EPITHELIX
In vitro respiratory solutions

In vitro primary human alveolar epithelium/endothelium and fibroblast model: a tool for investigating IPF and treatment strategies

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Introduction

Idiopathic pulmonary fibrosis (IPF) is a complex and lethal interstitial lung disease with median survival of only 3 years after diagnosis. However, the etiology of this Interstitial Lung Disease is yet to be unravelled.

The aim of the study is the development of an IPF model containing exclusively human primary cells. Here, known IPF biomarkers for Epithelial to Mesenchymal (EMT) transition (MMP-1, MMP-3) and Fibroblast to Myofibroblast transition (FMT) (α-SMA and collagen) were induced in a co-culture of lung fibroblasts and AlveolAir™, a tight epithelium made of pneumocytes of type I and II, and endothelial cells cultured at air-liquid interface.

Prevention of IPF markers was tested with reference antifibrotics – Nintedanib and Pirfenidone.

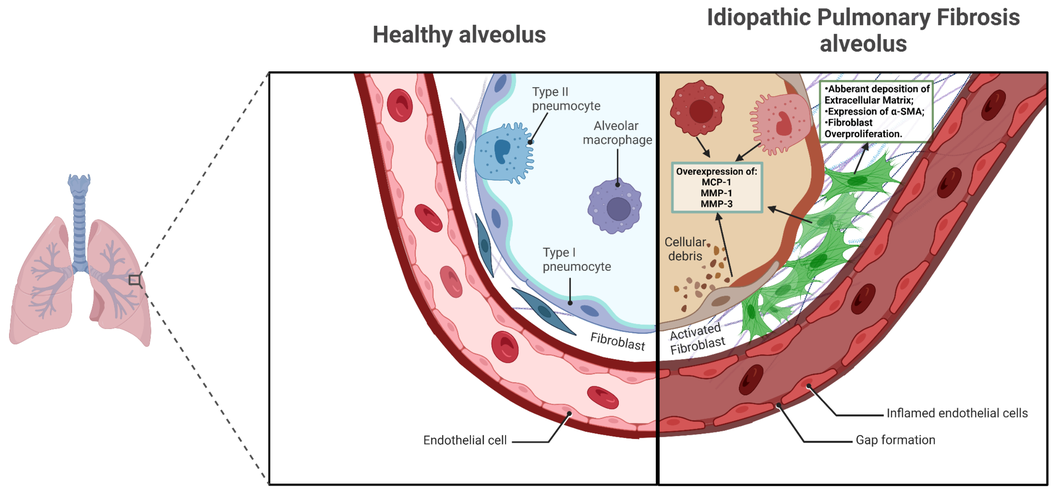

Fig. 1: Schematic representation of differences between healthy and Idiopathic Pulmonary Fibrosis alveolus. (Created with BioRender)

