EPITHELIX
In vitro respiratory solutions

Development of a novel in vitro model for the detection of profibrotic compounds based on primary human alveolar epithelium

Edmon Pira, Ghislaine Arib, Bernadett Boda, Song Huang, Samuel Constant

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a devastating lung disease with a median survival of only ~3-5 years after diagnosis. Its causes remain poorly understood, and current animal models—especially bleomycin-induced fibrosis in rodents—offer insights but lack full translatability to human disease.

To overcome these limitations, Epithelix aim to develop a human pulmonary fibrosis (PF) in vitro epithelium model based on the already existing AlveolAir™ model. It combines ATI and ATII cells at the air–liquid interface, co-cultured with endothelial cells, and enables the evaluation of profibrotic compounds using PF-specific biomarkers.

This platform provides a novel tool to study IPF pathogenesis and assess the fibrotic potential of candidate molecules with greater relevance to human biology.

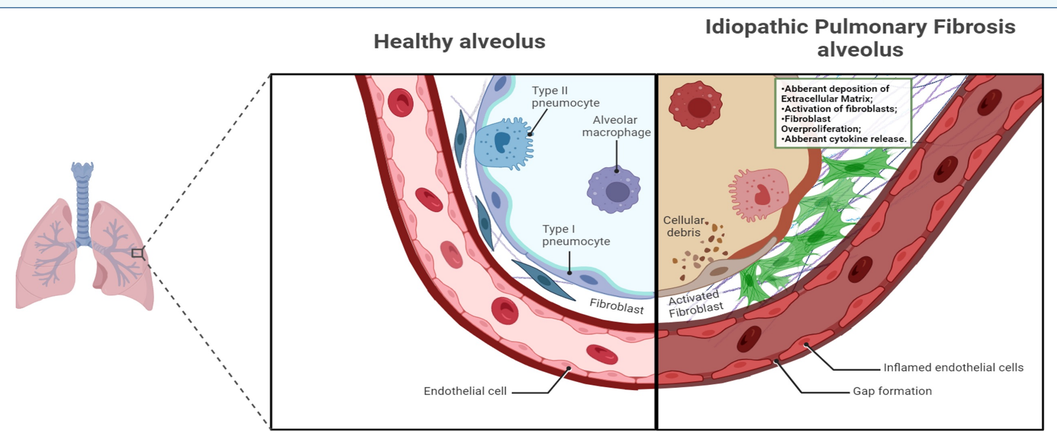


Fig. 1: Schematic representation of healthy and Idiopathic Pulmonary Fibrosis alveoli. (Created with BioRender)

TESTING STRATEGY FOR PF INDUCTION

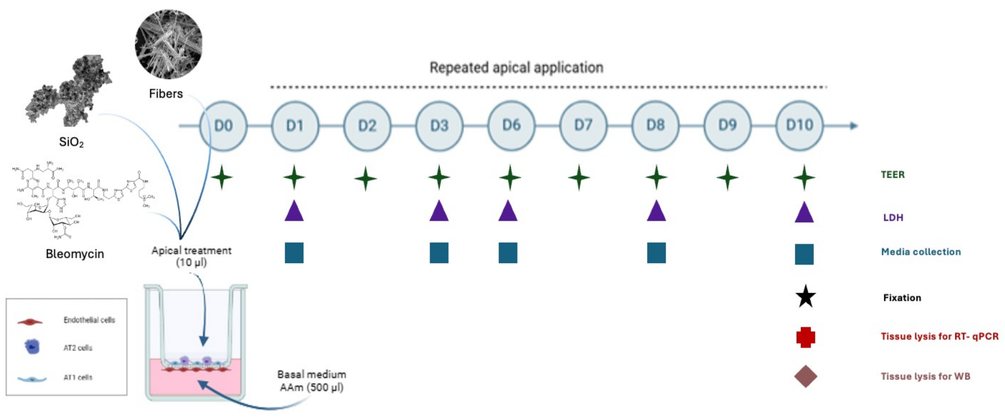


Fig. 2: AlveolAir™ was stimulated with profibrotic agents (Bleomycin 100 µg/ml, SiO_2 100 µg/cm², SiC 50 µg/cm² and Crocidolite 150 µg/cm²) and Epithelix profibrotic cocktail (EPC) (20 µg/ml of TGF-β1 and 50 µg/ml of TNF-α) for 10 days. PF development was monitored through multiple cytokines and gene expression changes.

