3D Airway Organoid Model to Assess Infectivity of Emerging Viruses using Corning® Matrigel® Matrix-3D Plates

Application Note

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Introduction

Organoids are organ-like tissues derived from pluripotent stem cells or isolated organ progenitor cells. The cells self-organize into the differentiated cell types that are present in the organ of interest and recapitulate the structural and functional characteristics of the native tissue. These 3D complex structures provide a unique opportunity to model human organ development in an *in vitro* culture system similar to *in vivo* conditions. Organoids therefore have the potential to be used for organ replacement, disease modeling, and drug testing[1-3]. In terms of virology, airway organoids have been derived from a variety of origin cell types including trachea, large airway basal, alveolar, human induced pluripotent stem cells (hiPSC), and embryonic lung[4]. These 3D structures can recapitulate the physiochemical and functional properties of the differentiated airway epithelium containing ciliated, goblet, and basal cells, and are effective tools for respiratory virus research and disease modeling[4].

The recent COVID-19 pandemic has triggered a global emergency spurring significant research on the infectivity and pathogenesis of the virus responsible, SARS-CoV-2. It has been reported that the spike (S) protein of SARS-CoV-2 plays a key role in host cell receptor recognition of angiotensin-converting enzyme 2 (ACE2), mediating cell viral entry. This has led to advancing studies in the structure, function, and development of antiviral drugs targeting the S protein[5-8]. ACE2 has also previously been identified as the host cell entry receptor of two other strains of human coronavirus, SARS-CoV and HCoV-NL63[9]. Airway organoid platforms have been established to study SARS-CoV-2-ACE2 interaction and subsequent virus infectivity, replication, and pathogenesis. These include hiPSC-derived lung organoids, human airway organoids from adult human lung stem cells, alveolar organoids from FACS- enriched primary distal lung epithelial cells, MRC5 human lung fibroblast cells, and human bronchial organoids (hBO) from commercially available cryopreserved human bronchial epithelial cells[4,10].

Influenza virus infection also represents a major remaining threat to public health worldwide. Airway organoid models have likewise proven to be useful tools to predict the infectivity of influenza viruses and study virus-host factors. A study by Zhou J, et al.[11] generated adult stem cell-derived human airway organoids and confirmed active replication of human-infective H7N9/Ah and 2009 H1N1 (H1N1pdm) viruses. Replication competence, tissue tropism, and host responses elicited by human and avian strains of influenza A virus have also been compared in lung parenchyma-derived airway organoids and in *ex vivo* human bronchus[12]. In terms of virus-host interactions, human influenza viruses are reported to bind to α2,6-linked sialic acids present in the upper respiratory tract to enable respiratory droplet transmission[13-14].

A variety of protocols using the biological hydrogel Corning Matrigel matrix have been used to generate airway organoids[15]. These include manually coated "dome" or "sandwich/on-top" methods with different concentrations of the extracellular matrix that provide support and biological cues to the developing organoid structures. In order to better utilize airway organoids in respiratory virus research and drug discovery, there is a need to establish convenient and consistent high throughput platforms for rapid assessment of emerging human virus infection.

In this study, we investigated the suitability of Corning Matrigel matrix-3D plates as a 3D virology experimental platform for high throughput generation of airway organoids (Figure 1). In this model, airway organoids demonstrated differentiated gene expression, expressed proximal epithelial ciliated, goblet and basal cells, and virus host receptors ACE2 and α2-6-linked sialic acids. Corning Matrigel matrix 3D-plates can therefore be used for high throughput study of human respiratory virus infectivity including SARS-CoV-2 and influenza viruses.