Testing strategy for IPF induction

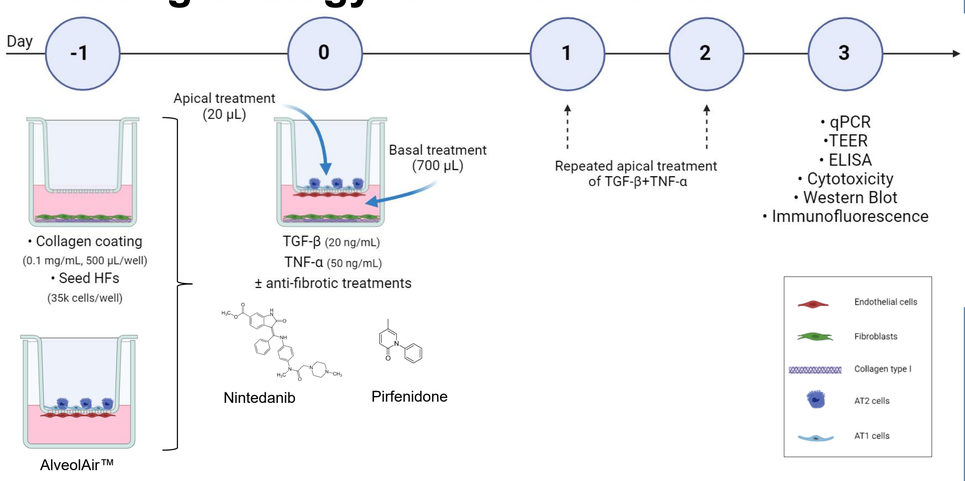

Fig. 2: AlveolAir™ co-cultured with fibroblasts were stimulated with TGF-β1 and TNF-α for 72 hours. Nintedanib and Pirfenidone, used as reference antifibrotics, were tested concomitantly with the stimulation in the basal culture medium. To monitor FMT, α-SMA and collagene gene expression was analyzed, while EMT was assessed by measuring multiple cytokines. To evaluate interindividual variability, response from 3 different donors (AL0822; AL0812 and AL0905) has been compared.

Characterization of the AlveolAir™ model

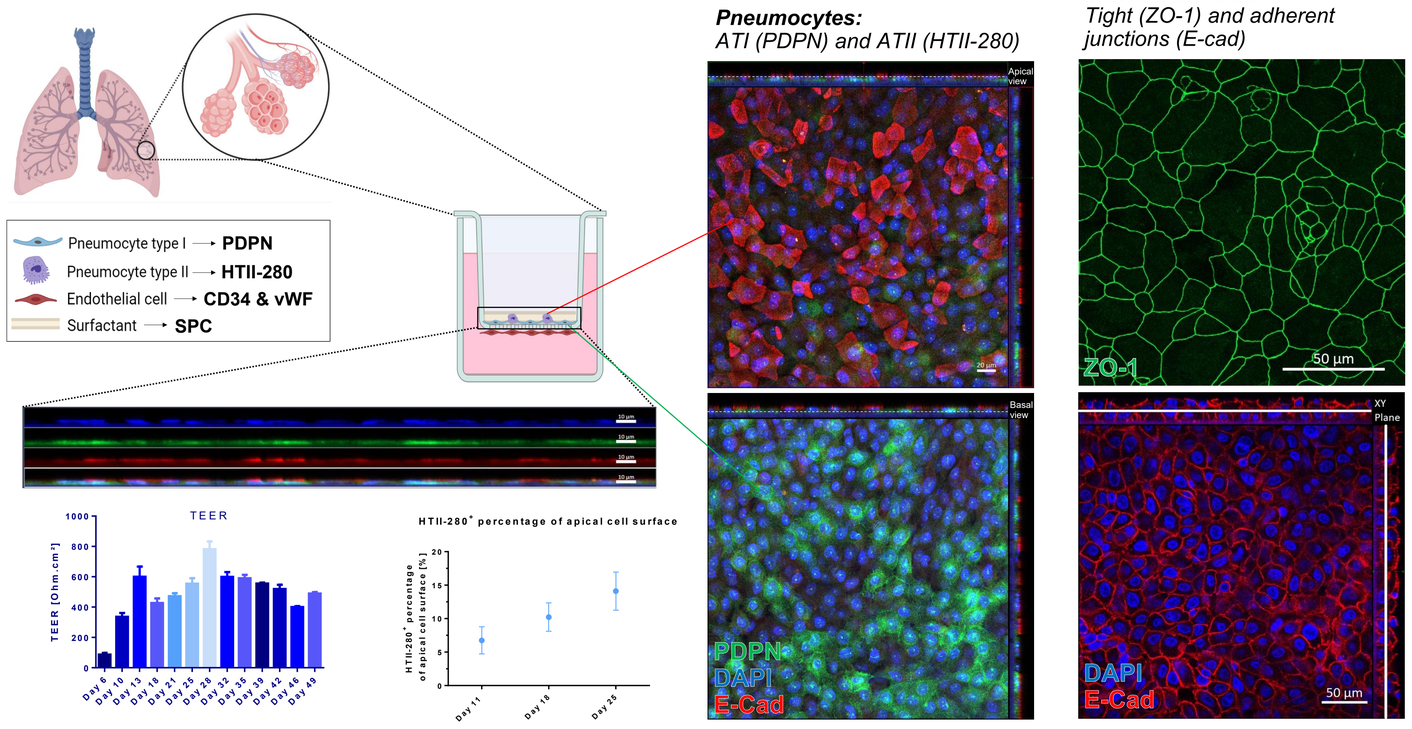

Fig. 3: AlveolAir™ is reconstituted with primary human type I and type II penumocytes with endothelial cells. The model is at the ALI interface and secretes surfactant. ATI cells are caracterized using PDPN while long term presence of ATII is confirmed by HTII-280 expression. Tight and adherent junctions are identified by ZO-1 and E-cad staining, respectively. Barrier integrity is evidenced by TEER measurement.

