

Successful generation of Three-Dimensional Human Precision-Cut Lung Slices for Pulmonary Fibrosis Modeling

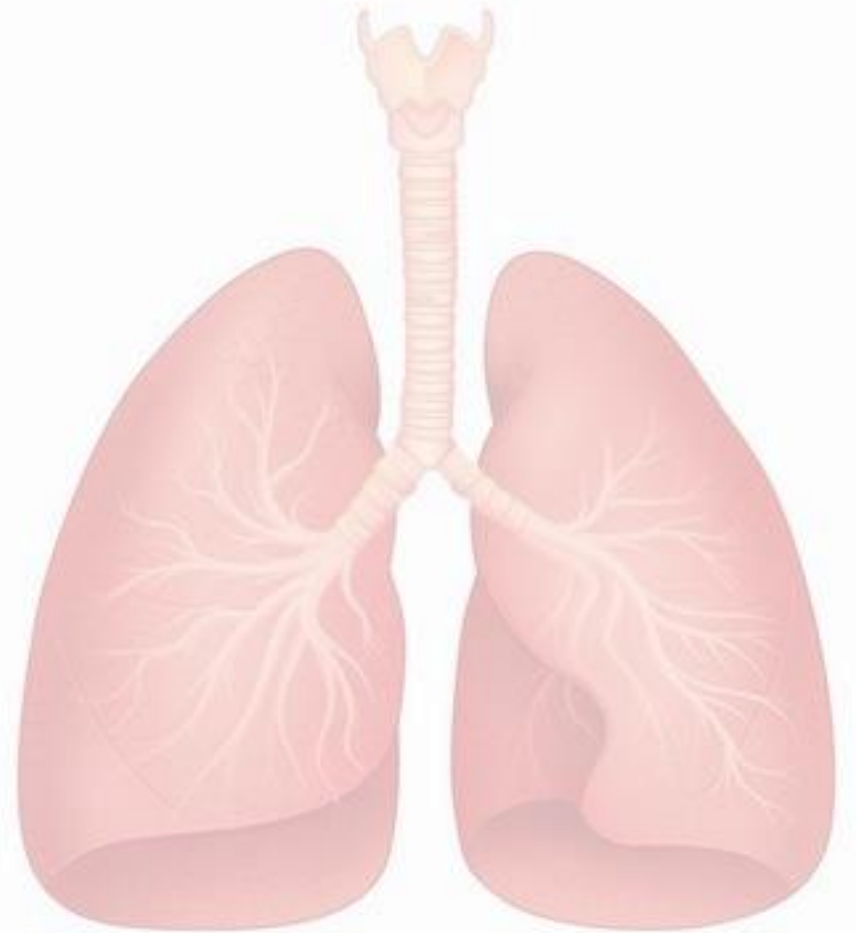
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22nd International Congress of the European Society of Toxicology In Vitro (ESTIV)
June 3-6, 2024 - Prague



Outline

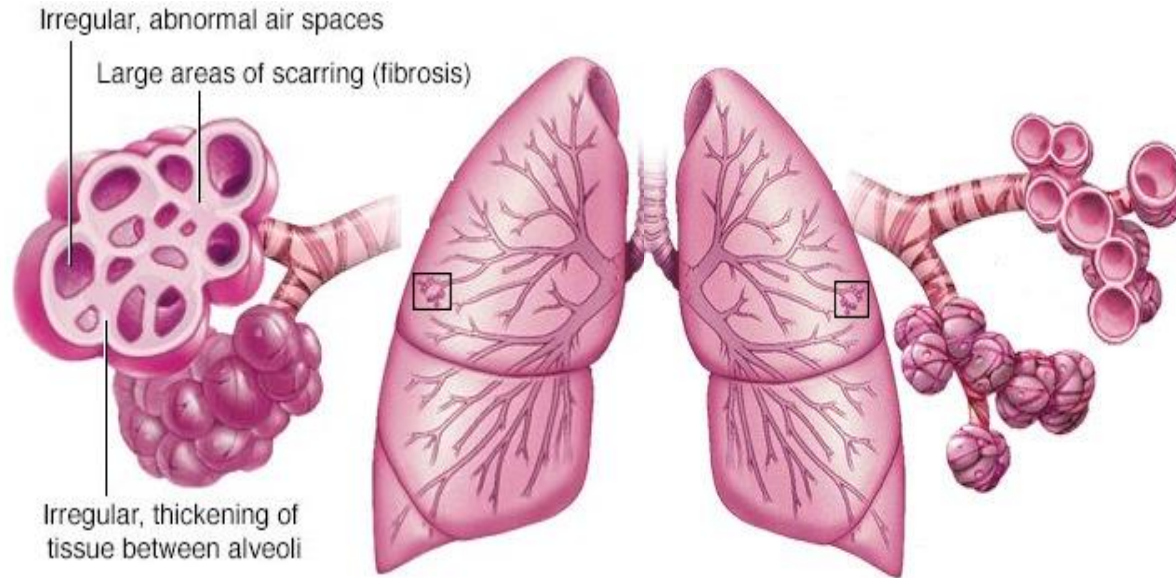
- Introduction
 - Pulmonary Fibrosis
- How to Model pulmonary fibrosis
 - 2D, 3D, *ex vivo* (PCLS)
- Human Precision-Cut Lung Slices (hPCLS)
 - What are hPCLS ?
 - Preparation of hPCLS
 - Characterization of hPCLS
 - Induction of lung fibrosis on hPCLS



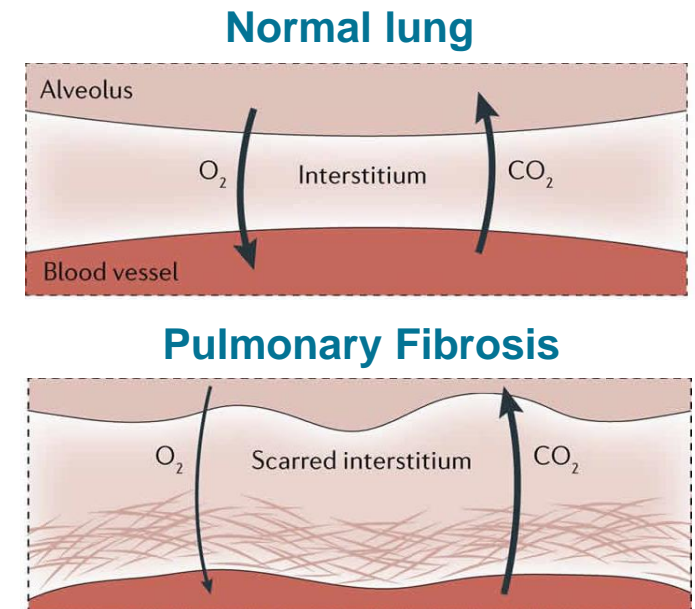
The Idiopathic Pulmonary Fibrosis (IPF)

Idiopathic Pulmonary Fibrosis (IPF)

- Chronic and progressive lung disease characterized by:



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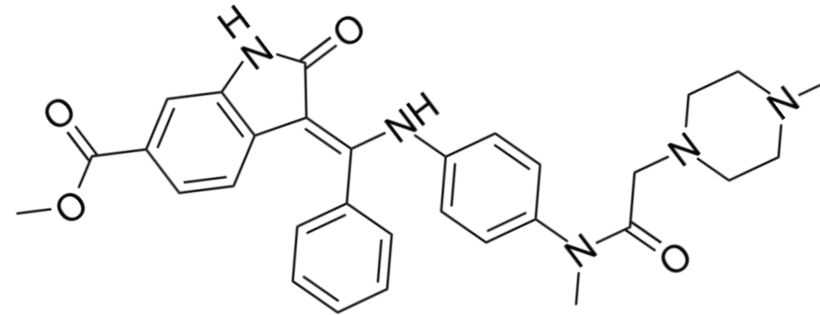


- Cause of pulmonary fibrosis : unknown (inflammation ?)

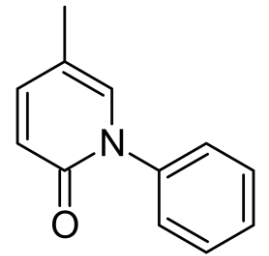
Idiopathic Pulmonary Fibrosis (IPF)

Treatments :

- Two FDA-approved drugs :
 - Pirfenidone (anti-inflammatory drug)
 - Nintedanib (tyrosine kinase inhibitor)



Nintedanib
MW: 539.6



Pirfenidone
MW:185.2

Limitations :

- Slow IPF progression
- Side effects
- Not effective for all patients

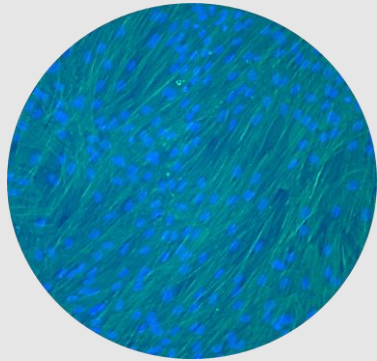
There is a need to develop new drugs to treat pulmonary fibrosis

A microscopic image of lung tissue, likely stained with a green fluorescent marker and a blue counterstain. The image shows a complex network of cells and fibers, with the green fluorescence highlighting specific structures, possibly collagen or other extracellular matrix components. The overall appearance is that of a dense, interconnected network of fibers and cells.

