Advances in *In Vitro* Modeling of Lung Diseases: Focus on Precision-Cut Lung Slices (PCLS) Model

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Symposium:

Innovations to Fight Respiratory Diseases

Prevention, research and treatments.

December 3-4, 2024 – Biocitech Paris-Romainville

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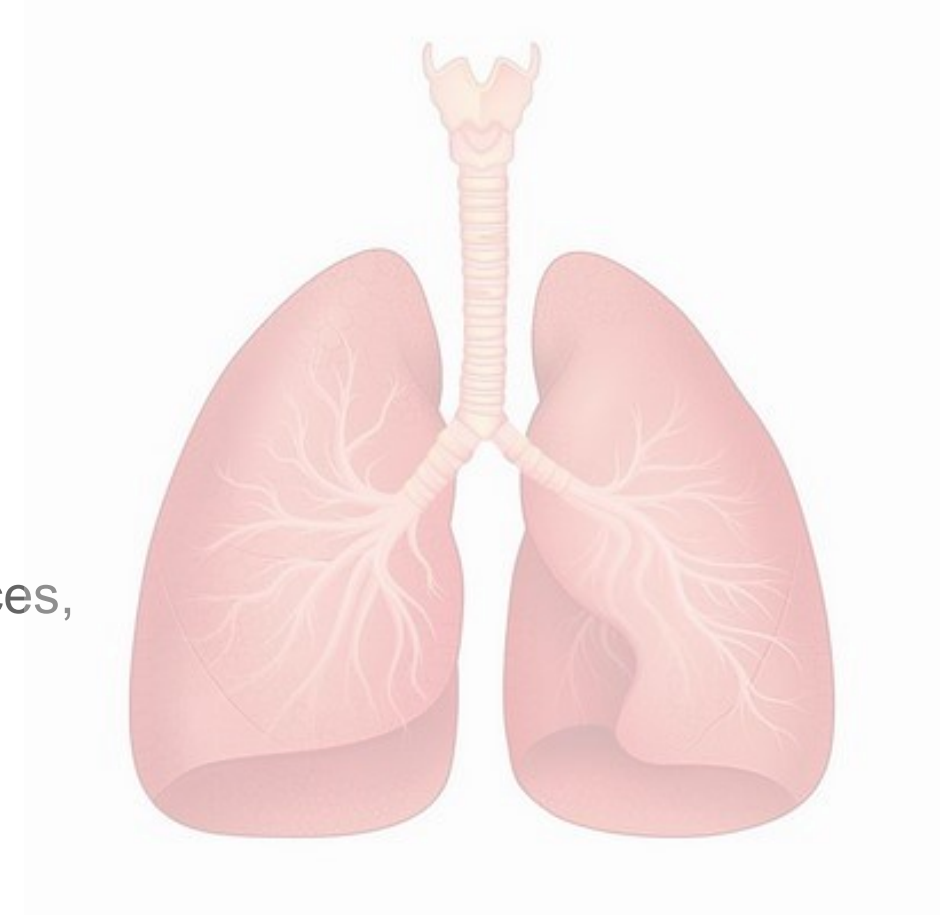
Outline

- Introduction :
  - Lung diseases / Lung fibrosis
- *In vitro* models used to model lung fibrosis
  - 2D, 3D, and *ex vivo* (PCLS)
- Case studies
  - Cell migration (2D model)
  - Inflammation / nebulizer system (3D lung model)
  - Induction of fibrosis (human precision-cut lung slices, hPCLS, model)



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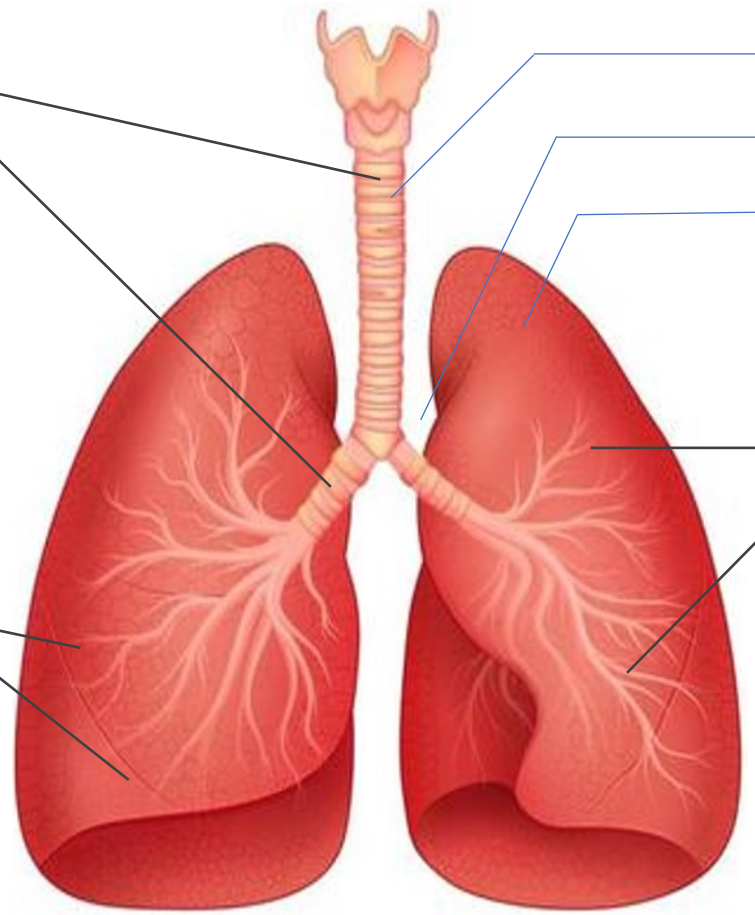
Most commun lung diseases

Airway Disease:
Air movement

Asthma, COPD, Bronchitis, emphysema

Lung tissue disease:
Damaged tissue from scarring or inflammation

Pulmonary fibrosis, sarcoidosis



Lung circulation disease:
Blood movement

Pulmonary hypertension, pulmonary edema

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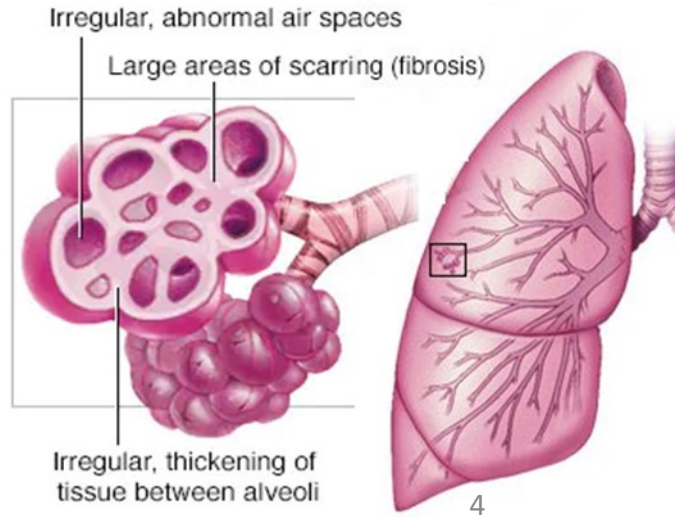
Pulmonary fibrosis

- Pulmonary fibrosis is a chronic and a progressive lung disease characterized by:
  - Excessive deposition of extracellular matrix (ECM) proteins in the lungs
  - This leads to scarring and thickening of lung tissues and loss of lung function, ultimately making it difficult for oxygen to pass through
  - Common symptoms: Cough, shortness of breath, fatigue …
- The exact cause of pulmonary fibrosis is often unknown (*i.e.* idiopathic)

➔ But it is believed to be a result of inflammation in the lungs

Alveoli in normal lung

Alveoli in pulmonary fibrosis

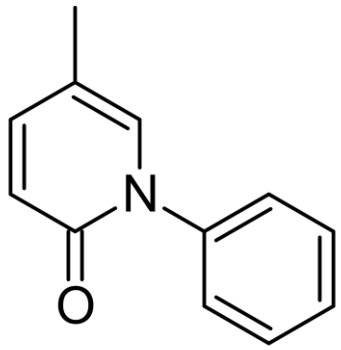


MAYO Foundation for medical education and research

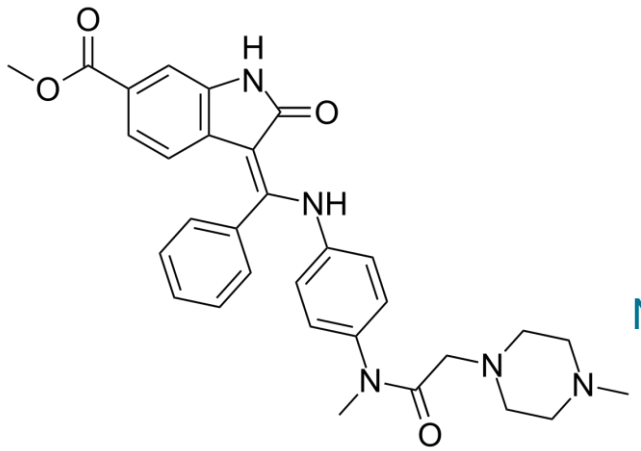
Pulmonary Fibrosis: Treatment Options

- Two FDA-approved drugs for the treatment of idiopathic pulmonary fibrosis (IPF):
  - Pirfenidone: an anti-inflammatory drug, approved for the treatment of mild-to-moderate IPF.
  - Nintedanib: a tyrosine kinase inhibitor, approved for the treatment of mild-to-moderate IPF.

- Pirfenidone and nintedanib have been shown to slow the progression of IPF down and improve lung function in clinical trials. However, they may have side effects and are not effective for all patients.



