

Introduction

- Drug-induced lung disease (DILD) has been reported for many compounds, for example amiodarone and afatinib, as a key factor in the discontinuation of drug therapy. *In vitro* pulmonary models are crucial tools for assessing the toxicity of compounds and pharmaceuticals, such as the 3D organoid model and the air-liquid interface (ALI) culture model.
- In this study, we established the 3D human bronchial organoid (HBO) model and the human ALI airway model, both static and perfused (bronchi-on-a-chip) a with demonstrated capacity to differentiate into mature mucociliary airway structures. These models serve as robust tools for *in vitro* pulmonary toxicity screening.

Methods

- The HBO model was first established under undifferentiated basal conditions for model characterization and drug-screening purposes. It was then differentiated for 14 days into mature airway organoids, which were characterized using immunofluorescence staining, immunohistochemical staining, and RT-qPCR analysis.
- The normal human bronchial epithelial (NHBE) cell ALI model was established by Transwell-based ALI culture for 28 days and was characterized by immunofluorescence staining and RT-qPCR analysis.
- CN-Bio's PhysioMimix system was used to establish the perfused bronchi-on-a-chip model using NHBE cells for 14 days, which was compared with the static NHBE model in terms of differentiation outcome by RT-qPCR analysis and histological staining.
- Drug screening using the reference compound afatinib, with reported pulmonary toxicity, was conducted in the differentiated 3D HBO model and NHBE ALI barrier model.

Conclusion

- Three-dimensional human bronchial *in vitro* model can be established as either a 3D organoid model or a Transwell-based barrier models, under both static and perfused condition with CN-Bio PhysioMimix system.
- Both models showed dose-responsiveness to compounds with reported pulmonary toxicity in terms of goblet-cell composition, which serve as potential tools for drug screening purposes for identification of side effects.