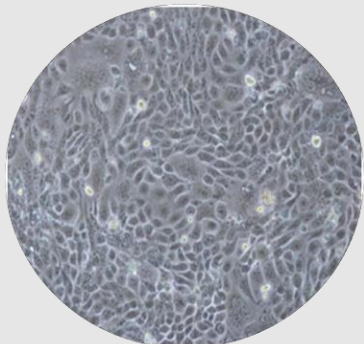
How to model Pulmonary Fibrosis ?

Biological systems to model lung disease

2D cell models

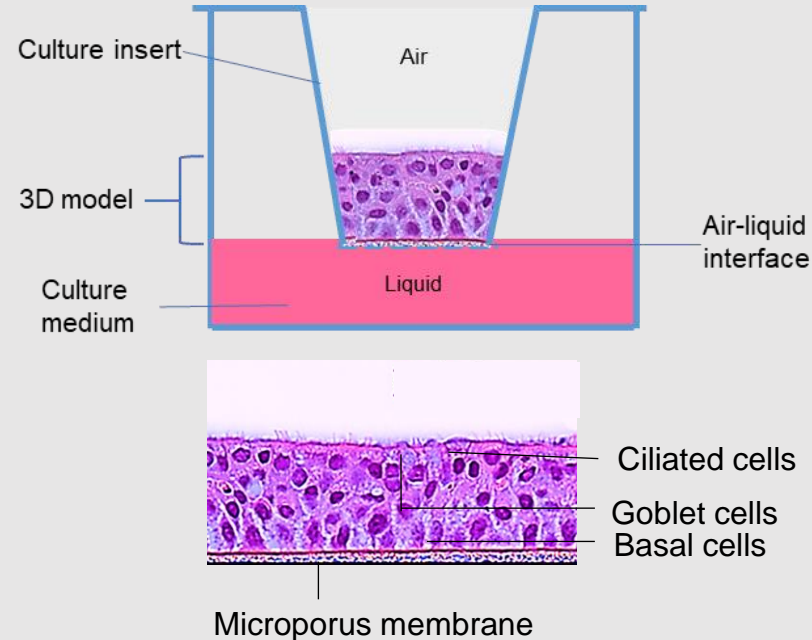


Primary Human lung fibroblasts



Calu-3 Human lung adenocarcinoma cell line

3D lung models



MucilAir™ (Epithelix)
EpiAirway™ (MatTek)

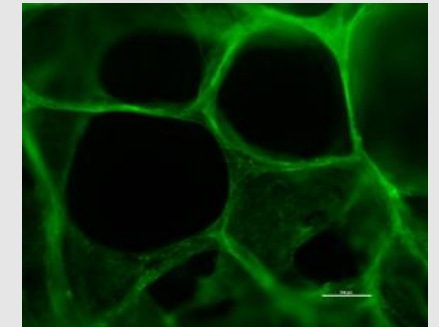
Both MucilAir and EpiAirway can be used to study lung fibrosis

Ex vivo models

Precision-cut lung slices (PCLS)



hPCLS (macroscopic)



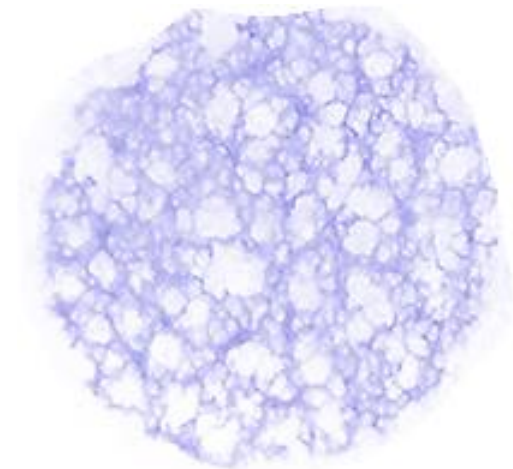
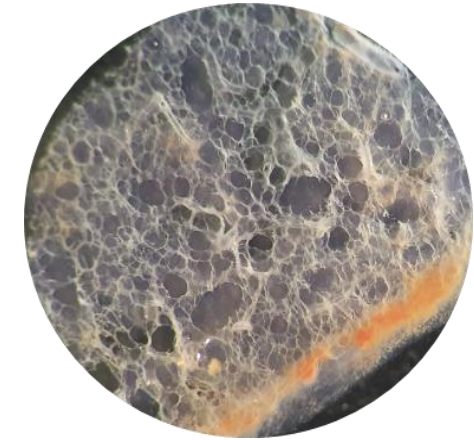
hPCLS (microscopic)

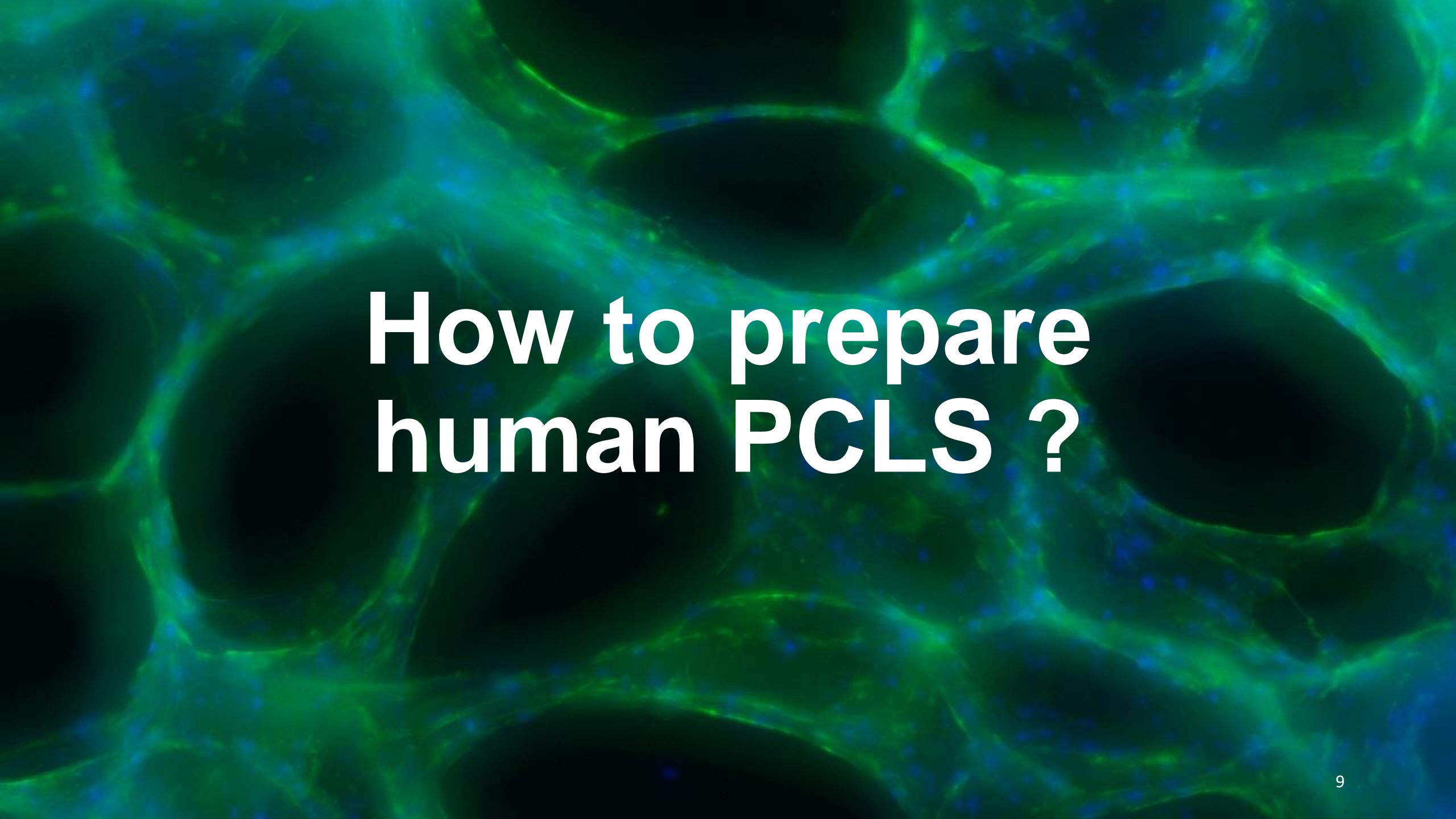
- Gold standard
- Prepared from fresh lung tissue
- Retain the native cellular architecture and extracellular matrix

Realistic representation of the lung microenvironment

Human Precision-Cut Lung Slices (hPCLS)

- Thin sections of human lung tissue (200-300 μm)
- Advantages of hPCLS:
 - Maintain the complex architecture (airways, alveoli, blood vessels) and cellular diversity
 - Responsiveness to various stimuli
 - Human relevance
 - Versatility (histological analysis, gene and protein expression studies, and functional assays)
- Applications of hPCLS:
 - Drug screening: efficacy and toxicity of new drugs
 - Model various lung diseases: infectious diseases, cancer, inflammatory conditions, and lung fibrosis
 - Toxicology studies of inhaled substances



A microscopic image of cells, likely human PCLS, showing a network of green and blue fluorescent structures. The green structures form a complex, interconnected web, while the blue structures are more discrete and scattered. The overall appearance is that of a highly organized, porous cellular network.

