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



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Science for Regeneration

BACKGROUND

Human precision-cut lung slices (hPCLS) are emerging as a sophisticated and physiologically relevant *ex vivo* model for respiratory research. These thin, precisely-cut tissues are obtained from fresh lungs using a vibrating microtome. The hPCLS retain the native 3D architecture of the lung. This unique feature, along with the presence of all lung cell types (including smooth muscle cells, epithelial cells, and fibroblasts), makes hPCLS an ideal model for investigating lung diseases and evaluating the safety, toxicity, and efficacy of novel therapies.

This study aimed to establish a protocol for generating high-quality, viable hPCLS suitable for modeling lung diseases, especially lung fibrosis.

METHODS

Human lungs resections (Biobanque CRB, Tumorothèque de Nice, BB-0033-00025) were perfused with Hanks' Balanced Salt Solution (HBSS) to remove excess blood. The lung resection was then infused with a 2% low melting point agarose solution at 40°C. The agarose was allowed to solidify at 4°C, then a biopsy punch was used to generate a lung cores of 8 mm diameter. Lung sections of 300 µm thickness were cut using the Compresstome® VF-300-0Z (Precisionary Instruments, USA). The obtained hPCLSs were cultured in a humidified cell incubator set at 37°C and 5% CO₂ for up to 14 days.

