EPITHELIX
In vitro respiratory solutions

Primary human in vitro co-culture model of alveolar epithelium/endothelium and parenchymal fibroblasts to study idiopathic pulmonary fibrosis (IPF) and related treatments

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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 (MCP-1, MMP-1, MMP-3) and Fibroblast to Myofibroblast transition (FMT) (α-SMA) 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.

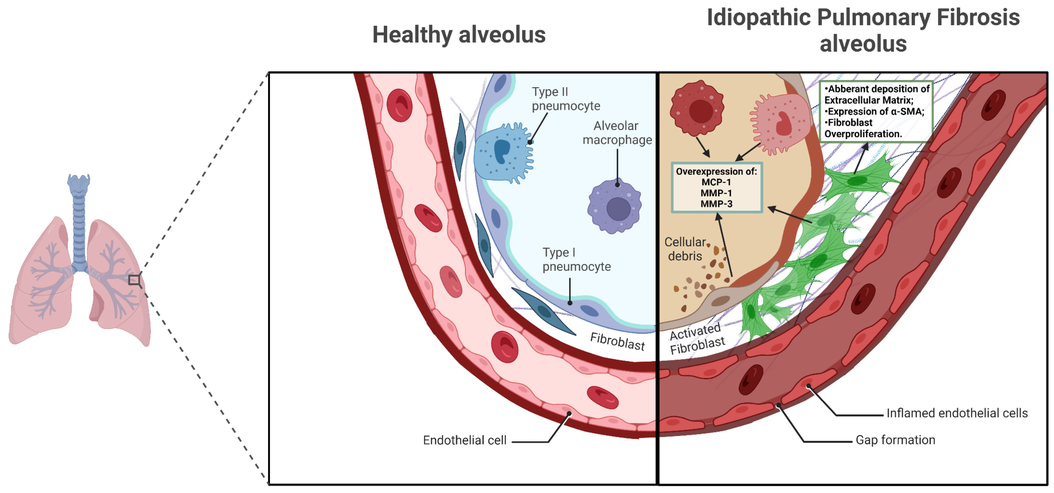

Fig. 1: Schematic representation of differences between healthy and Idiopathic Pulmonary Fibrosis alveolus. (Created with BioRender)

Testing strategy for IPF induction:

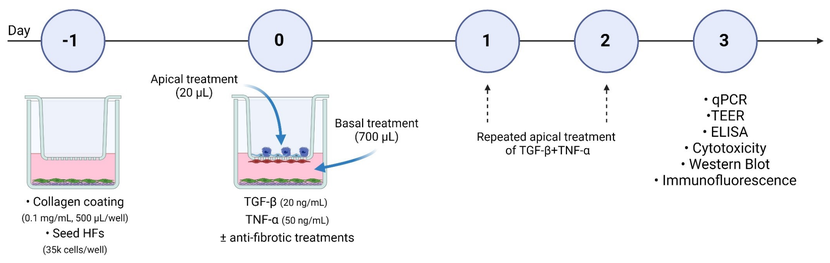

Fig. 2: Schematic experimental layout of the preparation, exposure and endpoints of the induction and prevention of IPF in the 3D alveolar model. (Created with BioRender)

Methodology:

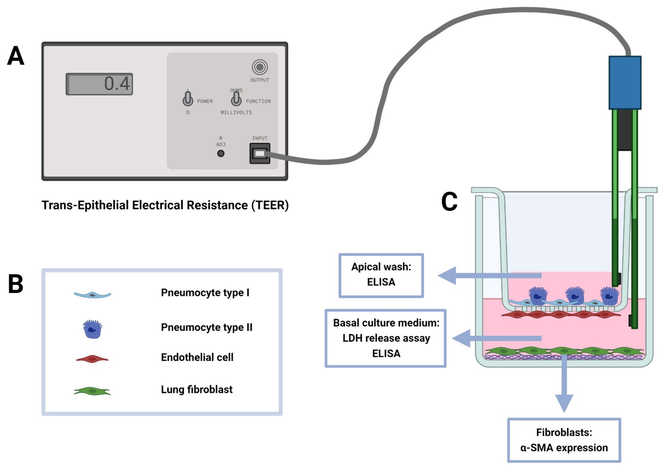

Fig. 3 A – TEER measurement for tissue integrity; B – Primary human cell types used in the IPF model; C – Experimental endpoints: ELISA (cytokines and chemokines secretion); LDH release assay (cytotoxicity); α-SMA expression through Immunofluorescence. (Created with BioRender)

