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Ask the Expert – Using Disease Models at the Air Liquid Interface to Advance Respiratory Disease Studies

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Air-liquid Interface (ALI) is ideal for studying respiratory tract epithelial cells because it exposes one side of the culture to liquid media and surrounds the other with air. ALI systems therefore allow researchers to more accurately mimic in vivo conditions compared to using conventional cell culture models. This makes 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). From asthma to COPD, ALI models have been critical in researching respiratory diseases. With the recent outbreak and severity of the COVID-19 virus, this technology is becoming even more critical in the race to understand and treat disease.

With the popularity of these more complex models growing, we have assembled a team of experts to answer your questions on disease modeling using ALI tools and systems.

What kind of cultureware do you recommend for Air Liquid Interface (ALI) cultures?

When generating air liquid interface or ALI cultures, you will need to create a dual compartment model that can be separated by a microporous membrane such as Transwell®, Falcon® or BioCoat® permeable supports.

ALI studies are performed on permeable supports typically coated with collagen type I, type IV, or a combination of collagen types I and III. Researchers can use pre-coated permeable supports, such as Corning BioCoat Collagen I cell culture inserts, or choose to apply the coating themselves. Permeable support inserts with a 0.4 µM pore size are

commonly used for ALI studies, although other pore size inserts may also be used depending on the application.

Regarding the choice of cultureware format it will depend on the end user's application. For example, if you are growing 3D organotypic skin equivalent model, a larger size formats, such as 6-well permeable support inserts, 100 mm dish or a rectangular tray would be best suited. Whereas for studies related to drug screening, using an HTS format, such as 96-well permeable supports would be an ideal format. The 24-well permeable supports are one of the most commonly used formats although the 12-well or 6-well formats are also used based on end-user's application. Multi-well companion plates, such as 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. Corning offers a permeable support selection guide which walks through how to select your membrane type, pore size, format and surface treatment most appropriate for your application.

Could you share any research that you are aware of that uses air liquid interface for COVID-19 research?

ALI models have expanded scientists' understanding of the virus that causes COVID-19. There have been many studies published in peer reviewed journals, and several more are to come.

Corning has compiled a citation summary for Transwell permeable supports on studying COVID-19 at the Air-Liquid Interface as well as published an application note on Human Airway Epithelial Cell Culture and COVID-19 Research. Coronavirus-related and COVID-19-related articles, including those using ALI research, have also been made accessible in public repositories such as PubMed through the NIH National Library of Medicine.

Regarding any studies on ALI used for COVID-19 research, the citation summary that Corning has put together highlights some of the recent publications in which Transwell permeable supports were used for ALI studies as an ex-vivo model and these studies demonstrate Transwell permeable supports as an effective tool to study the coronavirus infection and for the development of targeted therapies.

In fact, one of the first publications in The New England Journal of Medicine that came out right after the COVID-19 pandemic began, highlighted the use of Transwell permeable supports from Corning for an ALI model. In this model, the researchers cultured human airway epithelial cells on collagen coated Transwell permeable supports for virus infection from samples that were taken from patients and for subsequent virus propagation of human respiratory secretions onto human airway epithelial cell cultures to identify virus particles in the infected patients.

The citation summary mentioned above covers the various ways that ALI models can be used - from mechanistic studies as to which differentiated epithelial cell type does the coronavirus target for eg cilia and the replication of coronavirus to the study of drug compound potency on a COVID-19 strain, and the effect of drug combination therapies against COVID-19.

One of the citations listed highlights that 3D alveolar organoid models and 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.

In using the ALI model I have read that people have trouble achieving population doublings. What kind of doublings should be expected in an optimized culture and do you have any suggestions for reaching these numbers?

Population doubling is something that would be cell type dependent, as each cell type have their own growth rate pattern and this may vary on a permeable support, particularly if conditions are not optimized. As ALI is a long term (up to weeks or months) culture one should optimize initial cell seeding density and culture time for their specific cell type before performing the actual ALI culture. Typically, cells are seeded in the apical chamber, with media in both the apical and basolateral chambers, and maintained in growth media until they achieve the desired cell confluence.