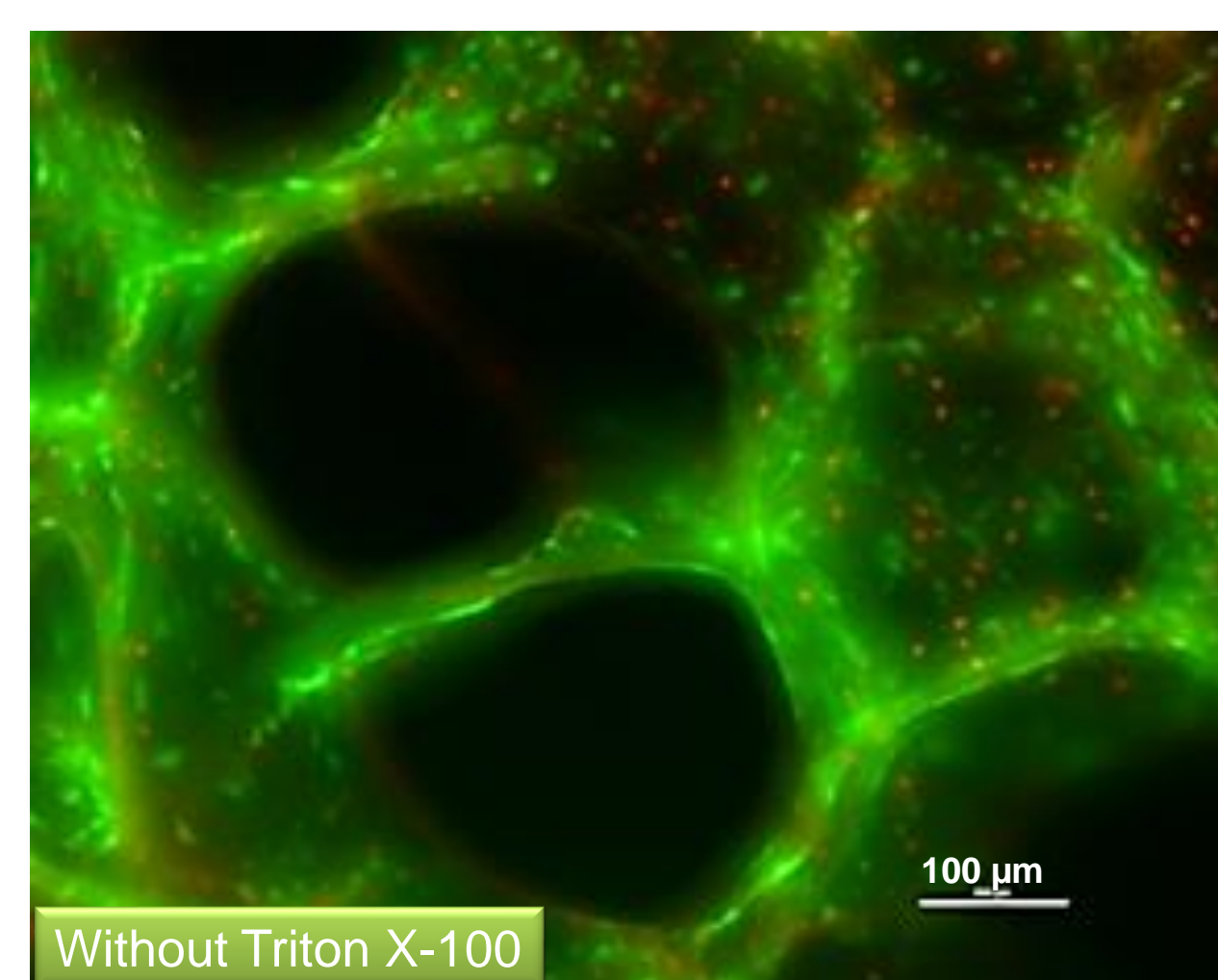
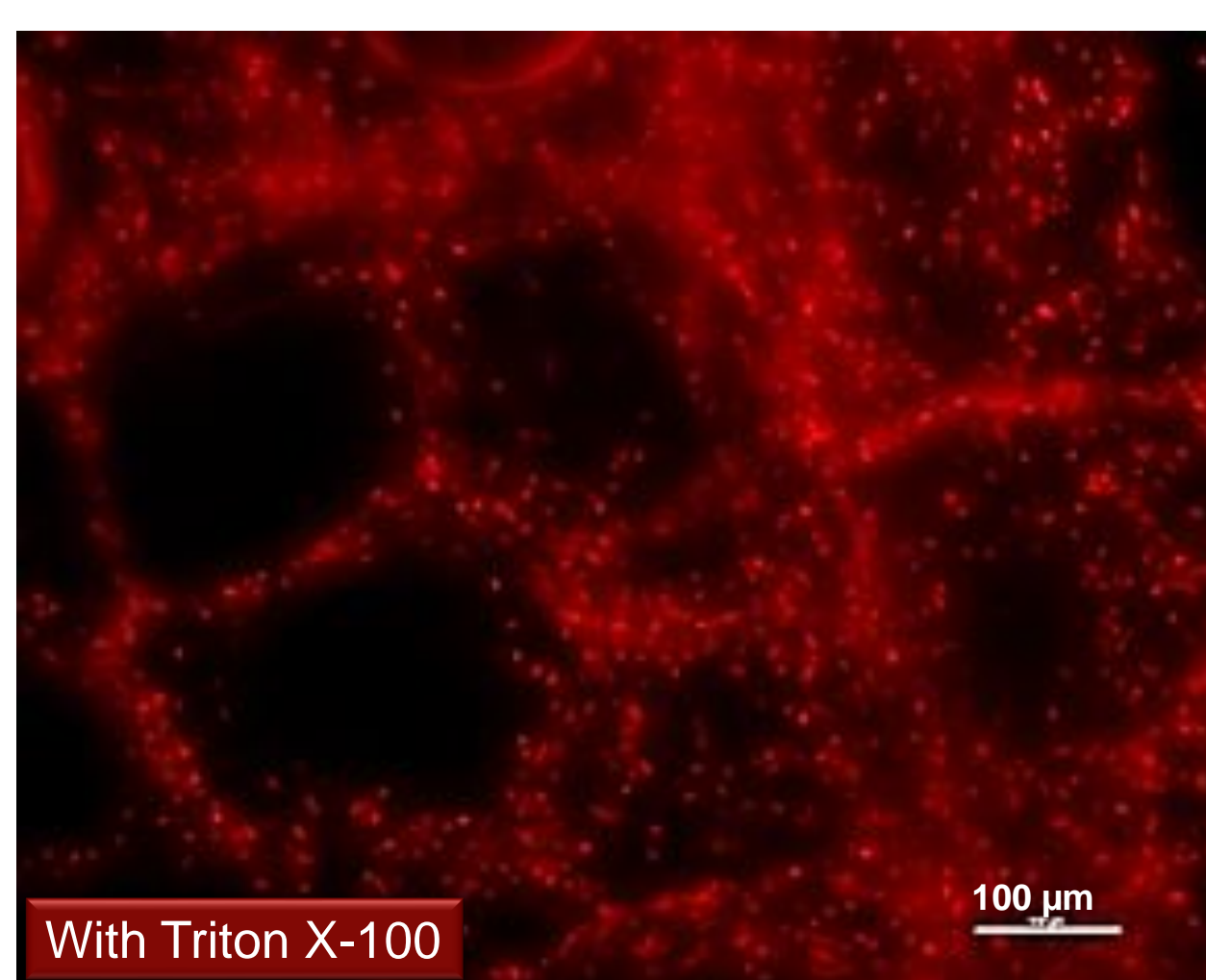
The viability of hPCLS was investigated using MTT and Live/Dead assays. These assays evaluated the metabolic activity and membrane integrity of the hPCLS, providing insights into their overall health. ActinGreen staining further examined the structural organization of the hPCLS using fluorescence microscopy with z-stacking. Following treatment with a profibrotic cocktail¹, the functionality of hPCLS was assessed by analyzing the mRNA expression of fibrotic markers through RT-qPCR.