ADVERSE OUTCOME PATHWAY 173

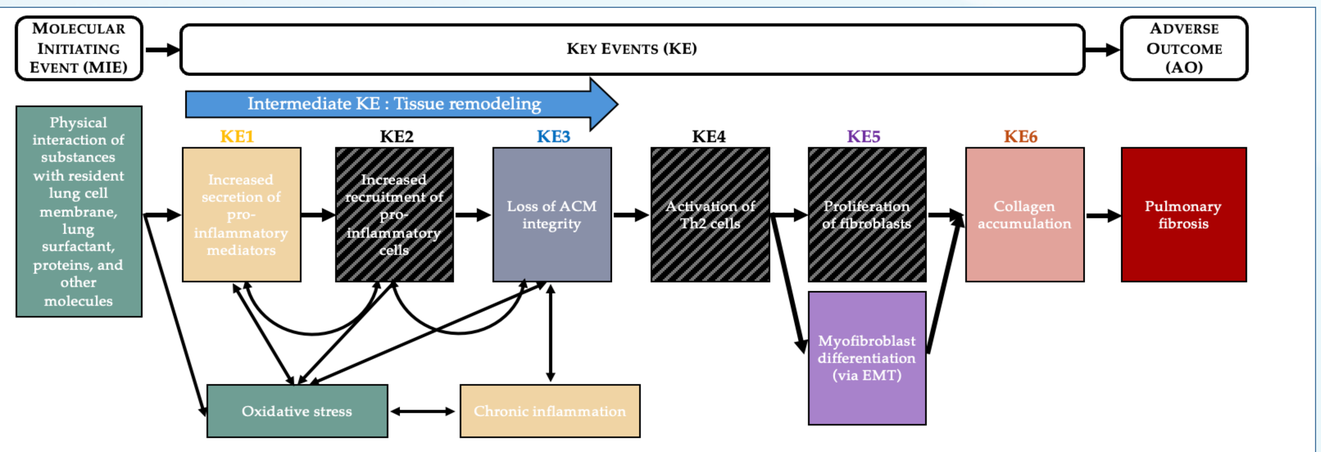


Fig. 3: Adverse Outcome Pathway describing pulmonary injury leading to fibrosis. The Molecular Initiating Event (MIE) involves the physical interaction of substances with resident lung cells. This triggers a cascade of Key Events (KE) that culminate in the adverse outcome of pulmonary fibrosis. Loops involving oxidative stress and chronic inflammation amplify the fibrotic process. KEs shaded in grey and black were not considered.

RESULTS

Alarmin (MIE) and Oxidative stress

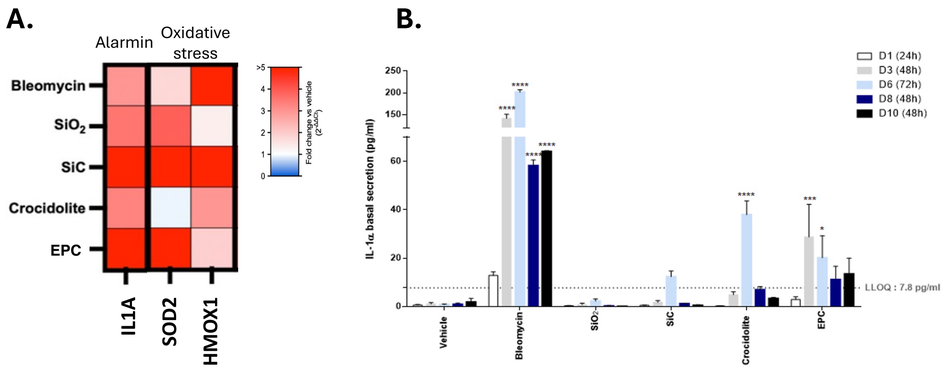


Fig. 4: (A) Gene expression at D10 of alarmin genes (IL-1α) and oxidative stress genes (SOD2 and HMOX1) in fold change vs vehicle (n=3-15). Data represent mean. (B) Dosage of IL-1α concentration through ELISA on basal side of AlveolAir™ (n=3-6). Data represent mean ± SEM.