How to prepare human PCLS ?

How to Prepare hPCLS ?



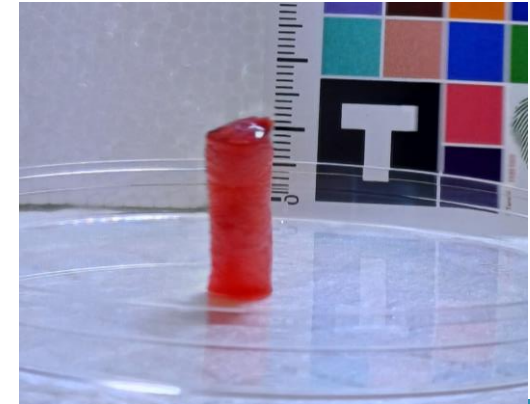
Lung resection



Low melting agarose inflation

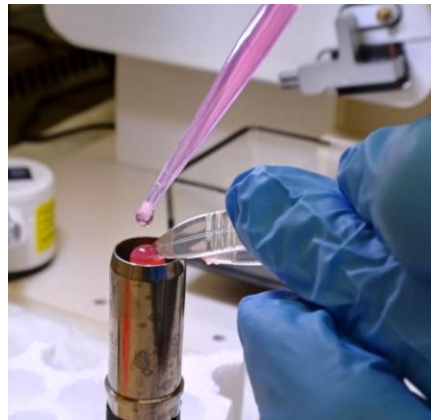


Biopsy of 8 mm Ø

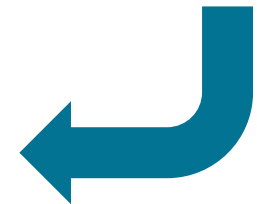
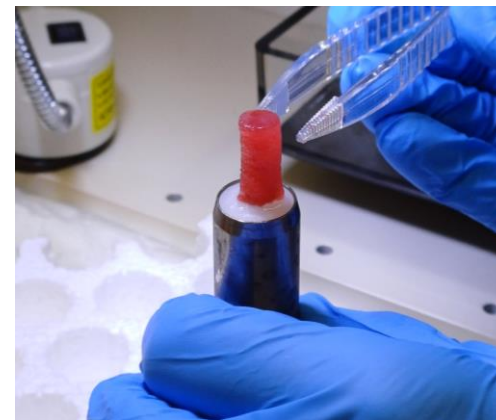


Lung core

Biobanque CRB
Tumorothèque de Nice
BB-0033-00025

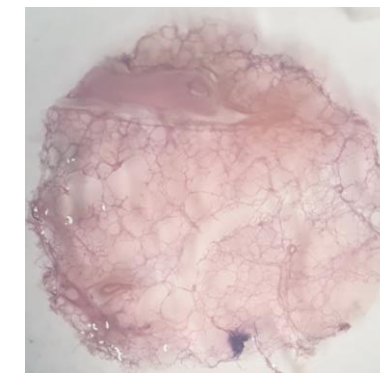
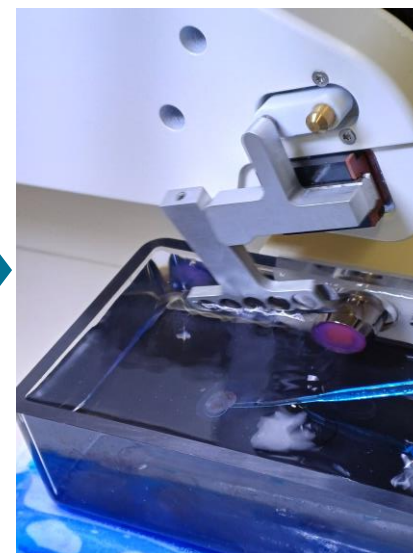
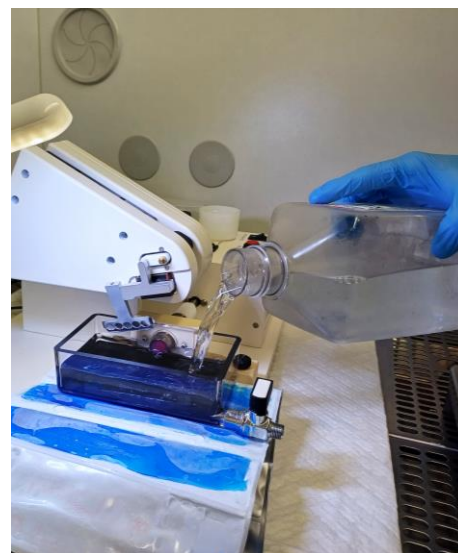
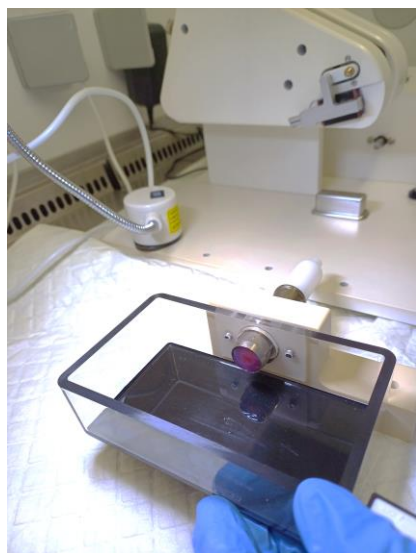


Mounting and gluing the lung biopsy on the holder



How to Prepare hPCLS ?

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



hPCLS
300 μ m thick

How to Prepare hPCLS ?

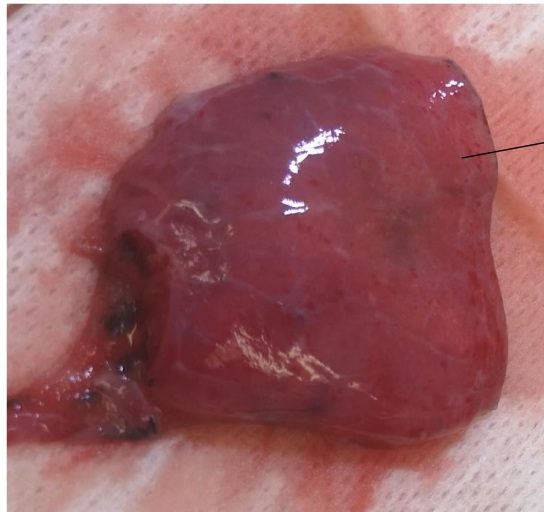


The background of the slide is a fluorescence microscopy image of hPCLS (human placental chorioallantoic membrane) cells. The cells are stained with a green fluorescent marker, likely highlighting the cytoplasm or cell membranes, and a blue fluorescent marker, likely highlighting the nuclei. The cells are arranged in a confluent monolayer, showing a typical epithelial morphology with some larger, more rounded cells and some smaller, more elongated cells. The overall appearance is that of a healthy, growing cell culture.