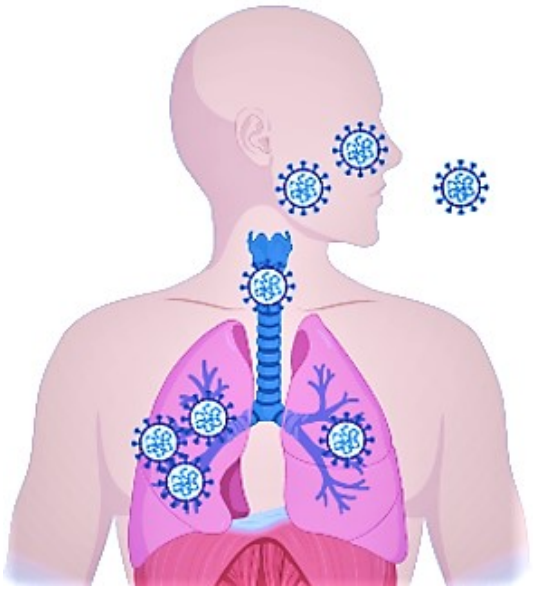
Pirfenidone
MW:185.2



Nintedanib
MW: 539.6

COVID-19: A Potential Risk Factor for Pulmonary Fibrosis

- About **44.9%** of COVID-19 survivors developed pulmonary fibrosis[1]
- Post-COVID-19 pulmonary fibrosis (PCPF) is one of the most worrying pulmonary complications as it causes permanent lung damage
- Cellular and molecular pathways contributing to PCPF are poorly-defined
- The pathogenesis of this emergent syndrome urgently needs to be studied



- Developing new treatments for pulmonary fibrosis is critical

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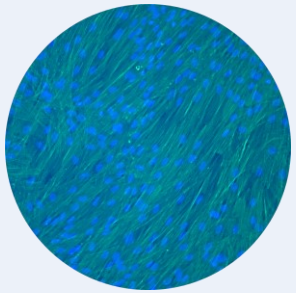
[1]*Hama Amin et al., Post COVID-19 pulmonary fibrosis; a meta-analysis study. Ann Med Surg (Lond). 2022 May;77:103590*

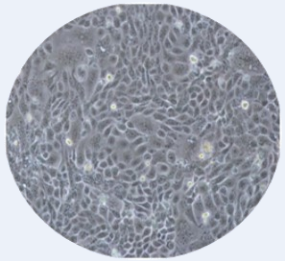
Biological systems to model lung disease

2D models



Primary Human lung fibroblasts

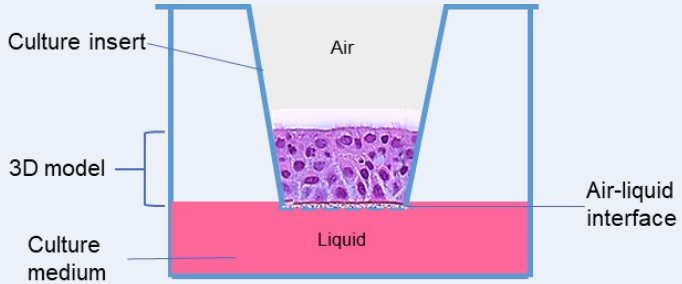
Exhibit characteristics of myofibroblasts.They respond to various stimuli that induce fibrosis

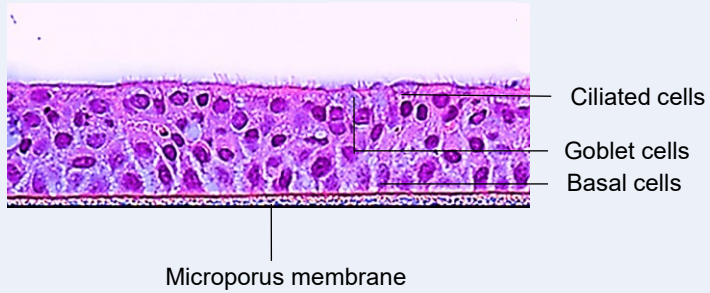


Calu-3 Human lung adenocarcinoma cell line.

Exhibit characteristics of both type II alveolar epithelial cells and submucosal gland cells

3D models



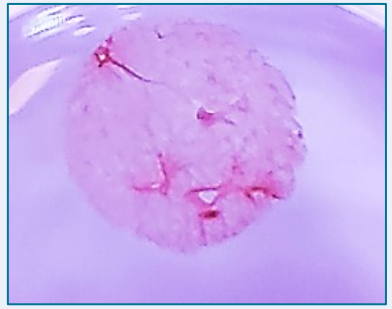


Epithelix, MatTek, etc

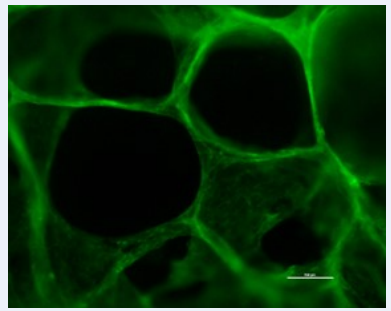
3D lung models can be used to study lung fibrosis and other diseases

Ex vivo models

Precision-cut lung slices (PCLS)



hPCLS (macroscopic)



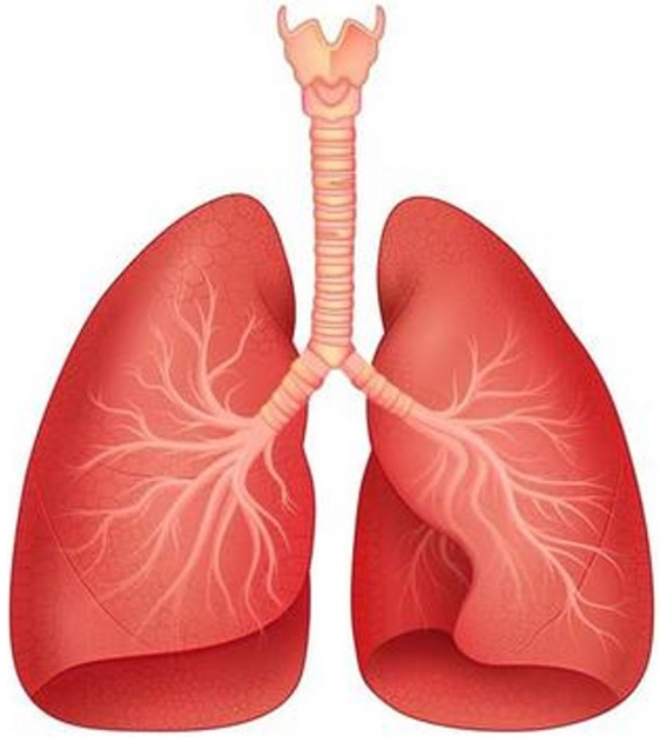
hPCLS (microscopic)

Realistic representation of the lung microenvironment

Gold standard. Retain the native architecture and all cell types of the lung

How to study Pulmonary fibrosis *in vitro*?

- Assessing the Therapeutic Potential of Antifibrotic Agents:
  - Investigate the effects of profibrotic stimuli
    - Transforming growth factor-β (TGF-β),
    - Profibrotic cocktail
    - Inflammatory agent
  - Assess the efficacy of potential antifibrotic drugs
    - Pirfenidone
    - Nintedanib
    - Beclospin
  - Multidimensional Endpoints
    - Cell migration (2D model)
    - Anti-inflammatory effects (3D model)
    - mRNA expression, proteomics, histopathology (PCLS model)



Case study - 2D model

Cell migration and expression of fibrosis markers

Cell migration – Scratch test

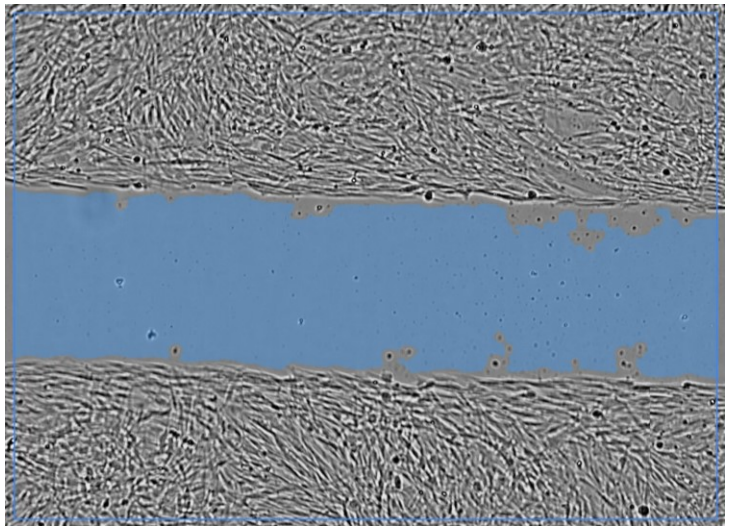
- **Rational:**
  - Lung fibrosis characterized by proliferation of fibroblasts with excessive accumulation of extracellular matrix (ECM) within the lung
  - Targeting fibroblast proliferation and ECM deposition represents a logical therapeutic strategy for both delaying disease onset and, potentially, reversal of disease pathology
- **Methods:**
  - Primary human lung fibroblasts
  - Treatments:
    - TGF-β
    - Nintedanib and Pirfenidone
    - Taxol as positive control
  - Live cell imaging of cell migration
  - mRNA expression of fibrosis markers:
    - LRRC15, ACTA2, FN1, MMP12, COL1A1, COL3A1

Ibidi® culture-insert

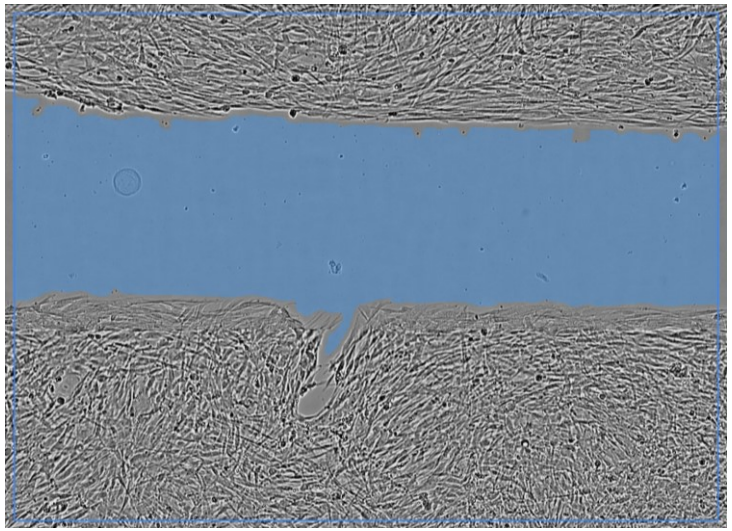
CytoSMART Omni brightfield device

Live-cell imaging of cell migration

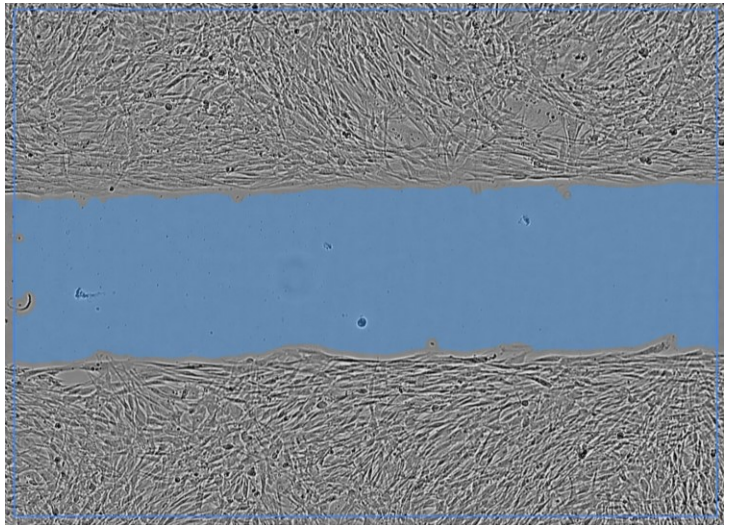
- Human lung fibroblasts treated with Pirfenidone or Nintedanib for 24 hours



