3D Lung Models for Studying Chronic Respiratory Diseases *in vitro*

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Statement of Purpose: Chronic respiratory diseases are the third leading cause of death worldwide, impacting 7.4% of the world's population[1]. The prevalence of fibrotic respiratory diseases, specifically, is expected to increase in light of the ongoing COVID-19 pandemic, as it and other similar diseases (SARS, MERS) have shown to provoke a fibrotic response within the lungs[2]. Treatments for these diseases are limited, and there is a pressing need for more robust, physiologically accurate *in vitro* cell culture platforms that will enable better research into the causes of and potential treatments for these diseases. Hydrogels are a unique and promising platform for developing *in vitro* disease models, because these materials can be engineered to mimic the properties of the pulmonary microenvironment. Here, we demonstrate that a 3D acinar model that mimics lung microarchitecture can be created using poly(ethylene glycol)-norbornene (PEG-NB) hydrogel microspheres. A PEG-NB embedding hydrogel was designed to mimic the mechanical properties of healthy or diseased lung tissue while facilitating degradation by cell-secreted enzymes and supporting cellular viability over 21 days.

Methods: *Fabrication of an in vitro acinar model:* PEG-NB based hydrogel alveolar mimics were fabricated through an emulsion polymerization process and crosslinked with an MMP-3 degradable crosslinker. Primary murine alveolar epithelial type II (ATII) cells were purified by magnetic column isolation and seeded onto the hydrogel microspheres at a density of 250 cells/sphere and then aggregated under a magnetic levitating drive for 3 days. *Formulation of tissue-specific PEG-NB embedding hydrogels:* Eight-arm 10 kg mol^{-1} PEG-NB hydrogels were formulated to recapitulate the mechanics of healthy (E = 1-5 kPa) and fibrotic (E = 15-20 kPa) lung tissue[3]. These hydrogels were polymerized in a UV-catalyzed reaction using an MMP-2 degradable crosslinker to enable degradation by fibroblasts. Hydrogel modulus was measured using a parallel plate rheometer and controlled by adjusting the weight percentage of PEG-NB backbone, as well as the PEG-NB:crosslinker ratio. *Embedding acinar mimics in tissue-specific hydrogels:* The cell-laden acinar microsphere clusters were embedded in 10 uL of embedding hydrogel mimicking either healthy (soft) or fibrotic (stiff) lung parenchyma. Primary murine pulmonary fibroblasts were added to the embedding hydrogel solution in a 1:1 ratio with the ATII cells. The embedding hydrogels were polymerized with UV light, and the alveolar mimics were incubated for 21 days.

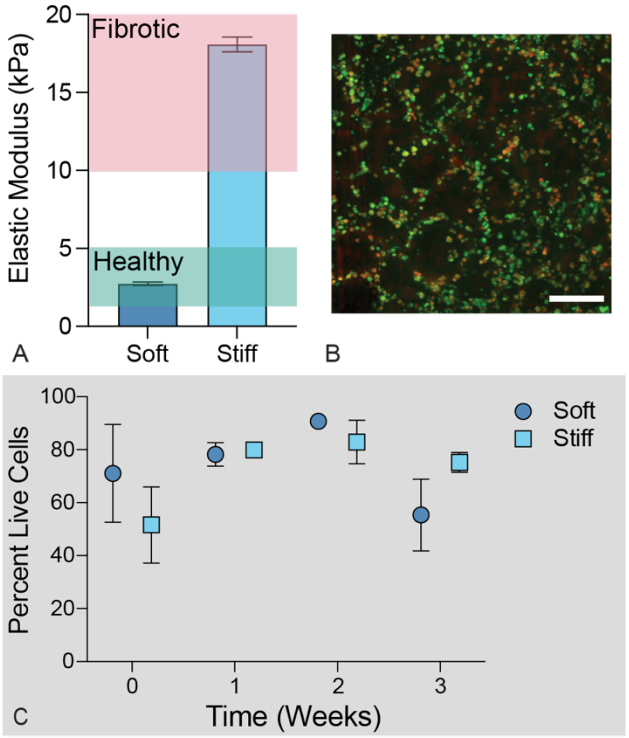

Figure 1. A) PEG-NB hydrogels recapitulate heathly and fibrotic lung tissue mechanical properties. B) Representative fluorescent image of live (green) / dead (red) staining of embedded cells after 3 weeks, scale bar 200 µm. C) Quantification of viability over time showed no significant differences in the proportion of live cells.

Results: The embedding hydrogels accurately recapitulated the elastic moduli of healthy (E = 2.73 kPa) or fibrotic (E = 18.1 kPa) lung tissue (Fig 1A). These embedding hydrogels effectively maintain the lung-like structure and arrangement of the magnetically aggregated microsphere/ATII cell clusters (Fig 1B). These hydrogel scaffolds provide a viable culture platform for primary murine ATII cells and fibroblasts out to at least 3 weeks, as shown by Live/Dead analysis (Fig 1C).

Conclusion: These results demonstrate that control over geometric and mechanical properties enables the creation of a more physiologically relevant *in vitro* cell culture platform than is currently available. Ongoing work is demonstrating the potential of these models to mimic cellular activation and cross-talk occurring in the pathogenesis of chronic lung diseases.

References: [1] Ritchie H, Roser M. Causes of Death. (2018). https://ourworldindata.org/causes-of-death. [2] Spagnolo P. *Lancet Resp Med*, 2020. [3] de Hilster RHJ. *APJ-Lung,* 318:L698-L704, 2020.