402-Excellence-8-bronchiole_organoids-Executive_Summary

Tissue Model of the Epithelial Mesenchymal Trophic Unit

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Background

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and chronic obstructive pulmonary disease (COPD) can cause damage to epithelial tissues of the lungs. This tissue injury triggers a fibrotic response in fibroblasts that results in further fibrosis. While some treatments such as bronchodilators and oral steroids alleviate symptoms, there is no cure for COPD. The development of pathogenesis during injury or disease is not fully understood, and an *in vitro* model that accurately recapitulates the tissue environment will allow for investigation of these mechanisms.

Problem and Competing Designs

Gaining an understanding of the ECM's influence on cellular responses in a wide range of lung diseases has become a focus of therapeutic research studies. 2D monolayer cell culture systems are commonly used to understand mechanisms that influence cellular behavior in a diseased state, but these systems lack the complex cell-ECM interactions of the lung microenvironment. The ultimate goal of the proposed 3D bioprinted gelatin methacryloyl (GelMA) hydrogel is to develop an *in vitro* model to examine the influence of fibrotic mechanical properties on co-cultured lung fibroblasts and epithelial cells.

Final Design

The hydrogel scaffold was created using the CELLINK BioX 3D Bioprinter and consists of the CELLINK GelMA bioink with 0.25% lithium phenyl(2,4,6-trimethylbenzoyl) phosphinate (LAP) as a photoinitiator. A CELLINK Temperature-Controlled Printhead was used to warm the bioink to a printable consistency, and the scaffold was photocrosslinked with a 405nm UV light. Research was conducted by the team to determine the necessary parameters to print scaffolds of both healthy (E = 2-5kPa) and fibrotic (E $\geq$16.5kPa) stiffnesses. Parameters such as printing temperature, printing pressure, heating temperature, temperature equilibrium time, and infill density were systematically varied to obtain an optimized bioprinting protocol over the course of the semester. UV crosslinking time was determined experimentally based on target stiffness. This protocol was used to create cylindrical hydrogels with tunable stiffness for lung epithelial cell research to be conducted in both conditions.

Design Validation

The GelMA hydrogels were validated through rheological testing for Young's modulus evaluation and LIVE/DEAD staining for encapsulated cell viability evaluation. Rheology showed that healthy gels had an average stiffness of 6.1 ± 3.9kPa, while the fibrotic gels had an average stiffness of 17.71 ± 2.4 kPa. The fibrotic gels met the design criterion for mechanical properties, but the healthy gels were over the set range. ImageJ analysis of representative LIVE/DEAD images showed that healthy gels had an encapsulated cell viability of 86.7 ± 6.7% after 1 week, while fibrotic gels had a viability of 85.1 ± 7.5% after 1 week. This meets the design criterion for supporting the encapsulation of fibroblasts.

Impact of Design

A tunable EMTU tissue model that accurately mimics native tissue would significantly enhance the comprehension of how inflammatory diseases affect lung epithelium and further the development of therapies that target disease-induced changes to lung tissue. Furthermore, a mimetic 3D *in vitro* model would reduce the need for *in vivo* animal models.