KE1 : Pro-inflammatory and pro-repair mediators

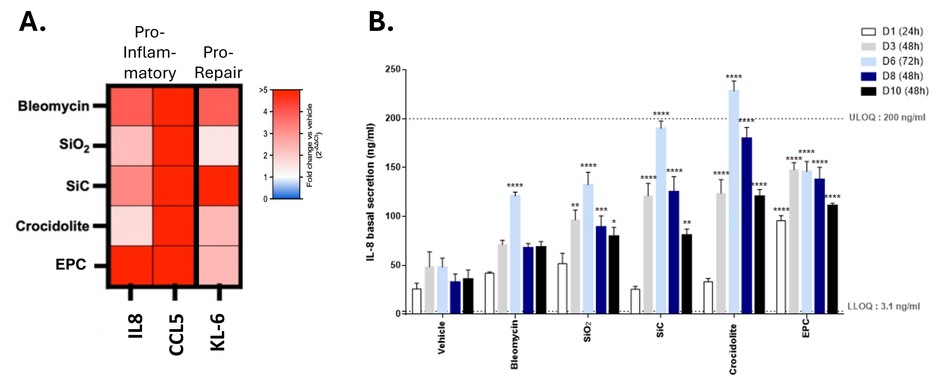
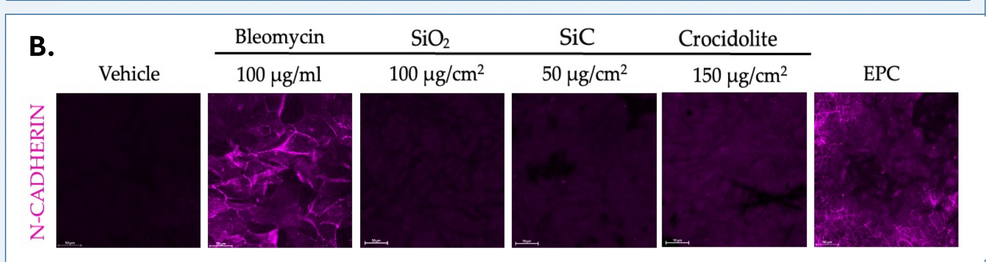


Fig. 5: (A) Gene expression at D10 of pro-inflammatory and pro-repair genes (IL-8, CCL5, KL-6) in fold change vs vehicle (n=2-10). Data represent mean. (B) Dosage of IL-8 concentration through ELISA on basal side of AlveolAir™ (n=3-6). Data represent mean ± SEM.



Intermediate KE: Growth factors and MMPS

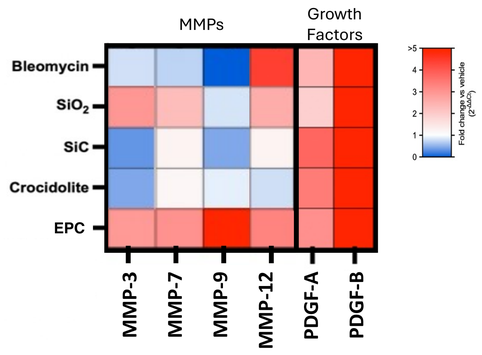


Fig. 6: Gene expression at D10 of MMPs (MMP-3, MMP-7, MMP-9, MMP-12) and growth factors (PDGF-A, PDGF-B) in fold change vs vehicle (n=2-6). Data represent mean.