Results

1. TEER

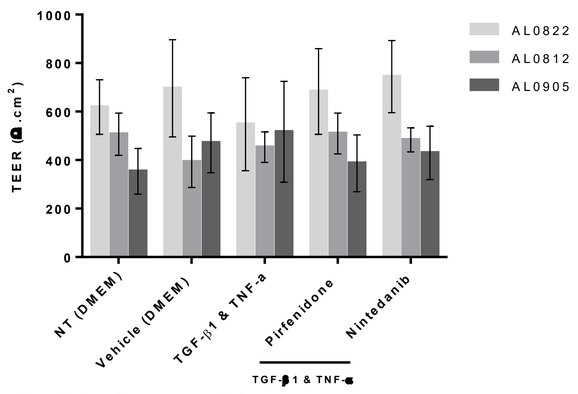

Fig. 4: Tissue integrity after (Day 3) (n=3, mean ± SD).

2. EMT Markers: MMP-1 and MMP-3

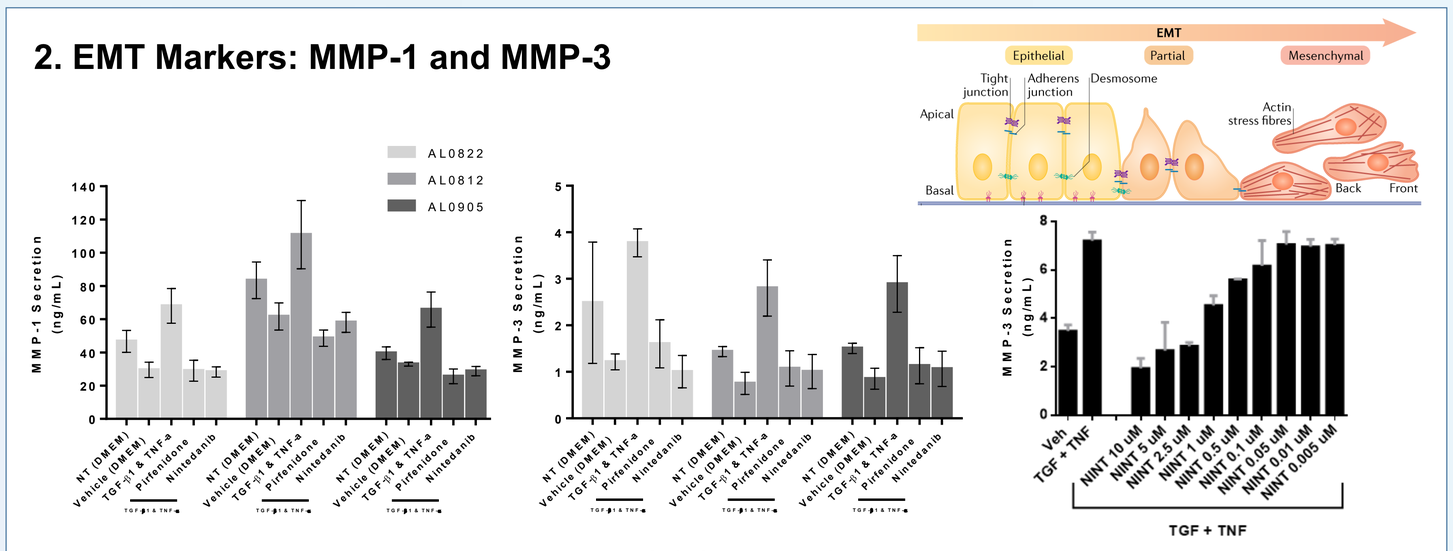

Fig. 5: Basolateral MMP-1 and MMP-3 secretion (n=4, mean ± SD). One-Way ANOVA followed by Dunnett's multiple comparison test against the TGF-β1 & TNF-α control. p values are indicated as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

