

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

Hanan Osman-Ponchet, Laurie Perdigon, Manon Barthe, Ines Metatla, Kevin Rogers, Ida Chiara Guerrera, Agnes Choppin, and Franck Chiappini



Lung In Vitro Event for innovative & predictive models
(LIVe 2024)
June 20-21, 2024 - Nice

LIVe 2024
LUNG IN VITRO event 20-21 JUNE
for innovative & predictive models

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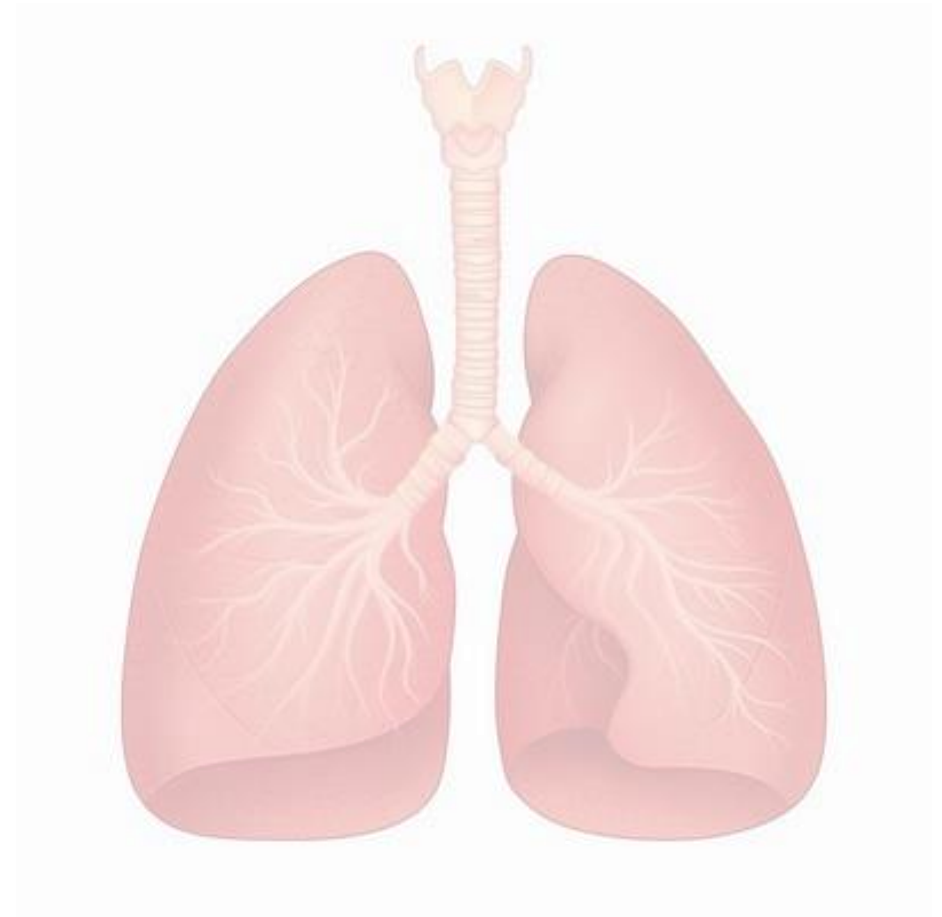
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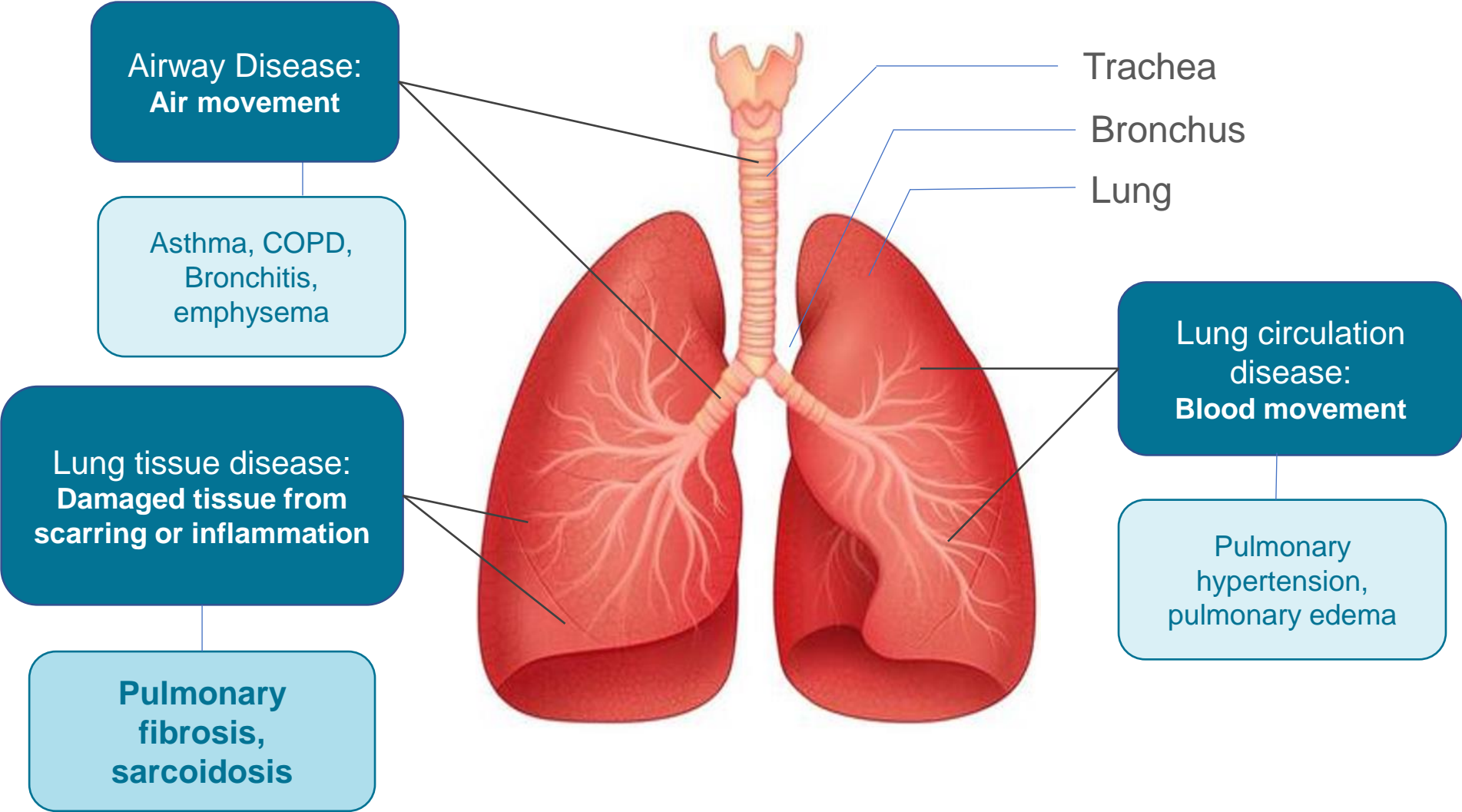
Outline

- 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 model)
 - Induction of fibrosis (hPCLS model)



<https://www.shutterstock.com/fr/search/bronchial-body-part>

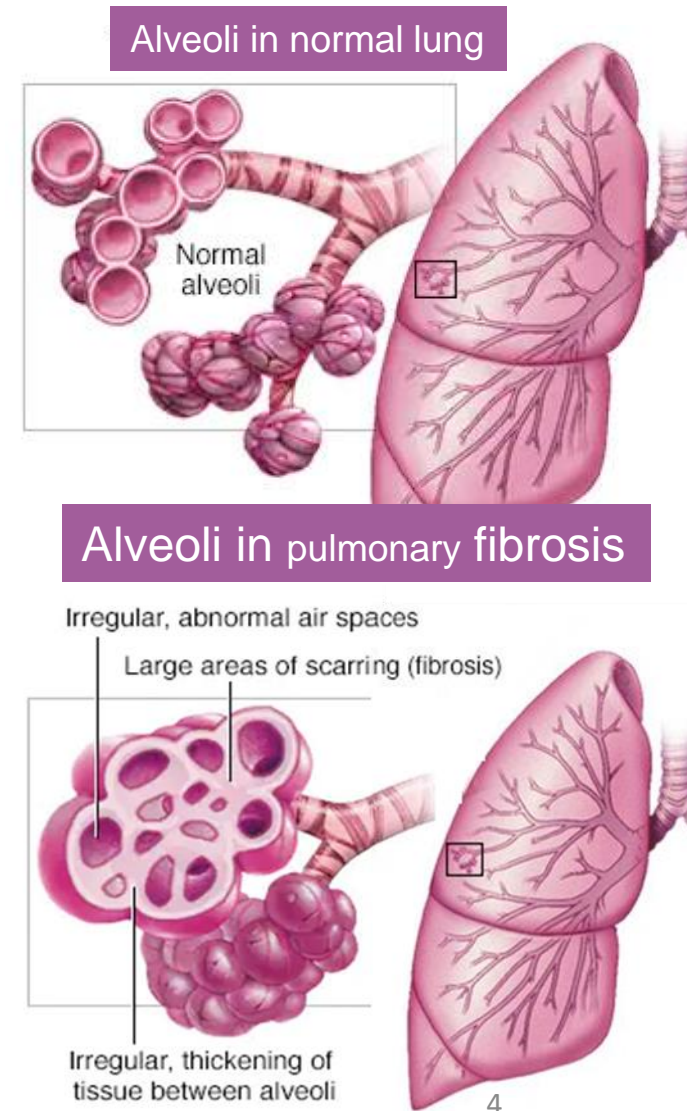
Most common lung diseases



Pulmonary fibrosis

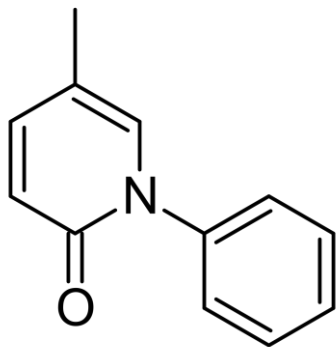
- Pulmonary fibrosis is a chronic and a progressive lung disease characterized by:
 - Excessive deposition of extracellular matrix (ECM) proteins in the lungs
 - This leads to scarring and thickening of lung tissues and loss of lung function, ultimately making it difficult for oxygen to pass through
 - 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

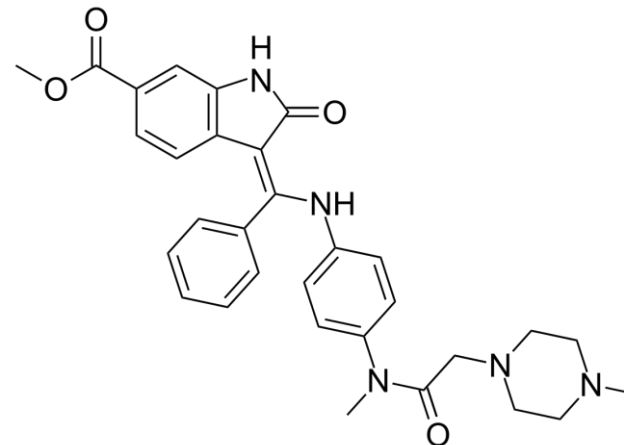


Pulmonary Fibrosis: Treatment Options

- 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.