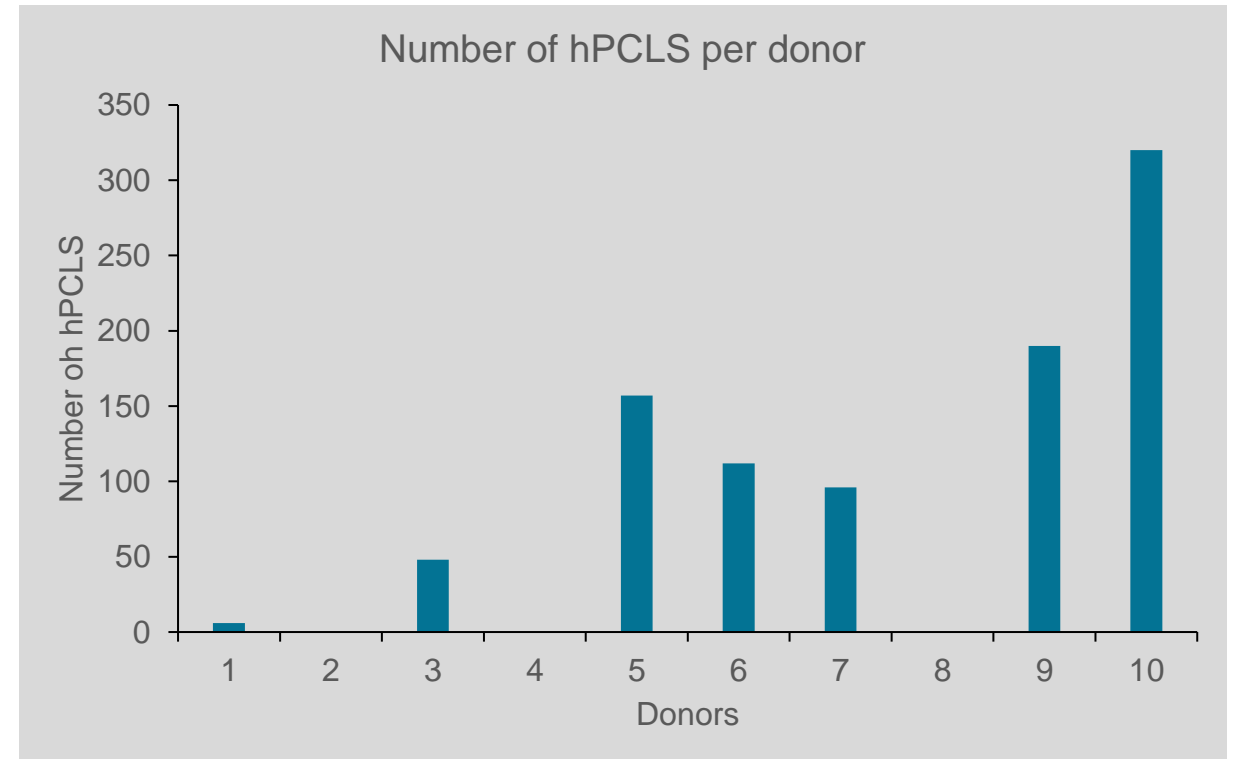
Characterization of hPCLS

Criteria for successful hPCLS

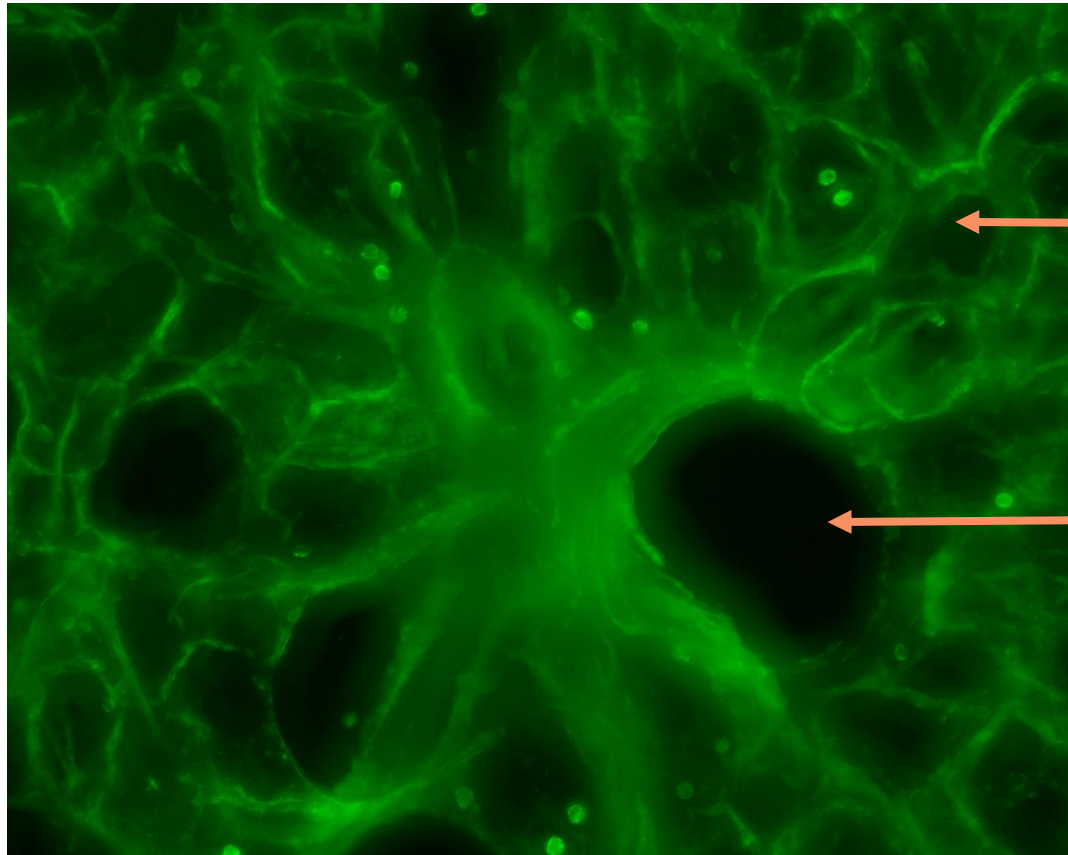
- Variable number of slice (from 0 to 300 hPCLS) depending on :
 - ✓ The size of lung resection
 - ✓ The thickness
 - ✓ The presence of a lung pleura



