Tissue Model of the Epithelial Mesenchymal Trophic Unit

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Spring 2024 Poster Presentation 04/26/2024
Acknowledgements: The team thanks Prof. TJ Puccinelli, the members of the Brasier Lab, and Dr. John Puccinelli

Problem Statement

- Lack of scaffolds that model the lung ECM and its changes due to cell injury from diseases (like COPD & pulmonary fibrosis)
- Dr. Brasier of the UWSMPH needs such a scaffold
  - Provide him with a tool to study lung inflammation and disease
  - Would aid in translational research such as therapies that target lung tissue changes induced by diseased state
  - Scaffold must be bioprinted

Small Airway ECM

- The extracellular matrix (ECM) is a network of proteins and macromolecules [1]
  - Provides support and mechanical/biochemical cues to cells
- The epithelial mesenchymal trophic unit (EMTU) is made of [1]:
  - Lung epithelial cells, surrounding ECM, subepithelial fibroblasts
- Chronic lung diseases injure lung epithelium [2]
  - Inflammatory response increases fibroblast activity
  - Fibroblasts produce more proteins such as collagen and fibronectin
  - The mechanical stiffness of the ECM increases

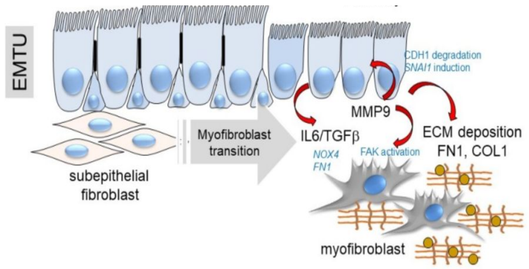

Figure 1: Schematic of EMTU response to injury [2]

Clinical Significance

- COPD is 3rd leading cause of death in the world [3]
- In the US, over 120,000 people die yearly of COPD [4]
- While not curable, current COPD treatments include:
  - Bronchodilators (inhalers) which relax and open the airways
  - Oral steroid medications
  - Pulmonary rehabilitation
  - Surgery (severe cases) [3]
- Tissue model would contribute to more thorough understanding of diseases and development of individualized treatments

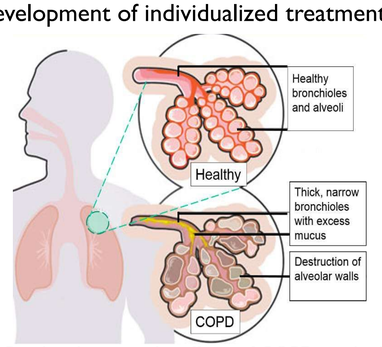

Figure 2: Graphic depicting the effects of COPD on the lungs [5]

Design Criteria

- Tunable mechanical stiffness
  - Healthy tissue: 2-5 kPa
  - Fibrotic tissue: ≥16.5 kPa
- Mimics biochemical properties of native lung ECM
  - Cell adhesive and enzymatically degradable for remodelling
- Support co-culture of fibroblasts and human small airway epithelial cells (hSAECs)
- <12 mm diameter to be compatible with an air-liquid interface (ALI)

Final Design

- ***Biomaterial:*** Gelatin Methacryloyl (GelMA)
  - **Pipette-based hydrogels**
    - 50% degree of functionalization
    - Cylindrical molds
      - 9 mm diameter
    - 365 nm UV light
      - 5 min for healthy stiffness
      - 15 min for fibrotic stiffness
  - **Bioprinted hydrogels**
    - CELLINK GelMA bioink w/ 0.25% LAP photoinitiator [6]
    - 3D cylindrical structure
      - 10 mm diameter x 2 mm thickness
    - Heating Temperature: 37 °C
    - Equilibrium Time: 30 min
    - Infill Density: 52%
    - Printing Temperature: 26.1 °C
    - Extrusion Pressure: 40 kPa
    - 405 nm UV light
      - 2 s for healthy stiffness
      - 13 s for fibrotic stiffness