Control

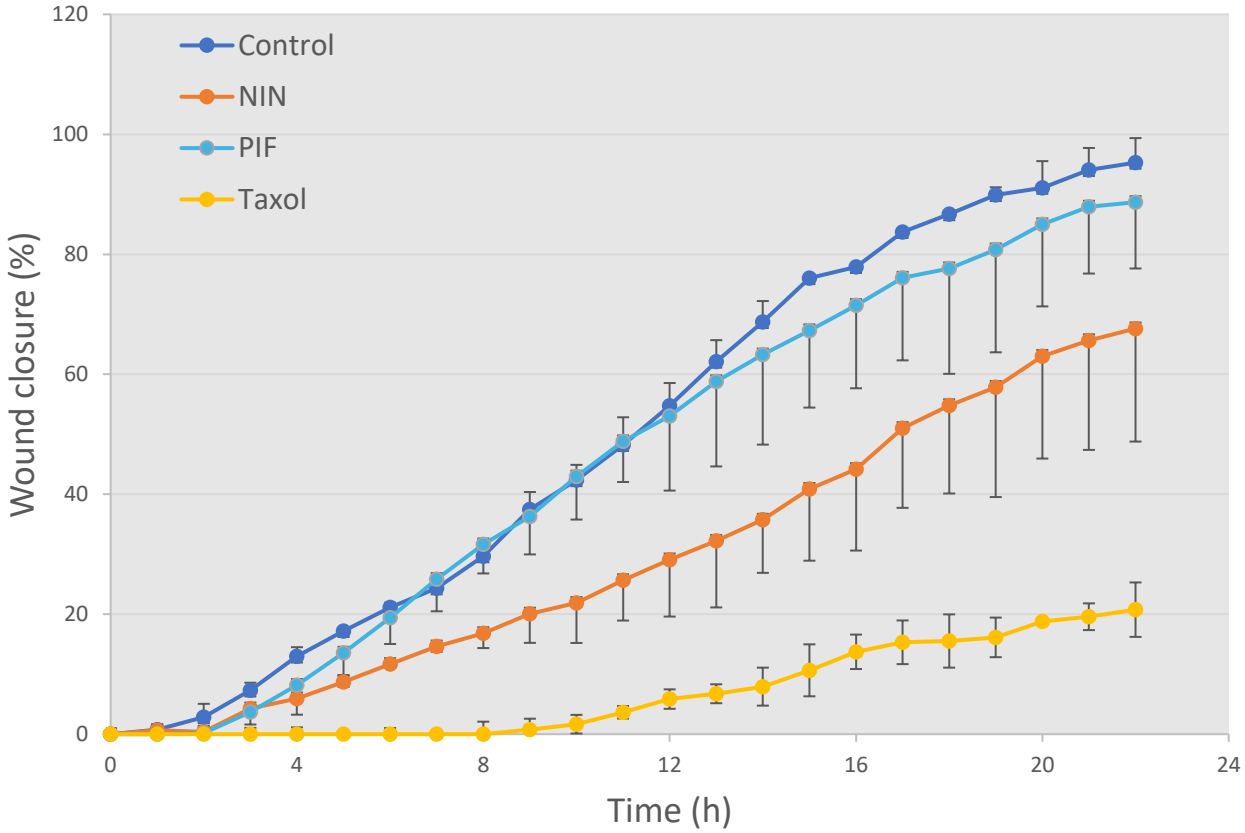


Pirfenidone 10 µM



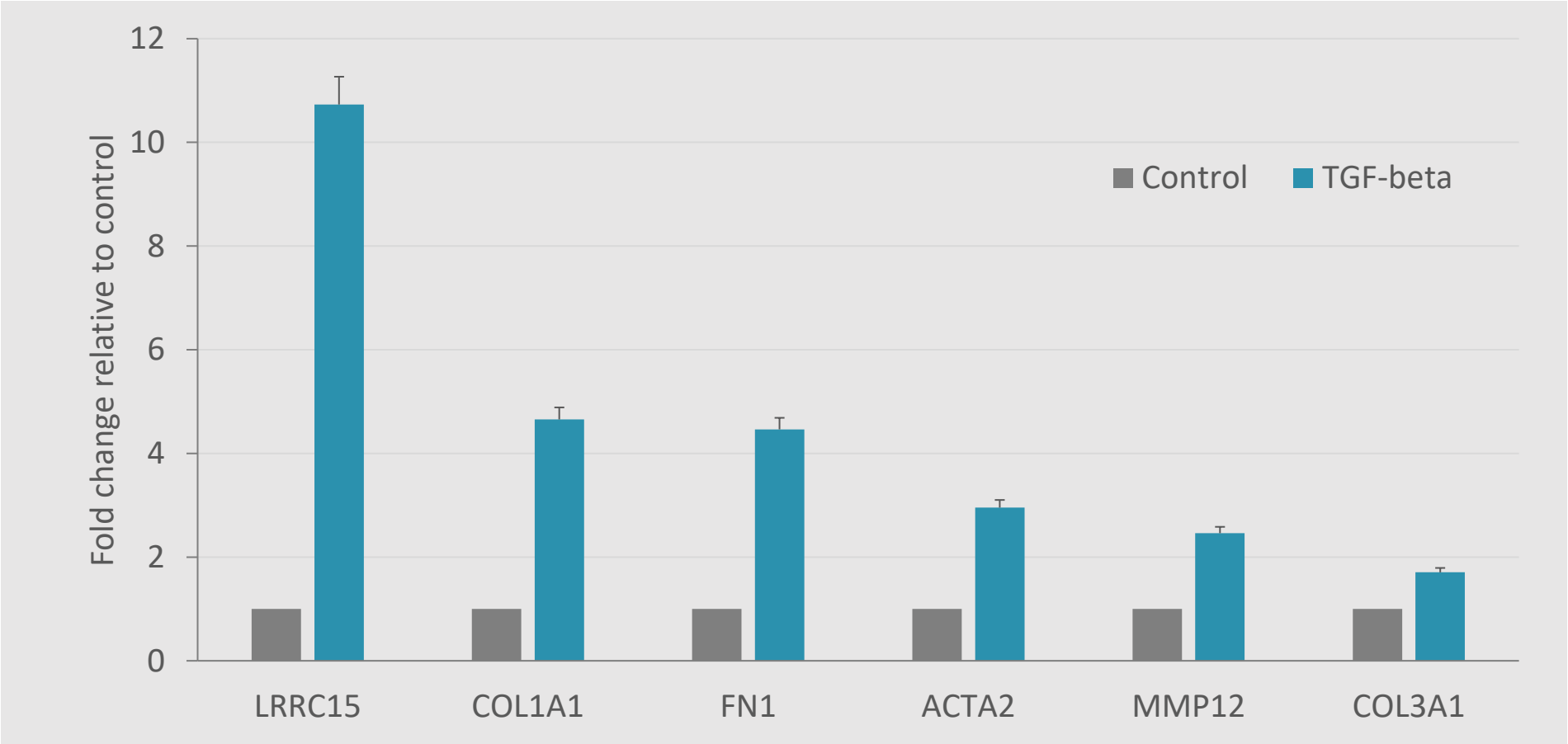
Nintedanib 1 µM

Effect of treatments on cell migration



- Nintedanib markedly decreased cell migration in human lung fibroblasts
- Pirfenidone had no or little effect on cell migration.

TGF-β increases the expression of fibrosis markers



- Treatment of human lung fibroblasts with TGF-β increased mRNA expression of pulmonary fibrosis markers: LRRC15, COL1A1, FN1, ACTA2, MMP12 and COL3A1

Case study - 3D model

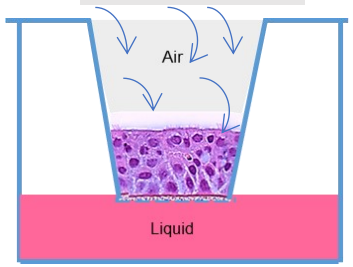
Expression of inflammatory markers

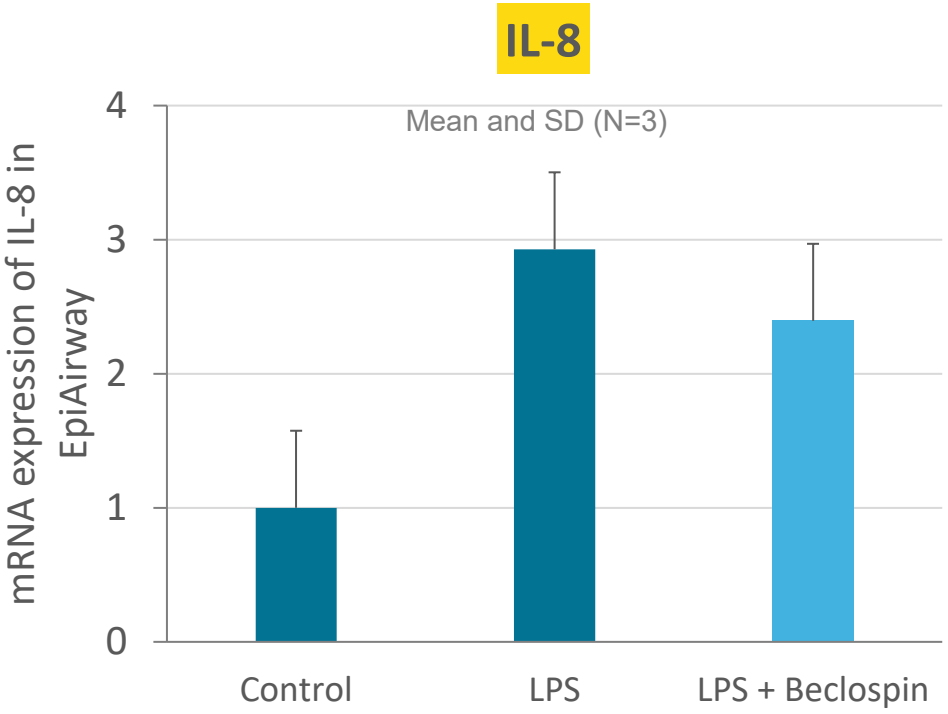
Case study – Inflammation assay

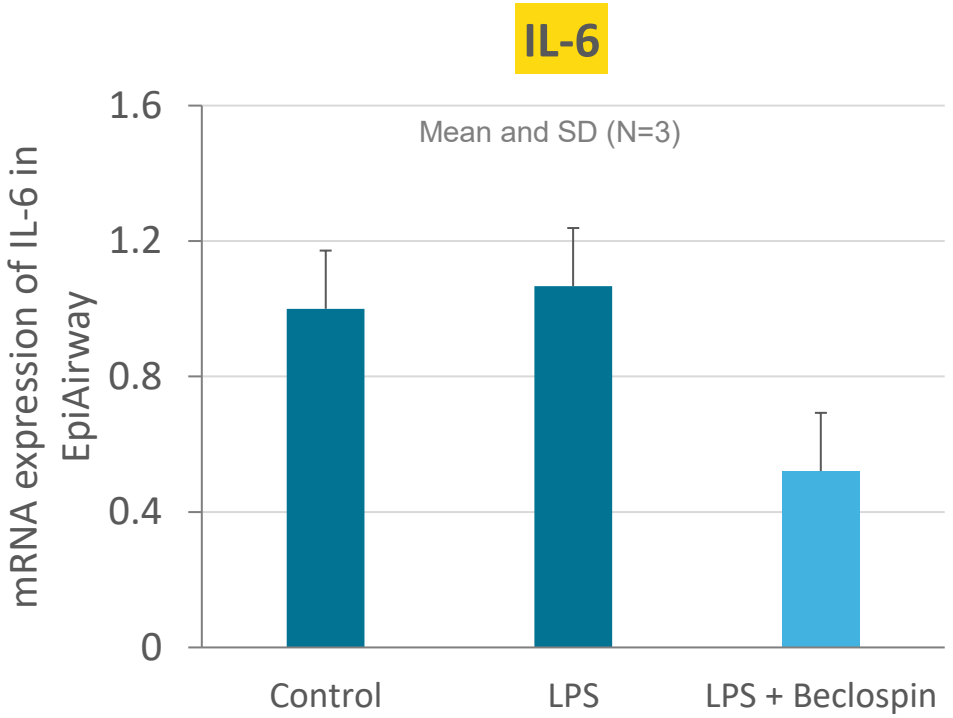
- **Rational:**
  - The exact cause of pulmonary fibrosis is often unknown, but it is believed to be a result of inflammation in the lungs.
  - Targeting lung inflammation represents a therapeutic strategy for delaying disease onset
- **Methods:**
  - EpiAirway™ (3D model)
  - Stimulation:
    - LPS
    - LPS + Beclospin (nebulizer system)
  - mRNA expression of inflammatory markers:
    - IL-8 and IL-6 (TaqMan RT-qPCR)

