3D Fibrotic Lung Extracellular Matrix Hydrogels Trigger Pro-Fibrotic Responses in Primary Lung Fibroblasts

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3D Fibrotic Lung Extracellular Matrix Hydrogels Trigger Pro-Fibrotic Responses in Primary Lung Fibroblasts

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Idiopathic Pulmonary Fibrosis (IPF) is characterized by the aberrant deposition and organization of extracellular matrix (ECM). Increased stiffness of fibrotic lung tissue is a major contributor to fibrosis by perpetuating fibrotic responses, through modulation of interactions between ECM-producing cells and fibrotic ECM. Human decellularized lung ECM-derived hydrogels can be used as a model for mimicking the native three-dimensional (3D) microenvironment, including recapitulating the mechanical environment in nonfibrotic (control) and fibrotic lung tissues. In this study, we aimed to characterize control and fibrotic human lung ECM-derived 3D hydrogels seeded with primary lung fibroblasts to investigate the cellular remodeling responses dictated by the origin of the microenvironment. IPF and control decellularized lung matrices were freeze-dried, ground to a fine powder, and mixed (pool of 7 donors per diagnosis) before pepsin digestion. Primary lung fibroblasts were isolated from lung tissue of patients with IPF (IPF) or macroscopically normal lung tissue derived from patients undergoing tumor resection (non-IPF). IPF or non-IPF lung-derived fibroblasts (n=6 each) were resuspended in pH-neutralized ECM solutions and hydrogels were allowed to form before being cultured at 37°C, 5% CO_2. Cell-seeded ECM-derived hydrogels were harvested on day 14 and their stiffness was measured using a Low-Load Compression Tester at 20% strain and compared with equivalent cell-free hydrogels. Cell-free IPF hydrogels were stiffer than cell-free control hydrogels on day 14. Time in culture did not result in a difference in stiffness of cell-free hydrogels. The stiffness of control hydrogels seeded with either IPF or non-IPF fibroblasts did not change over a 14-day culture period (Figure 1A, 1B). In contrast, the stiffness of IPF hydrogels was increased in the presence of non-IPF lung fibroblasts, compared to cell-free hydrogels over the 14 day period (Figure 1C) ($p = 0.016$). However, when IPF fibroblasts were seeded in IPF hydrogels the stiffness of the hydrogel did not change compared to the cell-free counterpart (Figure 1D). These results illustrate the importance of both the fibroblast-origin and the origin of the ECM in determining the response of the fibroblasts to the 3D ECM microenvironment. Specifically, the fibrotic microenvironment evoked a profibrotic response in non-IPF fibroblasts while IPF fibroblasts did not exhibit the same response in this microenvironment. Taken together, our data have implications for cellular therapies that rely on adding non-diseased cells to tissues to promote disease resolution, as well as revealing the complexity of the positive feedback between fibrotic ECM and fibroblasts.

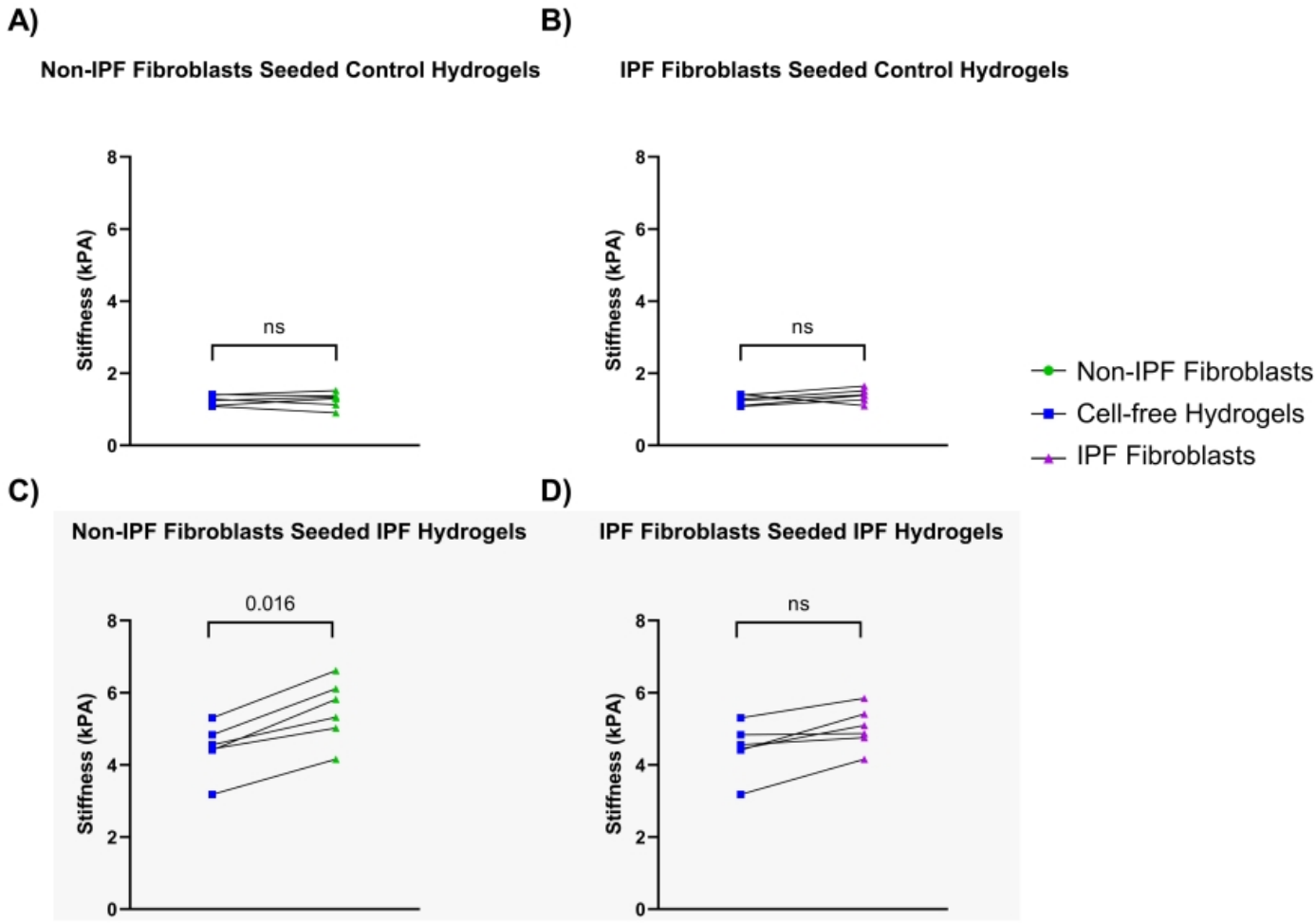

Figure 1 : Stiffness of human lung ECM derived hydrogels in the presence and absence of human lung fibroblasts. Non-IPF and IPF fibroblasts were seeded in control or IPF derived ECM hydrogels and cultured for 14 days. Hydrogel stiffness was assessed by low load compression testing. Stiffness measurements on control ECM hydrogels seeded with non-IPF **(A)** or IPF **(B)** fibroblasts. Stiffness measurements on IPF ECM hydrogels seeded with non-IPF **(C)** or IPF **(D)** fibroblasts. Each dot represents the mean of three independent measurement on the same hydrogel, fibroblasts from n = 6 different donors for each group. A paired mixed-effect analysis test was used to test for differences. ns: not significant, IPF Idiopathic Pulmonary Fibrosis

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