The desired cell confluence is the confluence required to obtain a monolayer of cells that have formed tight junctions. The amount of time to achieve desired confluence is dependent on cell type and seeding density, and some primary cells types may require higher cell seeding densities or longer culture times. The amount and type of the coating (eg. Collagen Type I Rat Tail) can also impact the ability for cells to form a monolayer and may require optimization if the desired confluence is not being achieved. There are several methods that can be used to determine when a cell monolayer has been achieved, but the most commonly used method is to take transepithelial electrical resistance (TEER) measurements. Typical TEER values vary by cell type and can be found in ALI literature publications. Another less common method is to stain the cells for tight junction marker proteins like ZO-1.

When the desired cell confluence is reached, the cells are air-lifted, which means the growth media is removed from both chambers and the basolateral chamber is filled with differentiation media. An example of this protocol is highlighted in this application note, "Development of an Air-Liquid Interface Model using Primary Human Bronchial Epithelial Cells and HTS Transwell®-24 Permeable Supports from Corning".

What components do you recommend for an optimized media, ROCK inhibitor, etc.?

The choice of media should be cell type and application dependent. Typically, growth media is required for cell expansion prior to seeding on permeable supports and for cell growth on permeable supports. When an optimal cell confluence is reached on the permeable supports, differentiation media in the basolateral chamber is required to achieve ALI. There are commercially available media for cell expansion and ALI culture. For example PneumaCult™-Ex Medium Serum- and BPE-free medium for expansion of primary human airway epithelial cells , PneumaCult™-ALI Medium Serum- and BPE-free medium for human airway epithelial cells cultured at the air-liquid interface, as well as supplements such as Hydrocortisone, are all available from StemCell Technologies. Scientists at Corning have experience in culturing primary airway epithelial cells for ALI model establishment using Pneumacult media with supplements Hydrocortisone and Heparin added to the medium and it has been effective during the process.

If you are working with stem cells (eg. iPSCs, organoids) you will need to include ROCK inhibitor in the culture media during permeable supports seeding as you would for single cell passaging. For specific cell types of interest literature available on the web would be a good resource for specific media component requirements.

Could you provide more details on differentiation and transferring cells from expansion to differentiation?

When cultured at the air-liquid interface (ALI), primary airway epithelial cells and some epithelial cell lines such as Calu-3 can polarize and form a pseudo-stratified epithelium containing progenitor providing basal cells, and columnar mucus-secreting goblet cells and ciliated cells. Bronchiolar epithelial differentiated models also contain cubic-shaped club cells. The differentiation process relies on environmental cues supplied by the presence of an ALI, the attachment of cells on a basement membrane extract (BME) such as collagen, tight cellular junctions, and the composition of the culture media. Cells first need to be expanded submerged in growth media on a BME-coated permeable support to form a monolayer with tight cellular junctions. Then, the growth media is removed and differentiation media is added only to the basolateral compartment of the permeable support system. The cells are maintained through regular media changes using differentiation media for 3-4 weeks. Within 2-3 weeks of the differentiation period, beating cilia can be observed and goblet cells begin secreting mucus. Some protocols recommend rinsing the apical chamber with buffer such as Dulbecco's Phosphate-Buffered Saline (PBS) to prevent mucus from accumulating on the apical side of the cells.

What type of airway epithelial cells have you cultured and do you have protocols that you could share?

Here are the links for some of the Corning technical literatures that includes the workflow/protocol.

- Bronchial epithelial model (Calu-3 cell line)
- Primary human bronchial epithelial cells
- Primary human bronchial epithelial cells

How many passages should you expect during expansion?

The number of passages you should expect during expansion will be cell type dependent particularly when working with primary airway epithelial cells. If you are using commercially available cells, the vendor will typically provide the maximum number of expected population doublings. For example, Lonza guarantees their cryopreserved human airway epithelial cells through 15 population doublings. You will need to find a balance between the minimum number of cell passages required to create a working cell stock and not reaching the cell population doubling limit prior to permeable support seeding. For studies involving testing many conditions, using a smaller permeable support format such as Corning HTS Transwell-96 permeable supports can help in minimizing the number of cells needed for your study.