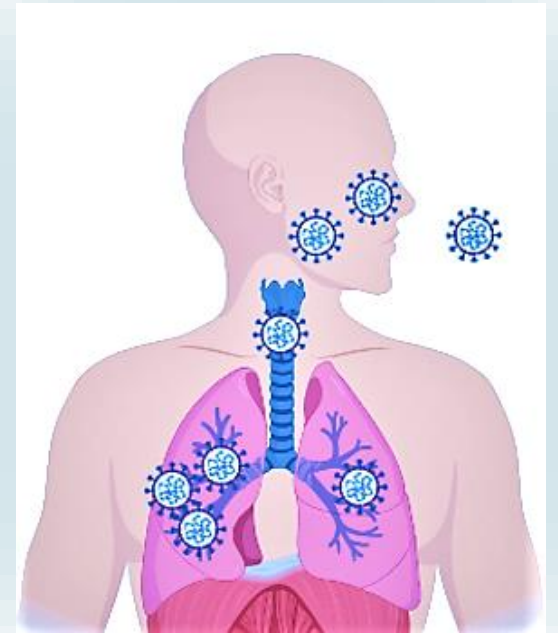
Pirfenidone
MW:185.2



Nintedanib
MW: 539.6

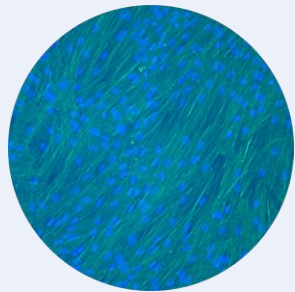
COVID-19: A Potential Risk Factor for Pulmonary Fibrosis

- About **44.9%** of COVID-19 survivors developed pulmonary fibrosis¹
- 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**



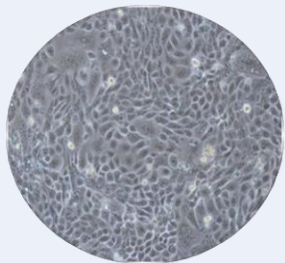
Biological systems to model lung disease

2D models



Primary Human lung fibroblasts

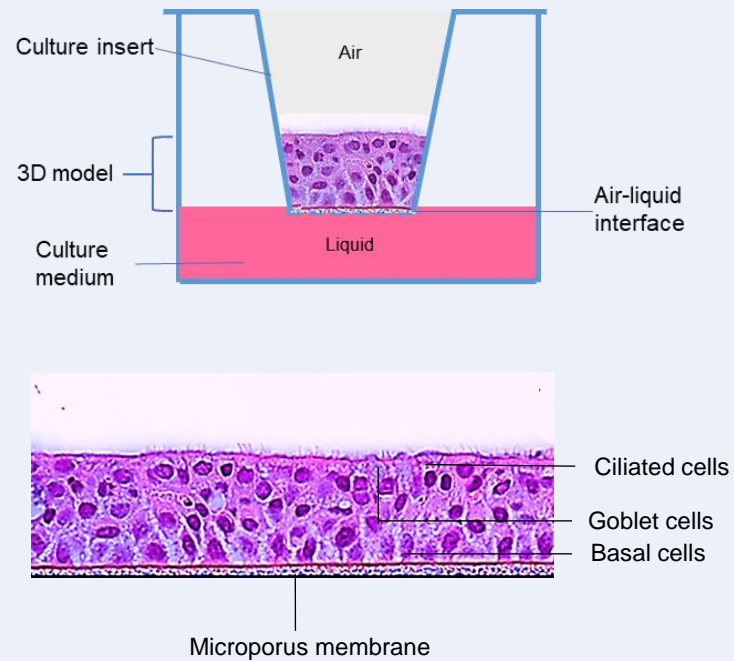
Exhibit characteristics of myofibroblasts. They respond to various stimuli that induce fibrosis



Calu-3 Human lung adenocarcinoma cell line.

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

3D models

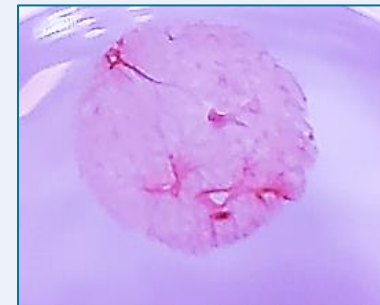


MucilAir™ (Epihelix)
EpiAirway™ (MatTek)

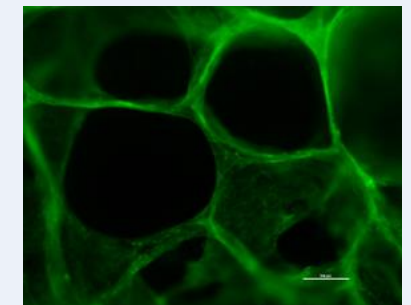
Both MucilAir and EpiAirway can be used to study lung fibrosis

Ex vivo models

Precision-cut lung slices (PCLS)



hPCLS (macroscopic)



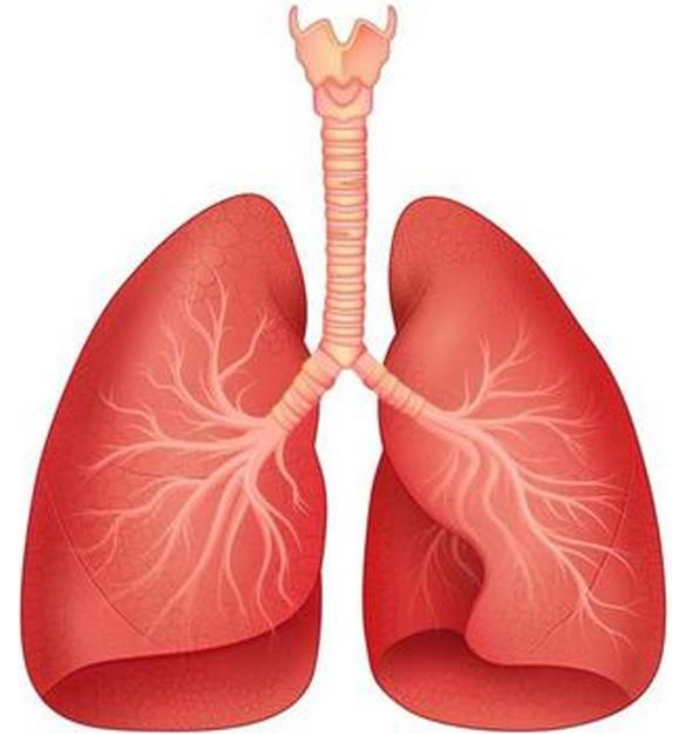
hPCLS (microscopic)

- Gold standard
- Prepared from fresh lung tissue
- Retain the native cellular architecture and extracellular matrix

Realistic representation of the lung microenvironment

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 of cell migration & fibrosis markers expression

2D model

Cell migration – Scratch test

- **Rational:**

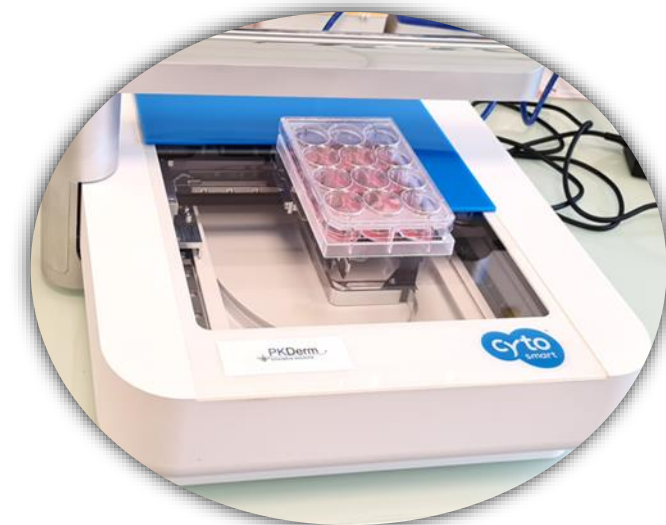
- Lung fibrosis characterized by proliferation of fibroblasts with excessive accumulation of extracellular matrix (ECM) within the lung
- Targeting fibroblast proliferation and ECM deposition represents a logical therapeutic strategy for both delaying disease onset and, potentially, reversal of disease pathology

- **Methods:**

- Primary human lung fibroblasts
- Treatments:
 - TGF-beta
 - Nintedanib and Pirfenidone
 - Taxol as positive control
- Live cell imaging of cell migration
- mRNA expression of fibrosis markers:
 - LRRC15, ACTA2, FN1, MMP12, COL1A1, COL3A1



