Establishment of a mechanically and biochemically tunable culture platform to probe key factors in macrophage responses during the initiation and progression of fibrosis

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Statement of purpose: Idiopathic pulmonary fibrosis (IPF) is a chronic fibrotic disease with unknown etiology thought to be initiated by repeated micro-injuries to the alveolar epithelium that results in deposition and accumulation of scar tissue and increased tissue stiffness[1]. Th2 cytokines (such as IL-13) are commonly upregulated in fibrotic lungs, which is hypothesized to aid in fibrosis progression by activating macrophages to profibrotic phenotype (M2 phenotype)[2]. Previous studies have examined the role of stiffness in activating fibroblasts[3]; however, it is unclear if presence of profibrotic cytokines or changes in ECM stiffness are the main drivers in accelerating fibrosis or if there is a synergistic effect that aids in fibrosis progression[4]. To investigate macrophage response in fibrosis progression, we present a biomaterial-driven approach utilizing well-defined hydrogel-based synthetic matrices.

Experimental Methods: Hydrogels were created utilizing photoinitiated thiol-ene click chemistry to achieve well-defined culture materials with bioinspired mechanical properties, biochemical content, and relevance for multi-dimensional cell cultures. Monomer solutions consisting of norbornene functionalized 4-arm PEG (2.5 wt% to 5 wt%), a degradable dithiol linker, monothiol integrin-binding peptides, and the photoinitiator Lithium acylphosphinate (LAP) were irradiated within geometries of interest to form culture substrates (10 mW/cm2 at 365 nm for 5 minutes). The resulting surface moduli of the hydrogels were assessed using microindentation to ensure the modulus mimicked that of the healthy and fibrotic diseased state. Murine alveolar macrophages (MH-S cells) were cultured on these substrates in comparison to controls (tissue culture polystyrene), and the role of both substrate stiffness and IL-13 stimulation (25 ng/mL) in the polarization of alveolar macrophage cells was assessed with flow cytometry, immunostaining, and RT-qPCR.

Results: Hydrogels with moduli ranging from healthy (Young's modulus (E) ~ 1.5 kPa) to fibrotic lung tissue (E~23 kPa) were prepared by varying crosslinking density, and the surface equilibrium swollen modulus was measured using microindentation. Relevant conditions were established to promote consistent macrophage attachment to these bioinspired substrates by independently tuning matrix mechanical properties and biochemical content. Substrate stiffness did not impact cell attachment and metabolic activity. The presence of IL-13 and increased substrate stiffness, both independently and synergistically, resulted in increased cell spreading. Additionally, increased surface stiffness significantly reduced the expression of pro-inflammatory markers assessed by flow cytometry (CD80 and CD86) and gene expression analysis (IL-1b). Further, increased CD206 expression with increased stiffness was observed in both gene expression and immunostaining **(Figure 1)**, suggesting the synergistic role of both IL-13 presence and increased stiffness in upregulating anti-inflammatory effects by macrophages that could drive fibrosis progression. We are further understanding the role of stiffness in mechanotransduction and macrophage polarization to investigate how aspects of these microenvironment cues impact the phagocytic capabilities of the cells, which might be key in developing better therapeutic delivery systems. Further engineering and application of these biomimetic systems provides exciting opportunities to investigate immune cell responses in both the initiation and progression of fibrosis.

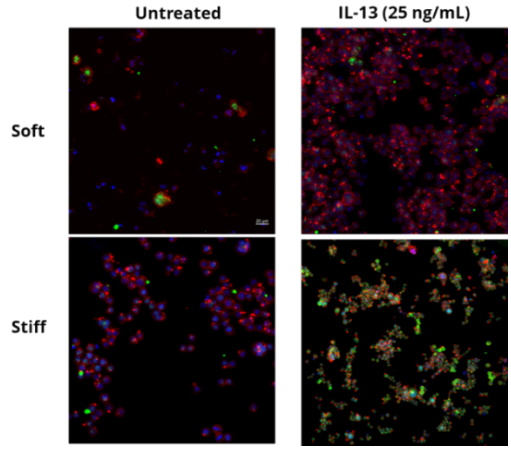

Figure 1: MH-S cells, seeded on different substrate, were immunostained with Nuclei (Blue), F-actin (Red), and CD206 (Green). Increased CD206 expression was observed with presence of IL-13 along with increased stiffness.

References:

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