Evaluation of *in vitro* human alveolar and bronchial microphysiological systems to predict the permeability and absorption of inhaled pulmonary medications.

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Introduction

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 3 of the top 10 leading causes of death across the globe, including chronic obstructive pulmonary disease (COPD) (3rd), lower respiratory infections (4th) and lung cancer (6th)[1]. However, the possibility of new therapeutics reaching the market is only 3% compared to 6-14% for other disease therapeutics[2] .

The disparity between requirement and therapeutic output can be, in part, explained by the lack of human relevant preclinical models which accurately predict responses to drugs. CN Bio's PhysioMimix™ OOC range of microphysiological systems (MPS) represent a novel but proven system for modelling the lung successfully *in vitro* by combining perfused primary cell coculture and air-to-liquid interface (ALI).

Methods

Primary human small airway or bronchial lung cells were cultured for 14 days at ALI on Transwells® in traditional static conditions, or under perfusion in the MPS (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 and cocultures analysed for tissue architecture and phenotype. Cocultures were subsequently analysed for apparent permeability (P_{app}) using Lucifer Yellow. Three pulmonary inhaled medications with varying properties were applied to alveolar cocultures in small volume liquid doses. Permeability and absorption were analysed using LC-MS. Results were validated against human clinical trial data compared to static *in vitro* and *ex vivo* data.

Results

Figure 1

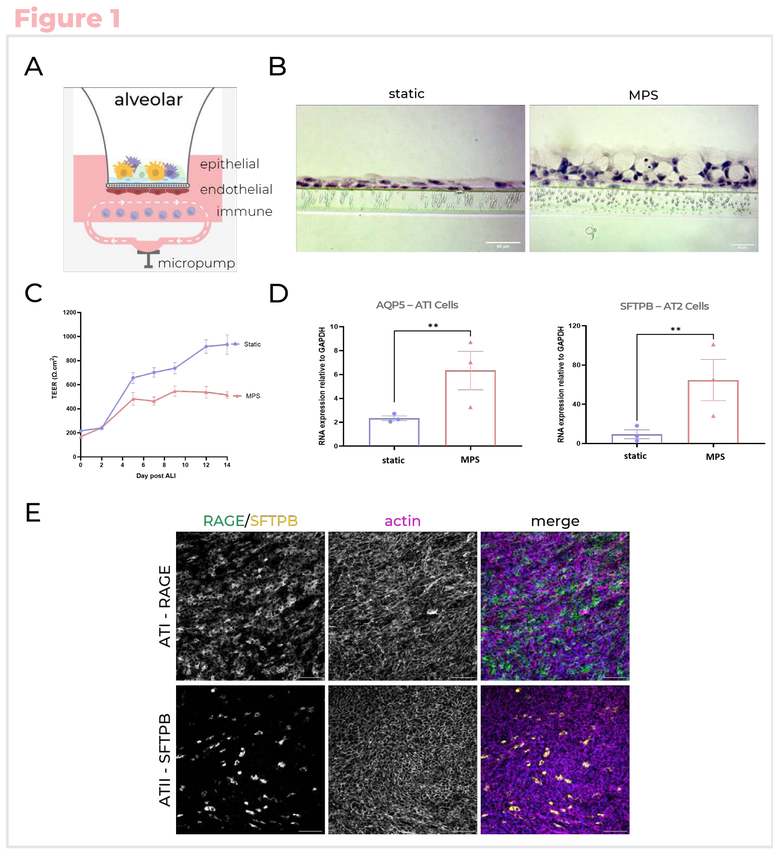


Figure 1. Alveolar MPS demonstrates increased biological and architectural relevance to human alveoli than static cultures. **A** Schematic of alveolar MPS using the PhysioMimix system with human primary pulmonary epithelial, endothelial and immune cells indicated. A micropump lies underneath each well which provides media circulation. **B** Static and MPS primary alveolar epithelial cell monocultures were sectioned and stained with H&E. Scale bar, 50 µm. **C** TEER analysis of static and MPS cultures over 14 days of ALI culture **D** qPCR analysis of alveolar cultures expression of AQP5 (ATI) and SFTPB (ATII). **E** Alveolar MPS cultures were fixed and stained for RAGE (ATI, green) or SFTPB (ATII, yellow), phalloidin (actin, magenta) and DAPi (DNA, blue). Scale bars, 100 µm.