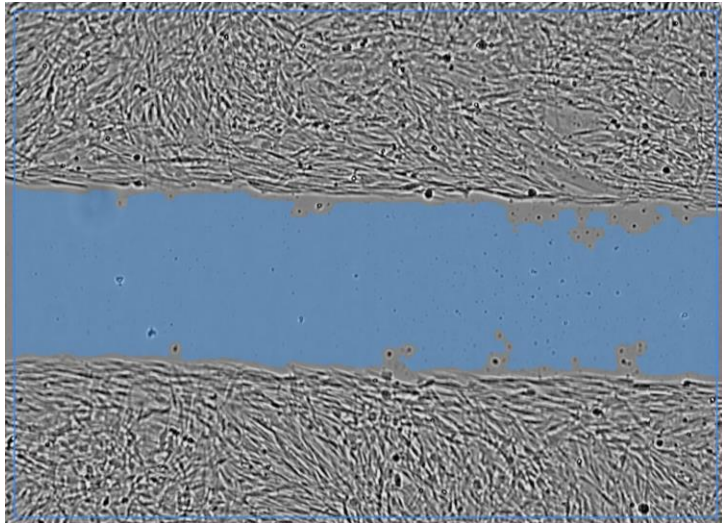
Ibidi® culture-insert



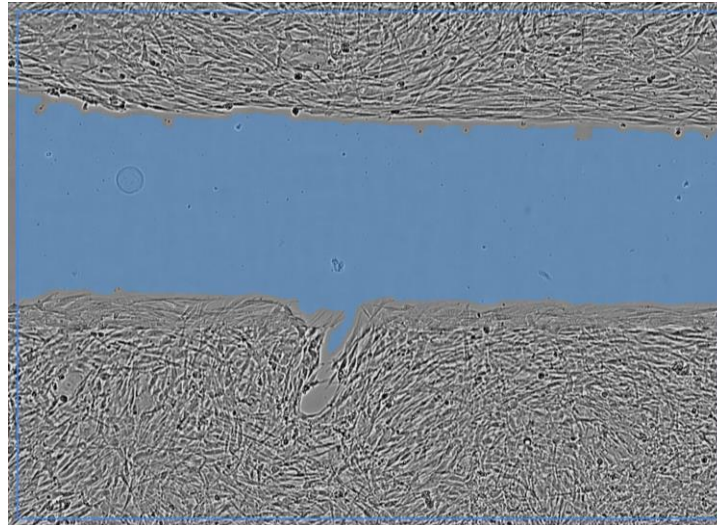
CytoSMART Omni brightfield device

Live-cell imaging of cell migration

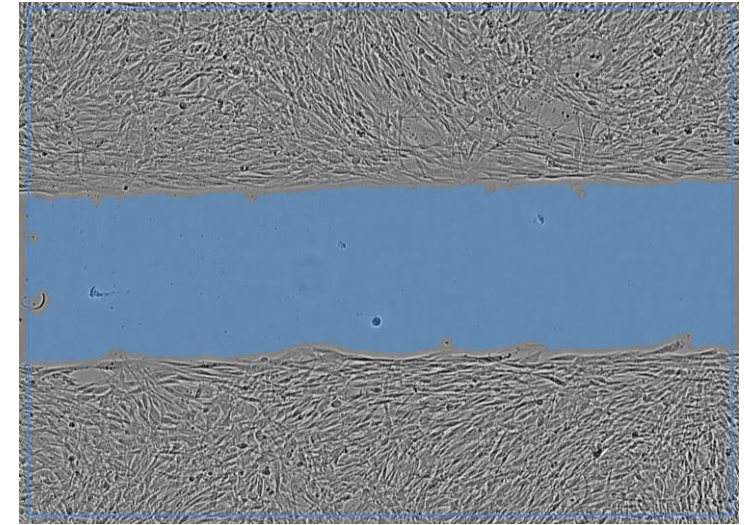
- Human lung fibroblasts treated with Pirfenidone or Nintedanib for 24 hours



Control

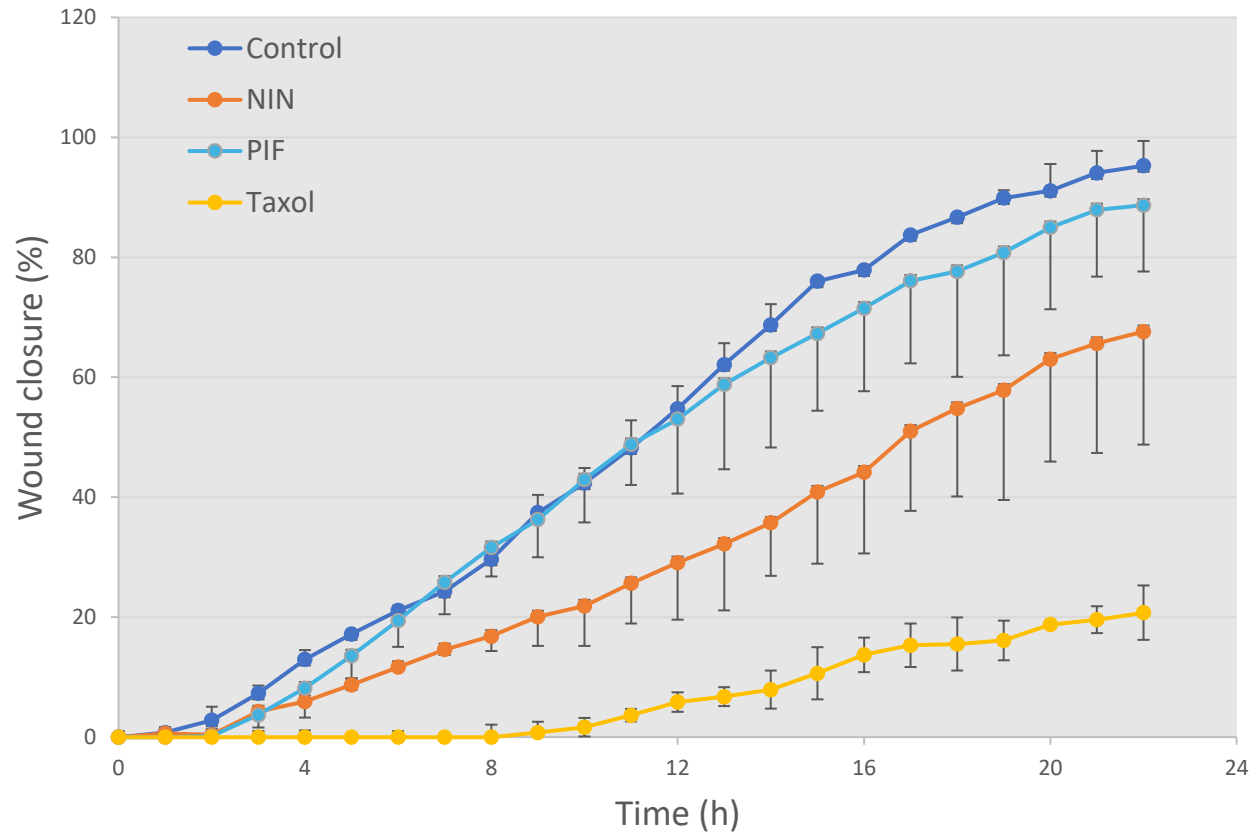


Pirfenidone



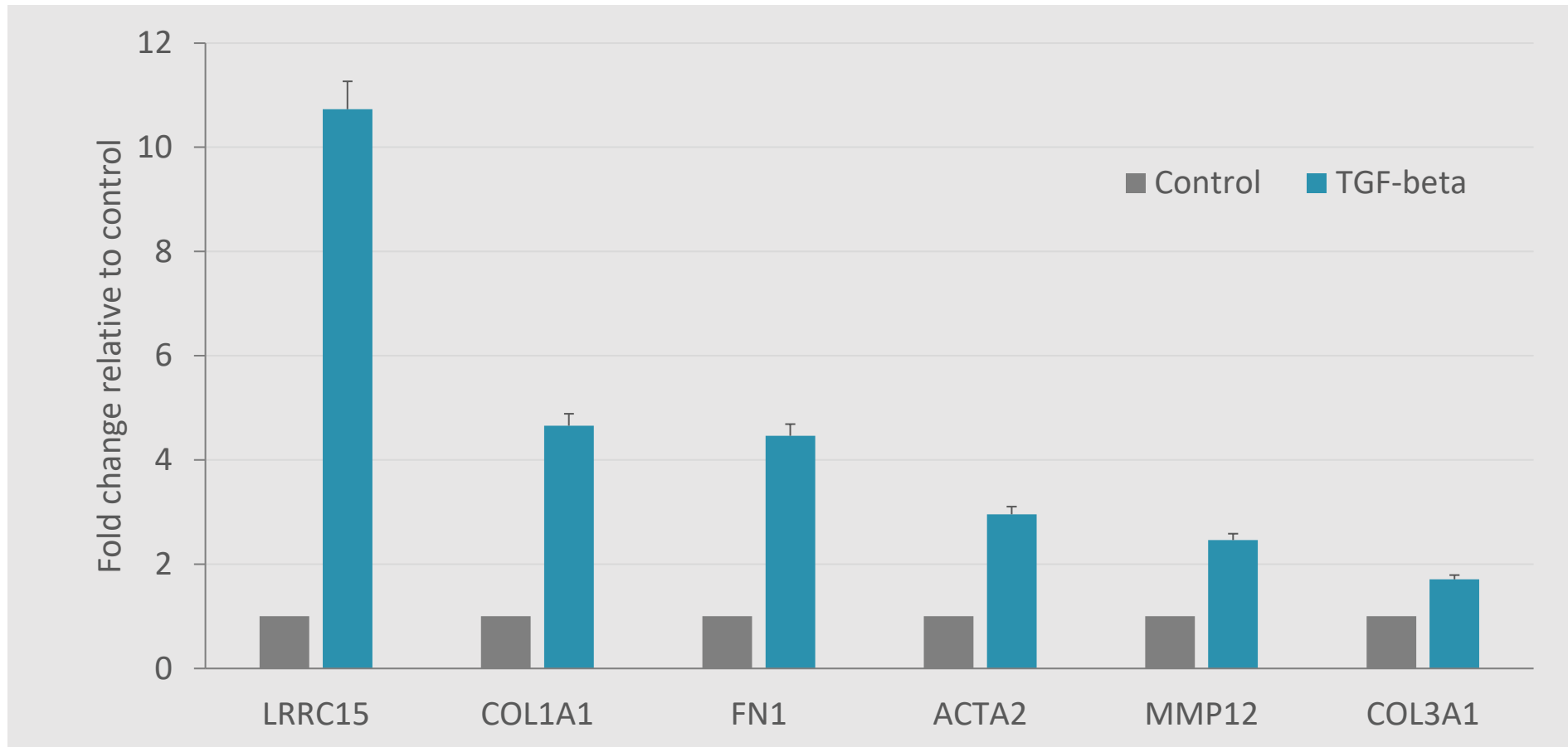
Nintedanib

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

Take-home message

- Primary cultures of 2D human lung fibroblasts offer a simple approach to modeling lung fibrosis.
- This model can be utilized in the early stages of drug development to evaluate potential anti-fibrotic therapeutic treatments.

For more details about the 2D model, don't miss the Manon Barthe poster!