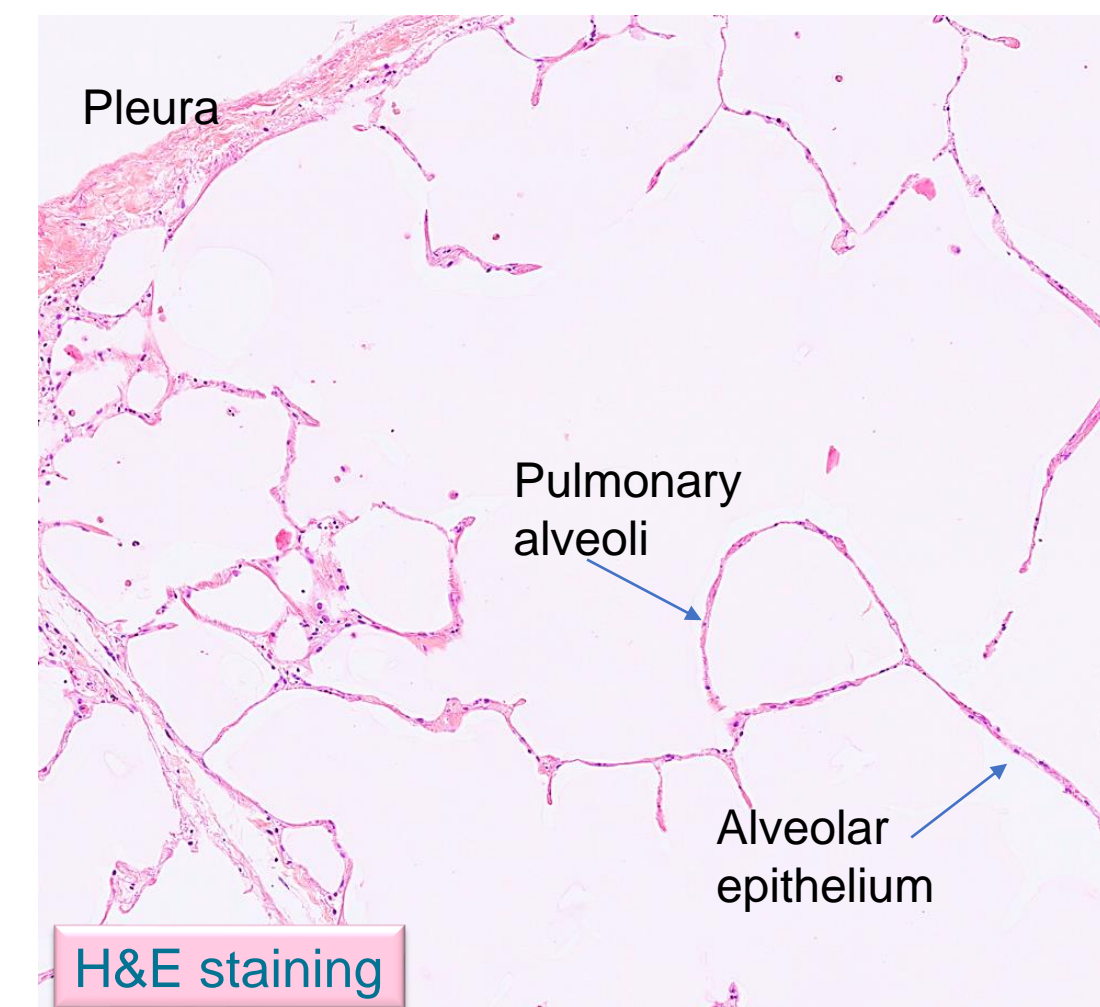
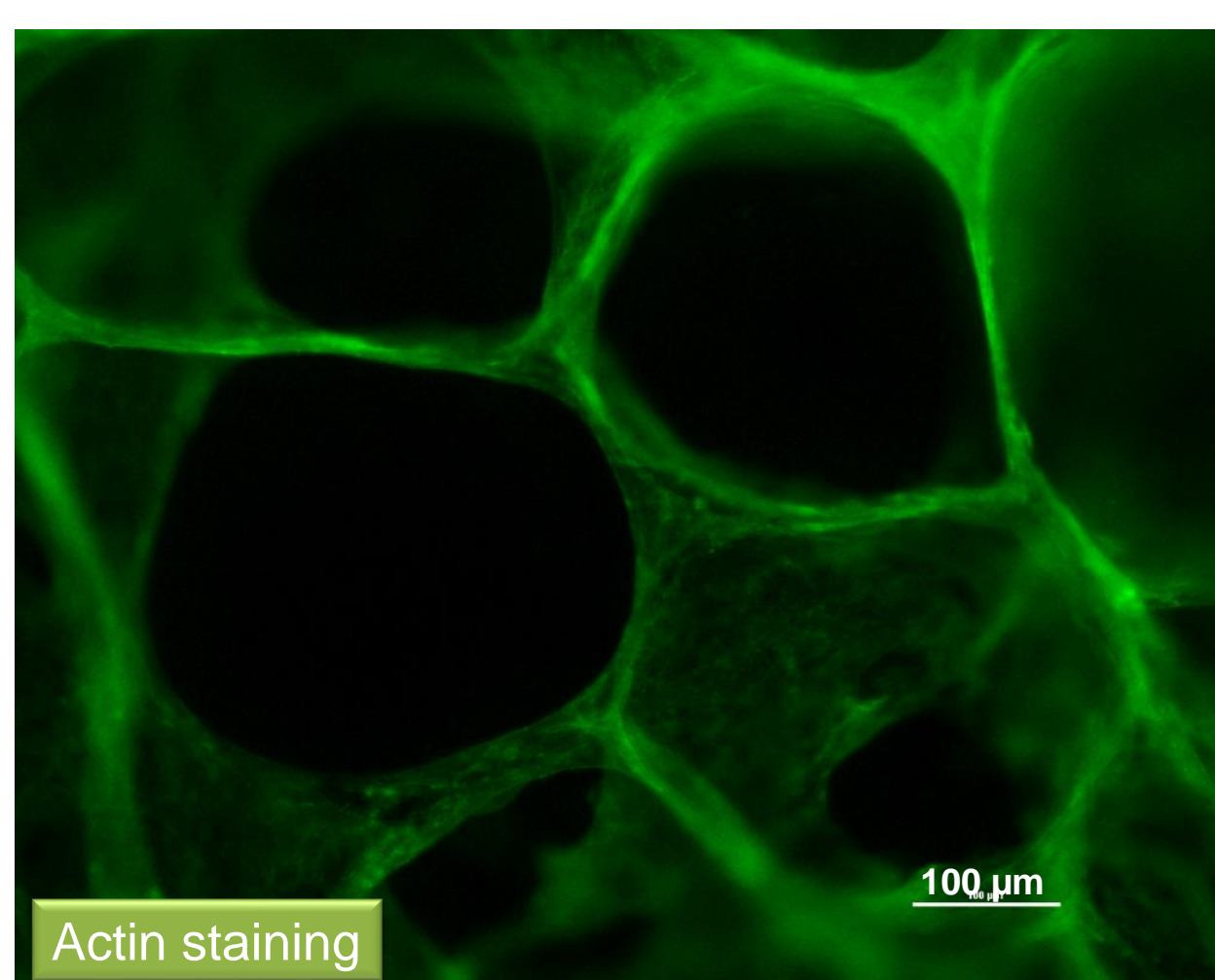
RESULTS