Lung pleura

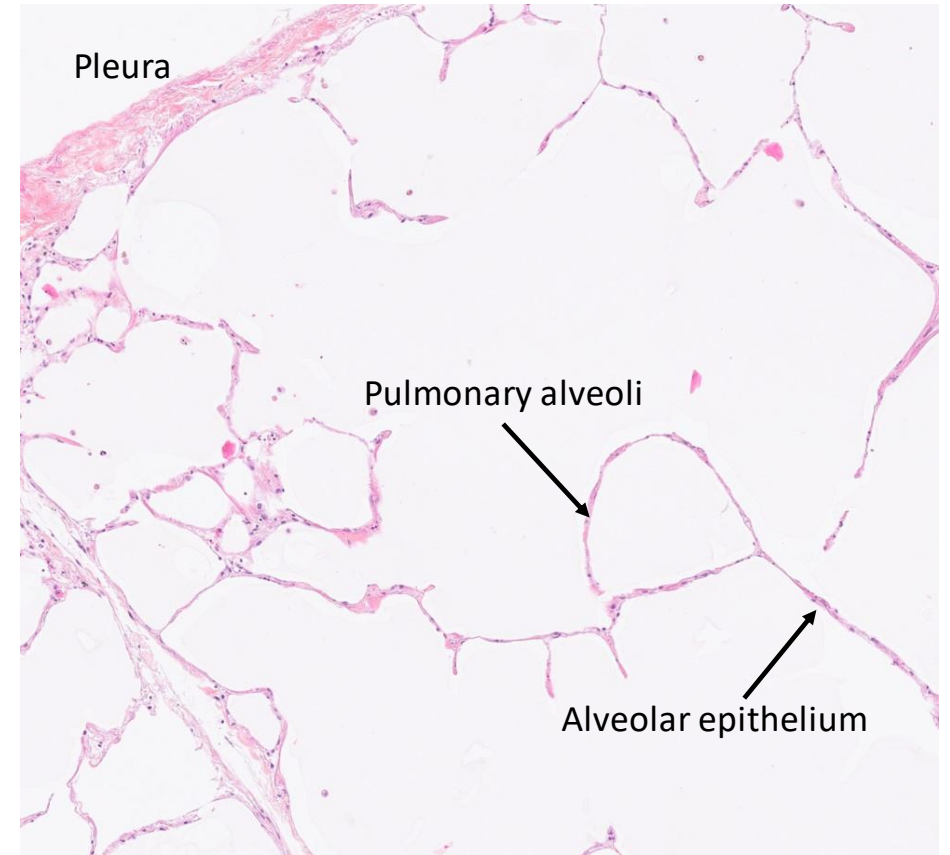


Architecture of PCLS



Alveoli

Bronchiole

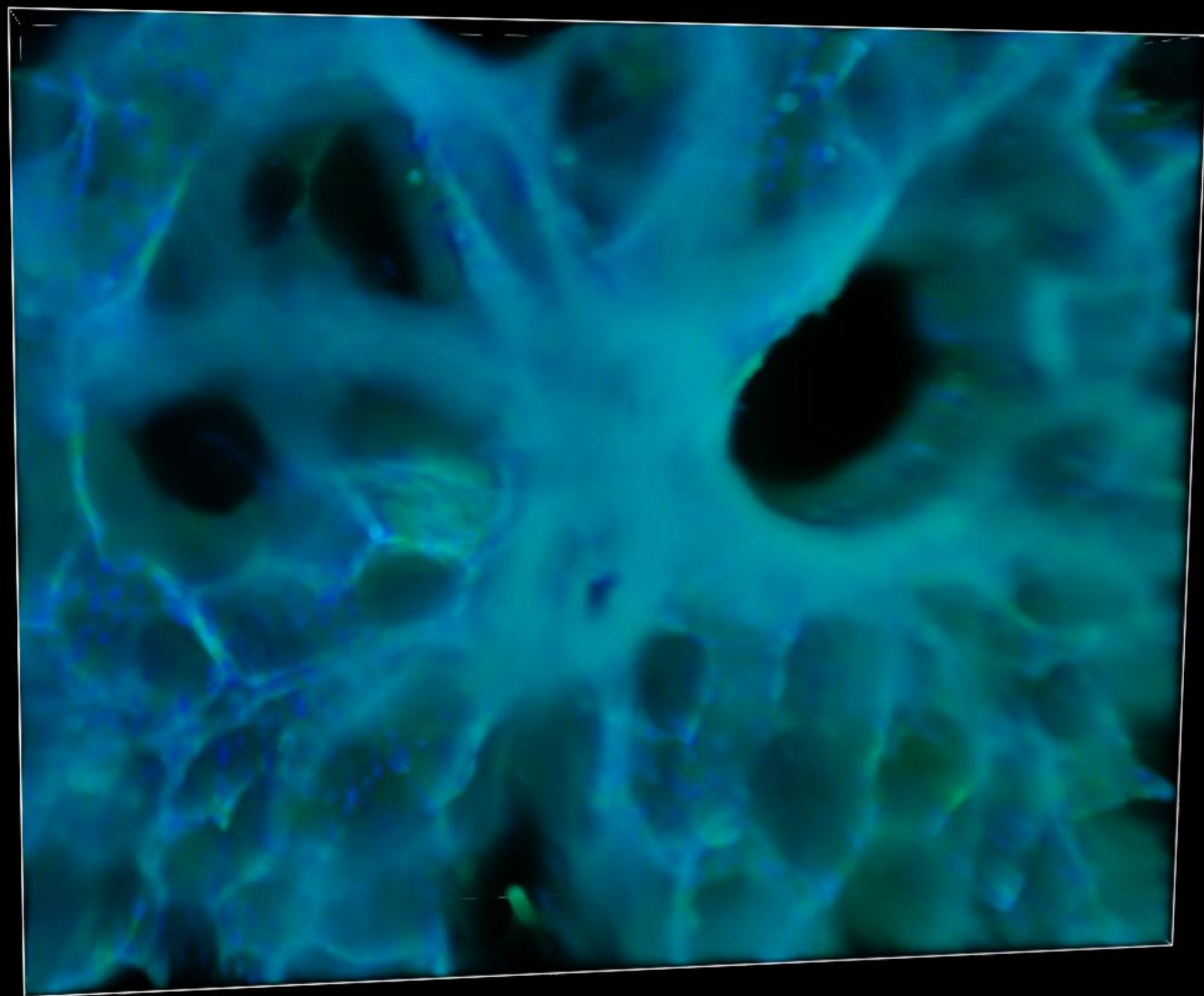


Pleura

Pulmonary alveoli

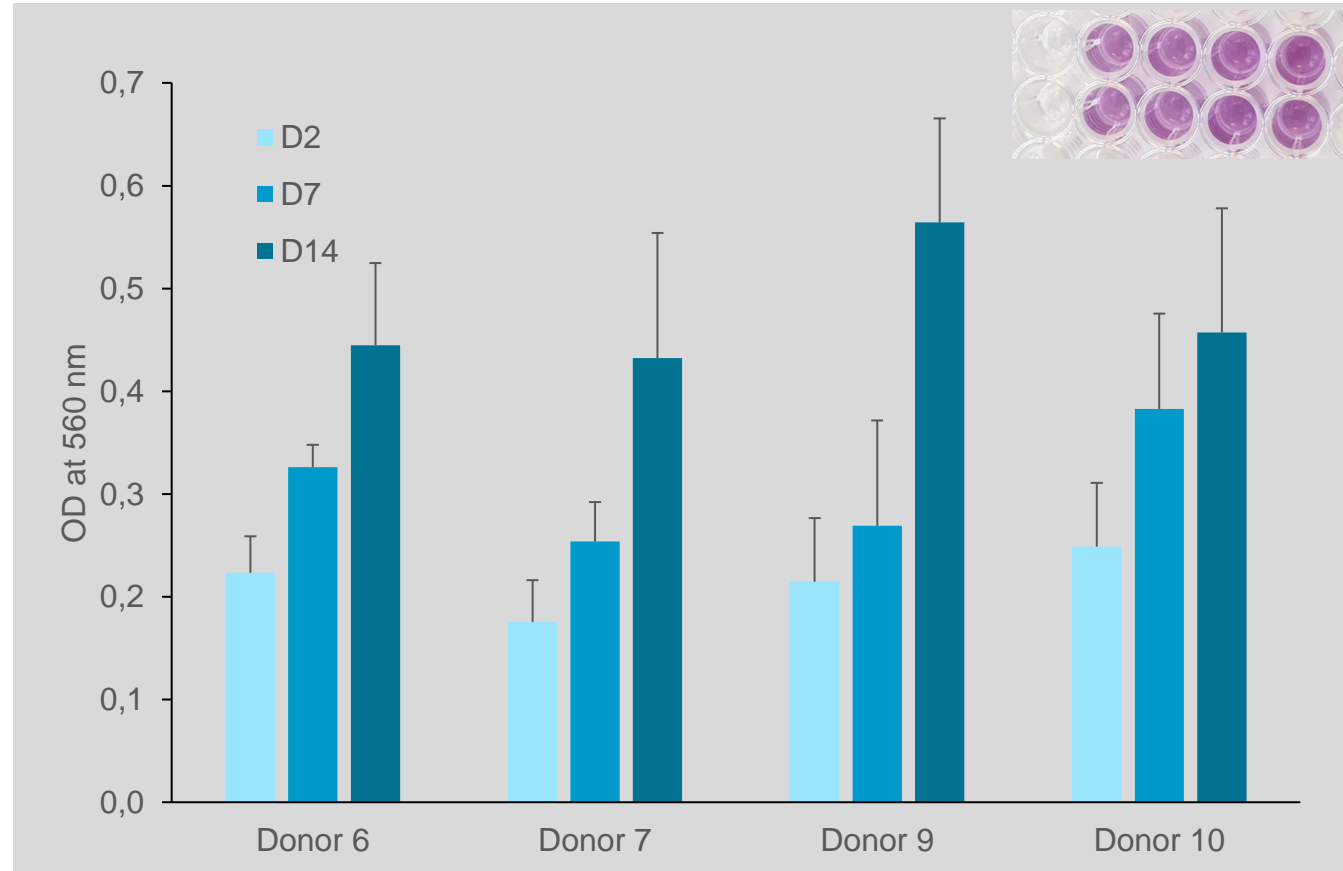
Alveolar epithelium

The lung architecture is well maintained in hPCLS model with well-defined alveoli



