APPLICATION NOTE

Alveolar and bronchial microphysiological systems for respiratory infection research and therapeutics evaluation

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Summary

The lung is the most vulnerable internal organ to infection and injury due to its constant exposure to inhaled particles and pathogens from the environment. Coinciding with this, respiratory diseases are a leading cause of death and disability. Respiratory diseases make up three of the top 10 leading causes of life expectancy reduction across the globe, including chronic obstructive pulmonary disease (3rd), lower respiratory infections (4th) and lung cancer (6th) (WHO Global Health Estimates, 2020). This has only been further exasperated by the COVID-19 pandemic, which has caused widespread disease and mortality.

Despite this, the frequency of new pulmonary therapeutics reaching the market is just 3% compared to 6-14% for other disease therapeutics (Barnes *et al*., 2015). In part, this extremely low attrition rate can be attributed to the poor preclinical models available. Models range from highly expensive, unethical and non-human *in vivo* experimentation to simple *in vitro* models, both of which are unable to recapitulate the complexities and intricacies of human lung biology and immunology. Therefore, there is an absolute and urgent requirement for new preclinical models which accurately mimic the human lung and predict reactions to novel therapeutics.

This application note described the development of two Innovate UK grant funded lung-on-a-chip, also known as microphysiological system (MPS), models to solve this issue. To map the distinct areas and physiology of the human lung, an alveolar and a bronchial airway model were constructed using the PhysioMimix® Single-organ System and Multi-chip Barrier plates. These models were compared to traditional air-liquid interface (ALI) cultures in static 24-well conditions. Additional complexity was added to the lung MPS models by including primary human pulmonary microvascular endothelial cells on the basolateral side of a perfused Transwell®. Monocytes were also incorporated, either basolaterally in the bronchial model to represent circulating immune cells, or on the apical side of the alveolar model to act as an alveolar macrophage-like cell. Together, the perfused multi-cell type MPS models accurately mimicked human lung tissue, demonstrating relevant tissue architectures and cellular differentiation.

To validate the use of these models for respiratory infection research and development, pseudotyped lentivirus expressing the SARS-CoV-2 Spike protein was used to infect the models to replicate COVID-19 infection. Infection and subsequent inflammatory responses were mapped, demonstrating the relevance of the model compared to patient inflammatory profiles in COVID-19 disease. Infection was inhibited using a

neutralising monoclonal antibody therapy against the SARS-CoV-2 Spike receptor binding domain (RBD), whereby the model responded in a dose-dependent manner. These data demonstrated the ability of the model to predict the efficacy of therapeutics against SARS-CoV-2, and to facilitate an understanding of COVID-19 disease progression.

Methods

Primary human small airway or bronchial epithelial cells were cultured for 14 days at ALI on Transwells in a 24-well plate (static) or in a PhysioMimix Multi-chip Barrier plate (perfused, Figure 1). Cultures were visualised using microscopy and cell differentiation analysed by qPCR. For the alveolar cultures, markers for alveolar type I (ATI) (aquaporin 5, AQP5) and alveolar type II (ATII) (surfactant protein B, SFTPB) were detected using antibodies (microscopy) or Taqman primers (qPCR). For bronchial cultures, markers for goblet cells (mucin 5AC, MUC5AC), ciliated cells (Forkhead box protein J1, FOXJ1, or acetylated α-tubulin), club cells (Secretoglobin family A1 member 1, SCGB1A1) were detected using the same detection methods.

Primary human pulmonary microvascular endothelial cells were added on the basolateral side of the Transwell in a coculture with epithelial cells. In both alveolar and bronchial cultures, THP-1 monocytes were added, to the basolateral side to mimic circulating monocytes. In alveolar cultures only, THP-1 cells were also added to the apical side to mimic alveolar macrophages (Figure 1). To test coculture inflammatory responses, cells were challenged with Lipopolysaccharide or poly(I:C). Media samples from the basolateral side were taken over 48 hours and analysed by ELISA for IP-10/CXCL10 secretion.

