—cells are often sensitive to the initial seeding density for good cell attachment

For further advice on using permeable supports for ALI culture, visit www.corning.com/ALI.

Corning offers a complete range of permeable supports to accelerate your ALI research, including Transwell®, Falcon®, and BioCoat® products. To find out more visit www.corning.com/lifesciences.

*Original Biocompare article is accessible here: https://www.biocompare.com/Bench-Tips/564894-Uses-and-Best-Practices-for-Air-Liquid-Interface-Systems/

Using Air-Liquid Interface in Your Research

Air-liquid interface (ALI) research has long been the gold standard in representing *in vivo* biology in an *in vitro* culture environment. Because it exposes one side of the culture to liquid media and surrounds the other by air, the technique is ideal for studying respiratory tract epithelial cells, which interact with both, liquid and air *in vivo*.

Though ALI experimentation has been vital in researching multiple respiratory diseases, such as asthma and chronic obstructive pulmonary disease, it's extremely critical now as scientists explore the respiratory underpinnings of the novel coronavirus. Already, ALI has expanded scientists' understanding of the virus that causes COVID-19; studies have been published in the New England Journal of Medicine and other elite medical journals, and several more are to come.

ALI is set to become even more pivotal for preclinical research. We spoke with Shabana Islam, MS, PhD, Product Line Manager at Corning Life Sciences, to understand why, and to learn best practices that researchers should consider as they apply ALI techniques in the lab.

The Mechanics of Air-Liquid Interface Cultures

ALI systems consist of apical and basolateral compartments, which are separated by a porous filter support, such as a precoated permeable support. Cells are cultured atop the apical surface; when they become confluent, they move across the basal-apical threshold. Because the media remains on the basal side, the cells on the apical interface are surrounded by air, after which they differentiate and generate an apical microenvironment.

The basal surface has access to the media so that it can feed and humidify the apical side via diffusion through the microporous membrane. As the cells differentiate, based on cell source, for example, airway epithelial cells, they secrete mucus into the apical compartment. This activity aligns well with respiratory research, Islam notes, because it physiologically mimics the behavior of the epithelium *in vitro*.

"Cell differentiation is a characteristic feature of airway epithelial cells," Islam says. "Therefore, models should include the ability of the cells to differentiate and maintain, and that can be achieved with air-liquid interface."

The Air-Liquid Interface Workflow, Explained

When establishing ALI cultures from primary cells, the first step is to isolate the cells from the tissue. Then, dilute the cells and seed them on a permeable membrane of a cell culture insert. Surfaces coated with an extracellular matrix such as collagen I or IV are good options for culturing cells.

Grow the cells for three to six weeks, depending on the cell type and the time needed for culturing. The epithelium cells will fully differentiate, forming the ciliated cells, goblet cells, and basal cells that represent *in vivo* human epithelial cells.

End-to-end solutions for this workflow can include uncoated transfers or precoated permeable supports. Multiwell companion plates, such as Corning's Falcon® 6-well Deep Well Plates, are long-term culture solutions that can reduce the frequency of media changes, save on labor, and minimize contamination risk.

ALI Applications

Because it can culture in vivo-like cells in in vitro models, air-liquid interfaces are commonly used to investigate lung diseases, both for cell-to-cell signaling and for disease modeling. Corning customer Epithelix, for example, has successfully used ALI methodology for its popular MucilAir *in vitro* cell model of the human airway epithelium.

ALI's potential applications in researching cystic fibrosis also hold intriguing promise, too. Cystic fibrosis is a rare pulmonary disorder affecting 70,000 people worldwide, the Cystic Fibrosis Foundation says, and its patient-to-patient treatment response is highly variable, driving a need for more research into personalized treatments. Nasal cells collected from patients and cultured with ALI could help fill those knowledge gaps.

"The nice thing about using nasal cells is that they're easy to obtain from patients to create ALI cultures," Islam says. Without ALI, she adds, researchers' ability to explore personalized approaches would be inherently limited.

"Animal models are very expensive and time-consuming, and there's considerable animal-to-animal variation," she says. "With air-liquid interface culture of nasal cells, you minimize that variability and can grow cells faster."
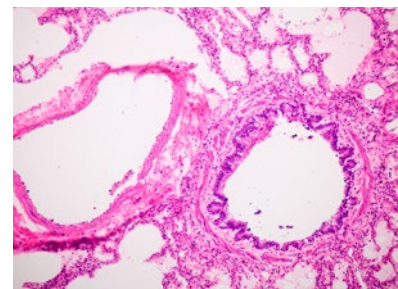
And yet ALI's possibilities are not limited to the respiratory system. Some studies, the journal Reproduction in Domestic Animals notes, have explored its potential uses in the female reproductive tract, including studies of the embryonic microenvironment and effects on assisted reproduction. Other studies have explored applications in the digestive tract and organotypic skin equivalence for wound healing, dermal remodeling, and cancer research.

Maintaining Consistency From Start to Finish

One significant benefit of ALI models is that they're less variable than animal models. To maximize that benefit, Islam advises consistency from end-to-end and recommends using cell models that are representative of the target tissue.

"Whatever the target tissue is in vivo and the endpoint aim, you should try to create cell models that are representative with relevant and comparable factors, for example, dose metrics and similar statistics comparisons to develop a standard," she says. "And when you're using the permeable supports, know that pore size, pore densities of the membrane, and choice of culture media are all variables that you can control."

There's a lot of momentum in the ALI cell culture space — and as the prevalence and profile of respiratory diseases rise as COVID-19 persists, that momentum will likely gain steam. Given ALI culturing's low cost and relatively fast uptake, opportunities abound for scientists who want to get involved — and there are more innovative supplies than ever to help accelerate your research.

Need help stocking your lab with the right equipment and supplies for your next ALI project? Contact Corning for customized recommendations or visit **www.corning.com/ALI.**

Related Resource: Permeable Supports Product Selection Guide (CLS-CC-027).

For more specific information on claims, visit www.corning.com/certificates.

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