Viability of hPCLS over culture time

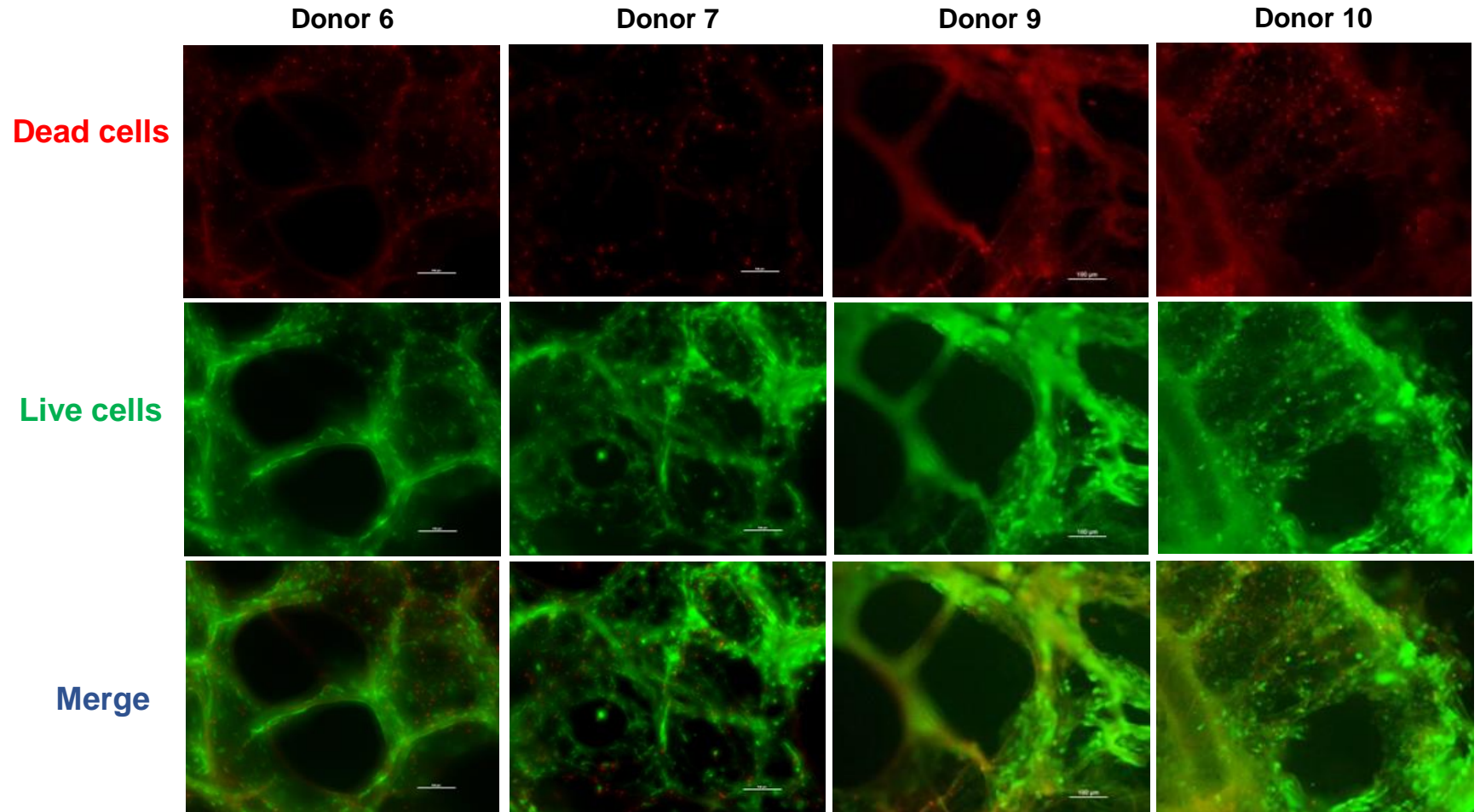
MTT assay



Viability of hPCLS is increasing between Day 2 and Day 14

hPCLS Viability of hPCLS at day 7

Live/dead assay



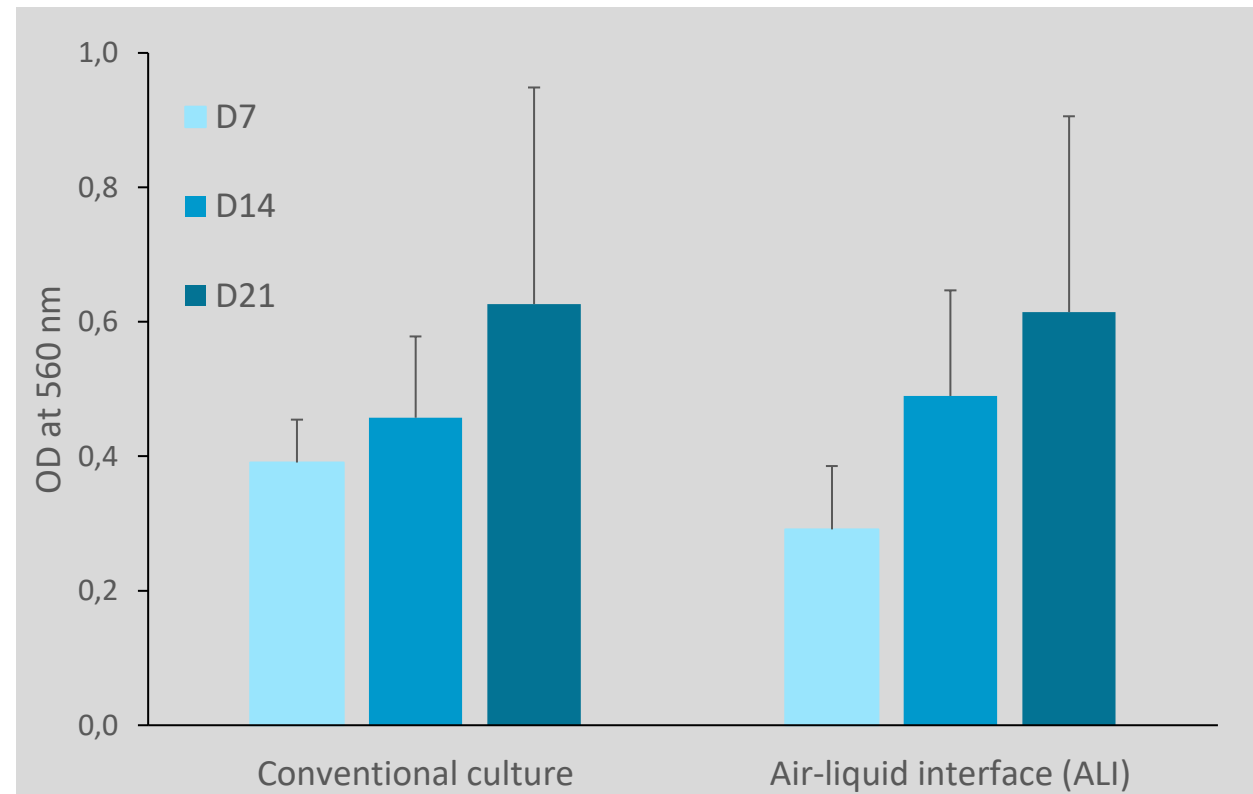
Viability of hPCLS is maintained after 7 days of culture

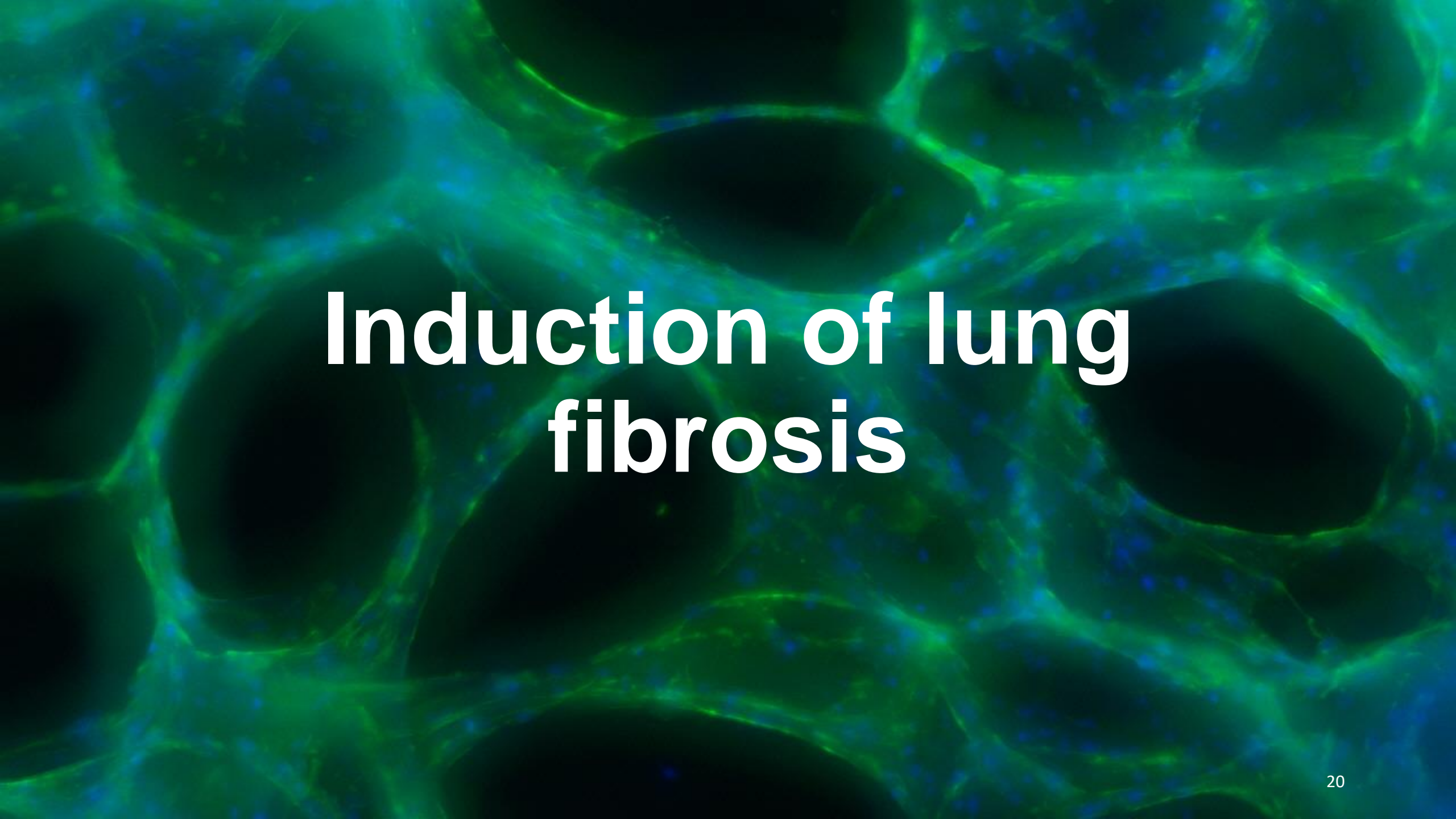
Viability of hPCLS in ALI conditions

- In Air-liquid interface culture conditions (ALI), hPCLS viability is maintained as in conventional culture conditions up to 21 days