Results:

1. TEER and LDH release

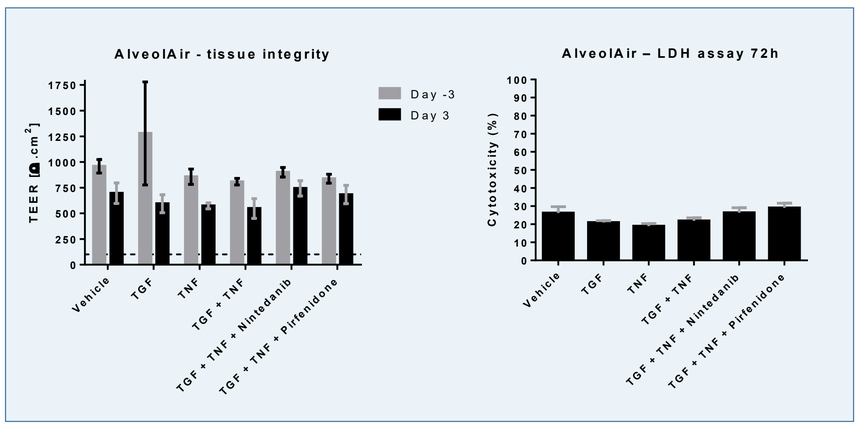

Fig. 4: A – Tissue integrity of AlveolAir before (Day -3) and after (Day 3) pro- and anti-fibrotic exposures (n=6, mean ± SEM); B – LDH release assay of the 3D model following pro- and anti-fibrotic exposures (n=6, mean ± SEM).