Expression of inflammatory markers after nebulized Beclospin

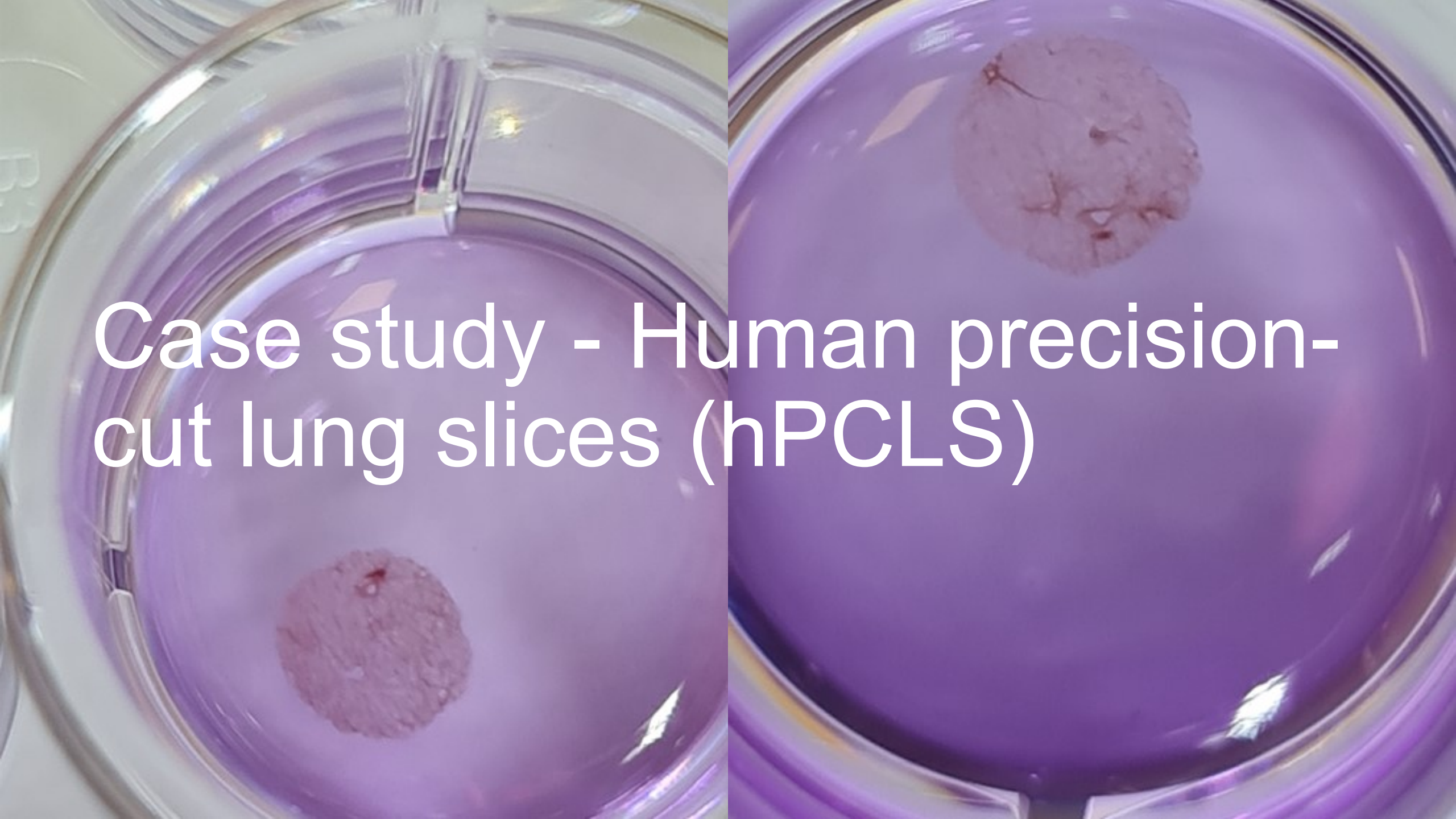
Beclospin







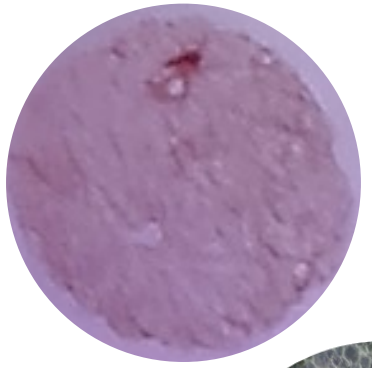
- Treatment with LPS increased IL-8 mRNA expression in EpiAirway™ tissues.
- The use of the nebulizer system to expose EpiAirway™ tissues to Beclospin resulted in a decrease in mRNA expression of inflammatory markers IL-8 & IL-6.



Case study - Human precision-cut lung slices (hPCLS)

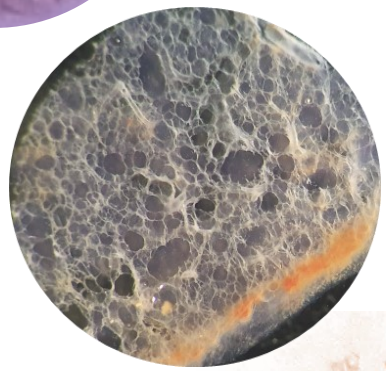
Human Precision-Cut Lung Slices (hPCLS)

- hPCLS are thin slices with precision thickness (200 – 300 µm) obtained from fresh lung tissue

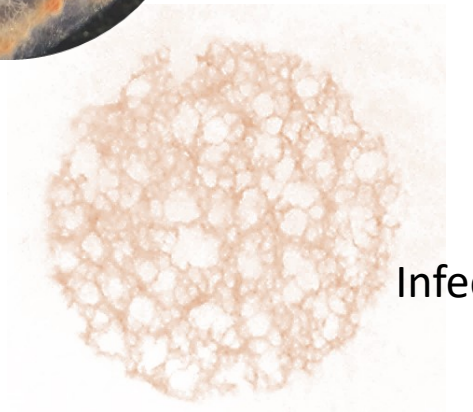


"Healthy" hPCLS

- Advantages and Applications of hPCLS:
  - Maintains lung architecture
  - Contains all types of lung cells
  - Reacts to different stimuli
  - Useful for drug screening
  - Models various lung diseases
  - Useful for safety testing of inhaled substances

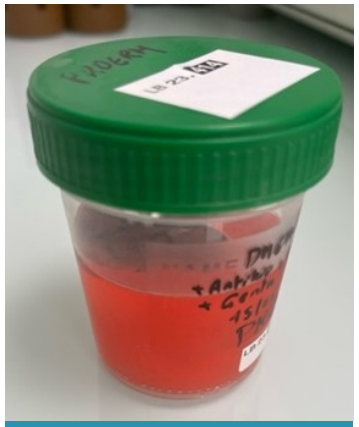


Fibrotic hPCLS



Infected hPCLS

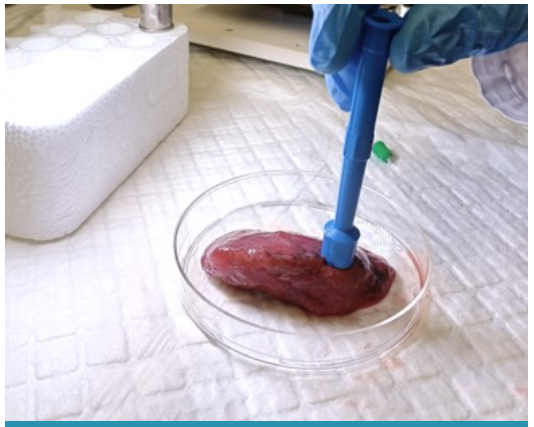
How to Prepare hPCLS ?