Pseudotyped lentivirus expressing the SARS-CoV-2 Spike (D614G) protein with an mCherry tag was applied to the apical side of the models for 48 hours to demonstrate the utility of the model for COVID-19 research. Media samples from the basolateral side of the model were taken over the 48-hour infection before tissues were fixed and stained with anti-SFTPB antibodies, phalloidin and DAPI. Tissues were analysed by confocal microscopy to determine infection efficiency. Monocytes were tagged with CellTracker™ Far Red dye and added to the cultures to determine immune cell interaction during infection. Media samples were analysed by ELISA for IL-1β and IL-6 secretion.

To determine the ability of the model to predict the efficacy of COVID-19

therapeutics, neutralising monoclonal antibodies against the Spike RBD were applied and infection compared over 48 hours. Media samples from the basolateral side of the model were taken over the 48-hour infection before tissues were fixed and stained with phalloidin and DAPI. Tissues were analysed by confocal microscopy and infected cells quantified using ImageJ/Fiji.

Lung MPS

FIGURE 1

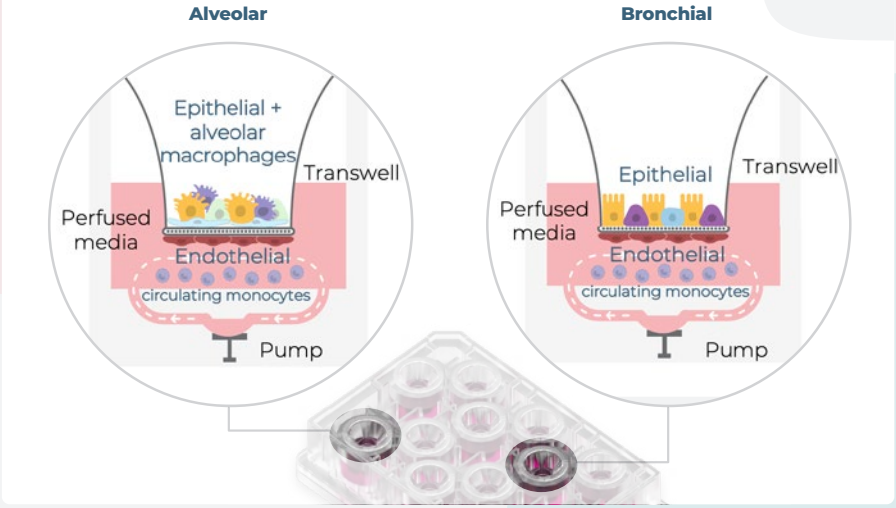


Figure 1. A visual representation of the alveolar and bronchial MPS models

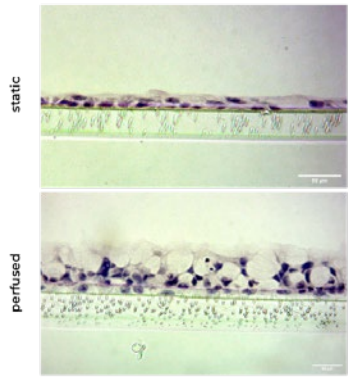
Using CN Bio's PhysioMimix® Single-organ System, alveolar and bronchial MPS models were made in Barrier plates. In the alveolar model, epithelial cells and THP-1 monocytes (alveolar macrophages) were cocultured on the apical side of the insert. In the bronchial model, epithelial cells were cultured on the apical side alone. In both models, lung endothelial cells were cultured on the basolateral side of the insert and THP-1 monocytes were circulated with the perfused media.