Materials and Methods

Airway Organoid Culture

Human Bronchial Epithelial Cells (HBEpiC; ScienCell Cat. No. 3210) were cultured in Bronchial Epithelial Cell Medium (BEpiCM; ScienCell Cat. No. 3211) per the manufacturer's instructions. The Corning Matrigel matrix-3D 96-well microplate (Corning Cat. No. 356259) was removed from -20°C storage and allowed to thaw at 4°C overnight on a flat surface. On the day of organoid initiation, the Corning Matrigel matrix-3D plate was incubated at 37°C/5% CO_2 for 30 to 60 minutes to allow the Matrigel matrix to polymerize. HBEpiCs were harvested with Accutase® (Corning Cat. No. 25-058-CI) and seeded on the polymerized Matrigel matrix-3D plate at a density of 7 x 10^3 cells per well in a volume of 80 µL per well of culture medium. Airway organoid culture medium consisted of complete PneumaCult™-ALI medium (STEMCELL Technologies Cat. No. 05001) containing 0.45 mg/mL Corning Matrigel matrix for organoid culture (Corning Cat. No. 356255), hydrocortisone (STEMCELL Technologies Cat. No. 07925), and heparin (STEMCELL Technologies Cat. No. 07980). Medium was exchanged every 2 to 3 days for 28 days.

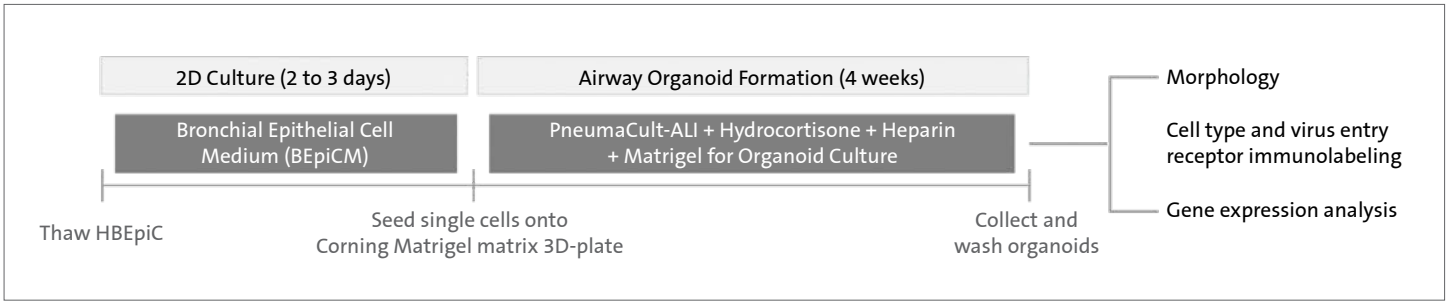

Figure 1. Schematic of the airway organoid generation workflow.

Gene Expression Analysis

After 28 days of culture, airway organoids were collected from the Corning® Matrigel® matrix 3D-plate by pipetting up and down with Axygen® wide bore tips (Corning Cat. No. TF-205-WB-R-S) and incubating with Corning Cell Recovery solution (Corning Cat. No. 354253) at 4°C for 20 minutes. Organoids were washed several times with cold phosphate buffered saline (PBS). RNA extraction was performed with the Magnetic Tissue/Cell/Blood Total RNA Kit (TIANGEN Cat. No. DP761). The One-Step TB Green® PrimeScript™ RT-PCR Kit (Takara Bio Cat. No. RR066A) with primers synthesized by GENEWIZ (Table 1) was used with the LightCycler® (Roche) for real-time PCR.

Table 1. Primers used for airway organoid gene expression analysis.

Gene	Target	Primer Sequence (5' to 3')
KRT5	Basal cells	AGCAGATCAAGACCCTCAACA
		GGTCCACTTGGTGTCCAGAA
MUC5AC	Goblet cells	ACCAATGCTCTGTATCCTTCCC
		CAGGTCTGGTTGGCGTATTTG
MUC5B	Goblet cells	CCCGTGTTGTCATCAAGGC
		CAGGTCTGGTTGGCGTATTTG
FOXJ1	Ciliated cells	GTGCTTCATCAAAGTGCCTCG
		GCCTCGGTATTCACCGTCA
ACE2	Human CoV receptors	GGATACCTACCCTTCCTACATCAGC
		CTACCCCACATATCACCAAGCA