Screening Anti-Fibrotic Drugs Using Primary Human Pulmonary Fibroblasts: A Cell Migration and Gene Expression Assay

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Case study of inflammation

3D model

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:**

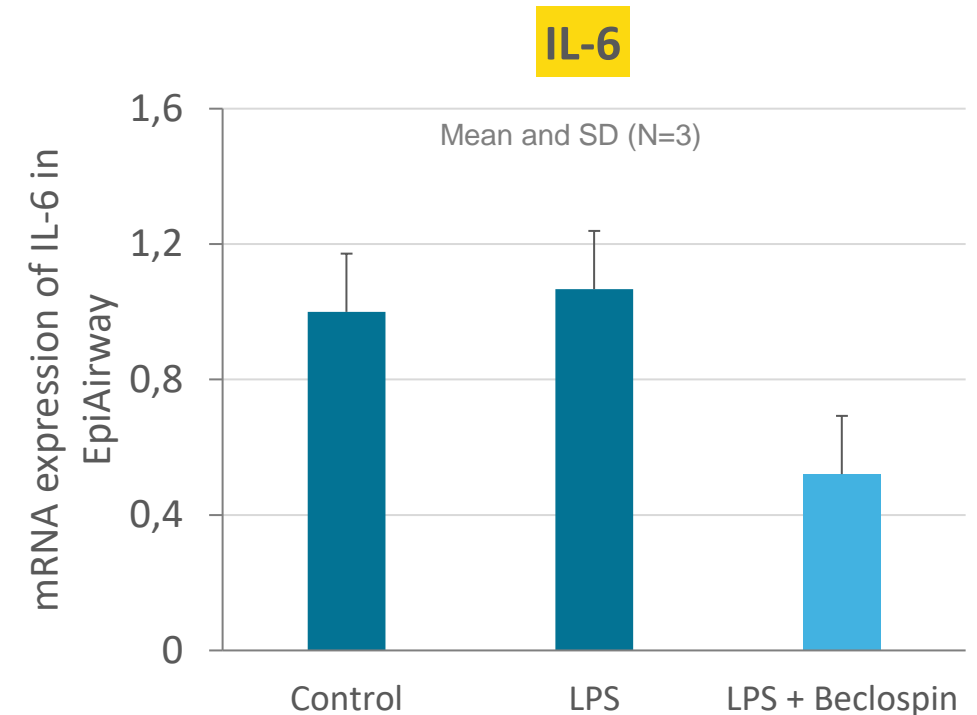
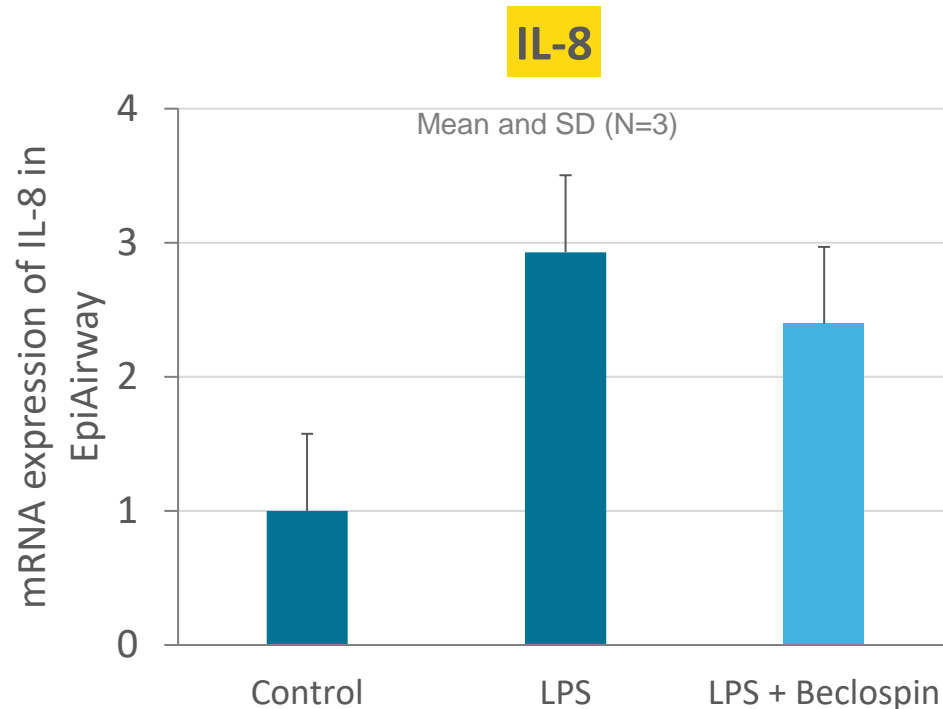
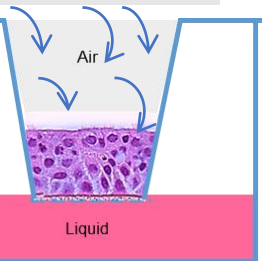
- EpiAirway™ (3D model)
- Stimulation:
 - LPS
 - LPS + Beclospin (nebulizer system)
- mRNA expression of inflammatory markers:
 - IL-8 and IL-6 (TaqMan RT-qPCR)



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.

Take-home message

- 3D lung tissue models with inhalation exposure provide a powerful tool to study inflammation and its role in pulmonary fibrosis.

The image is a composite of two photographs showing human precision-cut lung slices (hPCLS) in culture. The left photograph shows a single, roughly circular, pinkish-red slice resting on a white surface within a clear petri dish. A pipette tip is visible at the top, suggesting the addition of medium. The right photograph shows a similar slice in a petri dish, but with a more complex, branching network of vessels visible on its surface. The text 'Case study of Human precision-cut lung slices (hPCLS)' is overlaid in white on the left side of the image.

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

Human Precision-Cut Lung Slices (hPCLS)

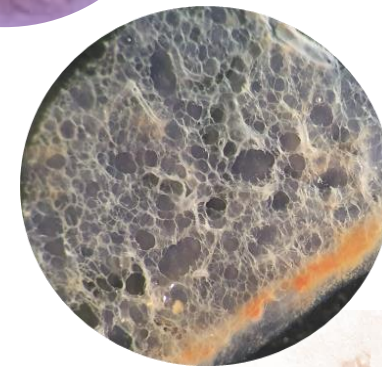
- hPCLS are thin slices with precision thickness (200 – 300 μm) obtained from fresh lung tissue

- Advantages and Applications of hPCLS:

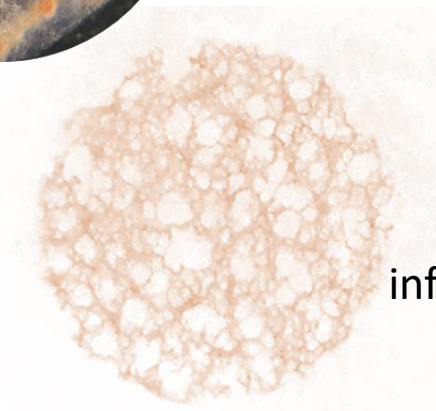
- Preservation of lung architecture
- Responsiveness to various stimuli
- Versatility
- Human relevance
- Drug screening
- Modeling various lung diseases
- Toxicology studies of inhaled substances



“Healthy” hPCLS



Fibrotic hPCLS



infected hPCLS

How to Prepare hPCLS ?

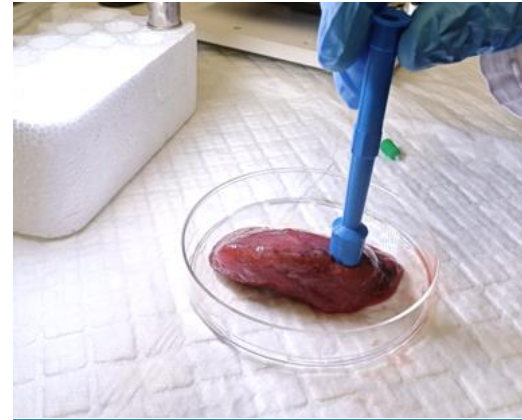


Lung resection

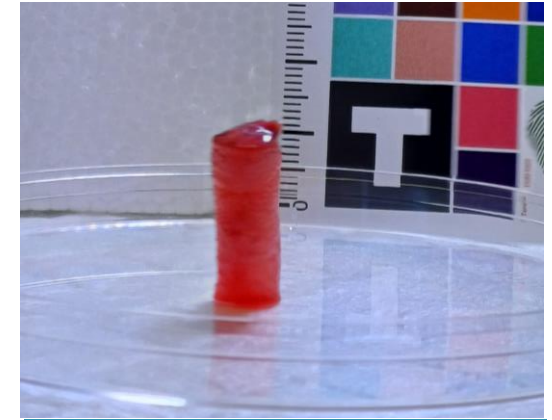
Biobanque CRB
Tumorotheque de Nice
BB-0033-00025