Figure 2

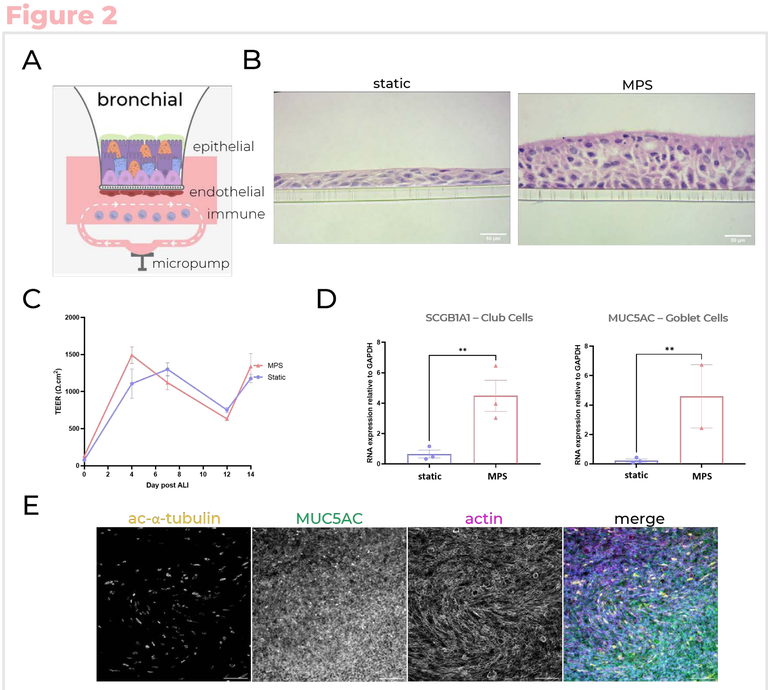


Figure 2. Bronchial MPS produces tissues with more human relevant cellular composition in a pseudostratified epithelium than static cultures. **A** Schematic of bronchial MPS using the PhysioMimix system with human primary pulmonary epithelial, endothelial and immune cells indicated. A micropump lies underneath each well which provides media circulation. **B** Static and MPS primary bronchial epithelial cell cultures were sectioned and stained with H&E. Scale bar, 50 µm. **C** TEER analysis of static and MPS cultures over 14 days of ALI culture **D** qPCR analysis of bronchial cultures with expression of SCGB1A1 (Club cells) and MUC5AC (Goblet cells). **E** Bronchial MPS cultures were fixed and stained for acetylated αtubulin (cilia, yellow)), MUC5AC (mucus, green), phalloidin (actin, magenta) and DAPi (DNA, blue). Scale bars, 100 µm.

Figure 3

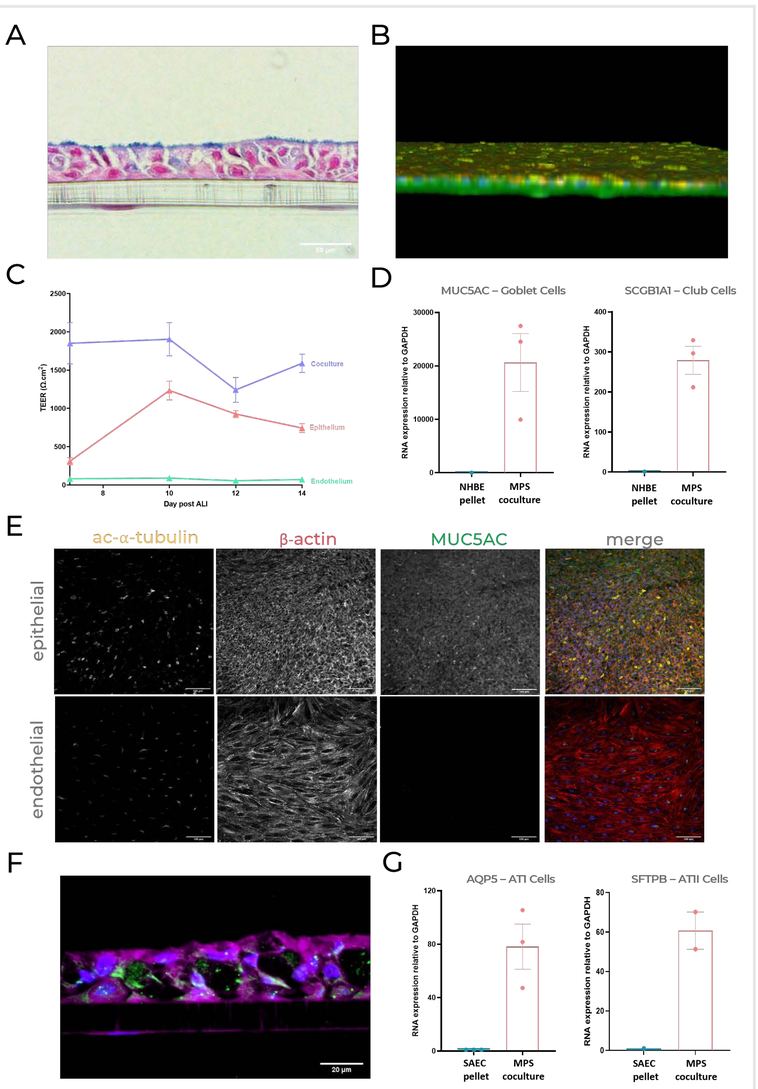
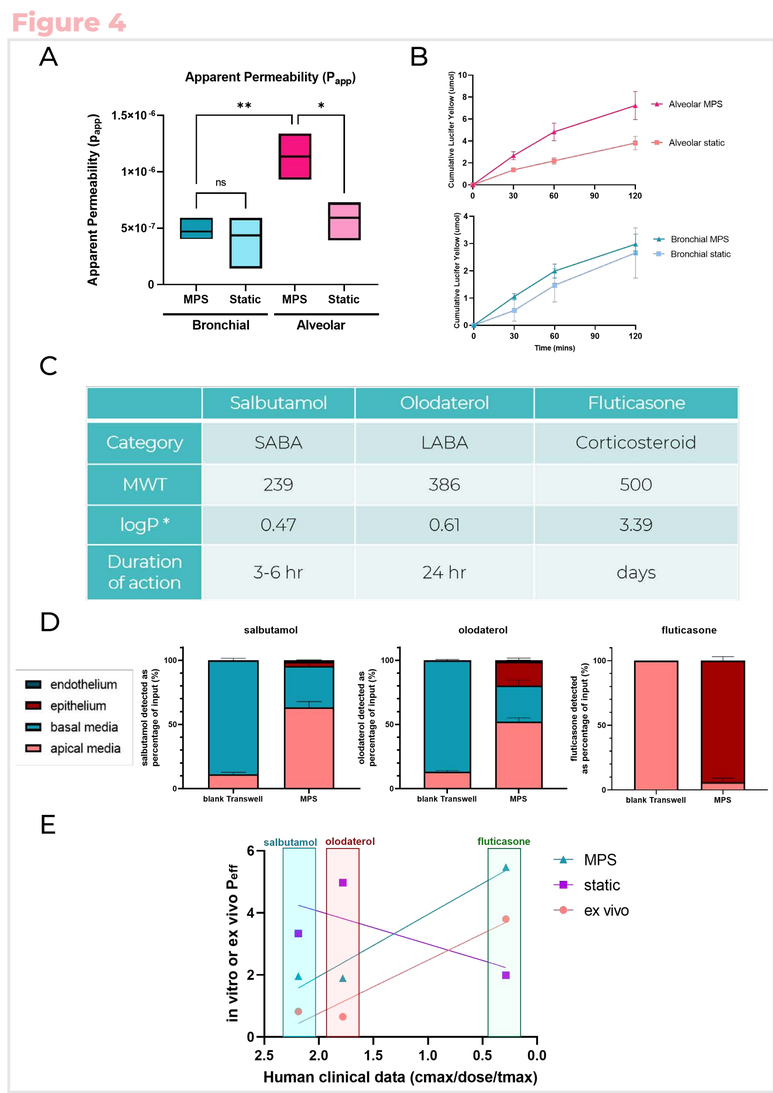


