

Induced Pluripotent Stem Cell Derived Alveolar Lung Organoids

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Introduction

Idiopathic Pulmonary Fibrosis

- Chronic and progressive lung disease
- Causes fibrosis
- High morbidity and mortality
- Main symptoms
 - Shortness of breath
 - Dry cough
- Can lead to further complications
 - Lung cancer
 - Pulmonary emboli
 - Pulmonary hypertension

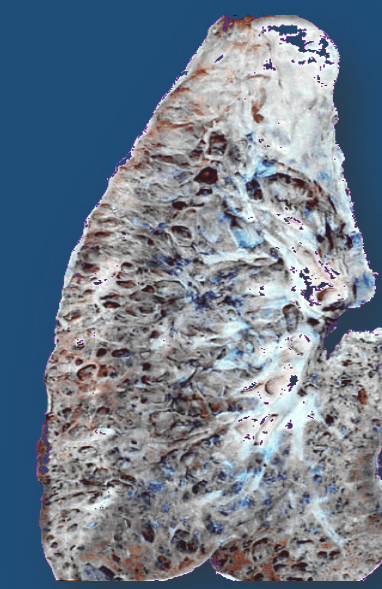


Figure 1.
IPF Lung
(Dr. K Watts)

There is no cure, only limited treatment options.

Our goal is to create a complex *in vitro* organoid model to address the lack of representative models.

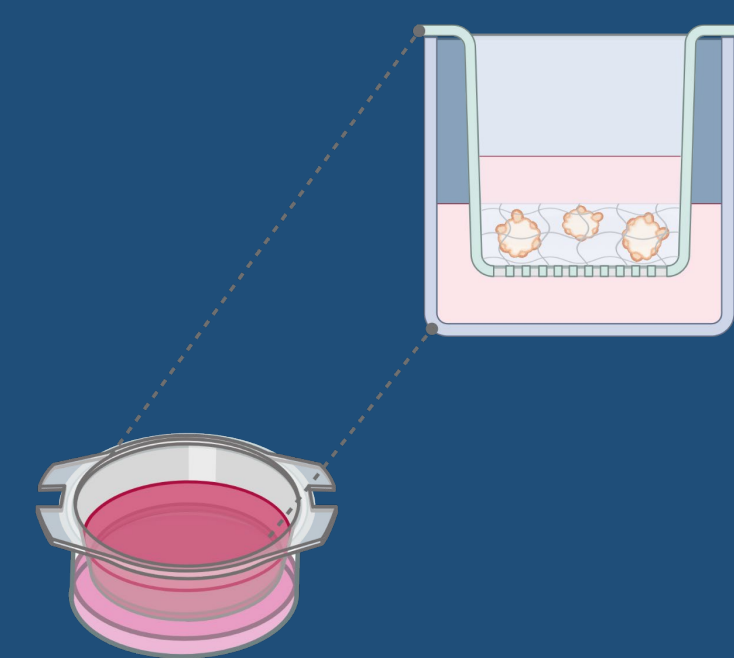


Figure 2. Alveolar Lung Organoid Culture Scheme

Methods

iPSCs were grown and differentiated into alveolar lung organoids using different cocktails of growth factors and cytokines; iPSCs were differentiated to endothelial cells by chemical induction. Each stage of differentiation was validated via flow cytometry, microscopy and relative gene expression analysis.

