Citations Summary for Transwell® 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 Transwell permeable supports from Corning were used for ALI as an *ex vivo* model. These studies demonstrate Transwell 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 Transwell-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 Transwell-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 Transwell 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 Transwell 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 Transwell 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.

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