Live/Dead assay at Day 7

Live cells are stained in green, and dead cells are stained in red

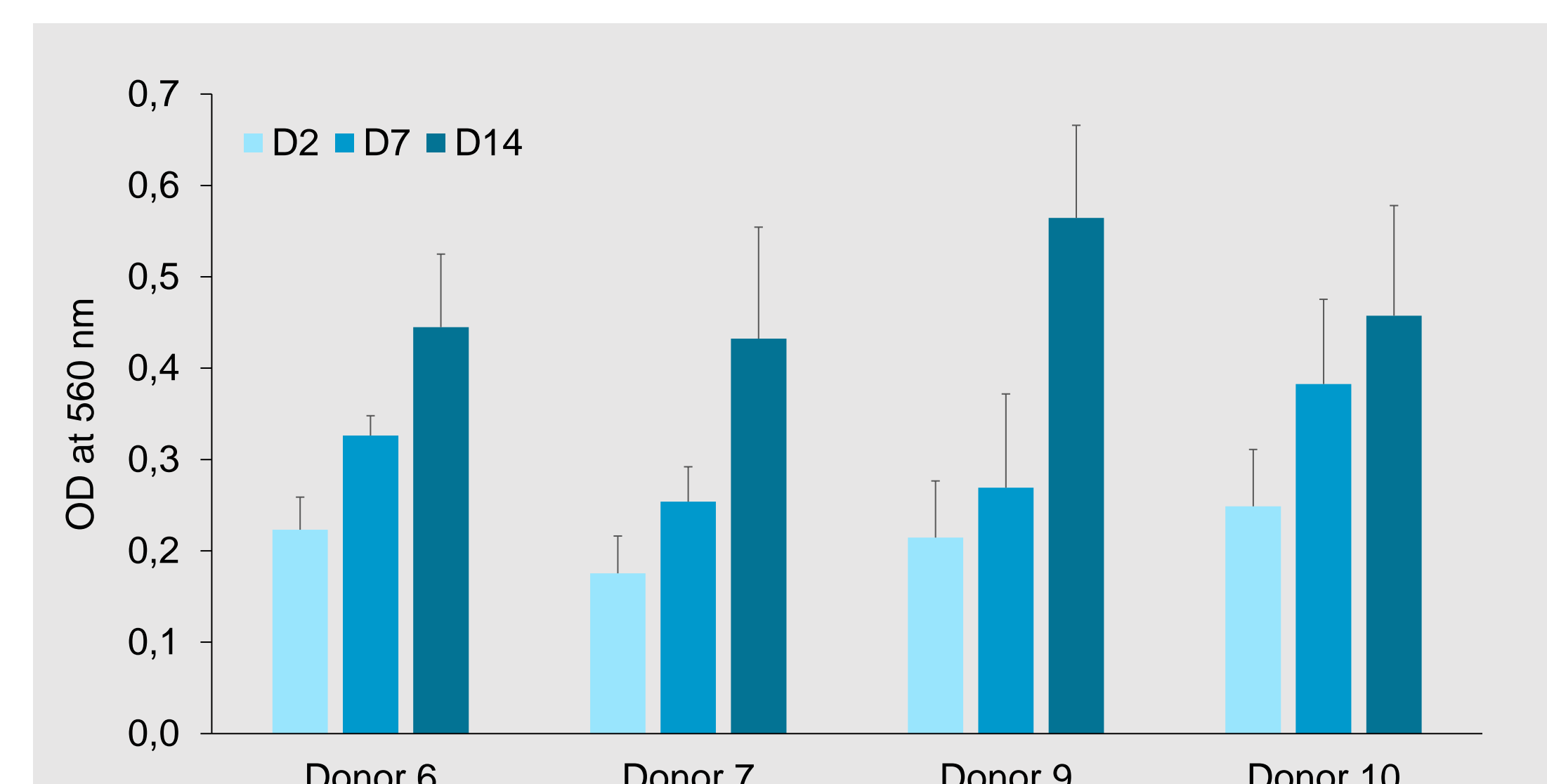


Actin, Hematoxylin and Eosin staining indicate well-preserved lung tissue architecture in hPCLS