Low melting agarose
inflation



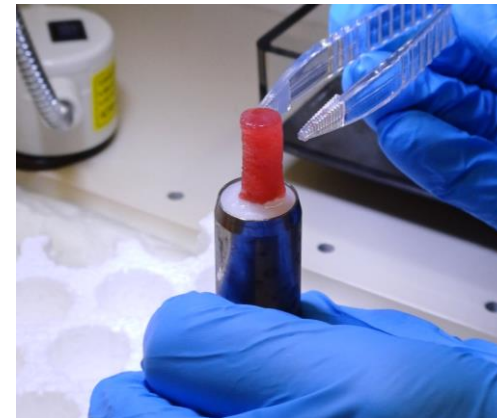
Biopsy of 8 mm Ø



Lung core

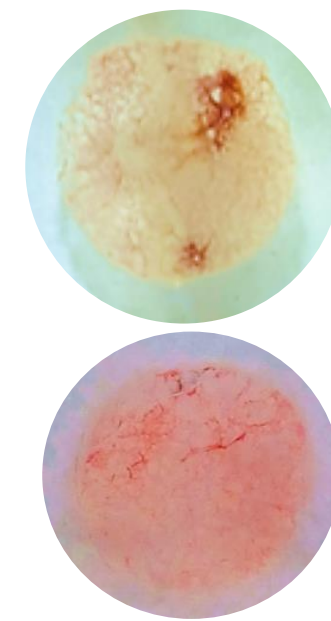
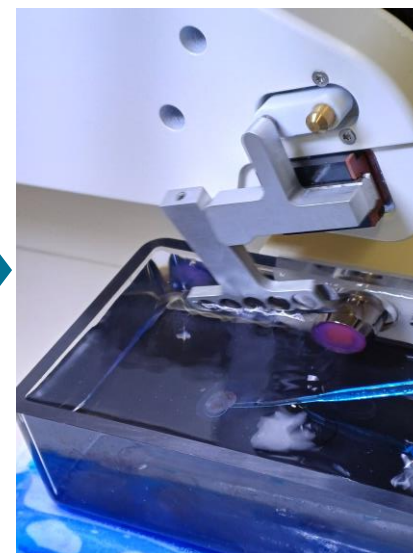
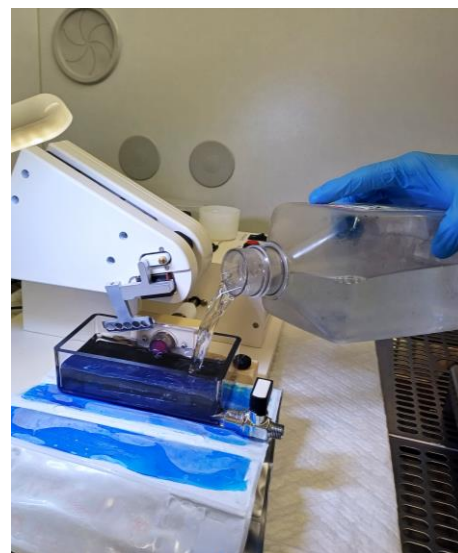
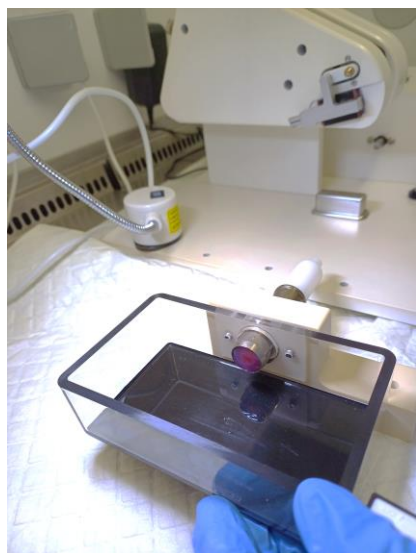


Mounting and embedding lung core on the holder



How to Prepare hPCLS ?

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



hPCLS 300 μ m thick

Preparation of hPCLs

Compresstome® VF-300-0Z

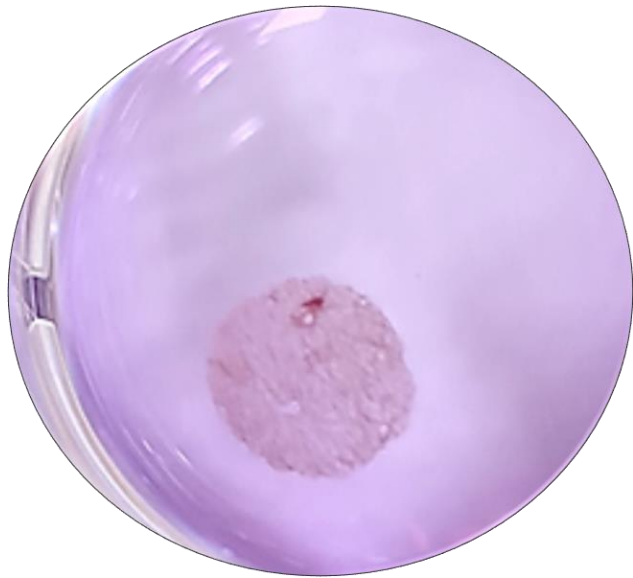


hPCLS

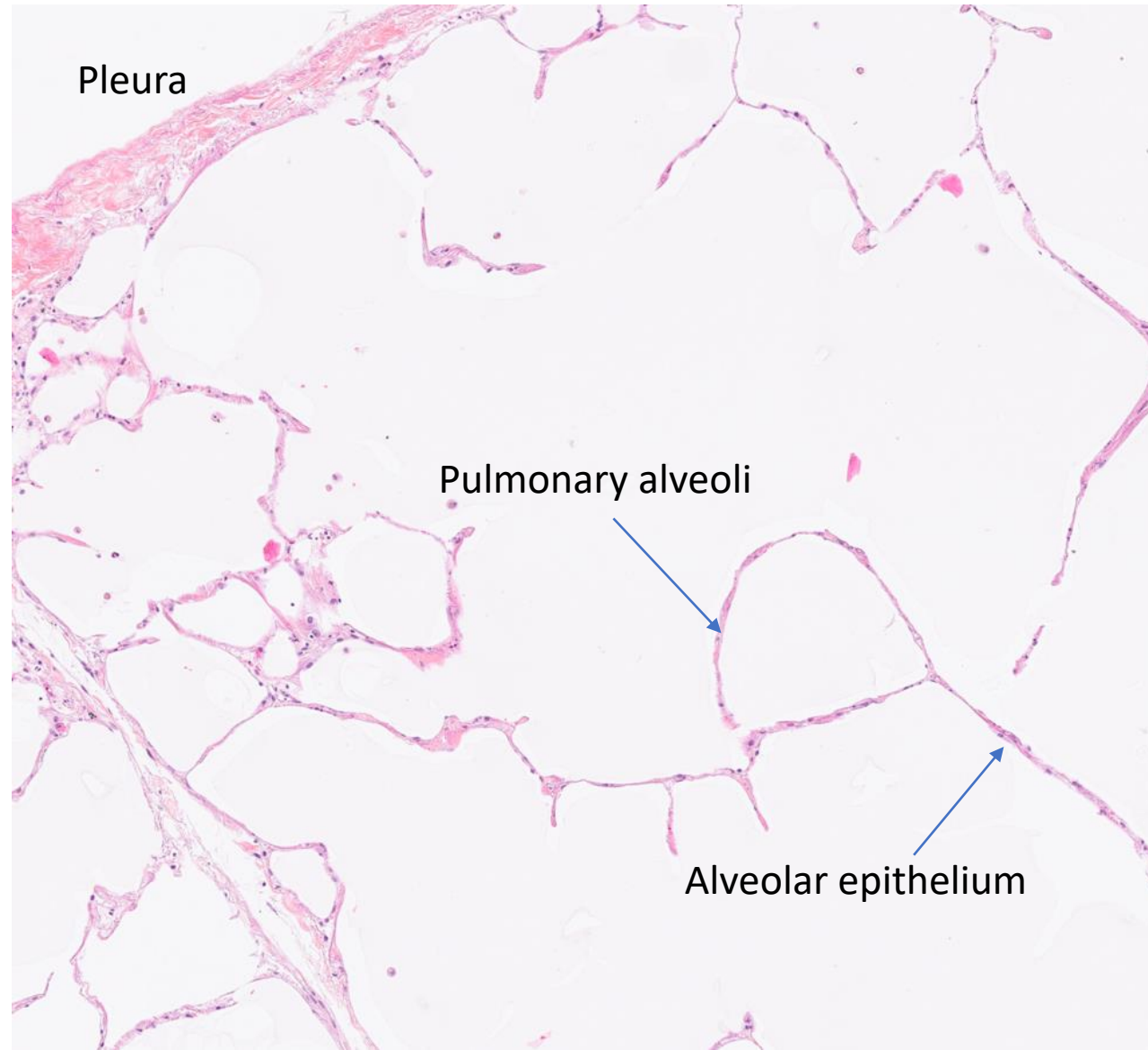
Multiple hPCLS of 300 μm can be successfully obtained from one lung resection