Results

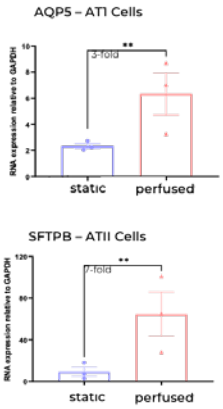
FIGURE 2

Alveolar

(A)

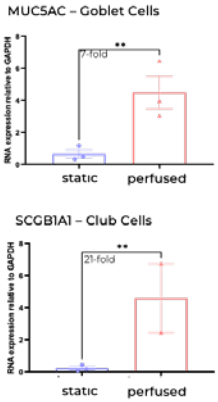


(B)



Bronchial

(C)



(D)

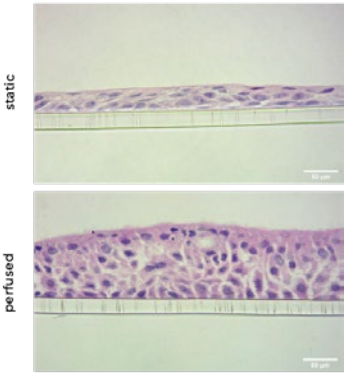


Figure 2. Alveolar and bronchial cells cultured in MPS display superior tissue formation and differentiate into physiologically relevant cell phenotypes

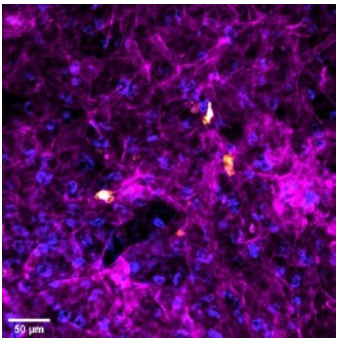
(A) Alveolar tissues were sectioned and visualised using H&E staining. **(B)** qPCR analysis of alveolar cultures expression of AQP5 (AT1 cells) or SFTPB (ATII cells). **(C)** qPCR analysis of bronchial cultures expression of MUC5AC (goblet cells) or SCGB1A1 (club cells). **(D)** Bronchial tissues were sectioned and visualised using H&E staining. Scale bar, 50 µm.

FIGURE 3

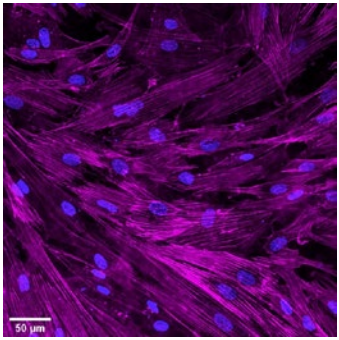
(A)

actin Mθ

Epithelium + monocytes

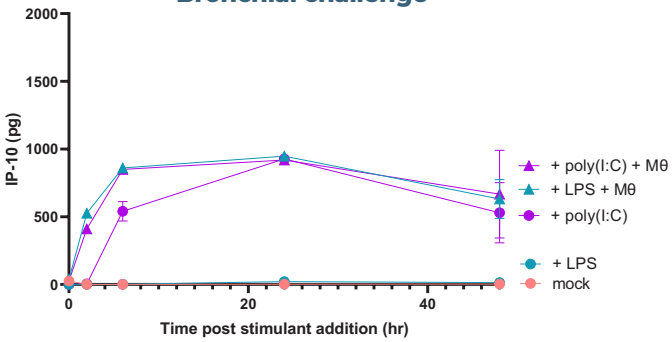


Endothelium



(B)

Bronchial challenge



(C)

Alveolar challenge

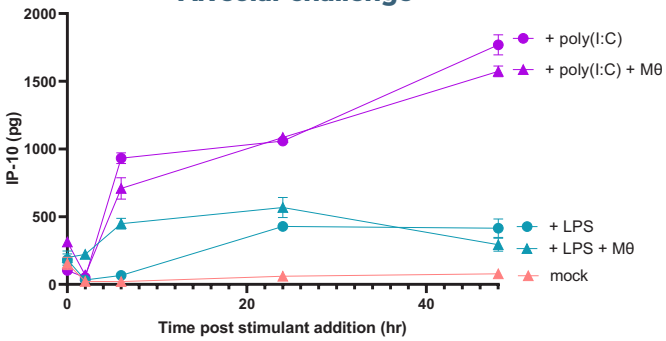
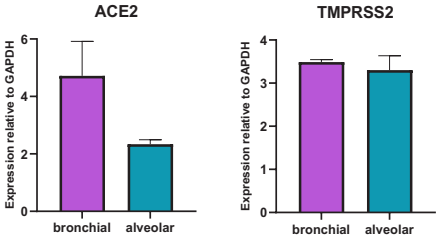