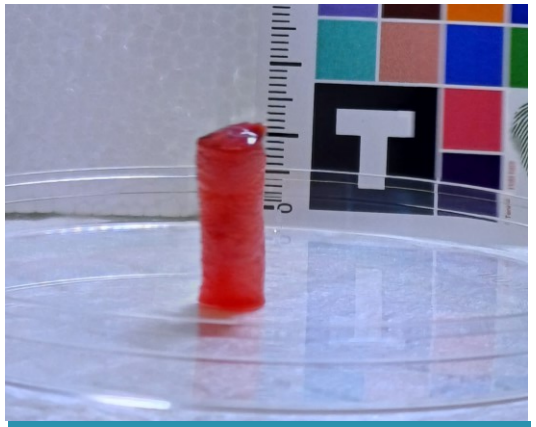
Lung resection

Biobanque CRB
Tumorothèque de Nice
BB-0033-00025

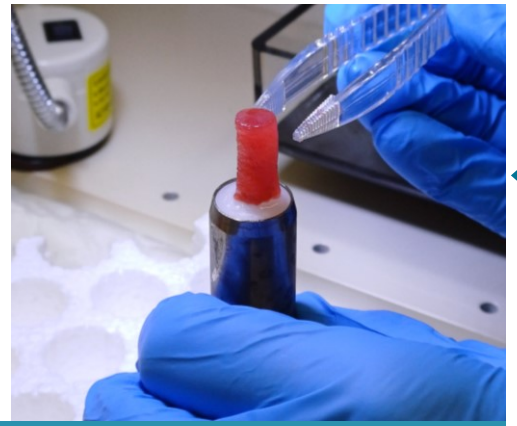
Low melting agarose inflation

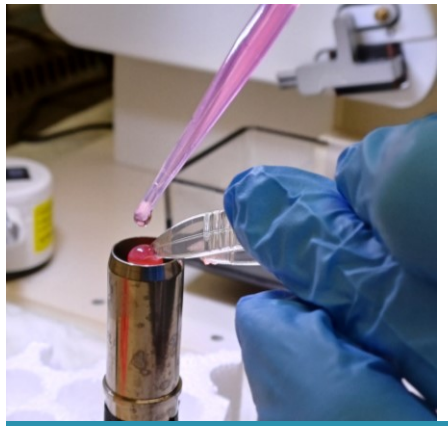


Biopsy of 8 mm Ø



Lung core

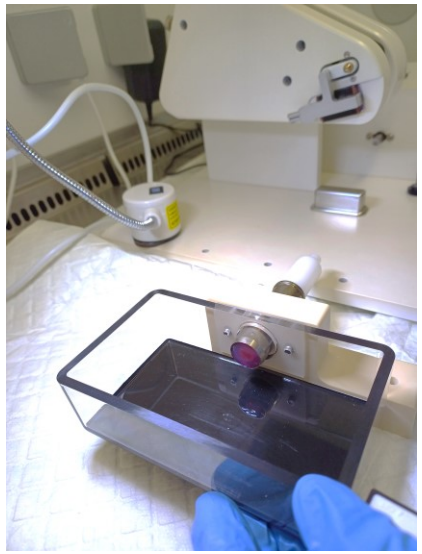
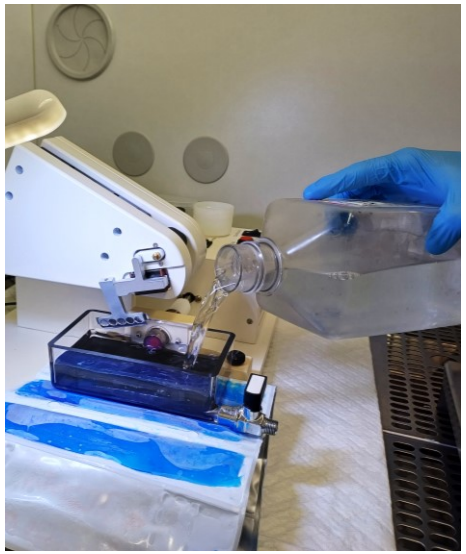
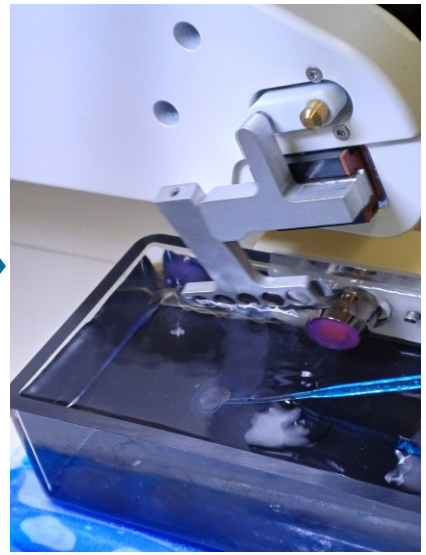
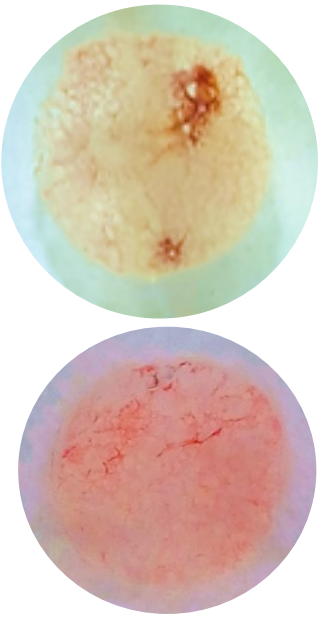




Mounting and embedding lung core on the holder

How to Prepare hPCLS ?