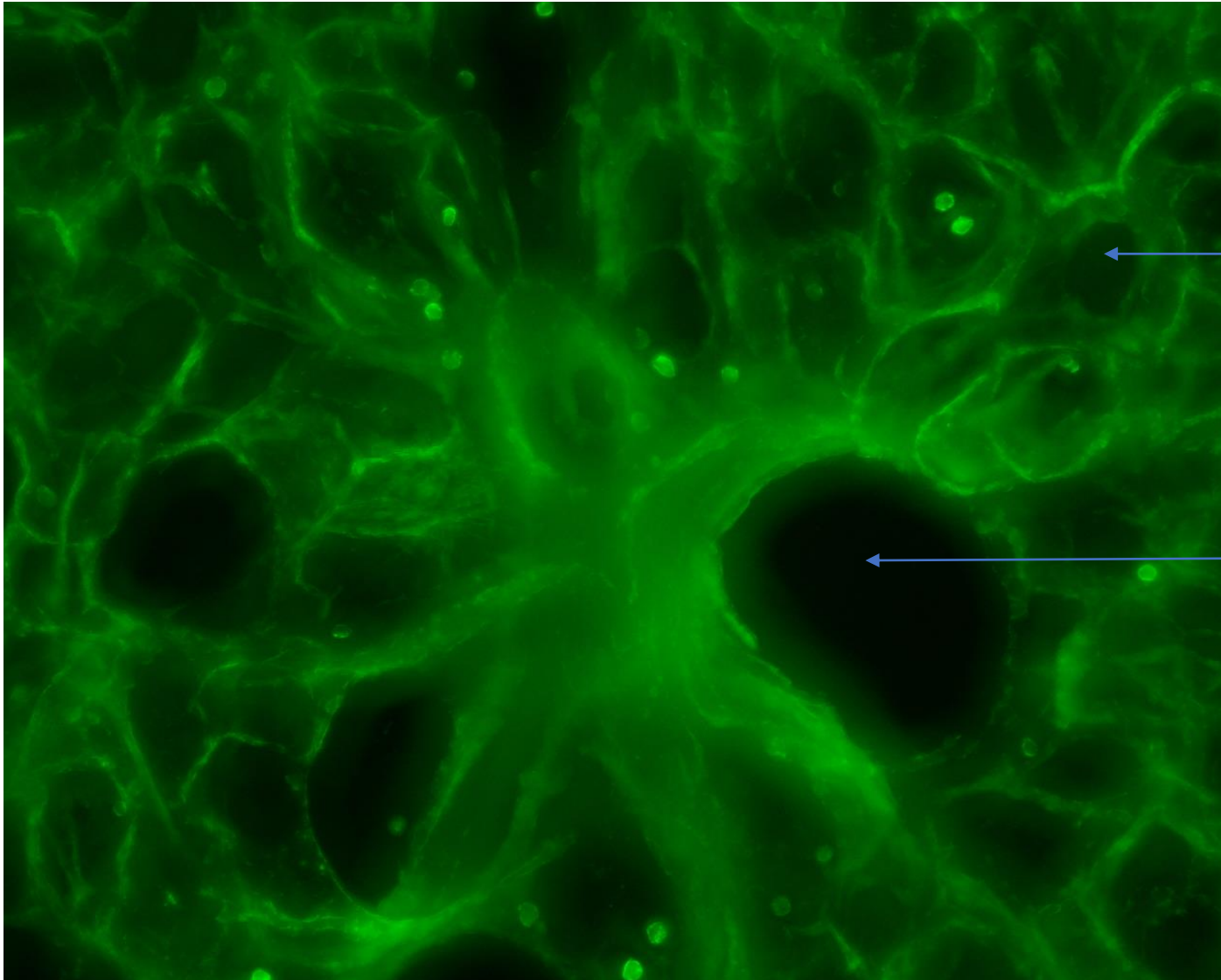
hPCLS maintains lung architecture



hPCLS in culture

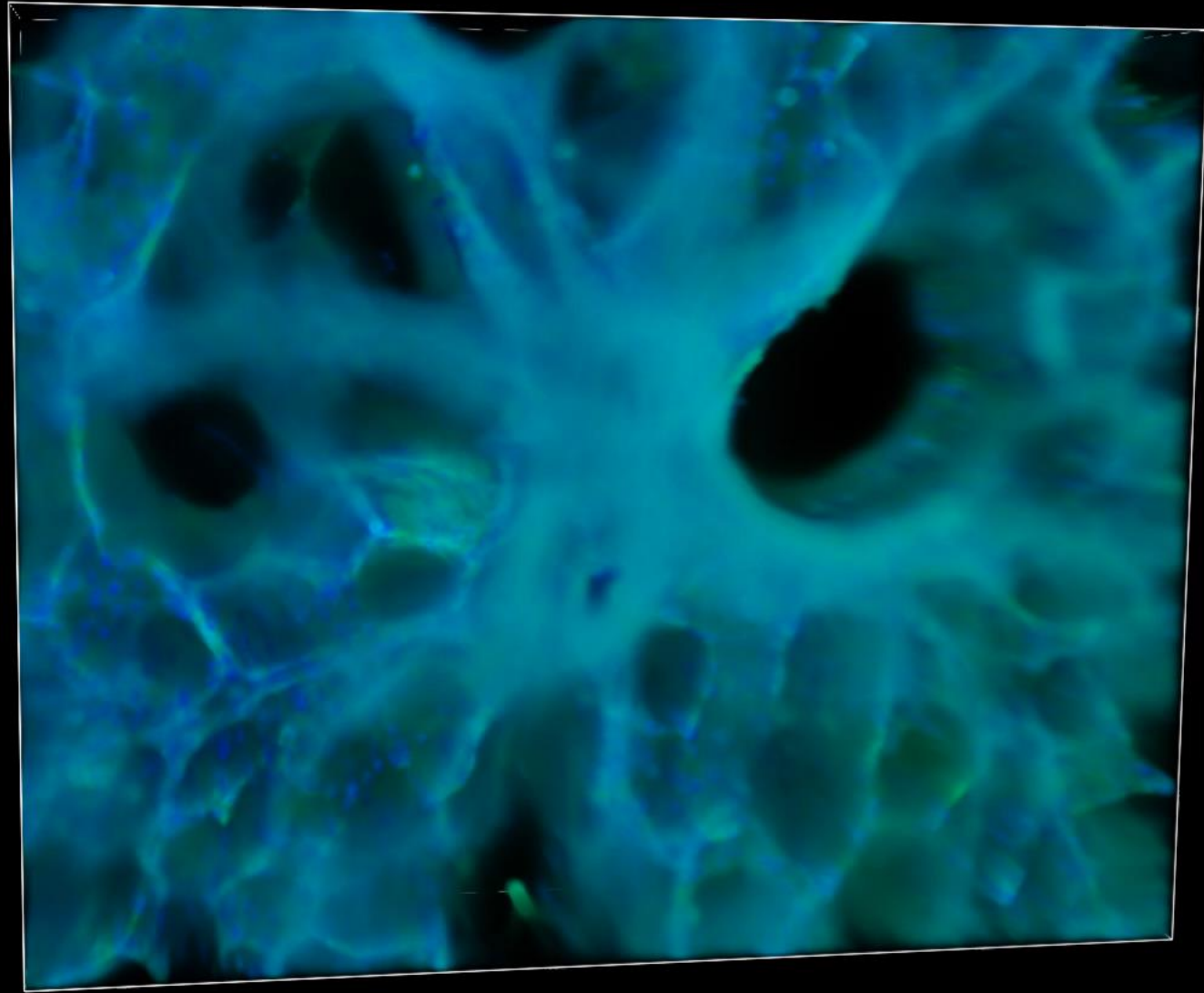


hPCLS maintains lung architecture



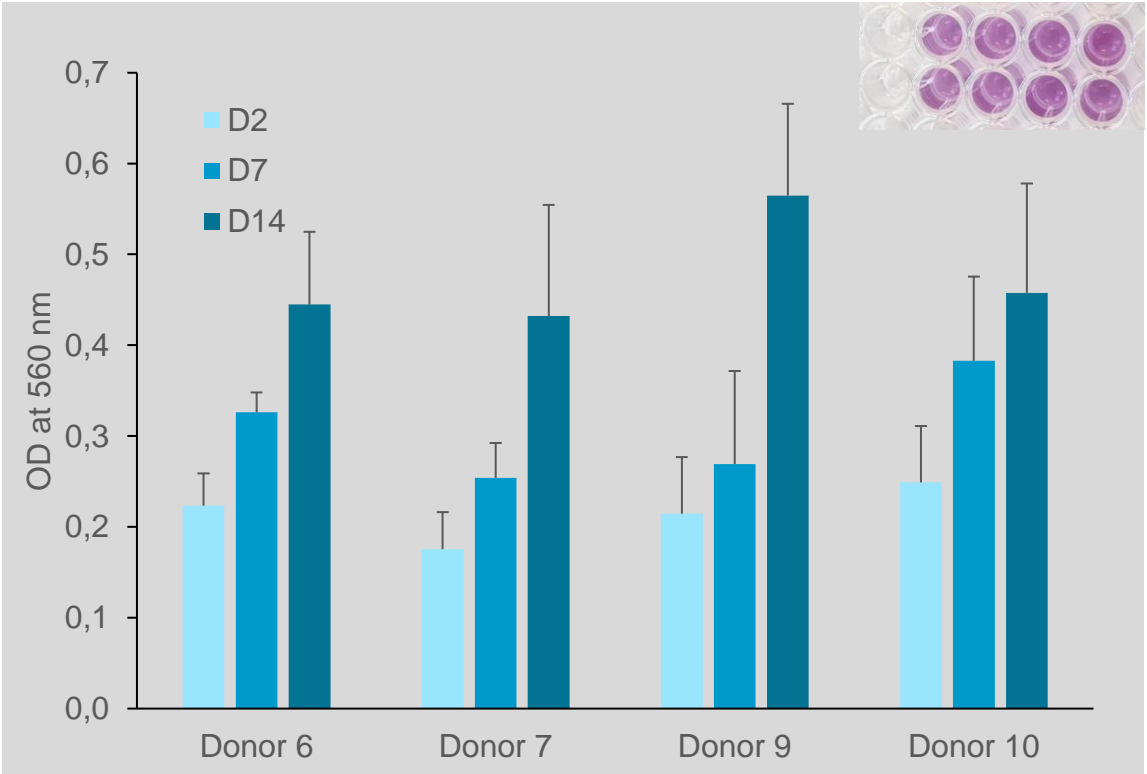
Alveoli

Bronchiol

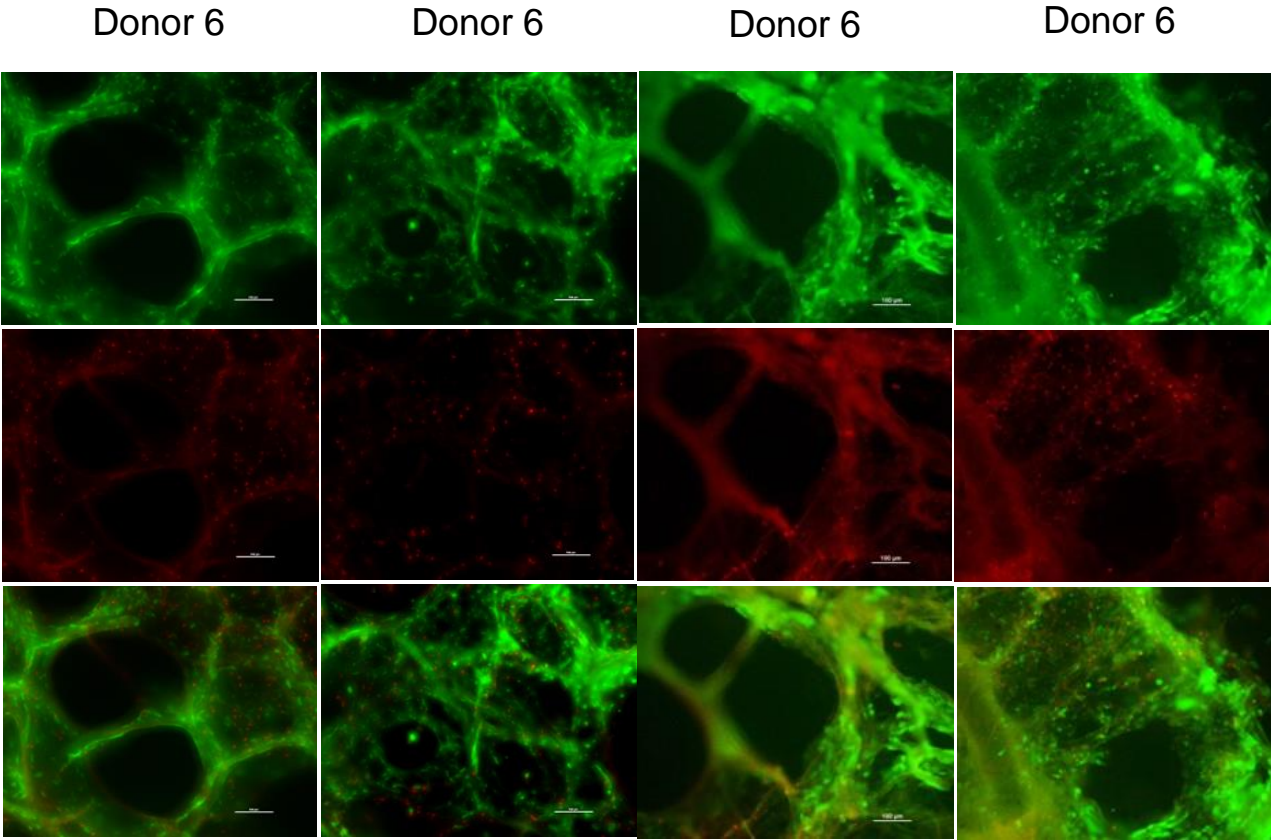


Viability of hPCLS is maintained over 14 days

MTT assay