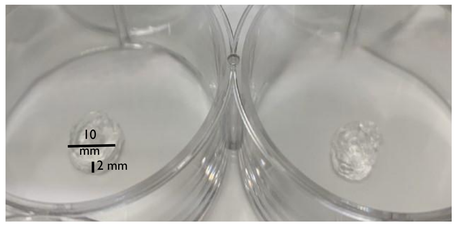

Figure 3: Bioprinted GelMA hydrogels

Figure 4: CELLINK BioX 3D Bioprinter [6]

Rheometry

- Mechanical stiffness of bioprinted hydrogels assessed via converting rheometry-obtained shear moduli (G) to Young's moduli (E)
  - $E = 2G(1+\nu)$
    - $\nu = 0.5$ for hydrogels [7]
- Frequency-sweep
  - Strain control - 1%
  - 0.1 Hz - 10 Hz

Condition (UV time)	Stiffness (kPa)
Healthy (2 s)	4.82 ± 0.6
Semi-Stiff (10 s)	12.31 ± 2.8
Fibrotic (13 s)	18.52 ± 3.6

Figure 5: Gels were swelled in cell culture media for 24 hr prior to testing

Cell Viability

- Encapsulated fibroblasts in pipette-based hydrogels to assess GelMA's ability to support cells
- Bright-field images show fibroblastic morphology
- LIVE/DEAD™ staining and imaging of cell-laden gels at four time points
- % cell viability after 1 week:
  - Healthy stiffness: 86.7 ± 6.7%
  - Fibrotic stiffness: 85.1 ± 7.5%

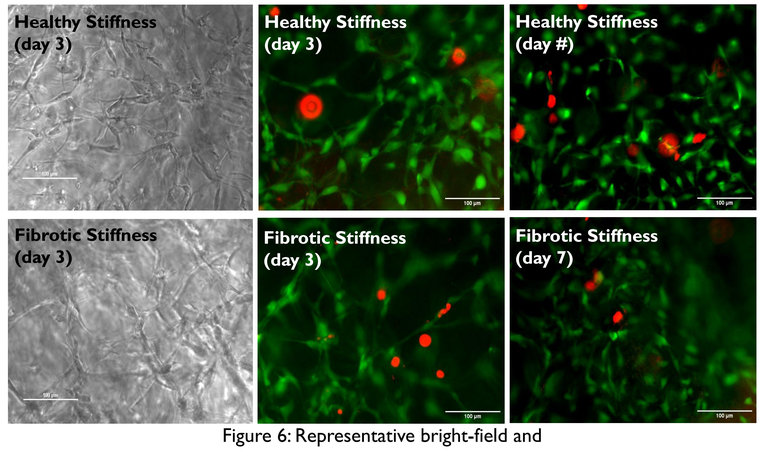

Figure 6: Representative bright-field and fluorescence images of cell-laden hydrogels

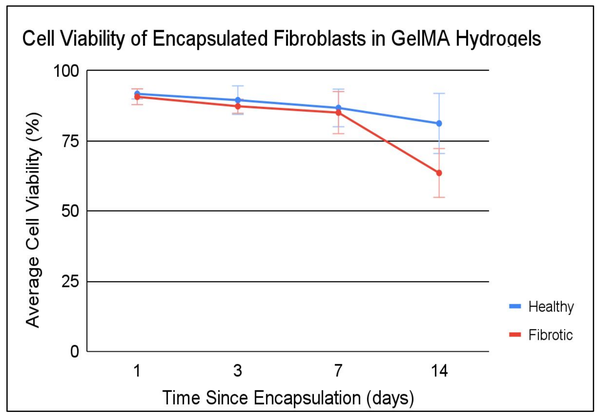

Figure 7: Plot of % cell viability for healthy and fibrotic stiffness hydrogels over time

Future Work

- hSAEC seeding on top of hydrogels to evaluate cell adherence
- Fibroblast/hSAEC co-culture (w/ cell viability testing)
- Incorporation of fibroblasts into bioink for bioprinting

References

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