Slicing of lung core using the vibrating microtome Compresstome® VF-300-0Z

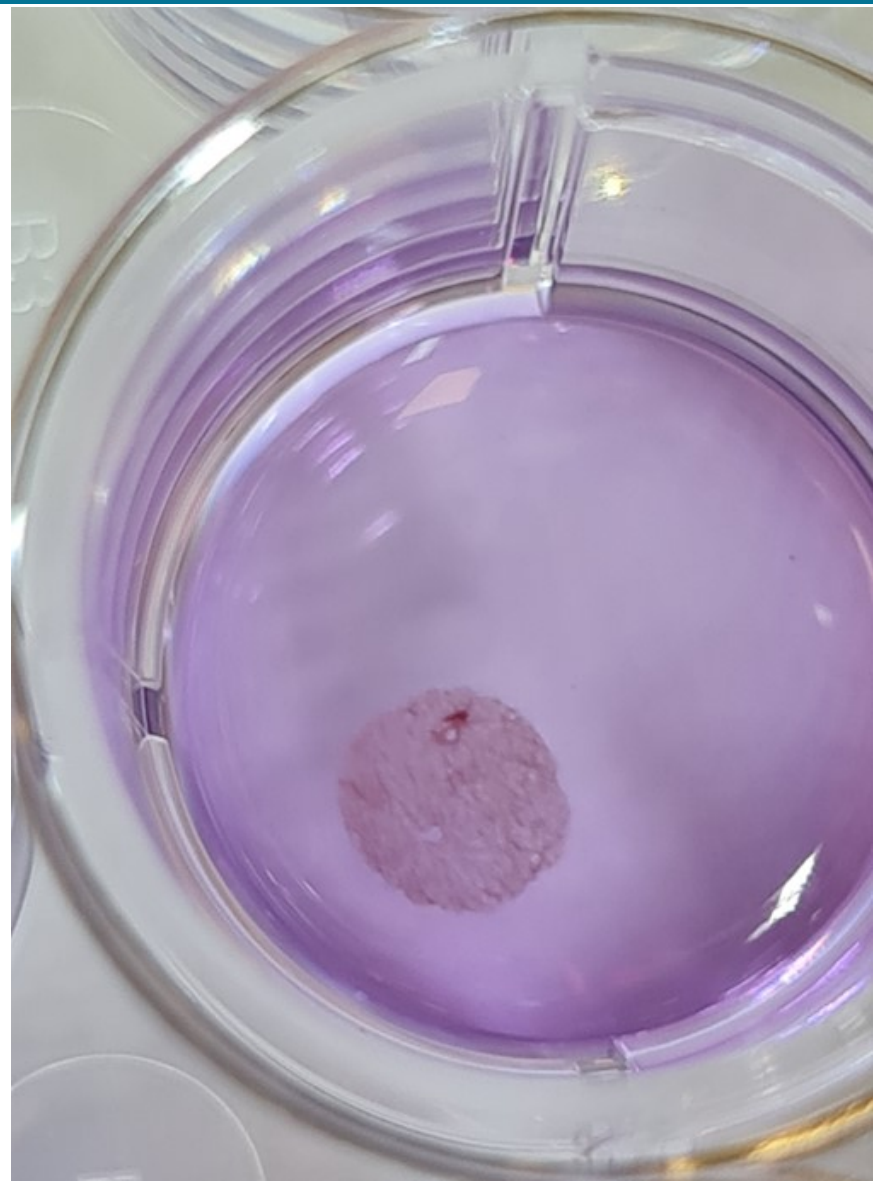
300 µm thick hPCLS

Preparation of hPCLS

Compresstome® VF-300-0Z

hPCLS

Multiple hPCLS of 300 µm can be successfully obtained from one lung resection



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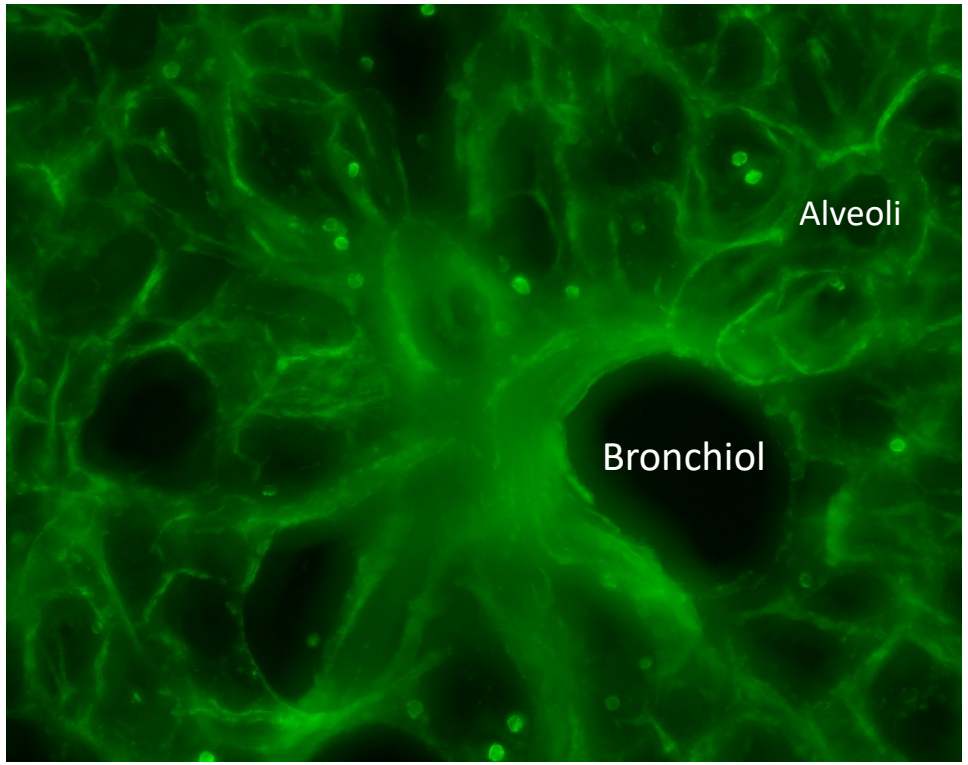
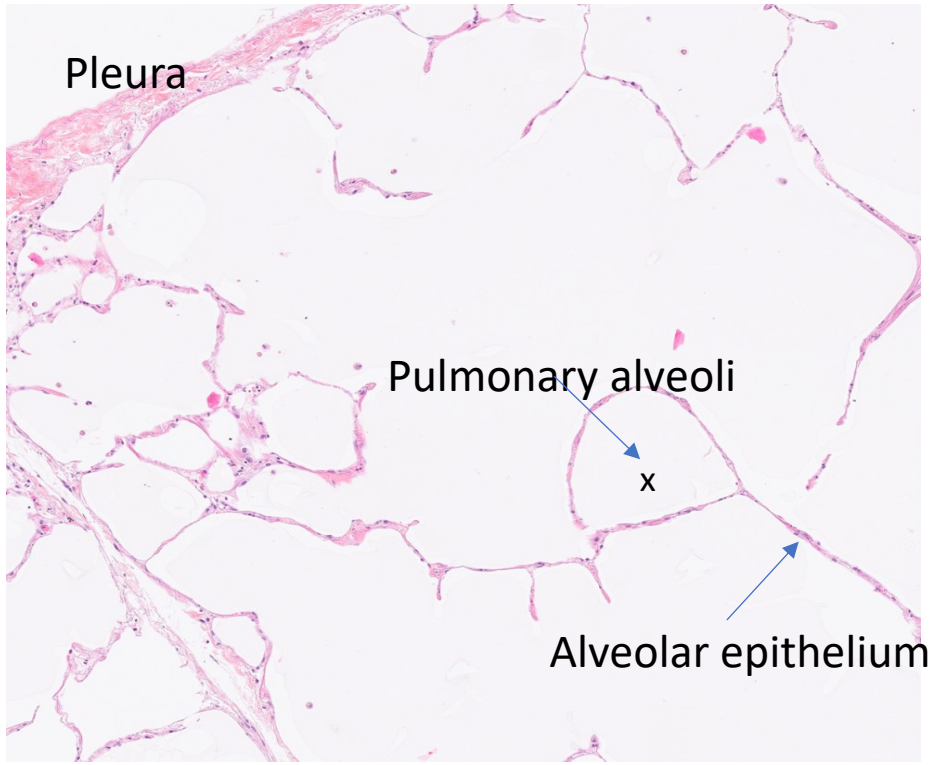
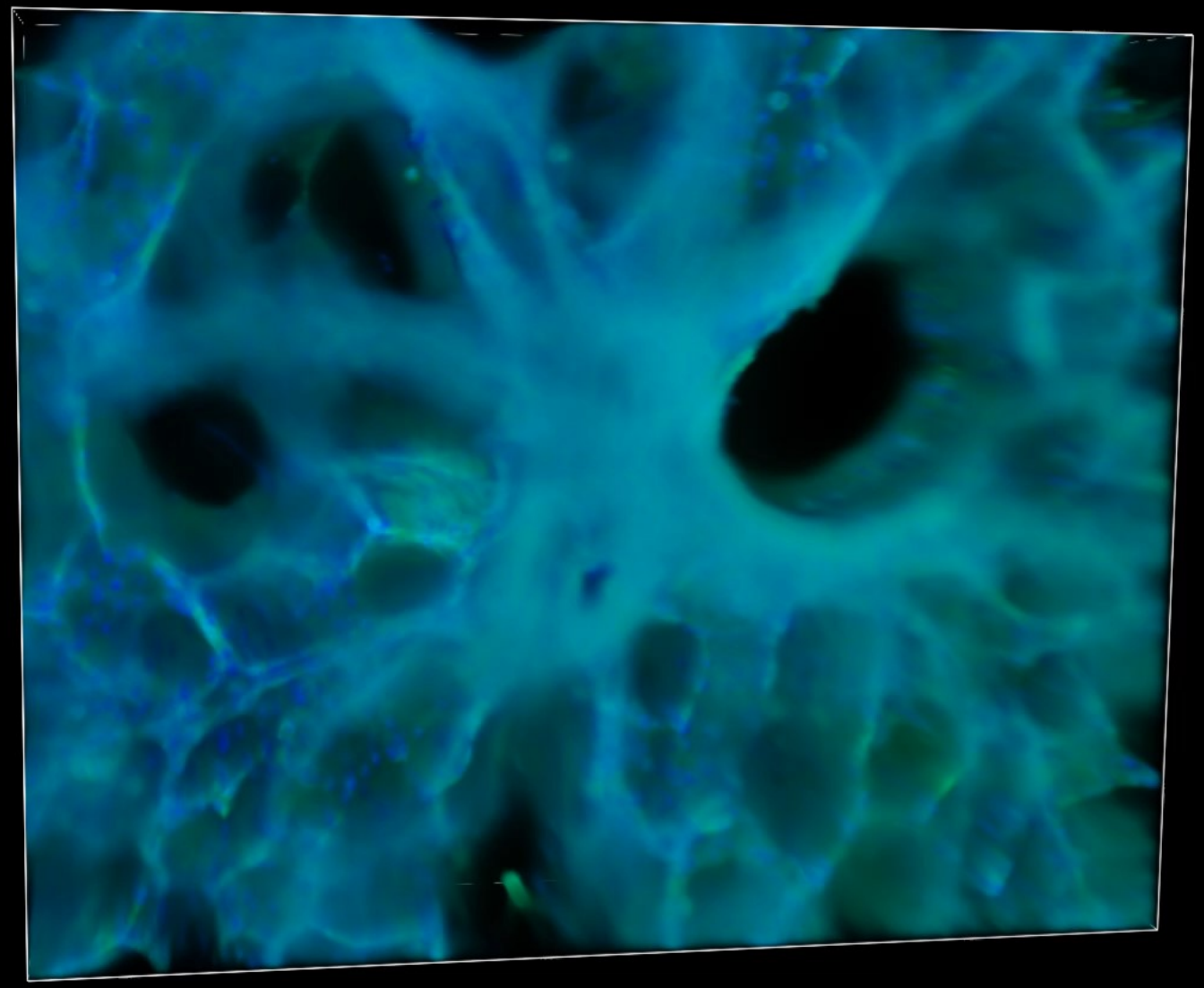
hPCLS maintains lung architecture