Airway Organoid Staining

Airway organoids were harvested and washed several times with cold PBS, and then fixed using 4% paraformaldehyde (PFA) at 4°C overnight. Airway organoids were washed with PBST (PBS + 0.05% Tween® 20) and permeabilized with 0.2% Triton X™-100 for 30 minutes. Organoids were washed several times with PBST prior to staining. For immunostaining, organoids were incubated with primary antibodies in 1:100 dilution at 4°C overnight. Cytokeratin 5 (KRT5; Abcam Cat. No. ab193894), MUC5AC (Abcam Cat. No. ab218714) and beta IV Tubulin (Abcam Cat. No. ab204034) were used to label basal, goblet, and ciliated cells respectively. ACE2 (Abcam Cat. No. ab89111) and *Sambucus nigra agglutinin* (SNA; Vectorlabs Cat. No. FL-1301-2) markers were used for SARS-CoV-2 (ACE2), and influenza (α2,6-linked sialic acids) virus entry receptor staining, respectively. The next day, organoids were washed with PBST, incubated with fluorescent secondary antibodies, and nuclei stained with 2 µg/mL of DAPI. Images were captured with the Olympus IX53 microscope, Yokogawa CQ1 Image Cytometer, and Leica THUNDER Imager 3D Cell Culture.

Results and Discussion

Airway Organoid Confirmation in Corning Matrigel Matrix-3D Plates

Within 3 days of culture, 3D structures began to appear in the wells followed by lumen formation in the center of the structures after 8 to 10 days (Figure 2A). After 28 days, airway organoids were approximately 150 µm in size and were relatively consistent in size between individual wells of the 96-well microplate (Figure 2B). The presence of proximal airway epithelial cells in the organoids was confirmed with positive staining for beta IV Tubulin, indicative of ciliated cells located on the apical surface facing inwards towards the air-filled lumen (Figure 3). The presence of goblet and basal cells was also indicated by positive MUC5AC and cytokeratin 5 staining, respectively (Figure 3).

Expression data was utilized to compare expression of cell type-specific genes for differentiated airway organoids relative to expression in the source bronchial epithelial cells prior to differentiation (Figure 4). The forkhead box protein J1 (FOXJ1) transcription factor, which is involved in ciliogenesis, was increased in the airway organoids relative to the source cells. MUC5AC and MUC5B that encode mucin proteins 5AC and 5B secreted by goblet cells were similarly upregulated in the organoids. Expression level of the basal cell marker KRT5 decreased in the airway organoids. Taken together, the data indicate that these organoids supported a more differentiated airway cell phenotype relative to the source cells.

Emerging Virus Receptors Expression Analysis

Immunofluorescence staining was performed to investigate the presence of virus-specific host cell entry receptors in the airway organoids. Experiments indicated the expression of ACE2, the receptor of SARS-CoV-2 (Figure 5), which was consistent with the upregulated transcriptional level of ACE2 (Figure 4). Higher ACE2 expression in airway organoids relative to 2D-cultured epithelial cells has been observed in other reports[10], supporting the utility of the airway organoid model for coronavirus research. *Sambucus nigra agglutinin* (SNA) is a lectin histochemical stain that binds preferentially to α2,6-linked sialic acids which are the entry receptors of human influenza virus. The binding of SNA indicated expression of α-2,6-linked sialic acids which is consistent with data reported in primary lung airway organoids and *ex vivo* cultures[12] (Figure 5). Co-immunostaining of ACE2 with the ciliated cell marker beta IV Tubulin and the basal cell marker KRT5 indicated a higher expression of the virus-specific receptor in basal cells (Figure 6). This data is comparable to Suzuki T, et al.[10] who reported that basal cells were positive for ACE2 with expression at the outer edge of the hBOs.