Figure 3. Epithelial and endothelial coculture MPS have increased barrier integrity whilst maintaining human relevant cellular phenotypes. **A** Human primary bronchial epithelial cells cocultured with pulmonary endothelial cells were sectioned and visualised with Alcian Blue. Scale bar, 50 µm. **B** Coculture bronchial MPS were fixed and stained with acetylated αtubulin (yellow), MUC5AC (red), phalloidin (green) and DAPi (blue). Confocal images were 3D-reconstructed using ImageJ/Fiji. **C** TEER values of epithelial alone, endothelial alone and coculture MPS cultures were analysed over 14 days post-ALI. **D** qPCR analysis of bronchial cocultures expression of SCGB1A1 and MUC5AC. **E** Coculture bronchial MPS cultures were fixed and stained for acetylated αtubulin (yellow), MUC5AC (green), phalloidin (magenta) and DAPi (blue) and imaged using confocal microscope. Scale bars, 100 µm. **F** Human primary small airway epithelial cells cocultured with pulmonary endothelial cells were sectioned and stained for SFTPB (green), phalloidin (magenta) and DAPi (blue). Scale bar, 20 µm. **G** qPCR analysis of coculture alveolar MPS cultures expression of AQP5 and SFTPB.

Figure 4



	Salbutamol	Olodaterol	Fluticasone
Category	SABA	LABA	Corticosteroid
MWT	239	386	500
logP *	0.47	0.61	3.39
Duration of action	3-6 hr	24 hr	days

Figure 4. Lung MPSs accurately predict the ADME properties of diverse pulmonary inhaled medications. **A** The apparent permeability (P_{app}) of Lucifer Yellow in bronchial and alveolar coculture MPS or static cultures. **B** The cumulative amount of Lucifer yellow in alveolar (top) or bronchial cultures (bottom) over 120 min . **C** Biochemical and clinical properties of pulmonary inhaled drugs used in the study – salbutamol, olodaterol and fluticasone. **D** Percentage of drug localised in Transwell alone or MPS culture compartments after 24 hr incubation, with endothelium (dark blue), epithelium (red), basal media (teal) and apical media (pink). **E** Comparison of the predictive ability of MPS, static or *ex vivo* data[3] compared to human clinical data[4,5]. Effective permeability (P_{eff}) from each dataset is plotted against clinical data relative to the C_{max}, dose and T_{max} of each drug.

Conclusions

- Lung MPS cultures (also known as Lung-on-a-chip) more accurately model human lung tissue biology and architecture than traditional static cultures.
- The alveolar MPS predicts ADME properties of inhaled compounds to a more precise manner than static or *ex vivo* preclinical methods.
- Future studies aim to explore a wider variety of compounds, and to understand the deposition/dissolution of drugs using aerosolization.

References

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