hPCLS in culture

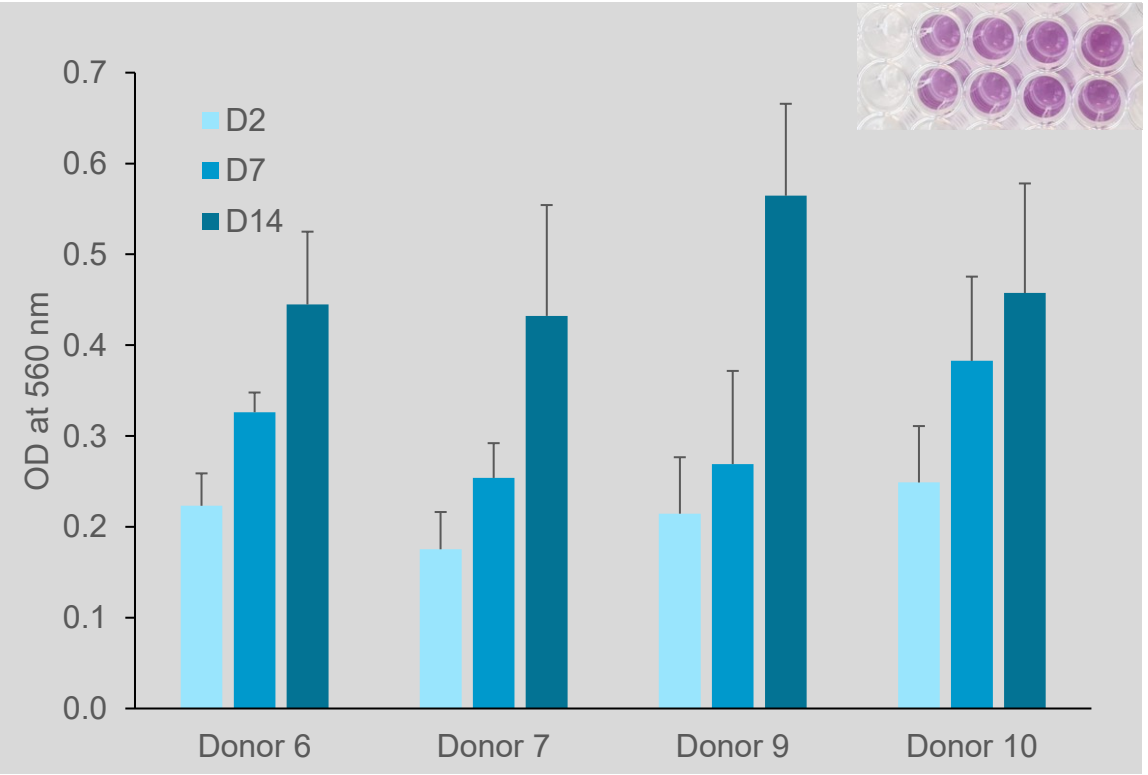




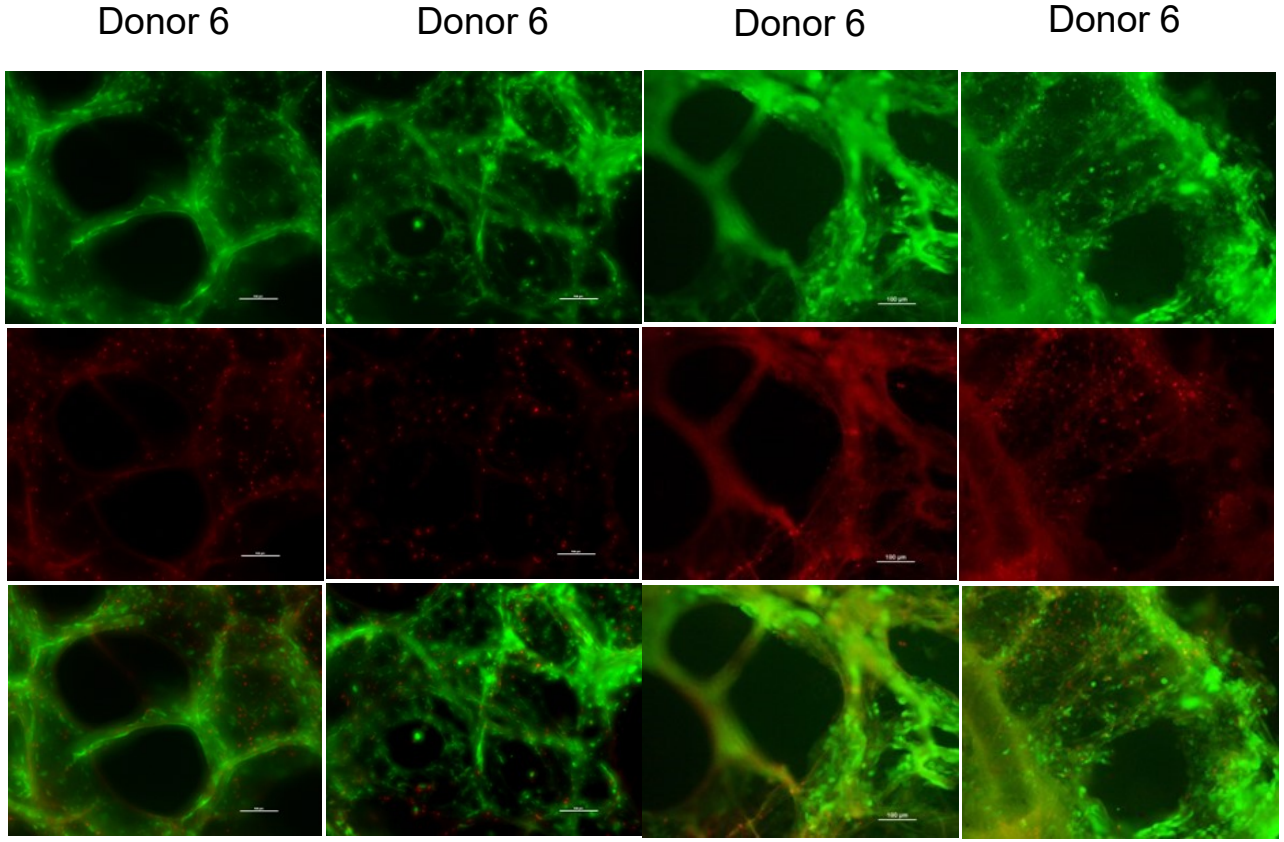


Viability of hPCLS is maintained over 14 days

MTT assay



Live/dead assay at Day 7



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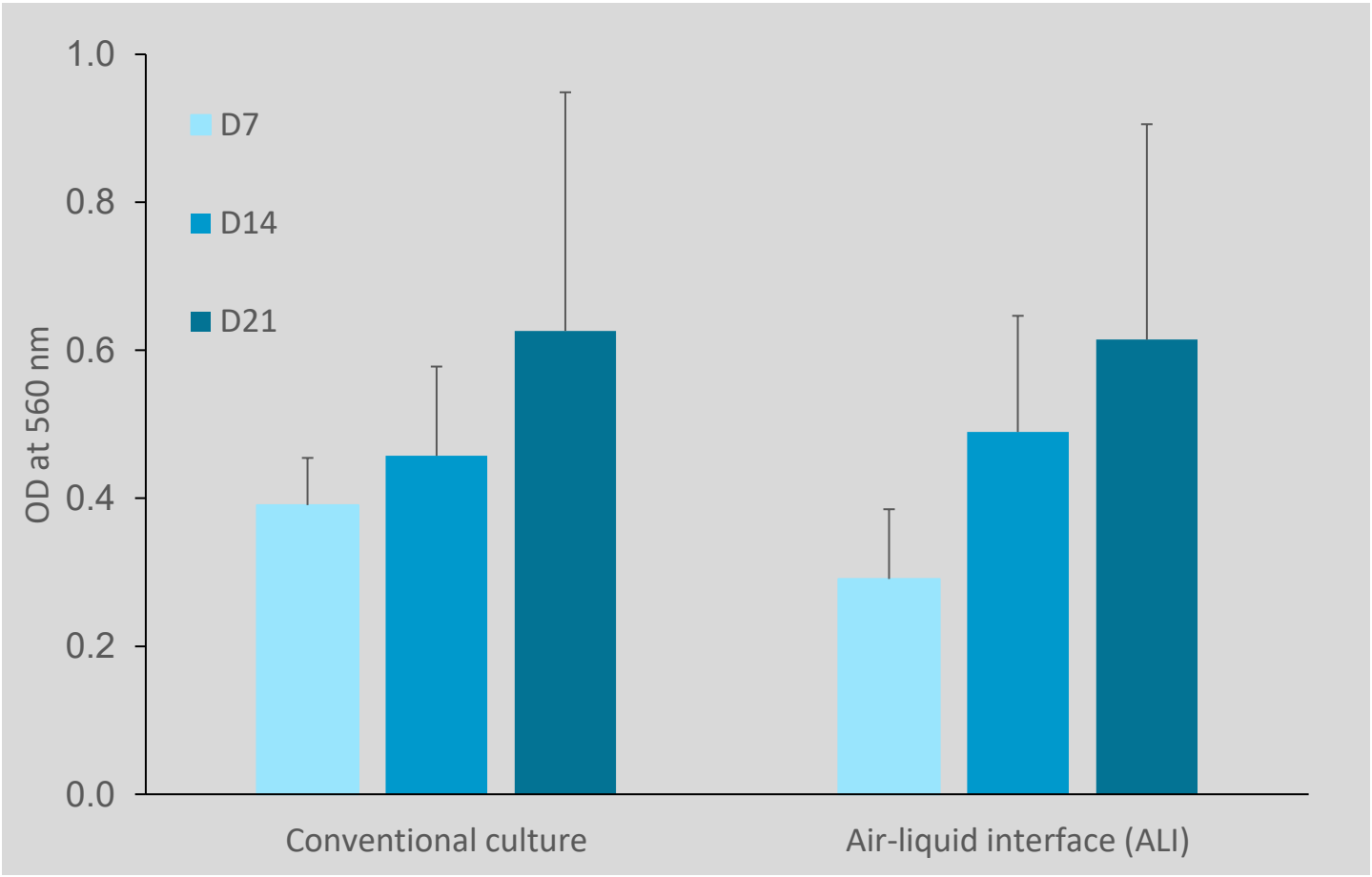
Viability of hPCLS in ALI conditions

- Viability of hPCLS is maintained up to 21 days when culture at the air-liquid interface

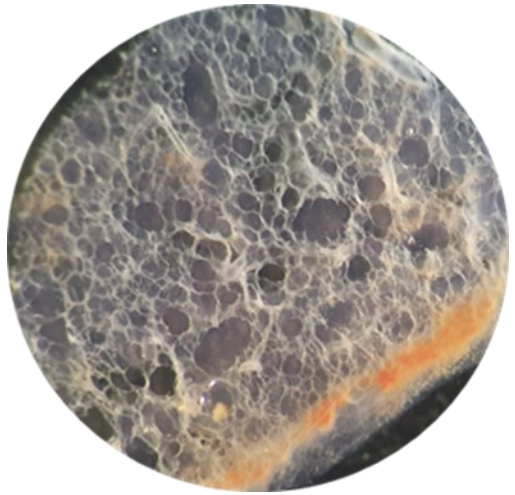
Culture of hPCLS at the air-liquid interface

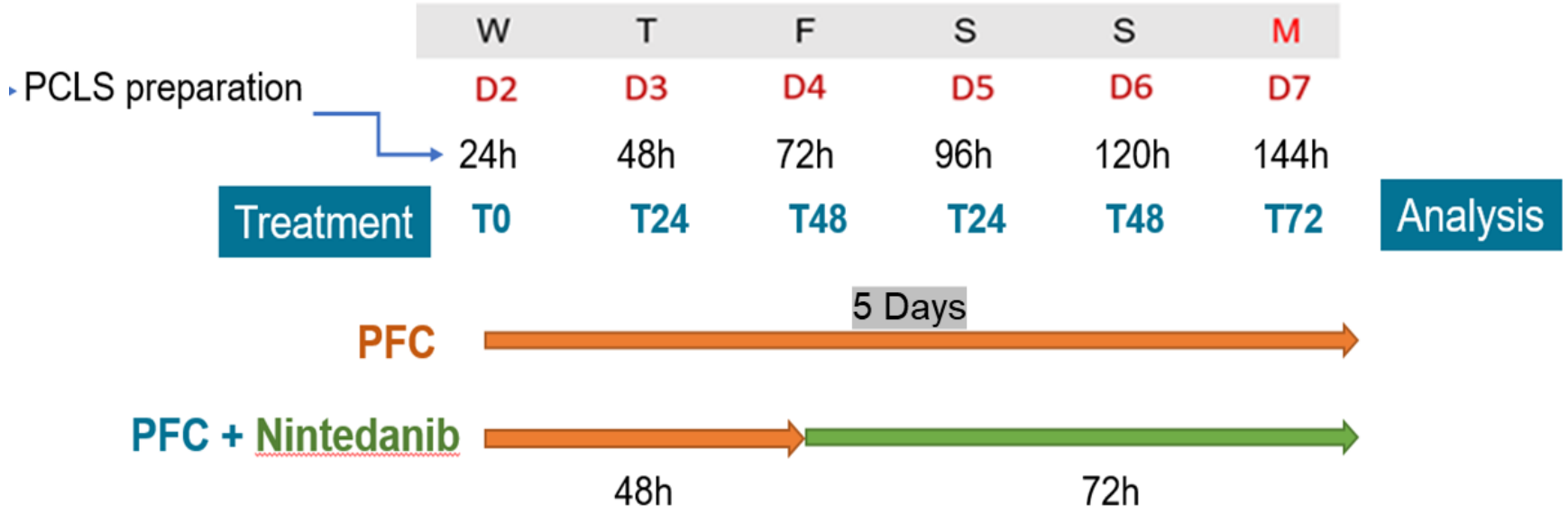


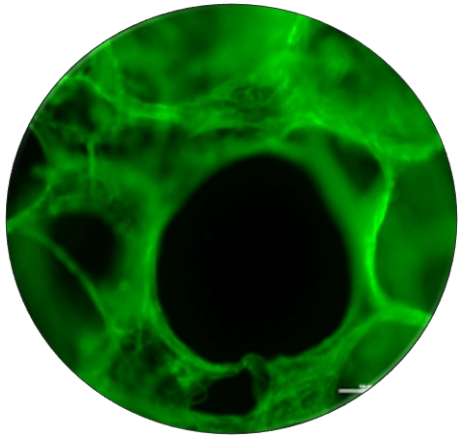
Induction of lung fibrosis in PCLS

Profibrotic Cocktail (PFC)[1]:

10 ng/mL platelet-derived growth factor-AB (PDGF-AB)
5 ng/mL recombinant transforming growth factor-beta (TGF-β)
10 ng/mL tumor necrosis factor-alpha (TNF-α)
5 µM lysophosphatidic acid (LPA)

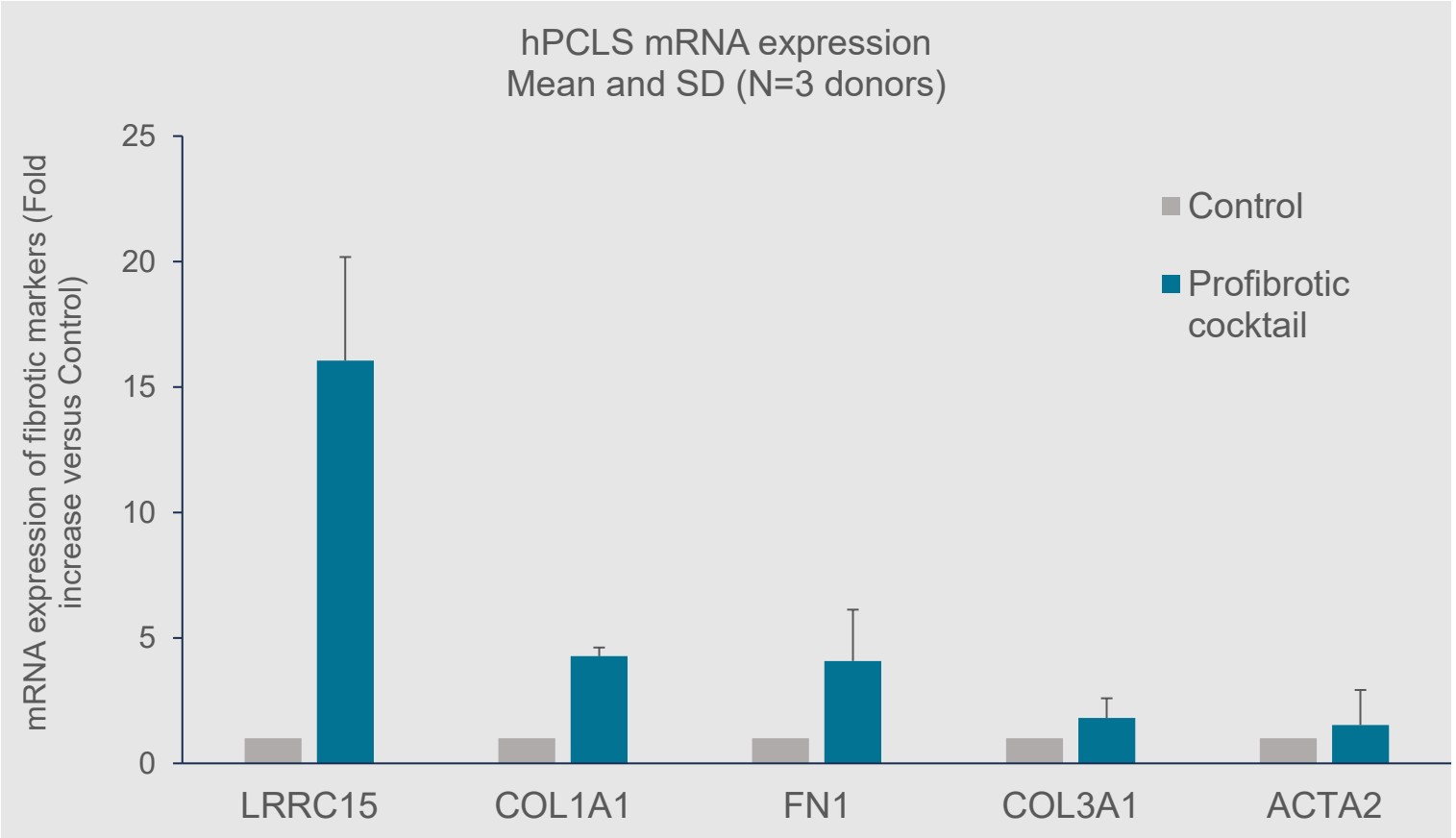






[1]Alsafadi et al., 2017

Expression of fibrotic markers in hPCLS



- Treatment of hPCLS with the profibrotic cocktail increases the mRNA expression of fibrotic markers, especially LRRC15 marker

