Assessment of Drug Permeability Using a Lung Microphysiological System

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Introduction

Background: Permeability and cellular uptake are important ADME determinants that influence the absorption and distribution of drugs. Being able to reliably predict these factors early in drug development increases the likelihood that drug candidates will be bioavailable once tested in vivo. However, few in vitro cell models are currently available which enable the reliable prediction of these parameters for inhaled drugs. Microphysiological systems (MPS) also known as organs-on-a-chip have been identified as a potential new alternative method (NAM) to traditional in vitro models. Recently a small-airway MPS has been developed that can recapitulate the physiological barrier between the air and vasculature in the lung. This microfluidic system contains two channels composed of polydimethylsiloxane (PDMS) where lung epithelial cells can be seeded into the apical channel and lung microvascular endothelial cells can be seeded in the basolateral channel. .

Purpose: The goal of this study is to evaluate the ability of the 3D human lung small airway MPS to serve as a permeability screening tool for inhaled drug candidates as well as to provide accurate permeability parameters for physiologically based pharmacokinetic (PBPK) models that may be used to support new and generic drug product development and approval.

Materials and Methods

The Emulate lung MPS was coated with collagen IV and seeded with human lung epithelial cells onto the apical channel and cultured for two weeks at the air liquid interface (ALI) to differentiate the cell monolayer into basal, ciliated and goblet cells. Following differentiation, endothelial cells were seeded onto the basolateral channel **(Figure 1)**.

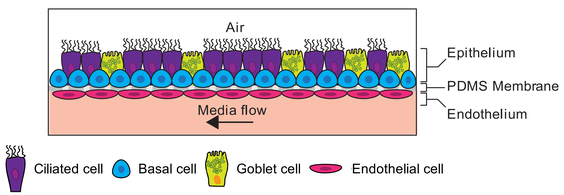

Figure 1. Diagram of lung epithelial and microvascular endothelial cells seeded on the chip.

Characterization
- The lung MPS was then characterized by confocal microscopy to assess differentiation and by fluorescent tracer assays to assess barrier integrity. Mucin levels were quantitated with Alcian blue assay.

Drug Adsorbance (Non-specific binding (NSB)) assessment
- Many drugs bind to polydimethylsiloxane (PDMS). Therefore, we conducted an adsorbance assay (NSB tests) to determine how much of the drug was binding to the chip (i.e., drug loss).

Drug Permeability
- Drug permeability was evaluated by passing the test compound at (10 µM) into the apical channel and measuring the concentration of drug that permeated into the basolateral channel over the time course using liquid chromatography-mass spectrometry (LC-MS/MS). We identified 10 µM as a physiologically relevant concentration based on estimates of regional lung surface area, mucus thickness, mucus volume, and drug deposition.

Results

Chip Characterization

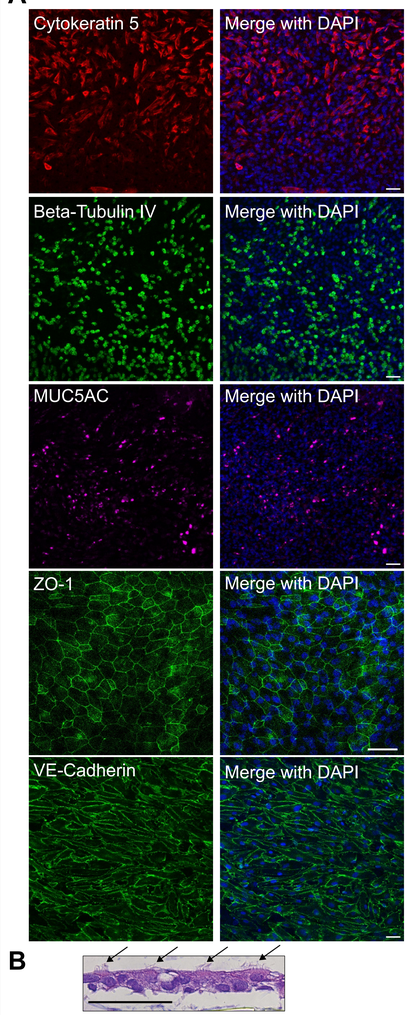

Figure 2 (A) Confocal micrographs of lung epithelial cells immunostained for ZO-1 – tight junction marker, beta tubulin IV – marker for ciliated cells, cytokeratin 5 – marker for basal cells, MUC5AC - marker for goblet cells, and VE-Cadherin - marker for vascular endothelial cells. Merge is DAPI and protein of interest **(B)** H/E stain of cilia (arrows) in the epithelial channel of the chips after 14 days of culturing the cells at the air liquid interface. Scale Bar = 50 µm

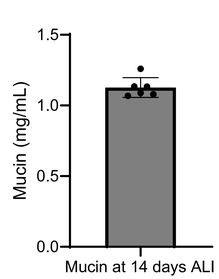

Figure 3. Cells secrete mucin after 14 days of differentiation at ALI on the chip. Chips were washed with PBS and the amount of mucin was quantitated with an Alcian blue assay n = 6 chips

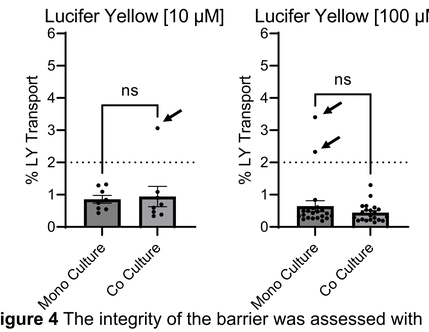

Figure 4 The integrity of the barrier was assessed with 100 µM or 10 µM Lucifer yellow (LY), a nontoxic fluorescent tracer. A higher % transport indicated a less intact cell barrier between the two channels indicated by arrows. ns = not significant, n = 8 - 21 chips. Mono Culture (epithelial cells only), Co Culture (epithelial and endothelial cells)