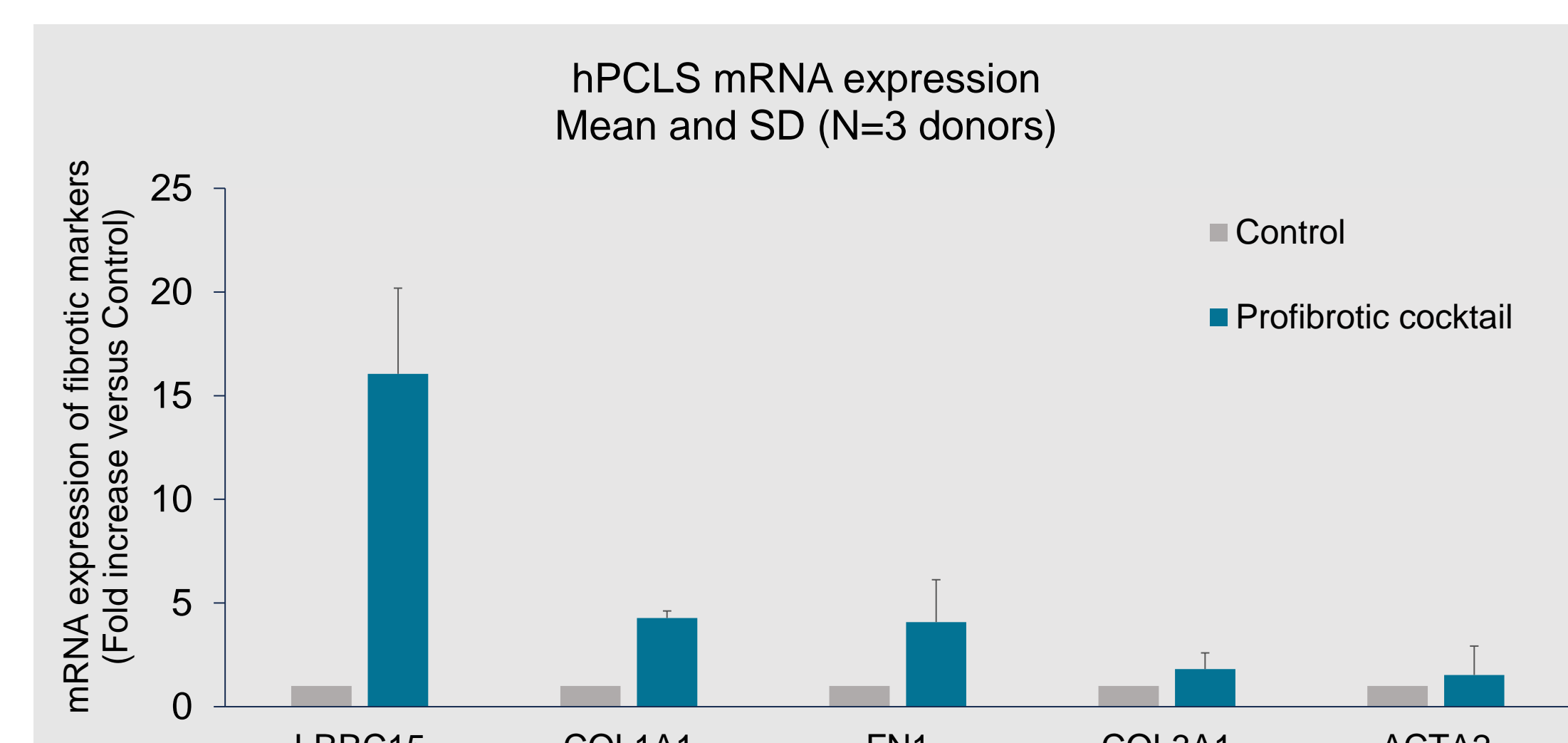


MTT assay

Cell viability of hPCLS is well maintained up to 14 days



Treatment of hPCLS with profibrotic cocktail increases the expression of fibrotic markers



CONCLUSIONS

Despite variability in the number of hPCLS obtained from lung resections, this study demonstrates excellent viability and well-preserved architecture of hPCLS for up to 14 days in culture.

Furthermore, the obtained hPCLS exhibit increased metabolic activity over time, retain alveolar cell features, and demonstrate functional responses to stimuli, as evidenced by the expression of fibrotic markers.

We successfully established a reliable protocol for generating high-quality and functional hPCLS model.

These hPCLS can be successfully used in modeling lung diseases, particularly lung fibrosis, and screening for antifibrotic drugs.

¹ Alsafadi et al., 2017