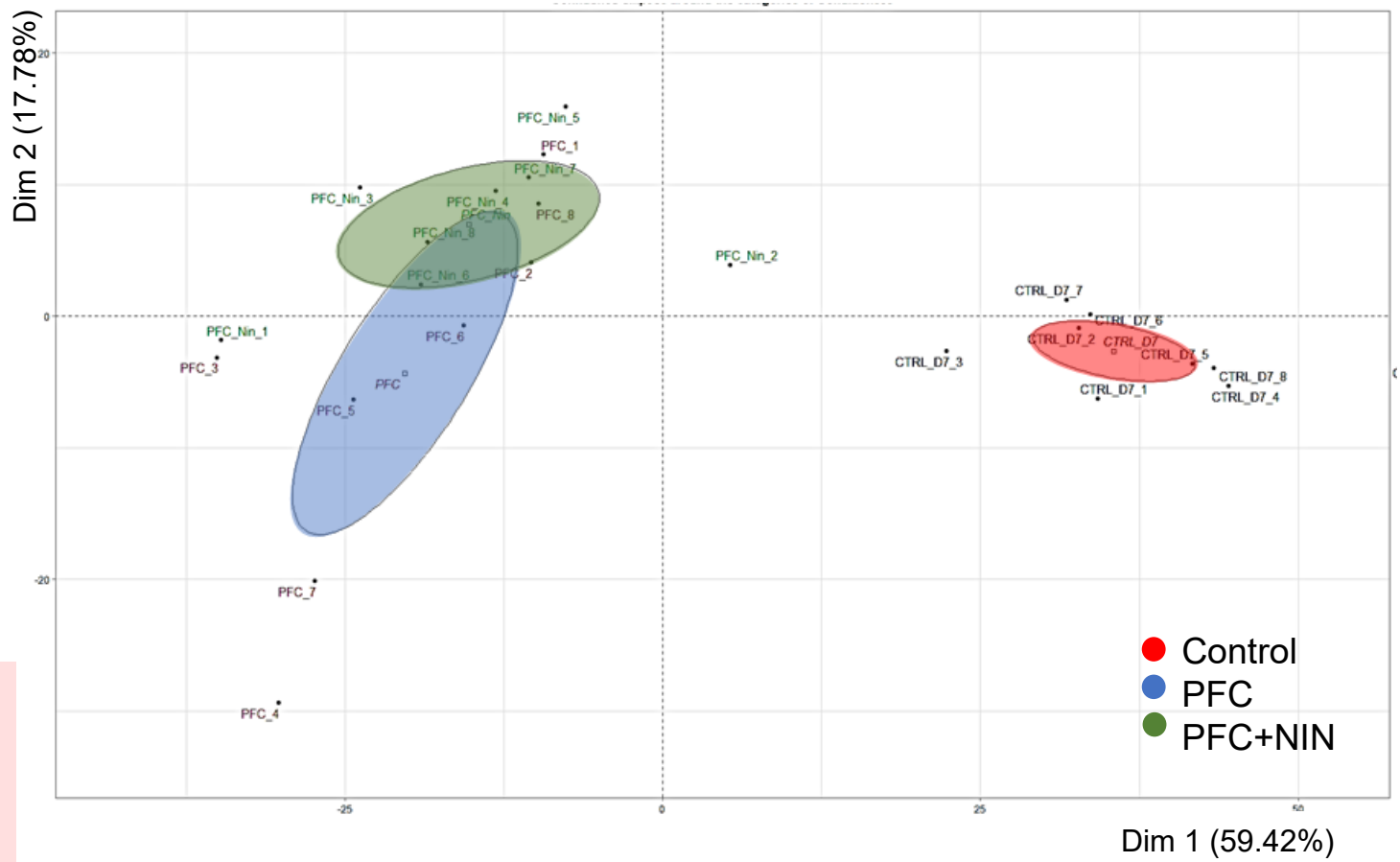
Proteomic analysis on hPCLS

- Treatment conditions:
  - 8 hPCLS control
  - 8 hPCLS treated with PFC
  - 8 hPCLS treated with PFC and nintedanib

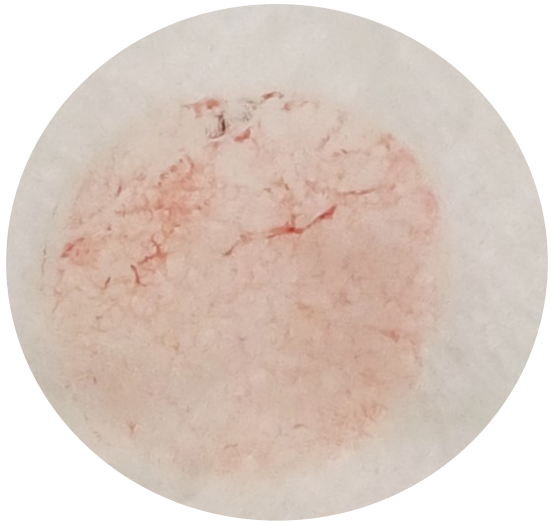
- Proteomic changes in hPCLS analysed by quantitative LC-MS/MS
  - 6586 proteins identified
  - 1216 proteins identified by Random Forest

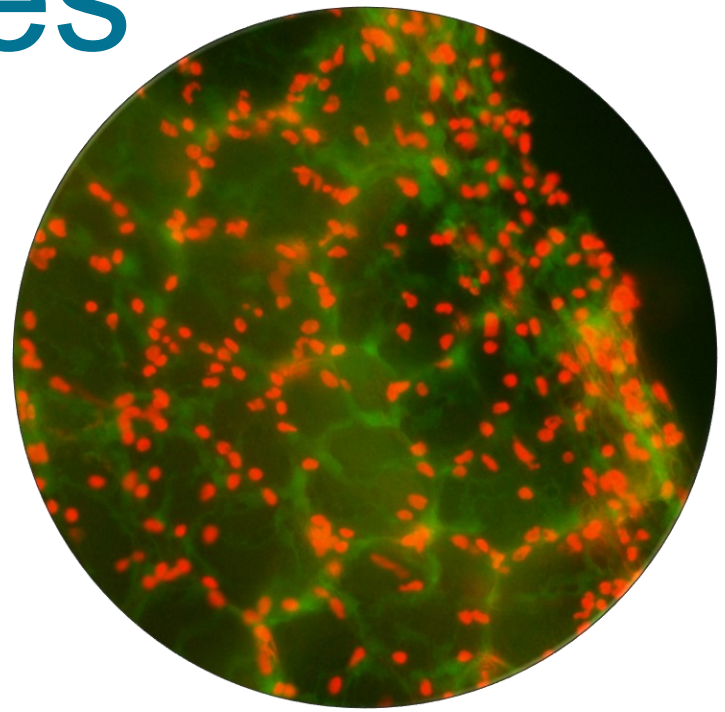
The global pattern of PCA in the proteome of hPCLS is clearly different between the three treatment groups

Principal component analysis (PCA) for proteome



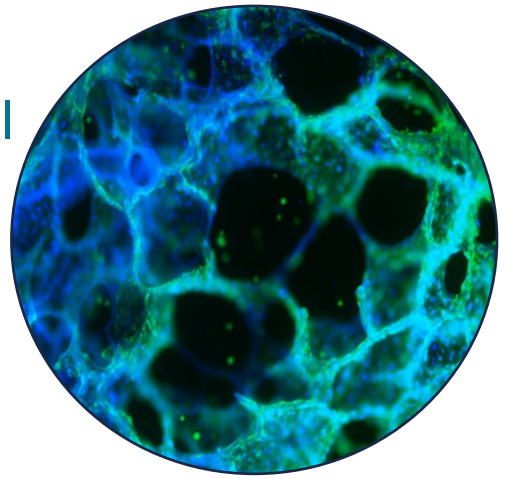
Take-home messages





Take-home messages

- 2D model of primary cultures of human lung fibroblasts offers a simple approach to modeling lung fibrosis.
- 3D lung model with inhalation exposure provides a powerful tool to study inflammation and its role in pulmonary fibrosis.
- The precision-cut lung slice (PCLS) model is currently considered the most relevant model for studying lung fibrosis as it preserves the architecture and cell types of the lung and responds to various stimuli.



- These models are useful for evaluating anti-fibrotic and anti-inflammatory therapies.
- While each model has its advantages and limitations, the key to successful research lies in selecting the most appropriate model for the research question at hand.

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Thank you!

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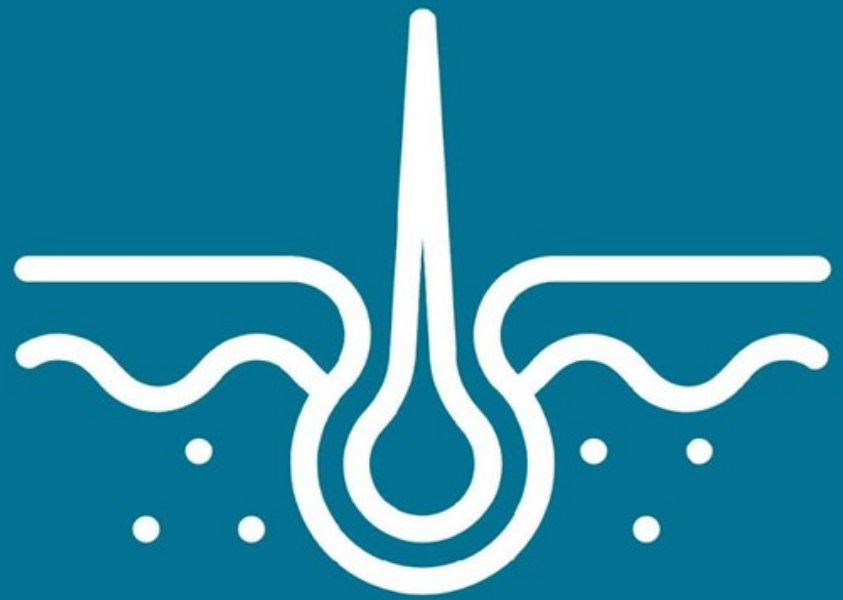

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