



An integrated multiomic and quantitative label-free microscopy-based approach to study pro-fibrotic signalling in *ex vivo* human precision-cut lung slices

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Multiomic and label-free imaging-based characterisation of *ex vivo* cultured human precision-cut lung slices (hPCLS) reveals that MMP signalling is a rate-limiting factor necessary for deposition of fibrillar collagen in ECM of hPCLS <https://bit.ly/3rcUa0e>

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Abstract

Fibrosis can affect any organ, resulting in the loss of tissue architecture and function with often life-threatening consequences. Pathologically, fibrosis is characterised by the expansion of connective tissue due to excessive deposition of extracellular matrix (ECM) proteins, including the fibrillar forms of collagen. A significant limitation for discovering cures for fibrosis is the availability of suitable human models and techniques to quantify mature fibrillar collagen deposition as close as possible to human physiological conditions.

Here we have extensively characterised an *ex vivo* cultured human lung tissue-derived, precision-cut lung slices (hPCLS) model using label-free second harmonic generation (SHG) light microscopy to quantify fibrillar collagen deposition and mass spectrometry-based techniques to obtain a proteomic and metabolomic fingerprint of hPCLS in *ex vivo* culture.

We demonstrate that hPCLS are viable and metabolically active, with mesenchymal, epithelial, endothelial and immune cell types surviving for at least 2 weeks in *ex vivo* culture. Analysis of hPCLS-conditioned supernatants showed a strong induction of pulmonary fibrosis-related ECM proteins upon transforming growth factor- β 1 (TGF- β 1) stimulation. This upregulation of ECM proteins was not translated into an increased deposition of fibrillar collagen. In support of this observation, we revealed the presence of a pro-ECM degradation activity in our *ex vivo* cultures of hPCLS, inhibition of which by a metalloproteinase inhibitor resulted in increased collagen deposition in response to TGF- β 1 stimulation.

Together the data show that an integrated approach of measuring soluble pro-fibrotic markers alongside quantitative SHG-based analysis of fibrillar collagen is a valuable tool for studying pro-fibrotic signalling and testing anti-fibrotic agents.