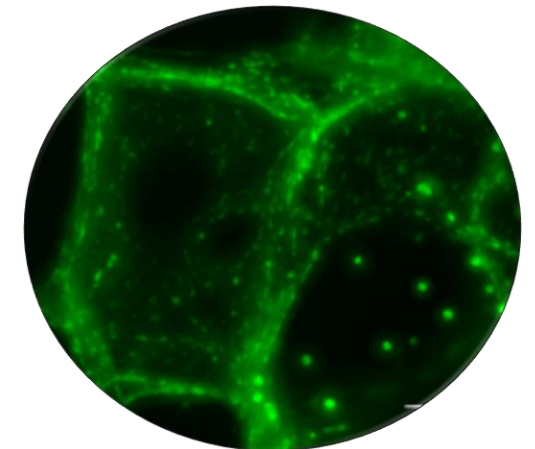
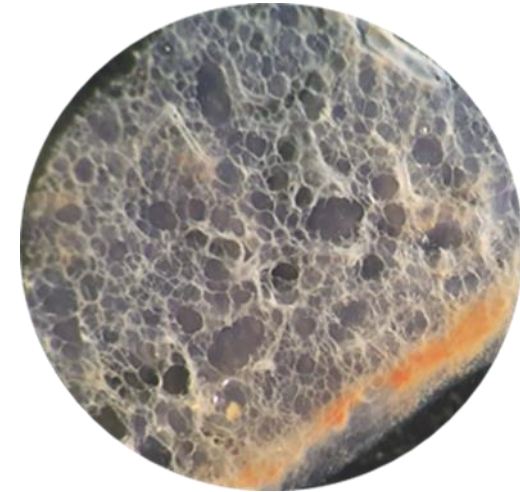
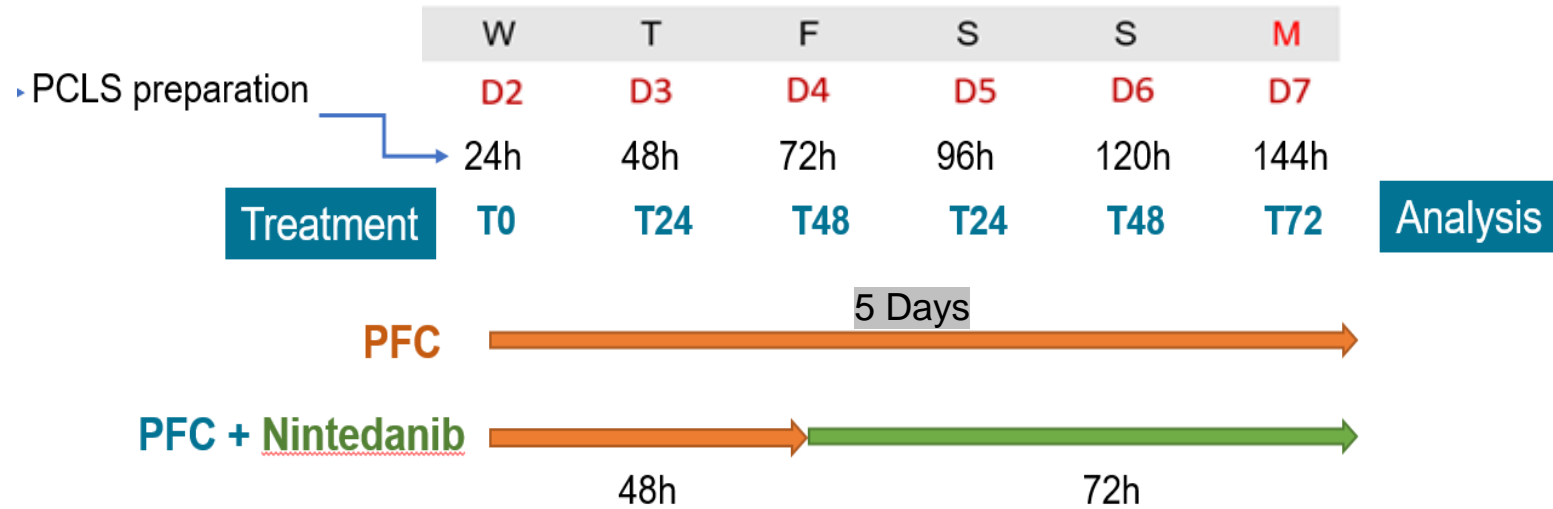
ALI conditions



The background of the slide is a fluorescence microscopy image of lung tissue. It shows a complex network of green-stained fibers and structures, likely representing collagen or other extracellular matrix components. Interspersed among these green structures are numerous small, bright blue spots, which are characteristic of DAPI-stained nuclei. The overall appearance is that of a dense, interconnected tissue structure.

Induction of lung fibrosis

Induction of lung fibrosis



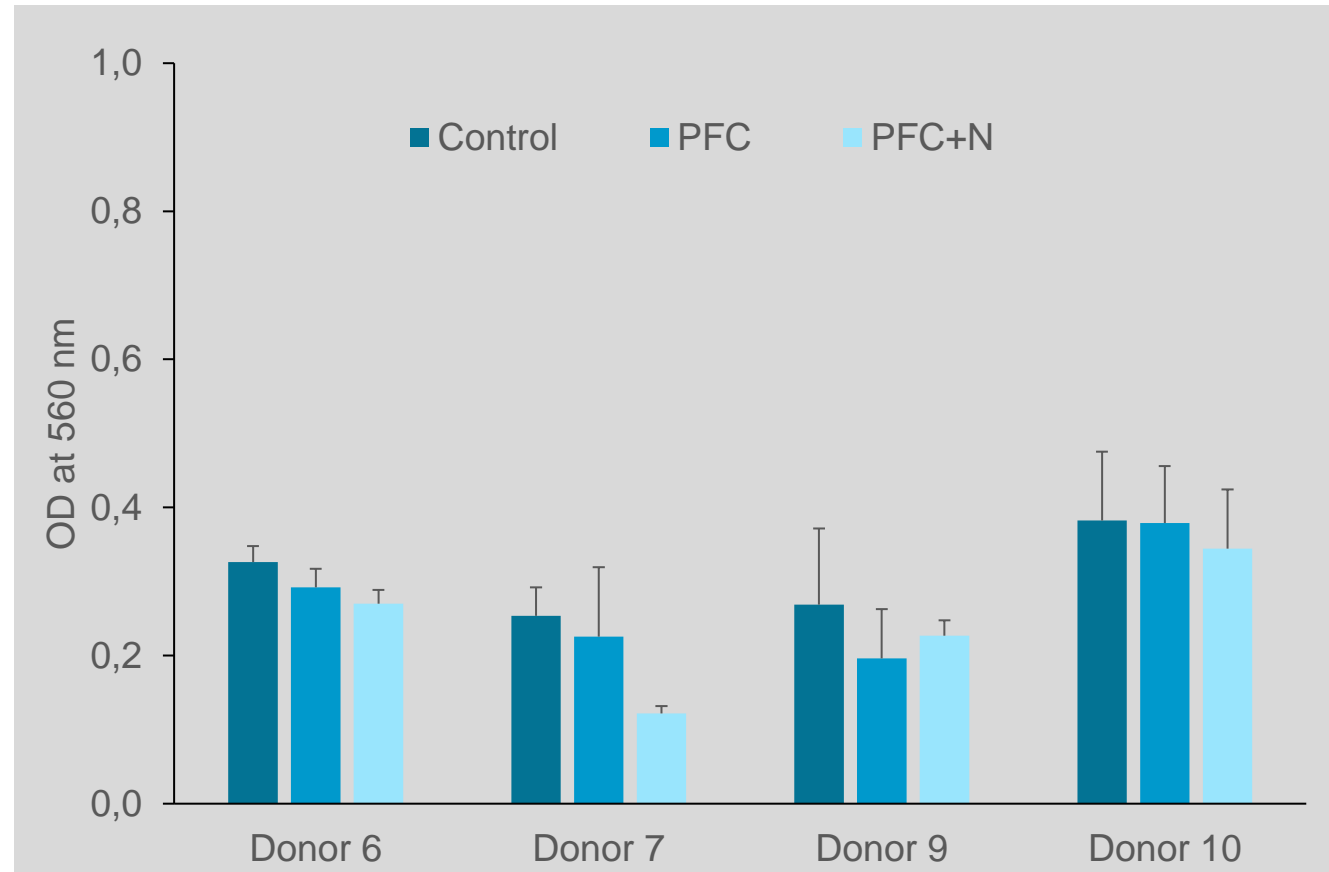
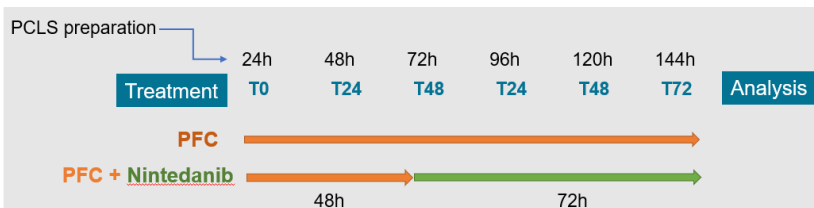
Profibrotic Cocktail (PFC)¹:

- 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).