Drug Stability and Adsorbance

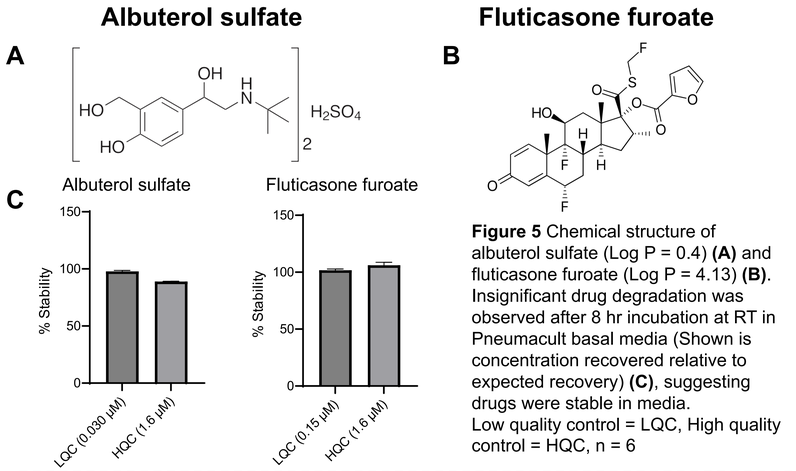

Figure 5 Chemical structure of albuterol sulfate (Log P = 0.4) **(A)** and fluticasone furoate (Log P = 4.13) **(B)**. Insignificant drug degradation was observed after 8 hr incubation at RT in Pneumacult basal media (Shown is concentration recovered relative to expected recovery) **(C)**, suggesting drugs were stable in media.
Low quality control = LQC, High quality control = HQC, n = 6

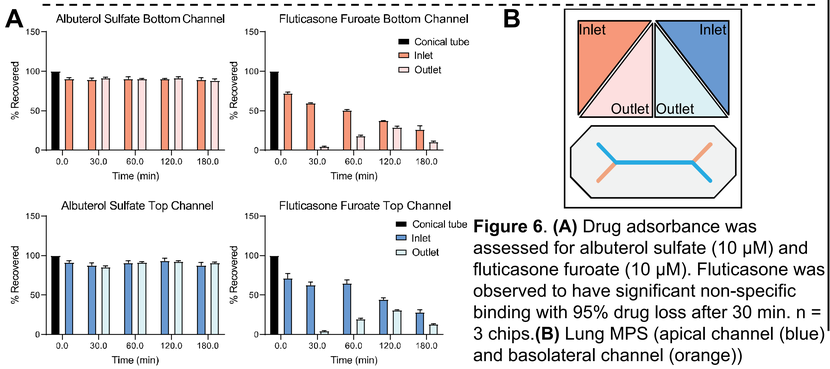

Figure 6. **(A)** Drug adsorbance was assessed for albuterol sulfate (10 µM) and fluticasone furoate (10 µM). Fluticasone was observed to have significant non-specific binding with 95% drug loss after 30 min. n = 3 chips.**(B)** Lung MPS (apical channel (blue) and basolateral channel (orange))

Drug Permeability Assessment

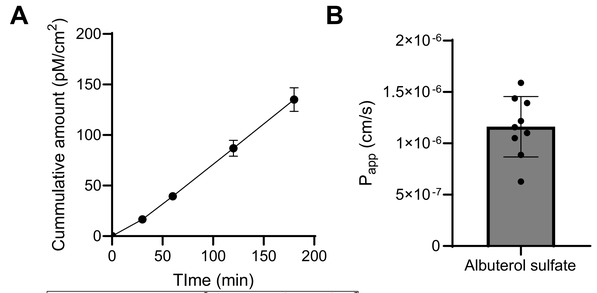

Figure 7 (A) The permeability of albuterol sulfate (10 µM) was assessed at 0, 30, 60, 120, 180 min by LC-MS/MS. The apparent permeability of $1.16X10^{-6}$ cm/s **(B)** was calculated based on the equation **(C).** The calculated apparent permeability is in line with other static in vitro model systems that use lung cell lines in a Transwell system **(D).** n = 9 chips

D

P_{app} (cm/s)	Author
2.88×10^{-6}	Dutton et al., 2020
1.99×10^{-6}	Ehrhardt et al., 2005
2.77×10^{-6}	Ehrhardt et al., 2005
1.1×10^{-6}	Cingolani et al., 2019

Conclusions

- After 2 weeks of culture at ALI, cilia beating, basal, ciliated and goblet cells were observed on the chip.
- Most chips were observed to have an intact monolayer after culturing at ALI and addition of endothelial cells. However, some chips were observed to be patchy as identified by the fluorescent tracer assay indicated by the arrows.
- Fluticasone furoate was observed to have severe drug loss due to high non-specific binding to the PDMS chip. Consequently, no further permeability studies were conducted.
- Preliminary data suggest that the apparent permeability of albuterol sulfate ($1.16X10^{-6}$) is in line with other in vitro data.
- Results obtained during this project will help inform drug developers and regulators of the quality controls necessary to determine if the chip can be used to assess drug permeability. Drug permeability data from the chip can be used to help inform PBPK model to support new and generic drug development.

Acknowledgements

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Disclaimer: The findings and conclusions in this poster have not been formally disseminated by the U.S. Food and Drug Administration (FDA) and should not be construed to represent any Agency determination or policy.
Disclosures: Alexandre Ribeiro is currently employed by Hovione PharmaScience Ltd