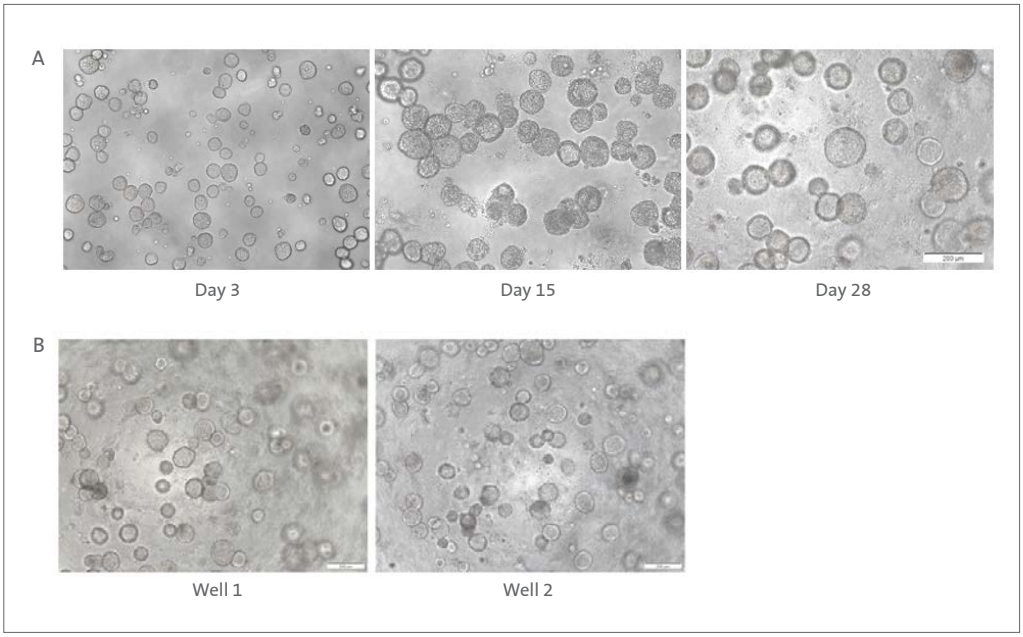

Figure 2. Airway organoid formation on Corning® Matrigel® matrix-3D plates. (A) Representative photomicrographs of airway organoids after 3, 15, and 28 days in culture. (B) Organoids (at day 28) were relatively consistent in size between wells. Images were captured at 100X magnification with an Olympus IX53 microscope. Scale bars are 200 µm.