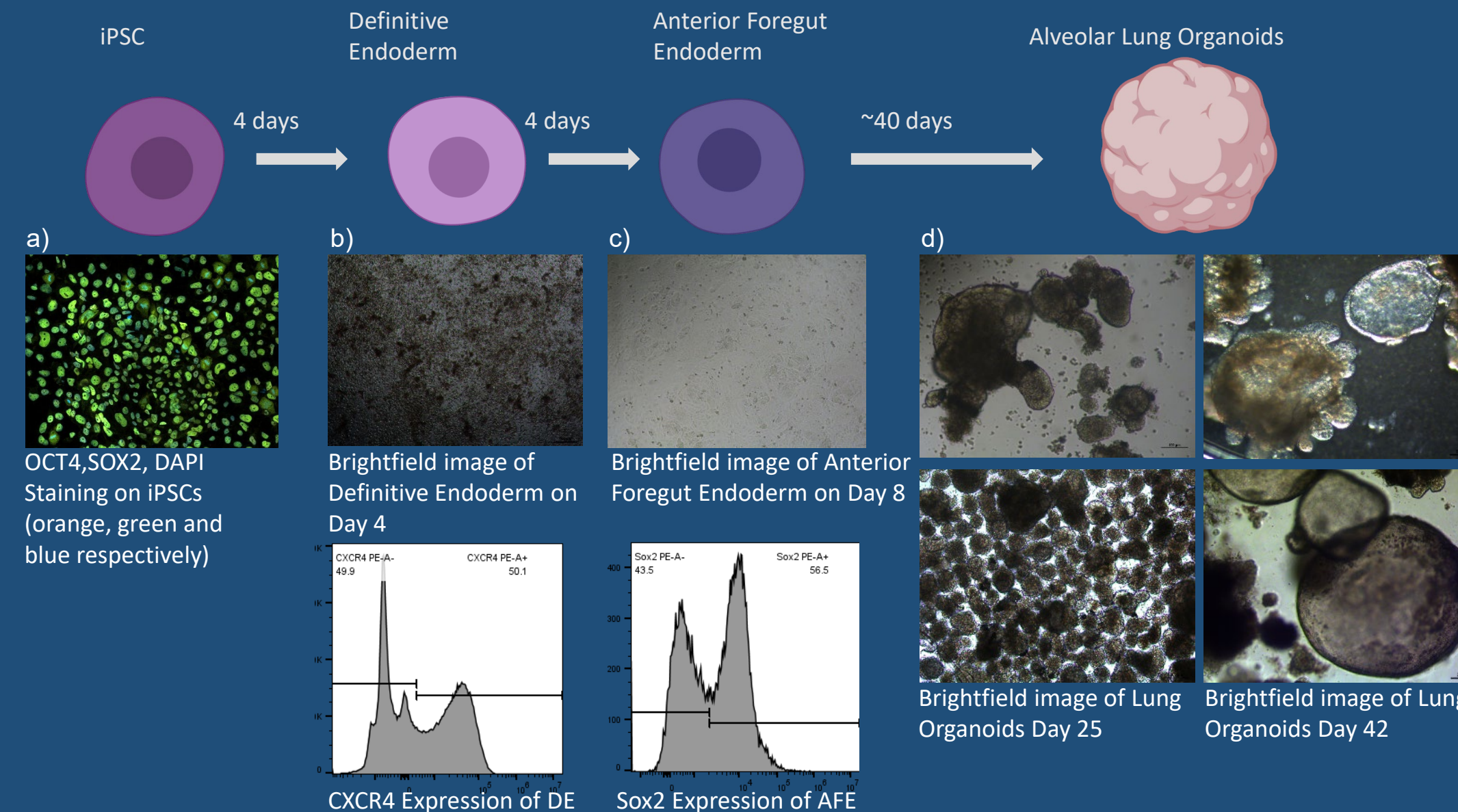


Figure 3. Alveolar Lung Organoid Differentiation. a) iPSC, b) Definitive Endoderm, c) Anterior Foregut Endoderm, d) Alveolar Lung Organoids

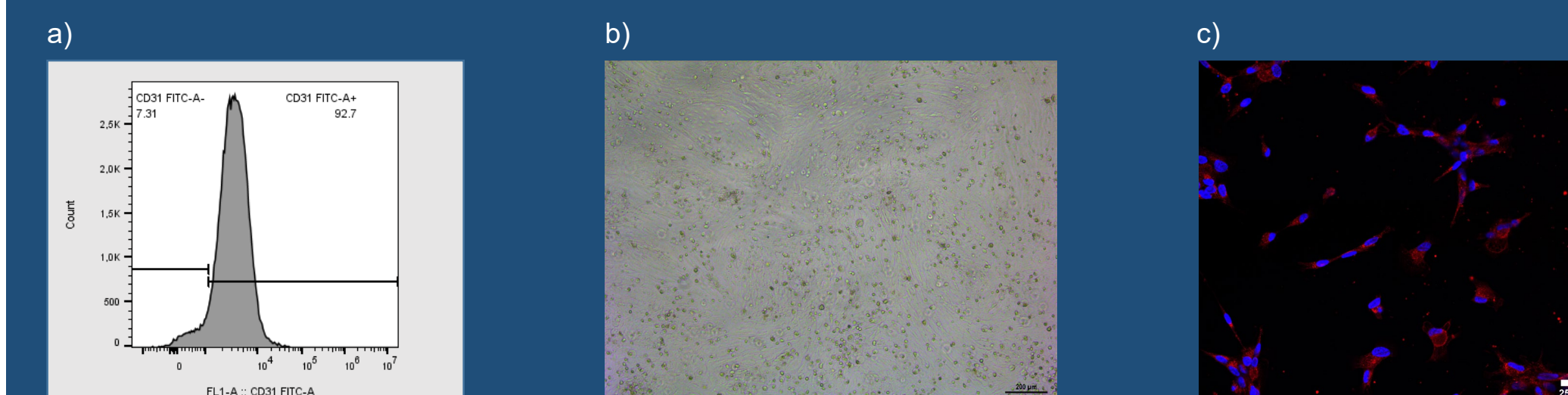


Figure 4. Validation of iPSC Derived Endothelial Cells. a) CD31 expression of Endothelial Cells after sorting Endothelial Progenitor Cells, b) iPSC Derived Endothelial Cells after one round of selective passaging, c) Immunofluorescence image of Endothelial Cells with von Willebrand factor (Red) and DAPI (Blue)

Results

iPSCs cultured in defined media were first differentiated into Definitive Endoderm; media was changed directing the cells towards Anterior Foregut Endoderm (AFE). AFE were differentiated into Alveolar Lung Organoids in two stages with branching structures emerging in matrigel after d35, and maturing after d40. Mature ALOs had AEI and AECII markers with multiple cell types.

iPSC were separately differentiated via chemical induction into endothelial cells as validated by CD31 staining on the endothelial cells.