KE3: Barrier Integrity

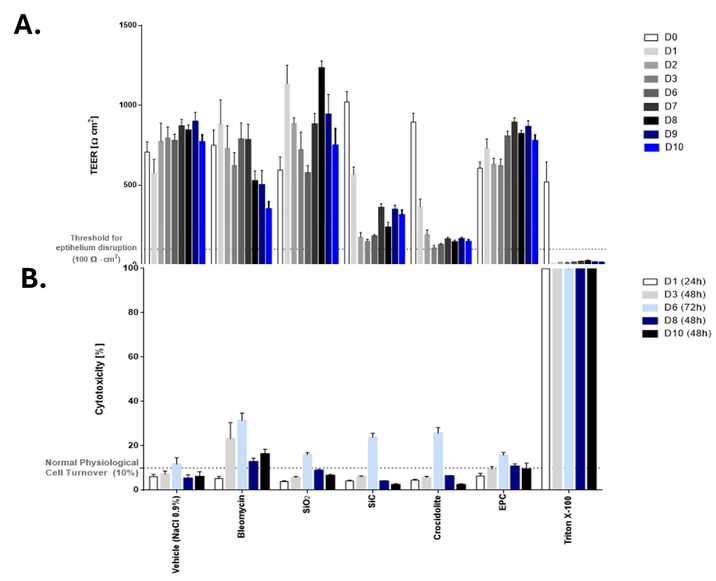


Fig. 7: Tissue integrity after repeated treatment to profibrotic agents for 10 days (n=5-12 for TEER (A), n=3-6 for LDH (B)). Data represent mean ± SEM.

KE5: Epithelial-to-Mesenchymal Transition

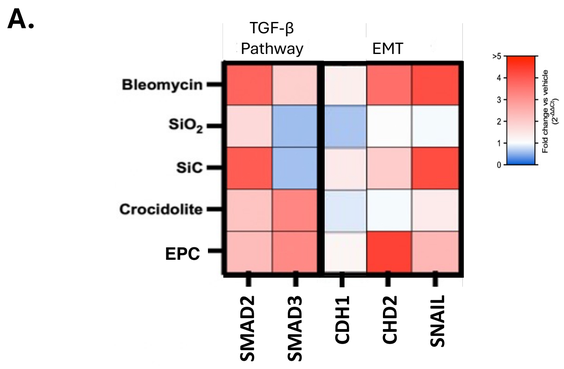


Fig. 8: (A) Gene expression at D10 of EMT-related genes (SMAD2, SMAD3, CDH1, CDH2 and SNAIL) in fold change vs vehicle (n=2-15). Data represent mean. (B) Immunostaining of alveolar epithelial cells with mesenchymal marker N-Cadherin (CDH2).

KE6 : Extracellular Matrix (ECM) accumulation

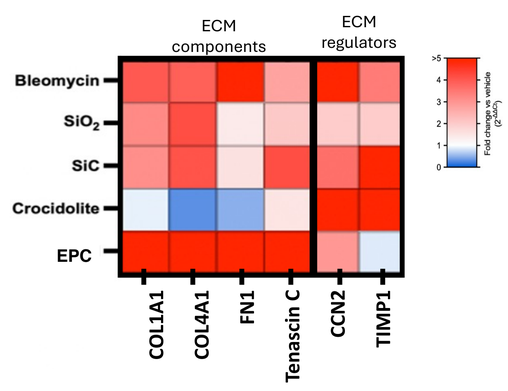


Fig. 9: (A) Gene expression at D10 of ECM components (COL1A1, COL4A1, FN1, TNC, CCN2 and TIMP1) in fold change vs vehicle (n=2-6). Data represent mean.

CONCLUSION

Our PF AlveolAir™-based model successfully recapitulates key mechanisms involved in the onset and progression of pulmonary fibrosis. The model responded robustly to diverse fibrogenic stimuli – including profibrotic cytokines (TGF-β1 and TNF-α), the chemotherapeutic agent bleomycin, silica nanoparticles (SiO_2) and fibrous nanomaterials such as SiC nanowires and crocidolite. Our long-term PF model holds promise for advancing preclinical antifibrotic screening and profibrotic agents screening, deepening the understanding of molecular mechanisms in IPF.