3. FMT Marker: α-SMA

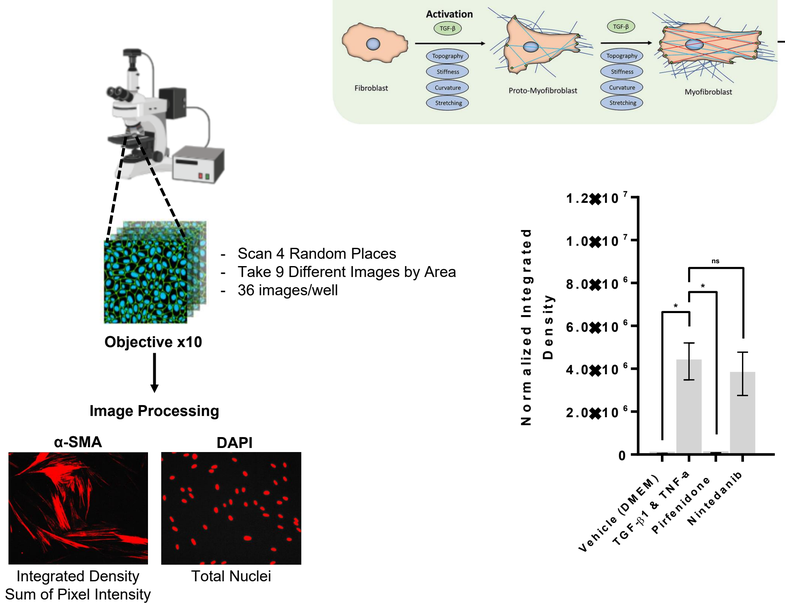

Fig. 6: Quantification of immunofluorescence of α-SMA in fibroblasts (n=3, mean ± SEM).

4. FMT Markers: Collagen genes

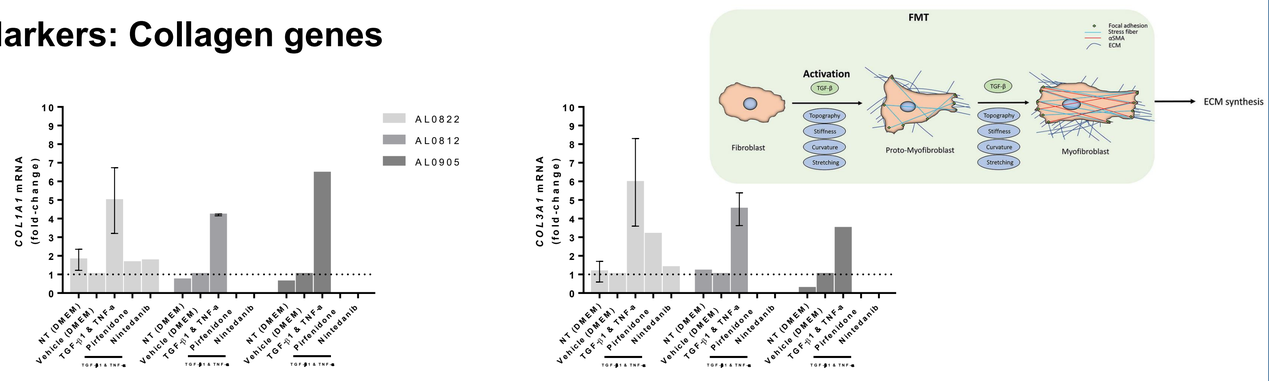

Fig. 7: Quantification of COL1A1 and COL3A1 genes in fibroblasts (n=2, mean ± SD).

Conclusions and Summary

We developed a stable and reproducible model of (TGF-β + TNF-α) induced alveolar fibrosis based on fully primary human cells (AlveolAir™-HF) combining epithelium/endothelium and fibroblasts. The simultaneous fibrotic stimulation and exposure to approved anti-fibrotic drugs (Nintedanib and Pirferidone) decreased the EMT (MMP-1 and MMP-3) and FMT (COL1A1 and COL3A1) markers of IPF. The induced IPF model using AlveolAir co-cultured with parenchymal fibroblasts holds promise for advancing preclinical antifibrotic screening and deepening the understanding of molecular mechanisms in IPF.