Figure 5. Endothelial Tube Formation Assay on Matrigel on iPSC derived Endothelial Cells after 6 hours

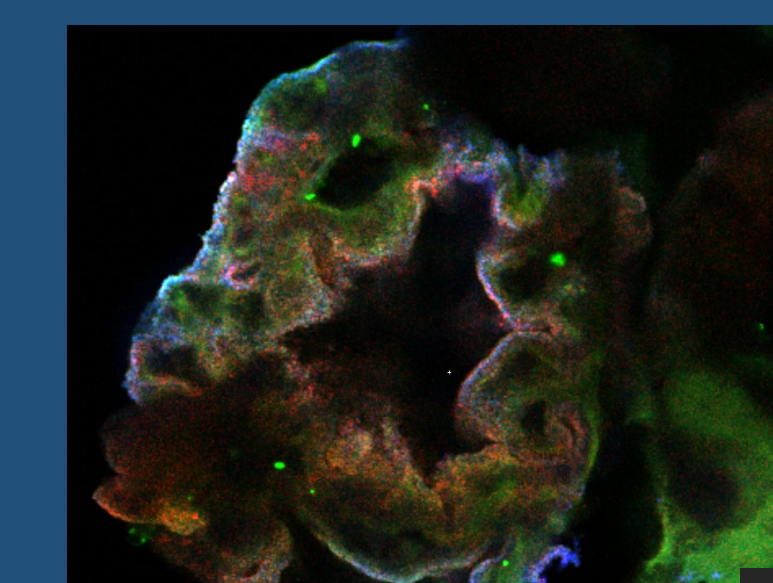


Figure 6. Immunofluorescence image of Alveolar Lung Organoid. SP-C (Green), SP-B (Red), DAPI (Blue)

Conclusion

iPSC differentiate into self organizing alveolar organoids that have both alveolar epithelial type I and alveolar epithelial type II cells when grown in matrigel in defined media.

Further investigations are ongoing to combine alveolar epithelial cells and endothelial cells into a complex organoid.

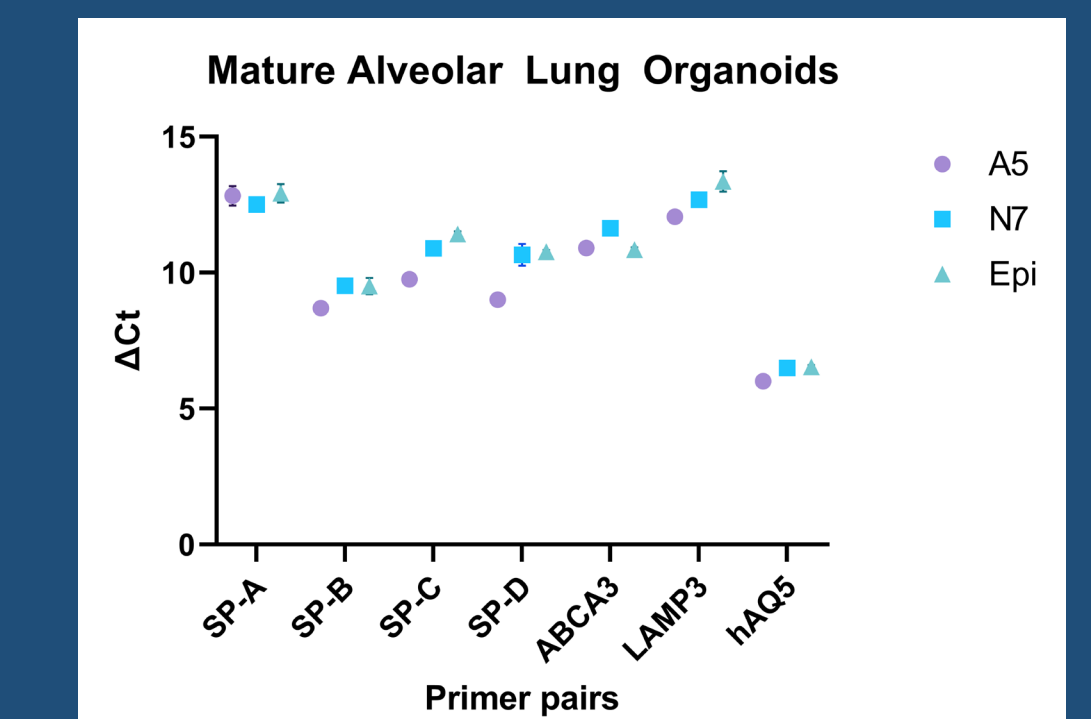


Figure 7. qPCR results showing mRNA expression of Alveolar Epithelial Type I and Type II marker expression in mature Alveolar Lung Organoids,

References

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 Illustrations created with BioRender (Fig 2, 3 & 7)