Live/dead assay at Day 7

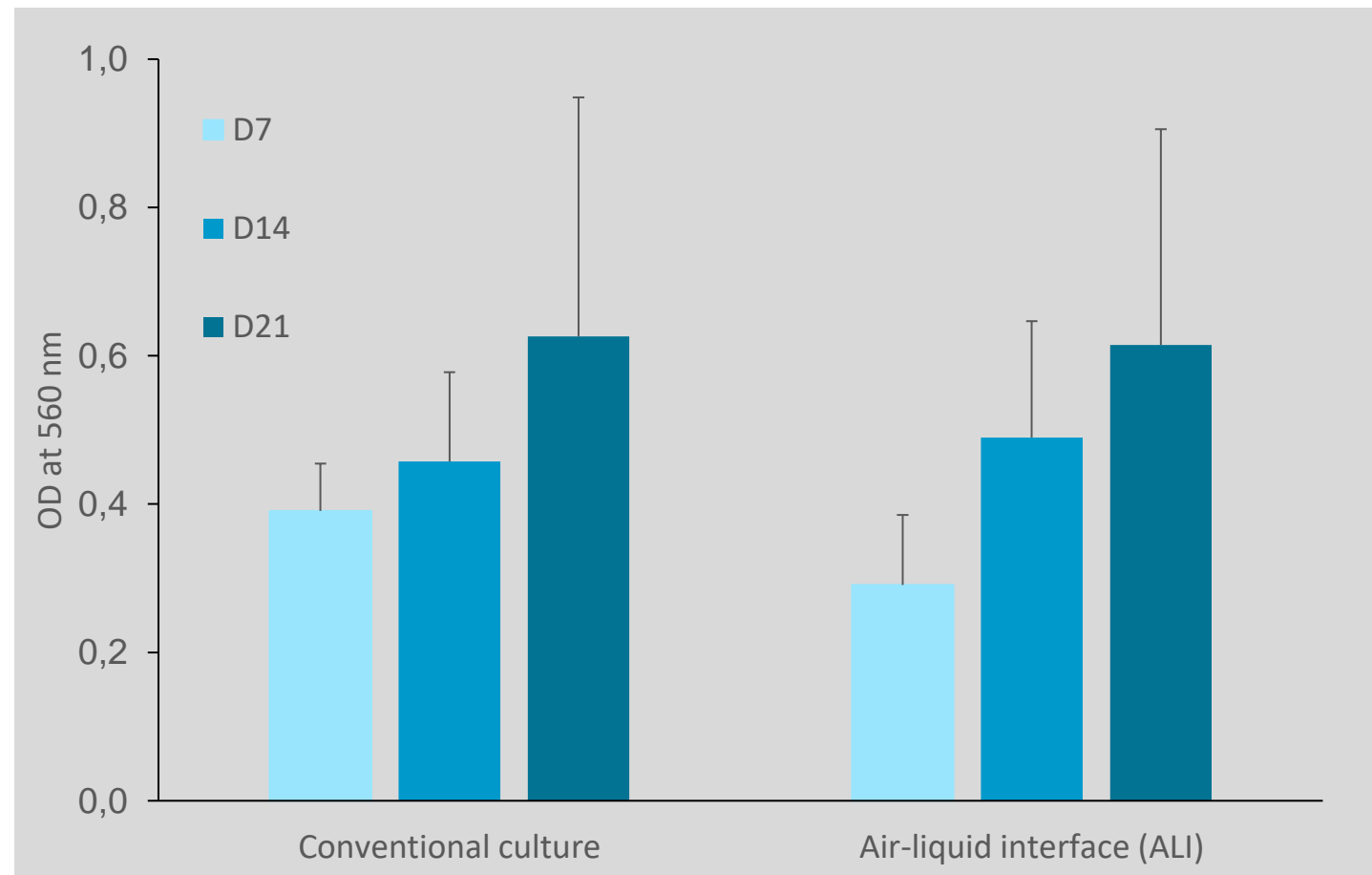


Viability of hPCLS in ALI conditions

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



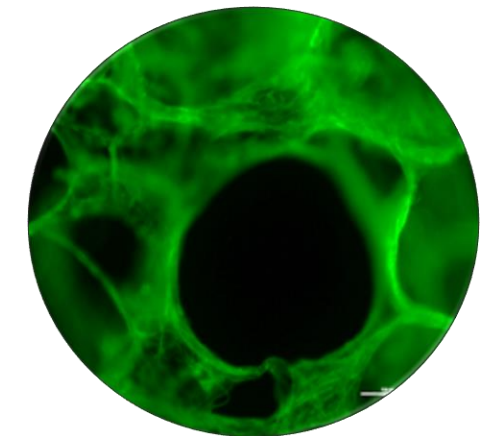
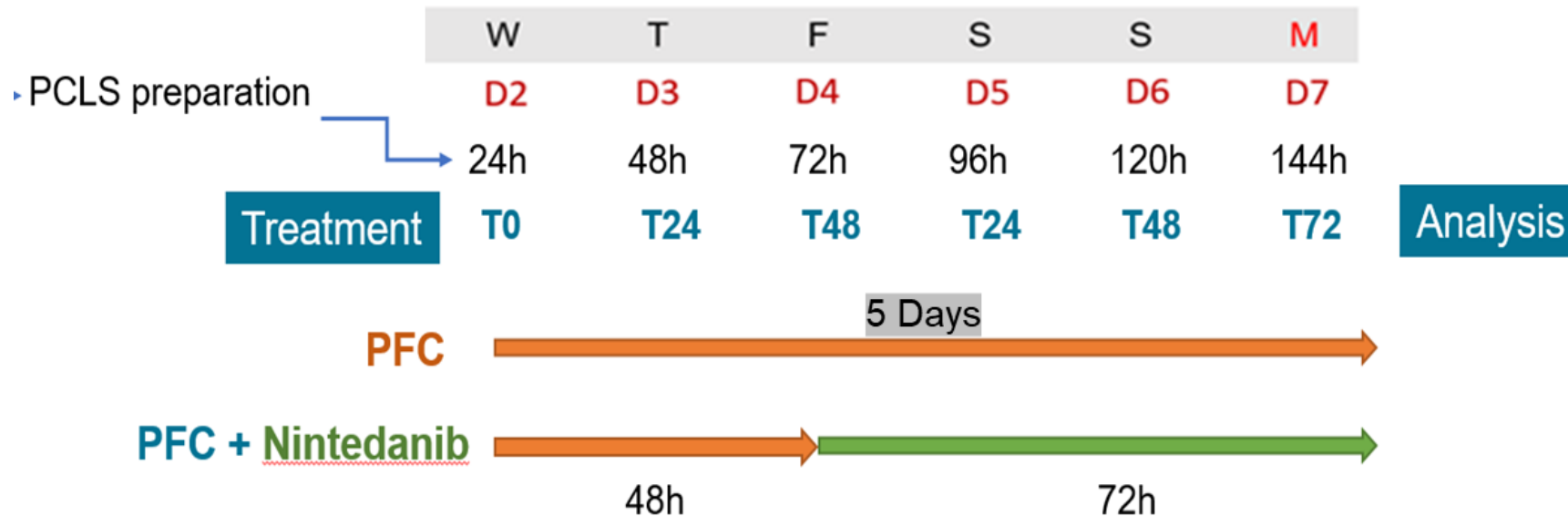
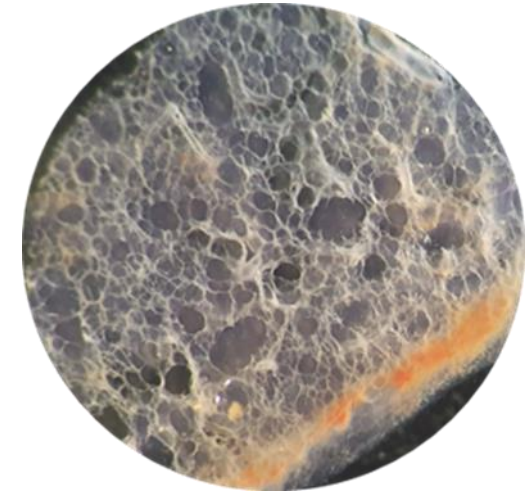
Culture of hPCLS at the air-liquid interface