2. ELISA – EMT biomarkers of IPF following stimulation and antifibrotics testing

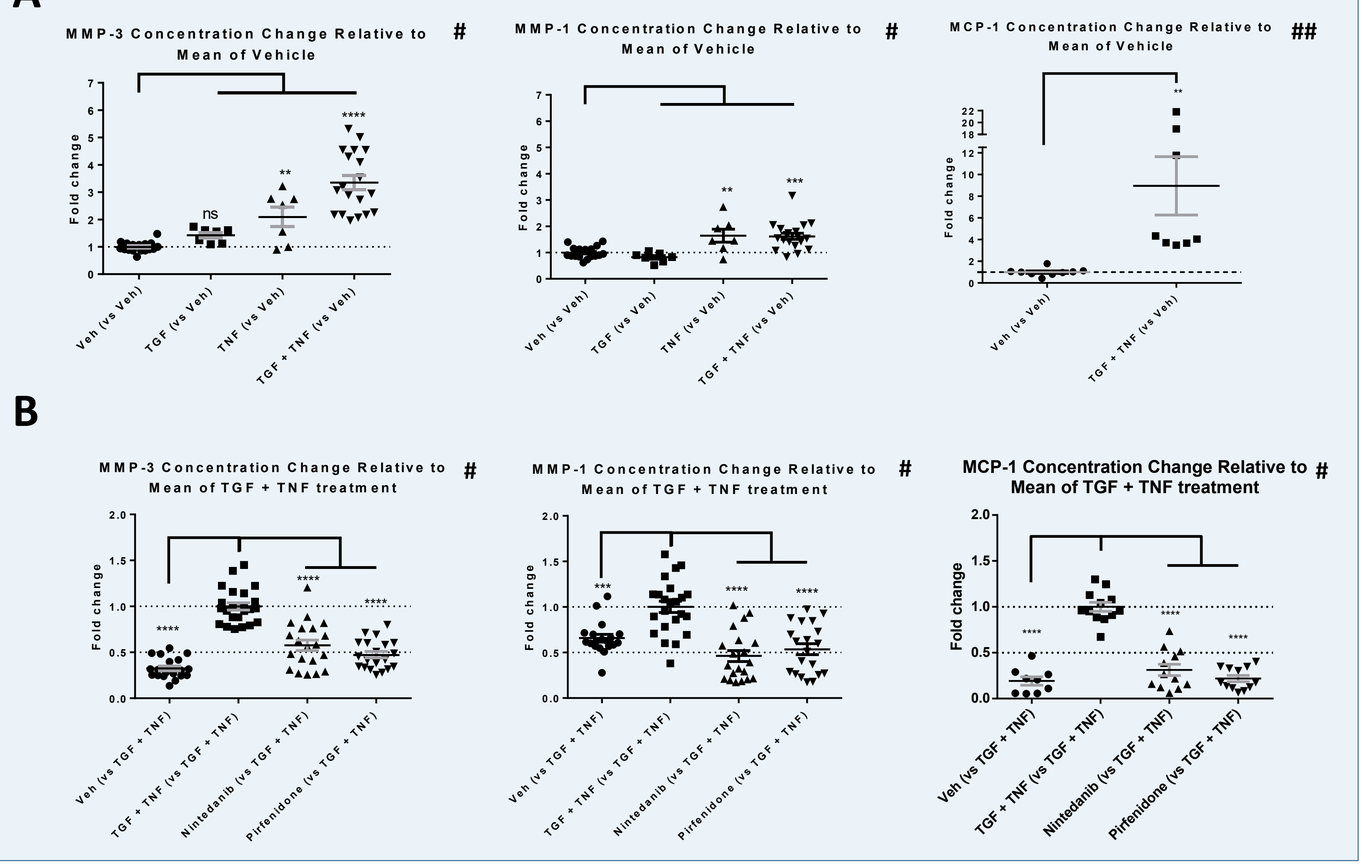

Fig. 5: A - Fold change of secreted cytokines concentrations relative to the mean of secreted cytokines in the Vehicle condition for each assay. B – Fold change of secreted cytokines relative to the mean of secreted cytokines in the double stimulation conditions. (MMP-1 and MMP-3 dosage was performed using basal media, while MCP-1 dosage was done from apical wash). (n=6-16 mean ± SEM)

One-way ANOVA with Dunnett's multiple comparaisons test with a single pooled variance, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001 (GraphPad, Prism)

Unpaired t-test. , *p<0.05, **p<0.005 (GraphPad, Prism)

3. Immunofluorescence – FMT marker, α-SMA expression in Human Fibroblasts

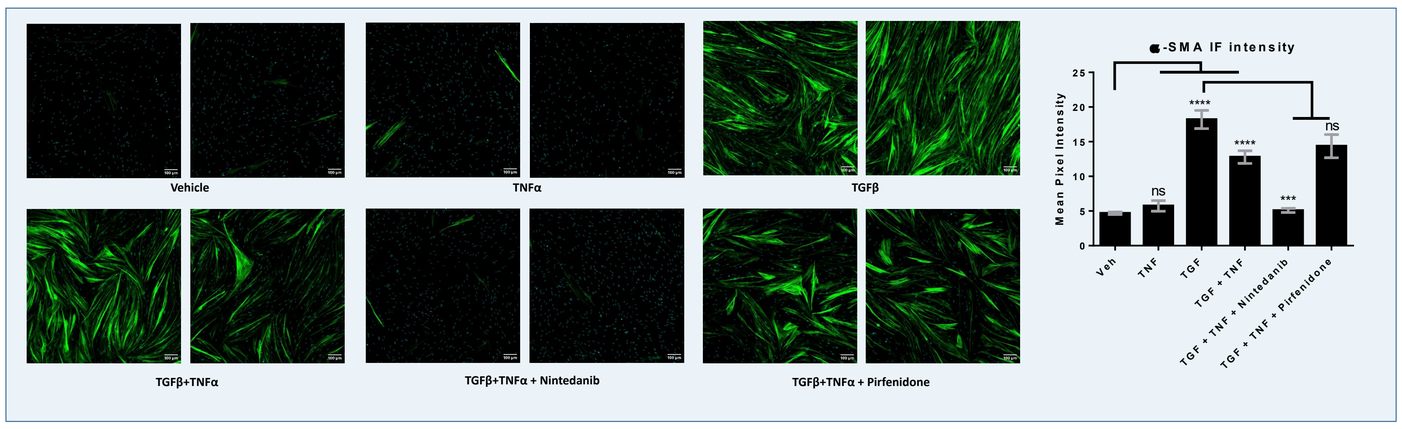

Fig. 6: A – Immunofluorescence of α-SMA in primary human fibroblast in the 3D model; B – Mean intensiy of α-SMA signal in human fibroblasts among conditions, . (n=4 mean ± SEM)

One-way ANOVA with Dunnett's multiple comparaisons test with a single pooled variance, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001 (GraphPad, Prism).

Conclusions and Summary:

We developed a stable and reproducible model of alveolar fibrosis exhibiting the hallmarks of fibrosis. Additionally, the simultaneous firbrotic stimulation and exposure to approved anti-fibrotic drugs significantly decreased the EMT and FMT markers of IPF. The IPF model in AlveolAir co-cultured with parenchymal fibroblasts has the potential to catalyse further preclinical antifibrotic screening and helps to expand the understanding of molecular mechanisms in IPF.