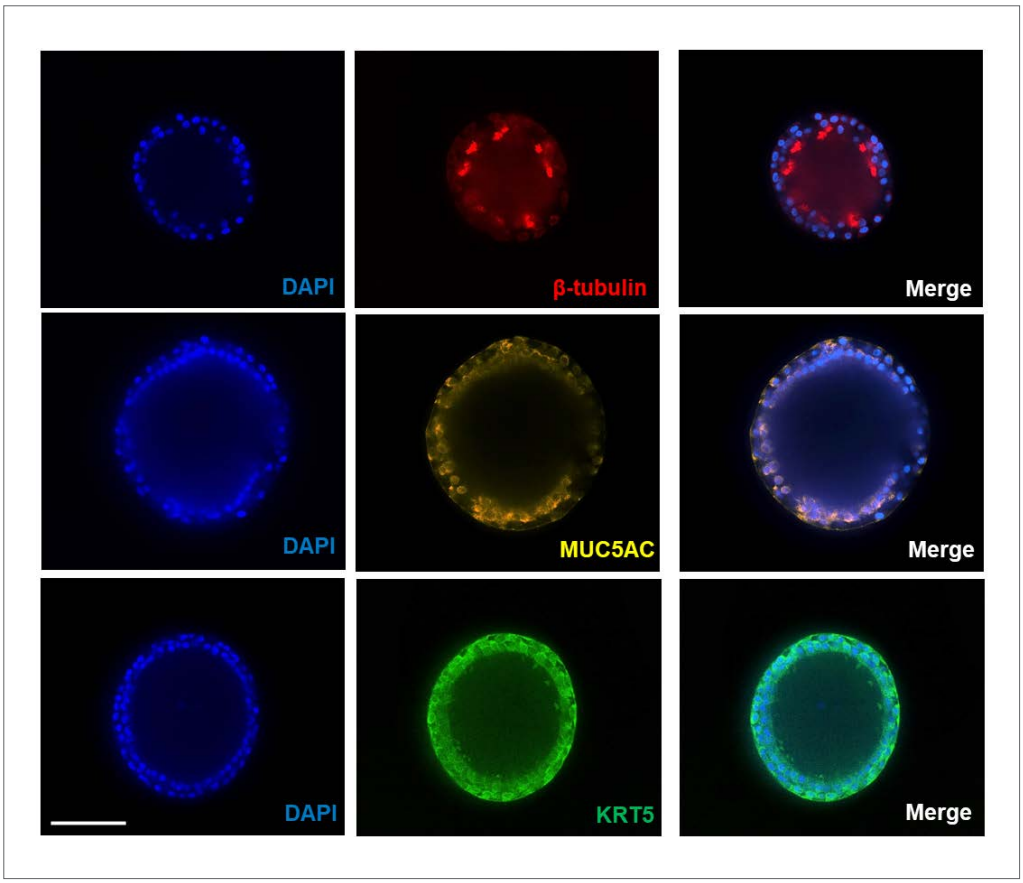

Figure 3. Different cell types in human bronchial epithelial cell-derived airway organoids. Fluorescent labels indicate specific cells types: ciliated cells (red; beta IV Tubulin), goblet cells (yellow; MUC5AC), basal cells (green; KRT5), and nuclei (blue; DAPI). Images were captured at 400X magnification with the Yokogawa CQ1 Image Cytometer. Scale bar is 100 µm.

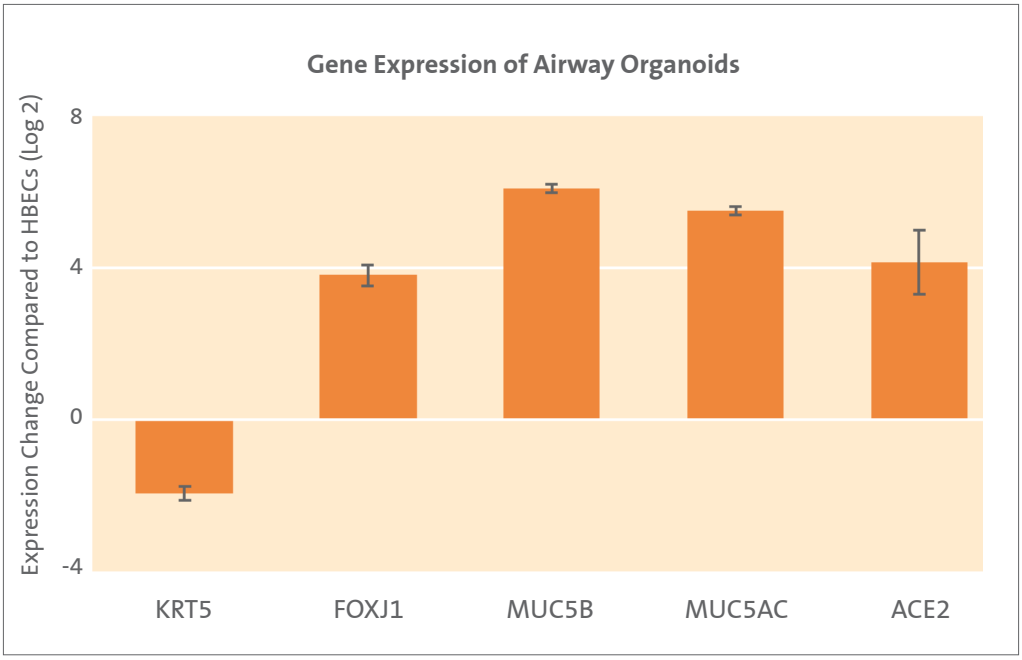

Figure 4. Expression change of genes associated with airway organoid generation. Fold change of gene expression of airway organoids compared to source human bronchial epithelial cells. KRT5 and FOXJ1 expression are associated with basal and ciliated cells, respectively. MUC5B and MUC5AC expression are associated with goblet cells. ACE2 is the entry receptor of SARS-CoV-2. Data represent mean ± SD.