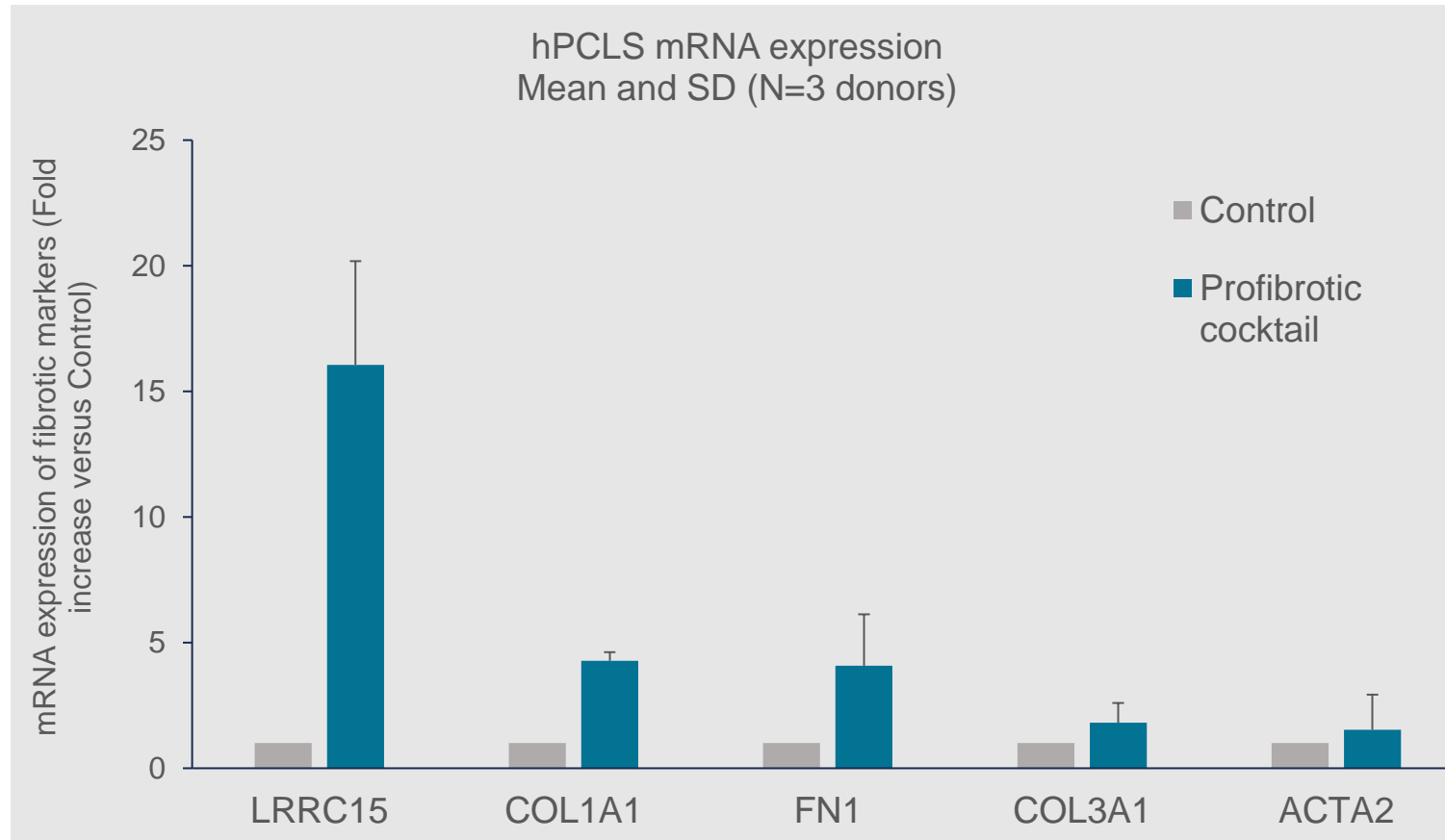
Induction of lung fibrosis

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 μ M lysophosphatidic acid (LPA)



Expression of fibrotic markers in hPCLS



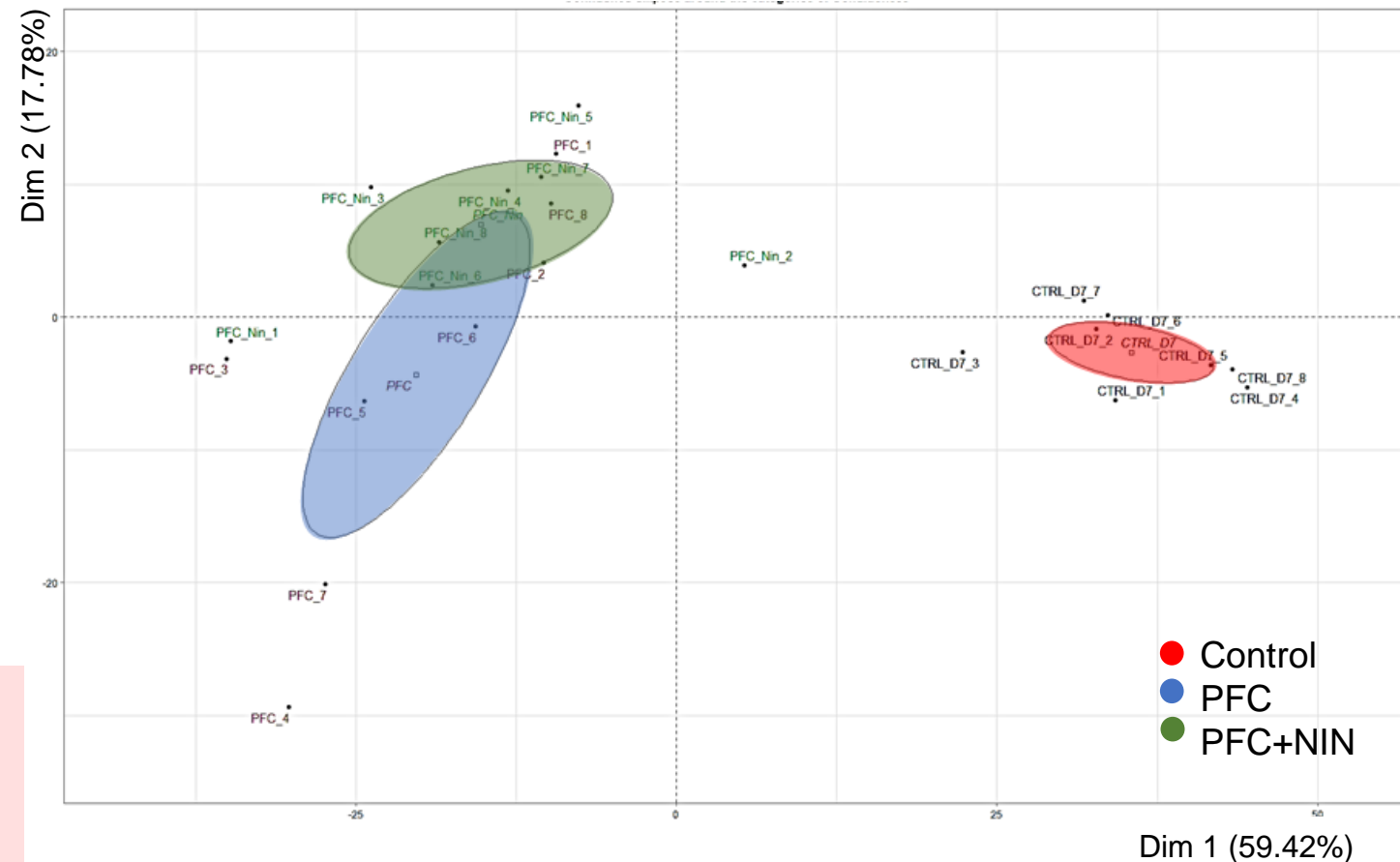
- Treatment of hPCLS with the profibrotic cocktail increases the mRNA expression of fibrotic markers, especially LRRC15 marker

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 message

- The preparation of hPCLS is a complex and technically challenging process that requires expertise in tissue handling and culture techniques
- However, hPCLS model provides a valuable tool for studying lung fibrosis due to its ability to recapitulate the *in vivo* environment and respond to various stimuli.

For more details about the PCLS model, don't miss the Laurie Perdigon poster !

Successful Development of Human Precision-Cut Lung Slice (hPCLS) Model for Studying Lung Diseases

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¹ PKDERM Laboratories, Grasse Biotech, 45 Bd Marcel Pagnol, 06130 Grasse, France.

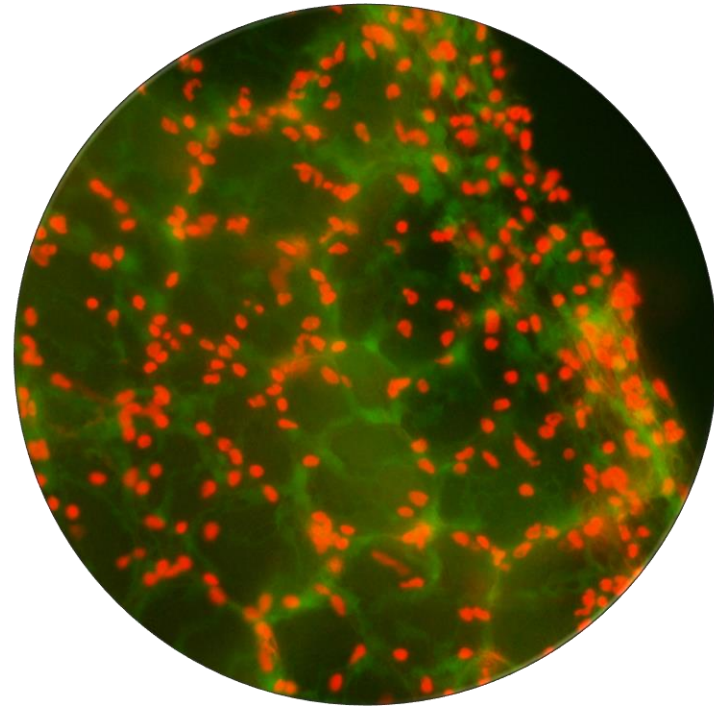
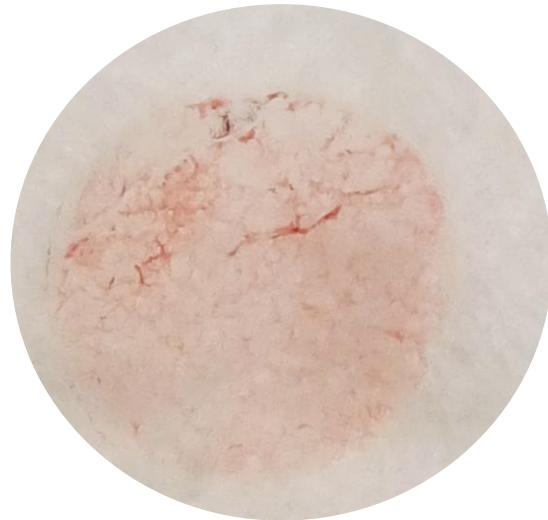
² OTR3, 4 rue Française, 75001 Paris, France.



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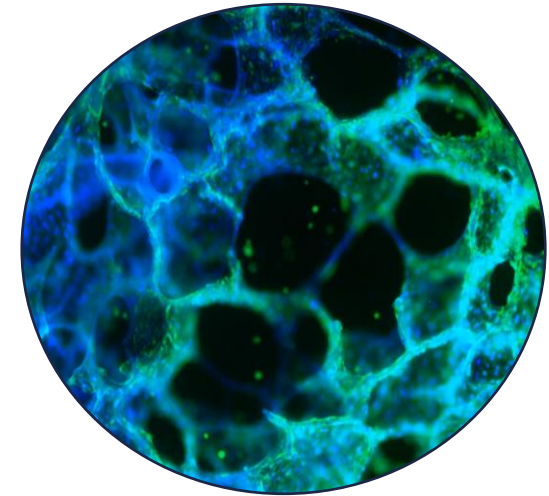


Summary



Summary

- *In vitro* models are a valuable tool for assessing the efficacy of treatments for pulmonary fibrosis and other lung diseases.
- 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.
- The precision-cut lung slice (PCLS) model is currently considered the most relevant model for studying lung diseases as it preserves the architecture and cell types of the lung and responds to various stimuli.





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Thank you!

PKDERM Team

Manon Barthe

Laurie Perdigon

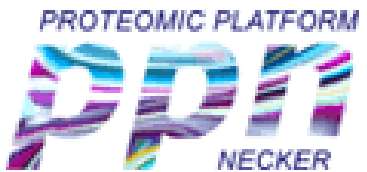
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