Figure 3. Addition of endothelial cells and monocytes allow mapping of disparate inflammatory responses

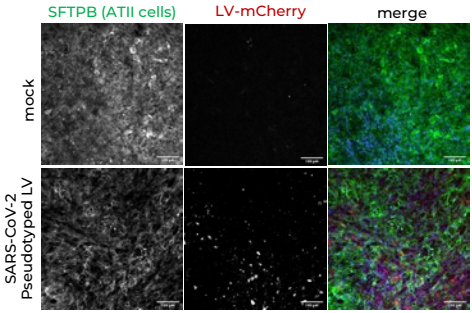
(A) Confocal images of the cell layers in the alveolar model, with epithelial and monocyte (Mθ) on the apical side (top) and endothelial cells on the basal side (bottom). Monocytes were stained with CellTracker™ Far Red (orange) and all cell stained with phalloidin (magenta) and DAPi (blue). Scale bar, 50 µm. **(B)** Bronchial cocultures with or without monocytes (Mθ) were challenged with LPS or poly(I:C) and IP-10 expression analysed using ELISA over 48 hrs. **(C)** Alveolar cocultures with or without monocytes (Mθ) were challenged with LPS or poly(I:C) and IP-10 expression analysed using ELISA over 48 hrs.

FIGURE 4

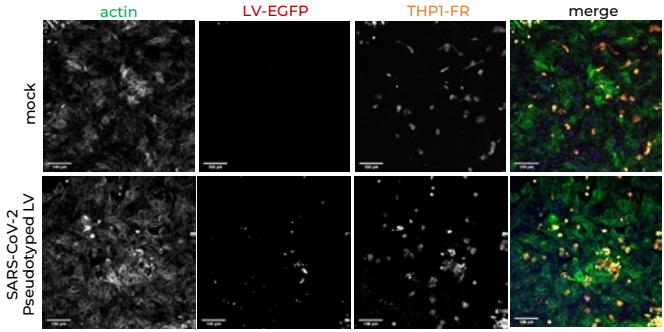
(A)



(B)



(C)



(D)

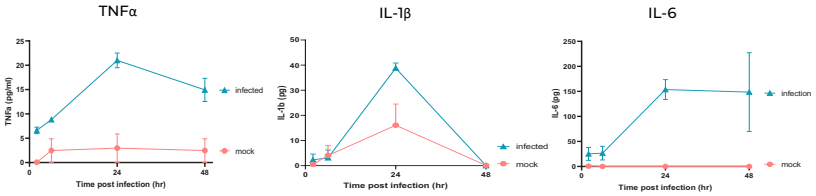
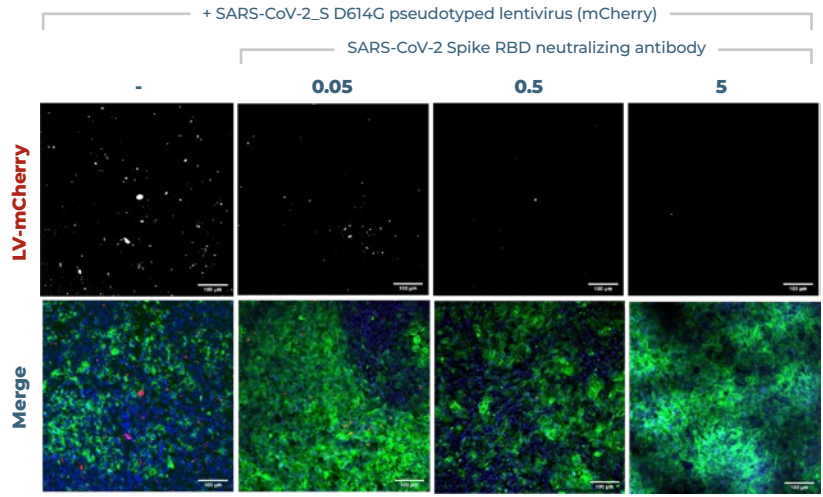