¹Alsafadi et al., 2017

hPCLS Viability after PFC treatment

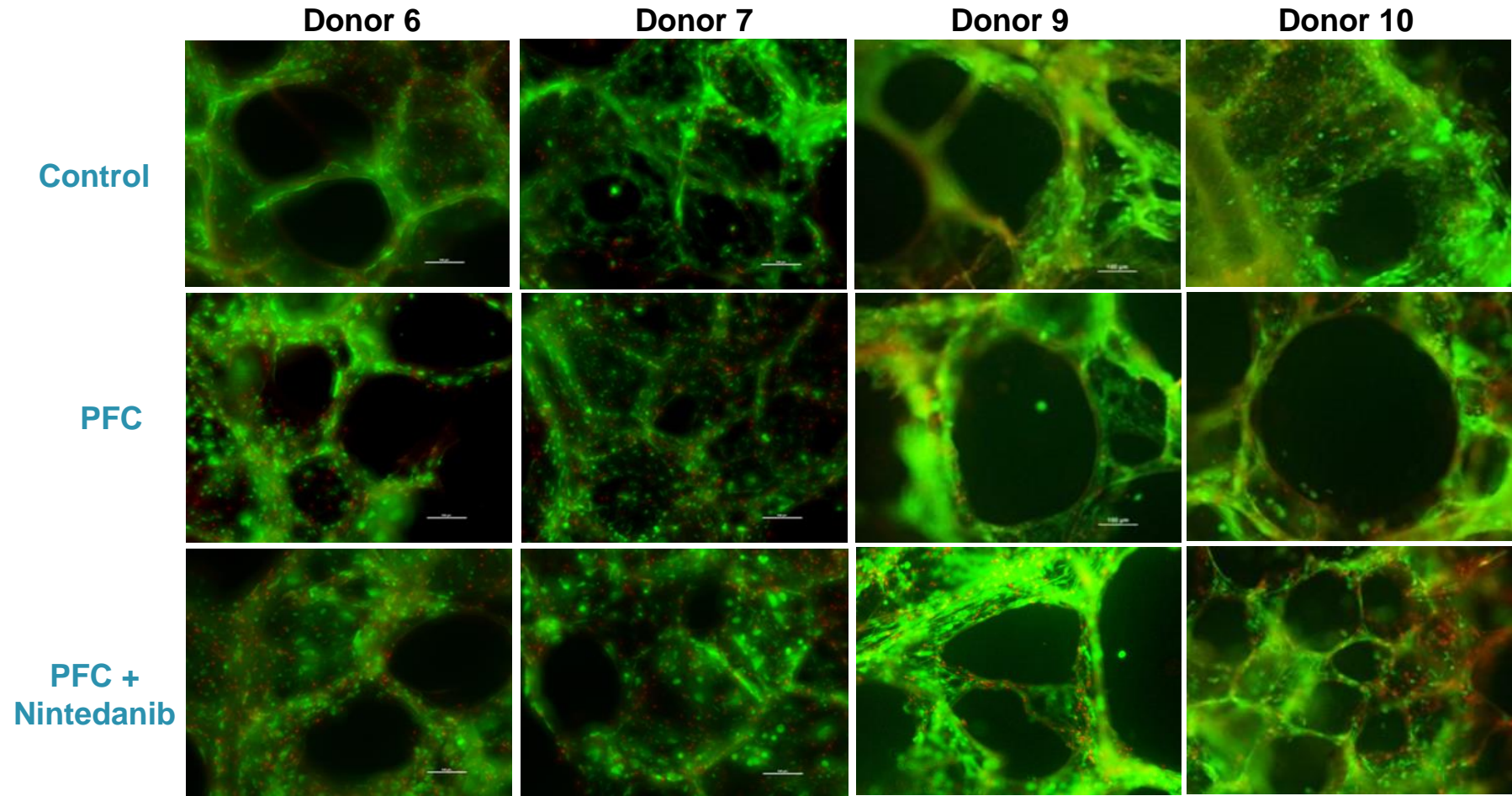
MTT assay



Cell viability of hPCLS is well maintained after profibrotic cocktail treatment

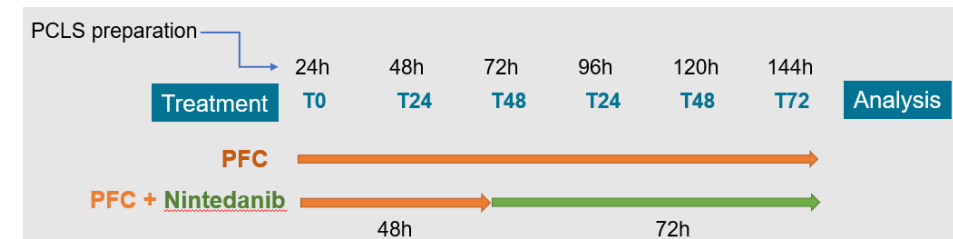
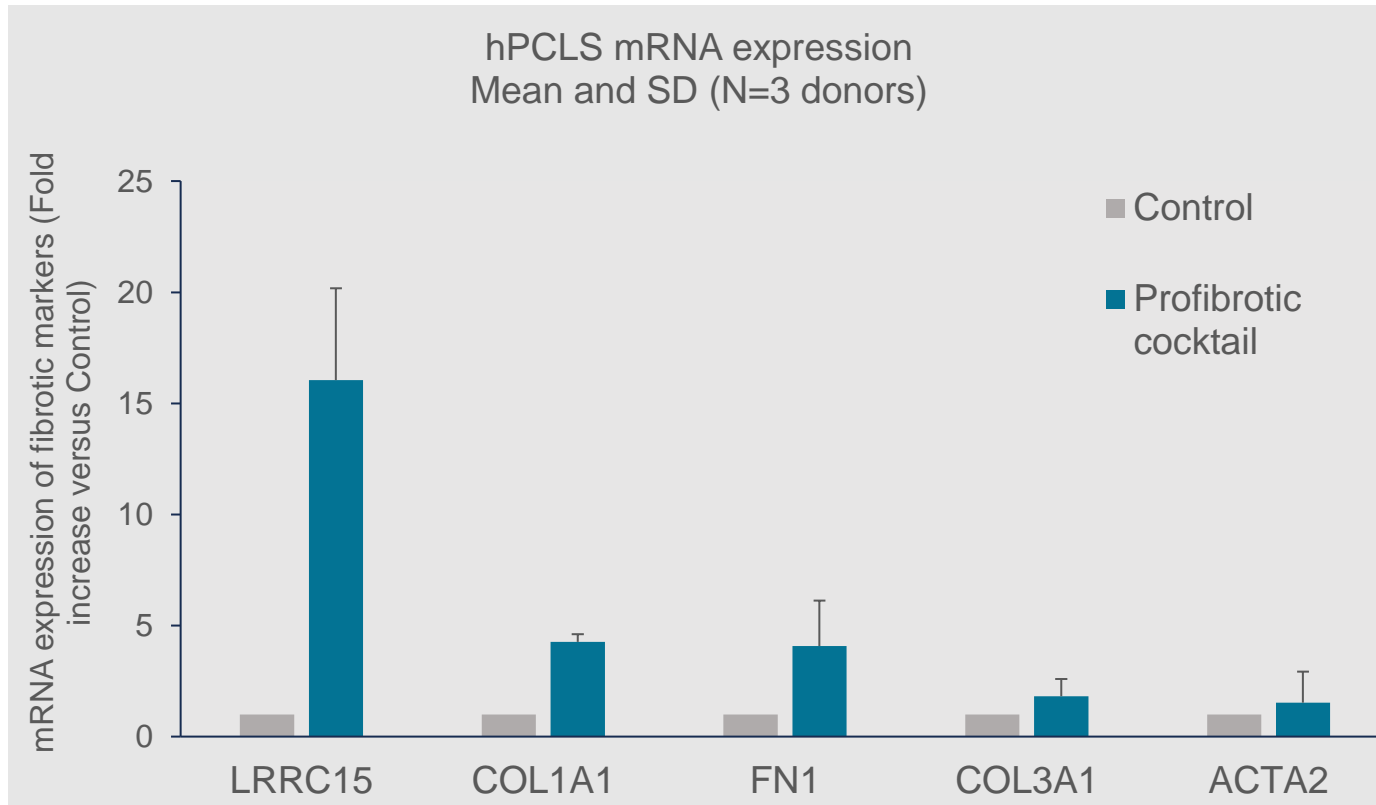
hPCLS Viability after PFC treatment

Live/dead assay



Cell viability of hPCLS is well maintained after treatment with profibrotic cocktail

Expression of lung fibrosis markers in hPCLS



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

A microscopic image of plant cells, likely from a leaf, showing a network of green and blue fluorescent structures. The green structures form a complex, interconnected web, while the blue structures are more discrete and scattered. The overall appearance is that of a highly organized, cellular network.

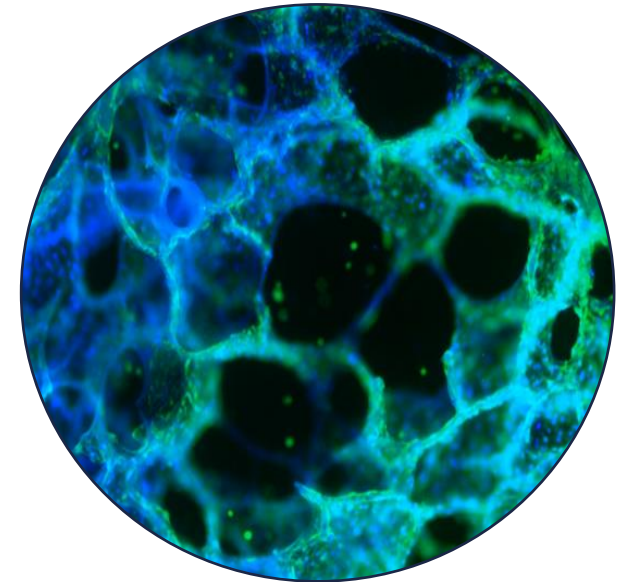
Conclusion

Conclusion

- Preparation of hPCLS is a complex and technically challenging process that requires expertise in tissue handling and culture techniques
- hPCLS model provides a valuable tool for studying lung fibrosis due to its ability to recapitulate the *in vivo* environment, preserves the architecture and cell types of the lung and responds to various stimuli.

On going analysis :

- ✓ Proteomic analysis of hPCLS treated with profibrotic cocktail
- ✓ Characterization of the Air/Liquid interface culture model





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Thank you!

PKDERM's Team

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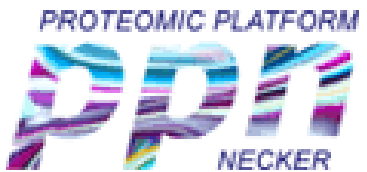
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