Can automation be used to increase throughput? I am just thinking for screening purposes, it could be limiting if it isn't high throughput.

Yes. Corning has used HTS Transwell-24 permeable supports for the generation of multiple ALI cultures in an automation-friendly format for throughput cell-based assay of both healthy and diseased airway epithelium. Literature is available on miniaturized 96-Transwell air–liquid interface human small airway epithelial model using semi-automated cell seeding and automated long term maintenance of ALI cultures in 96-Transwell plates.

Do you need different expansion and differentiation media for culturing these models?

Yes, different expansion and differentiation media are needed for culturing these models. For example, undifferentiated airway epithelial cells are initially expanded in a cell cultureware vessel for several passages using cell growth media. It is typically recommended to harvest the cells when they reach 60-80% confluence. The cells are then seeded onto and expanded in porous inserts such as Transwell, Falcon or BioCoat permeable supports typically coated with collagen I or other collagen types. The expansion media, which is added to both the apical and basolateral compartments of the inserts, contain growth factors (eg. bFGF) to promote cell adhesion and growth. In commercially available airway epithelial growth media, these growth factors are supplied by the presence of bovine pituitary extract (BPE), such as in Bronchial Epithelial Cell Growth Medium or Small Airway Growth Media by Lonza, or by human plasma-derived components such as in PneumaCult™-Ex Medium or PneumaCult™-Ex Plus Medium.

Once the cells have formed a tight monolayer, the expansion culture media is gently removed from both the compartments. For cell differentiation, media such as PneumaCult™-ALI Medium is then added to the basal compartment only leaving the apical surface of the cells exposed to the air (air lifting).

How long does it typically take to obtain a differentiated fully functional model and how long can you keep the model viable in culture?

It will depend on the cell type as well as the origin and disease state of the cells being used. For example, the amount of time can depend on whether you are using freshly isolated primary cells or commercially available cells and whether the cells have been cryopreserved. This can impact how long it takes for the cells to form a tight monolayer before the ALI can be established. Once the cells have been air-lifted, it typically takes 3-4 weeks for a fully differentiated pseudostratified epithelium to be generated. The culture media and permeable support coating can also impact this timing. For example, when using human airway epithelial cells at the air liquid interface (ALI) on collagen I-coated Transwell inserts using Stemcell Technologies' Pneumacult Ex plus and Pneumacult ALI medium, a fully differentiated pseudostratified epithelium is generated in 4-5 weeks.

As to how long can you keep the model viable in culture again it will depend on the cell type and

source. For example, commercially available ALI models can have a long lifetime (3–12 months). Long term maintenance of the cultures relies on regular media changes and the use of good aseptic technique to ensure that contamination is not introduced. Examples of commercial ALI human airway models include EpiAirway developed by MatTek Corporation, and OncoCilAirTM and MucilAirTM from Epithelix.

This information is specific to air-liquid interface culture of airway epithelial cells on permeable supports. Organoid models are also increasingly being used to model the human airway. References for organoid airway models can be found here.

Session Experts

Audrey Bergeron is an applications scientist for Corning Life Sciences. She evaluates new products and develops protocols and technical documents using Corning cell culture products. She provides product training to Corning employees and customers. Working with the Corning scientific support team, she also engages with customers to help troubleshoot their research.

Shabana Islam, PhD supports Corning Life Sciences' global commercial Permeable Supports business. She has been with Corning Incorporated since 2009 where she previously worked as a Senior Scientific Support Specialist. In her current role she works very closely with cross-functional teams to drive revenue growth of Permeable Supports, identify customer needs and translate voice of customer, as well as writes technical documents. Her postdoctoral experiences are in cell and molecular biology where she has worked in different areas of research. She holds Masters and Ph.D. degrees in Biochemistry from Aligarh Muslim University, India.