Figure 4. Lung MPS are infected by SARS-CoV-2 and produce an inflammatory response

(A) ACE2 and TMPRSS2 mRNA expression in alveolar and bronchial MPS cultures were measured using qPCR. **(B)** Pseudotyped lentivirus expressing the SARS-CoV-2 Spike (D614G) protein tagged with mCherry was used to infect alveolar cultures. Tissues were fixed and stained for SFTPB (green), DNA (blue) and mCherry (red). Scale bar, 100 μm. **(C)** Pseudotyped lentivirus expressing the SARS-CoV-2 Spike (D614G) protein tagged with mCherry was used to infect alveolar cultures with THP-1 monocytes (tagged with far-red Cell Tracker (orange)). Tissues were fixed and stained for mCherry (red), actin (green) and DNA (blue). Scale bar, 100 μm. **(D)** TNFα, IL-1β and IL-6 expression were measured using ELISA over 48 hrs during infection.

FIGURE 5

(A)


+ SARS-CoV-2_S D614G pseudotyped lentivirus (mCherry)
SARS-CoV-2 Spike RBD neutralizing antibody
-
0.05
0.5
5
LV-mCherry
Merge

(B)

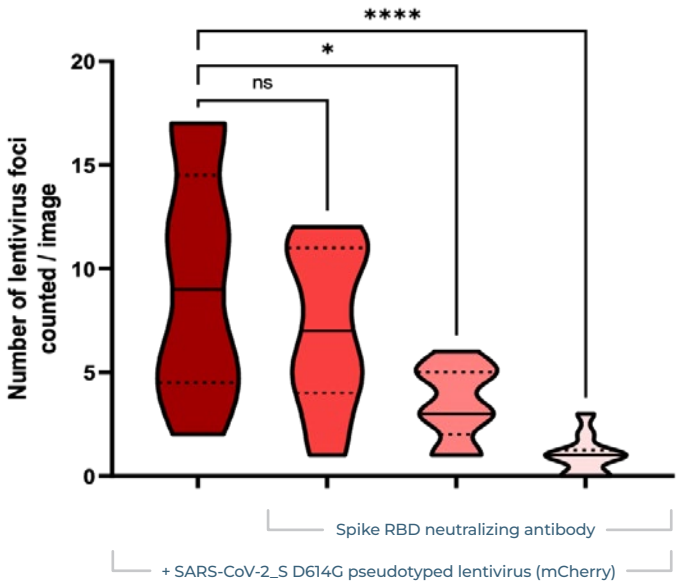

****
*
ns
20
15
10
5
0
Number of lentivirus foci counted / image
Spike RBD neutralizing antibody
+ SARS-CoV-2_S D614G pseudotyped lentivirus (mCherry)

Figure 5. Lung MPS predict efficacy of COVID-19 neutralising antibodies

FIGURE 5

(A) Alveolar cultures were incubated with increasing concentrations of monoclonal antibody against the Spike RBD (0.05, 0.5, 5 µg/ml), before being infected using pseudotyped lentivirus expressing SARS-CoV-2 Spike (D614G) protein. Cultures were incubated for 48 hr before being fixed and stained for actin (green), DNA (blue) and mCherry (red). Scale bar, 100 µm. **(B)** Number of infection foci per image (10 images/condition).

Conclusion/Discussion

Through funding granted by Innovate UK, two lung-on-a-chip models have been developed to replicate the alveoli and bronchial airways for use in respiratory infection research and drug development. Compared to traditionally cultured cells at ALI, the perfused models demonstrated superior tissue formation and differentiation into key cell types found in each region of the human lung (Figure 2).

