Micro-Environmental Attributes Regulate Lung Fibroblast Phenotypes

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a non-resolving and progressive disease that causes excessive collagen deposition leading to lung stiffening, breathing problems and ultimately death. Fibroblasts are the main effector cells in fibrosis, driving matrix deposition and remodeling of the lung. In vitro culture methods for fibroblasts use hard culture plates to isolate and expand cells from tissue samples. This method, while practical, influences fibroblast response and behavior, ultimately widening the divide between in vitro research and in vivo outcomes. To explore this issue, we investigated the influences of culture conditions and extraction method on fibroblast phenotype.

Methods: Cells were collected from minced or enzymatically digested murine lung tissue and seeded on hard 2D culture plates or encapsulated in 3D collagen hydrogels. Cultures were then passaged within their same condition or transferred to the opposite condition and changes in gene expression, cellular contractility, cytokine expression, and ECM remodeling were assessed (Fig 1).

Results: Fibroblasts extracted via collagenase digestion significantly upregulated fibroblast activation marker α-smooth muscle actin (αSMA) as well as ECM components collagen 1A, collagen 3a, fibronectin, and matrix degradation enzyme, MMP3, when compared to mechanical mincing methods for extraction (Fig 2A). Culturing fibroblasts within a 3D hydrogel reduced fibroblast activation in both extraction methods as shown by a significant decrease in αSMA (Fig 2B) and reduced cellular contraction of the hydrogel ECM when compared to 2D conditions (Fig 2C). Cells cultured in 2D then transferred into 3D reduced expression of αSMA (Fig 2B) and CCL2 production, a monocyte chemokine, (Fig 2D) suggesting a decreased activation phenotype when compared to 2D culture. Interestingly, production of CCL2, which is elevated in IPF patients, was elevated in fibroblasts cultured on a 2D plate compared to those cultured in a 3D hydrogel (Fig 2D).

Conclusions: These results demonstrate that in vitro culture conditions regulate gene expression and function of fibroblasts. Establishing better in vitro cultures that mimic the in vivo microenvironment of the lung are needed to identify dysregulated signaling pathways that can serve as targets for inhibiting fibrosis.

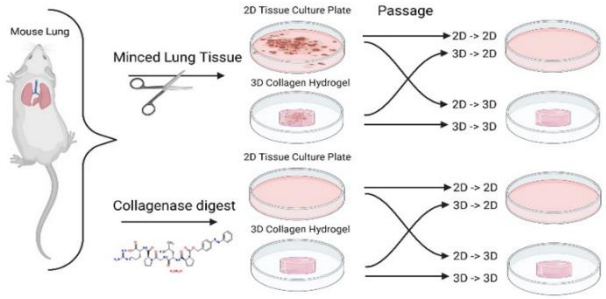

Fig 1. Experimental setup: pulmonary fibroblasts were isolated using either mechanical mincing or enzymatic digestion of murine lung tissue then placed on 2D culture plates or encapsulated in 3D hydrogel constructs. Cells were then passaged into 2D or 3D culture and assessed for changes in gene expression and phenotype functionality.

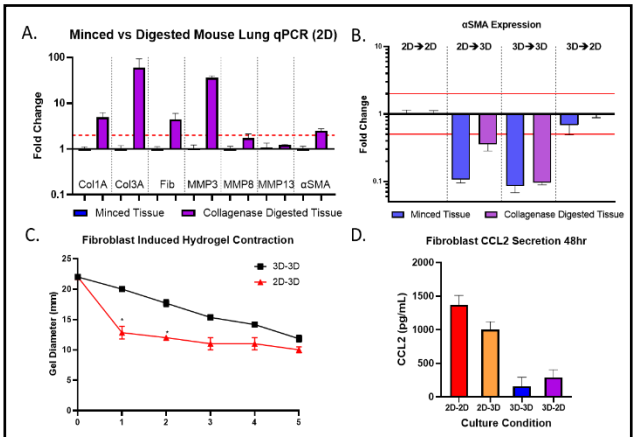

Fig 2. Culture dimension and method of extraction influences fibroblast gene expression (A, B), as well as ECM contractility (C), and cytokine secretion (D).