Alveolar and bronchial human microphysiological systems for use in respiratory infection research and therapeutics evaluation

Summary

Requirement for better preclinical lung models:

- Lung diseases are a leading cause of death yet only 3% of new inhaled medications reach the market[1,2]. The COVID-19 pandemic has highlighted the need for more efficient therapeutic evaluation.
- Traditional 2D cell models are cost effective but not physiologically relevant, whereas animal models are costly, time consuming and not human representative.

The solution:

- Lung microphysiological system (MPS) models with an open-well format to allow physiologically relevant infection and therapeutics evaluation.
- Cells differentiate into human-relevant phenotypes and are able to predict responses to infection and drug treatment.

Methods

Primary human small airway or bronchial lung cells were cultured for 14 days at air-liquid interface (ALI) on Transwells® in traditional static conditions or in the perfused MPS system (PhysioMimix™ OOC & Barrier (MPS-T12) plates). Cultures were visualised using microscopy and cell differentiation analysed by qPCR. An endothelial cell layer was added on the Transwell® basolateral side. THP1 monocytes were added to the apical (alveolar culture) side to mimic alveolar macrophages and basolateral (alveolar and bronchial) side to mimic circulating monocytes. Cultures were challenged with LPS or poly(I:C) and inflammatory responses measured over 48 hrs. Pseudotyped lentivirus expressing the SARS-CoV-2 Spike (D614G) protein was used to infect the models and disease relevant inflammatory markers were mapped over 48 hrs. Monoclonal neutralising antibodies against the Spike receptor-binding domain (RBD) were applied and infection measured.

Results

Figure 1. Alveolar and bronchial cells cultured in the MPS display superior tissue formation and differentiate into physiologically-relevant cell phenotypes versus static cultures.

(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 1

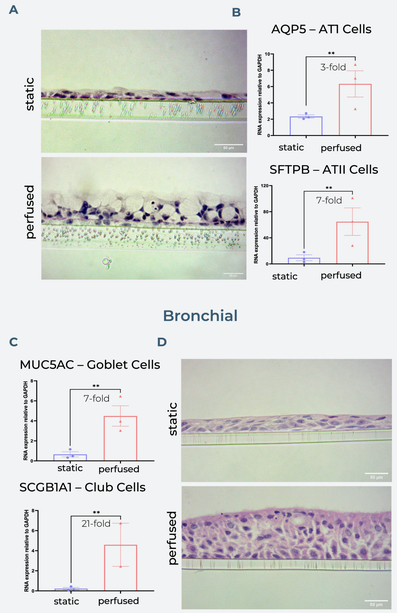
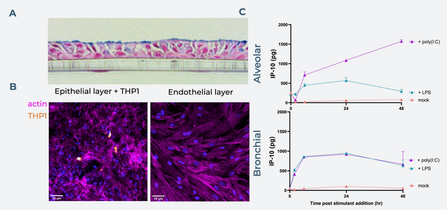


Figure 2



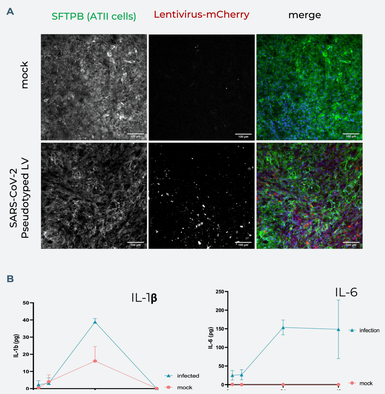
The addition of endothelial cells and monocytes allow mapping of disparate inflammatory responses.

(A) The bronchial model, stained with Alcian Blue, with epithelial cells on the apical side and endothelial cells on the basolateral side.

(B) The alveolar model with epithelial and THP1 on the apical side (left) and endothelial cells on the basolateral side (right) with actin (magenta), DNA (blue) and THP1 (orange) staining. Scale bar, 100 µm.

(C) IP-10 expression of the alveolar and bronchial models over 48 hr in response to challenge with LPS or poly(I:C).

Figure 3

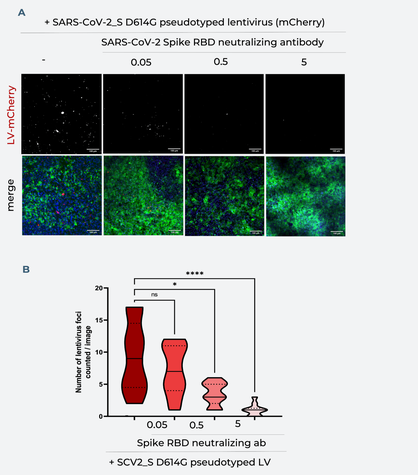


The Lung MPS model is responsive to infection

(A) 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.

(B) IL-1β and IL-6 expression were measured over 48 hrs during infection.

Figure 4



Lung MPS predict the efficacy of COVID-19 neutralising antibodies.

(A) Alveolar cultures were incubated with monoclonal antibody against the Spike RBD before being infected using pseudotyped lentivirus expressing SARS-CoV-2 Spike (D614G) protein. Cultures were incubated for 48 hrs 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).

Conclusions

- Culturing lung primary cells in the MPS system improves the differentiation of alveolar and bronchial cells into physiologically-relevant phenotypes and provides superior tissue formation compared to static ALI cultures.
- Discrete differences in inflammatory responses to stimuli can be distinguished between culture methods.
- MPS models can be infected by SARS-CoV-2, produce physiologically-relevant inflammatory responses and can be used to test novel COVID-19 therapeutics.

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References

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