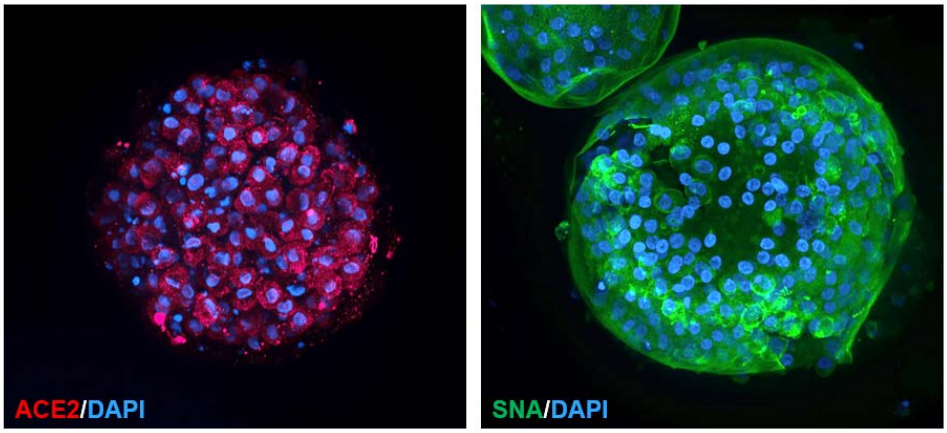

Figure 5. Respiratory virus receptor immunostaining in airway organoids. Immunostaining shows the presence of virus-specific entry receptors ACE2 (red) and α2-6-linked sialic acids (green) with counter nuclei (blue) staining. Images were captured at 400X magnification with the Yogokawa CQ1.

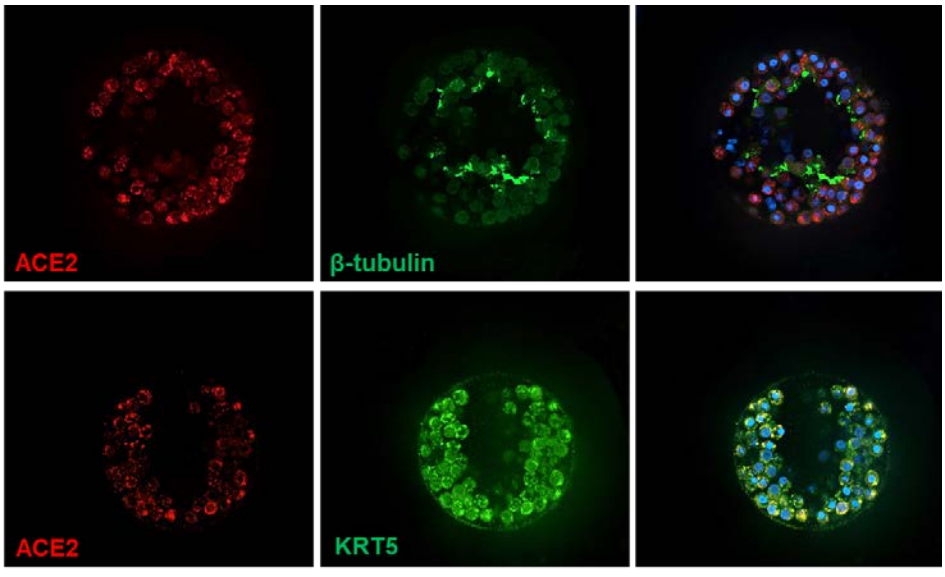

Figure 6. Cell-specific expression of ACE2 in airway organoids. Histochemical staining indicates the presence of (upper) SARS-CoV-2 receptor ACE2 (red) in beta IV Tubulin+ ciliated cells (green) and (bottom) SARS-CoV-2 receptor ACE2 (red) in cytokeratin 5+ (KRT5+) basal cells (green) with counter nuclei (blue) staining in both studies. Images were captured at 200X magnification with the Yogokawa CQ1.

Conclusions

- Corning® Matrigel® matrix-3D plates provide a convenient platform for the differentiation of bronchial epithelial cells into airway organoids.
- Airway organoids express proximal epithelial ciliated, goblet and basal cells, and the virus entry receptors ACE2 and α2-6-linked sialic acids.
- The airway organoid model can be used for high throughput, rapid assessment of human respiratory virus infectivity including SARS-CoV-2 research.

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