Alveolar MPS tissues formed alveolar sac-like structures and presented cellular markers of both ATI and ATII cells (Figure 2 A.). Other primary cell or MPS models will often construct tissues which are biased towards ATI cells, a sign of cellular stress due to trans-differentiation of ATII cells to ATI, however no cellular bias towards an ATI alone phenotype was detected (Figure 2 B.). Bronchial MPS formed multi-layered, pseudostratified epithelium with thicker and more polarised tissue than static cultures (Figure 2 D). Cells differentiated into a range of bronchial cell types, including functionally relevant multiciliated cells and mucus-secretory cells (Figure 2 C).

Addition of pulmonary microvascular endothelial cells further increased the physiological relevance of the models through crosstalk of physical and chemical cues (Figure 3). Immunocompetence of the models was demonstrated by addition of monocytes, which circulated in media on the basolateral side or cocultured with the alveolar epithelial cells on the apical side to form alveolar macrophage-like cells (Figure 3). When challenged with TLR agonists, the MPS models were shown to have disparate inflammatory reactions, correlating with *in vivo* and *in vitro* data (Errea *et al*., 2015; Ritter *et al*., 2005). Bronchial MPS cultures showed a complete lack of response to LPS, as per their lack of TLR4 receptors. Addition of monocytes altered the response of the bronchial cultures, whereby an inflammatory response was induced by the monocytes upon LPS challenge (Figure 3 B). Alveolar cultures responded to both challenges, however, a larger inflammatory response was observed following the viral simulated challenge (Figure 3 C).

The MPS models were tested for their utility in COVID-19 research. Firstly, the expression of the SARS-CoV-2 receptor proteins ACE2 and TMPRSS2 were measured in the lung MPS and determined to express levels shown in human lung patients (Hou *et al*., 2020, Figure 4A). The cultures were infected with pseudotyped lentivirus expressing the SARS-CoV-2 Spike (D614G) protein with an mCherry tag. Both cultures were successfully infected with the lentivirus and displayed physiologically relevant inflammatory responses to infection, with clustering of alveolar macrophage-like cells around areas of infection and secretion of key cytokines TNFα, IL-1β and IL-6 which are typically elevated in COVID-19 patients (Figures 4 B-D) (Del Valle *et al*., 2020; Huang *et al*., 2020).

To validate the use of the lung MPS for COVID-19 drug discovery, a neutralising anti-Spike monoclonal antibody was applied to the model during infection in three dose concentrations (0.05, 0.5 and 5 μg/ml). Infection of the lung MPS was inhibited in a dose-dependent manner, thus demonstrating the utility of the lung MPS for qualifying the efficacy of COVID-19 therapeutics (Figure 5). Incorporation of these lung MPS models into pharmaceutical workflows will allow for quicker, more cost effective and reliable screening of drug candidates and thus more rapid responses to novel pathogens.

The COVID-19 pandemic has been a wake-up call to the world of the danger of new and evolving pathogens. To combat this, as well as future threats, new methodologies which can more accurately model and predict diseases must be utilised. The lung MPS are a solution to this requirement. The two lung MPS model the human lung and demonstrate human-relevant inflammatory responses to infection by pathogens. Analysis of both tissue and cell culture media can be used to understand the biological mechanisms that lead to disease, as well as to predict human reactions to medications to combat disease. Together, the lung MPSs provide a solution to the requirement for more rapid identification and validation of clinical drug candidates, which will allow us to tackle emerging diseases with absolute precision.

Summary

- Alveolar and bronchial MPS lung models have been developed to mimic the human lung more precisely.
- Lung MPS tissues can be formed with cocultures of epithelial, endothelial and immune cell components.
- The alveolar and bronchial MPS develop tissues with physiologically relevant architectures and cellular compositions.
- The lung MPS are validated for COVID-19 research and can be applied to other infectious pulmonary disease research.
- Use of the lung MPSs allow for more rapid qualification of drug candidates for preclinical research.
- Both lung MPS can also be fluidically interconnected to a predictive human model of the liver (Liver MPS, or Liver-on-a-chip) using the PhysioMimix Multi-organ System and Dual-organ plate for further investigation of inflammatory mechanisms and organ-organ crosstalk during disease and therapeutic intervention.

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