Tissue Model of the Epithelial Mesenchymal Trophic Unit

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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

Background

- 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

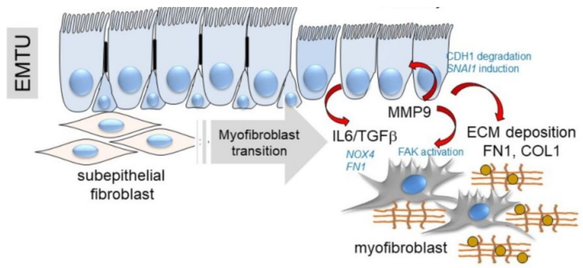

Figure 1: Schematic of EMTU response to injury [2]

Motivation

- 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 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

Design Criteria

- Tunable mechanical stiffness
  - Normal tissue: 2-5 kPa
  - Fibrotic tissue: ≥16.5 kPa
- Mimics biochemical properties of native lung ECM
  - Cell adhesive
  - Enzymatically degradable by matrix metalloproteinases (MMPs)
- <12 mm diameter to be compatible with an air-liquid interface (ALI)

Final Design

- ***Biomaterial:*** Gelatin Methacryloyl (GelMA)
  - **Pipette-based hydrogels**
    - GelMA 50% degree of functionalization
    - Cylindrical molds
      - 9 mm diameter
      - ALI compatible
    - Prior to photocrosslinking gels were allowed to cool/set at 4 °C
    - 365 nm UV light
    - Prior semester stiffnesses
      - Low stiffness: 4.08 +/- 0.56 kPa
      - High stiffness: 24.2 +/- 9.2 kPa
  - **Bioprinted hydrogels**
    - CELLINK GelMA [6]
      - Degree of functionalization not specified
      - LAP at 0.25% incorporated in cartridge
    - 3D cylindrical structure
    - 405 nm UV light

Figure 2: Pipette-based hydrogel tools (made with Biorender)

Figure 3: Bioprinted GelMA hydrogels

Figure 4: BioX CELLINK bioprinter [5]

Testing and Results

- **Pipette-based hydrogels**
  - Seeded at 8 million cells / mL
  - Preliminary cell viability test: 81% @ 24 hr
  - Visibly verified cell adhesion and morphology
  - Rheometry (at room temperature)

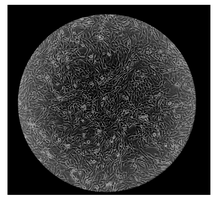

Figure 5: Bright field image after 24 hours in cell culture

- **Bioprinted hydrogels**
  - Printer Optimization
  - Increasing equilibrium time and using new GelMA bioink improved printability
    - Heating Temperature and Equilibrium Time
      - Initial: 37 °C and 10 min
      - Final: 37 °C and 20 min
    - Infill density: 35%
    - Printing Temperature
      - Initial: 27 °C
      - Final: 28 °C
    - Extrusion Pressure
      - Initial: 35 kPa
      - Final: 22 kPa
    - New GelMA Stiffness: 995 ± 915 Pa
      - Rheometry (at 37 °C)
      - UV light 2 sec

Pipette-Based Hydrogels	
Condition (4 °C, UV)	Stiffness (kPa)
3 min, 5 min	4.2 ± 0.92
5 min, 5 min	6.63 ± 2.6
10 min, 5 min	13.24 ± 2.8
5 min, 7 min	49.65 ± 22
5 min, 10 min	277 ± 155

Figure 6: Gels were set in 4 °C prior to UV crosslinking

Future Work

- Identify and utilize new quantitative cell viability assay
- Longer experimental timescale - matrix remodeling
- Bioprinter optimization
  - Utilize new GelMA bioink
  - Use CAD file to 3D print cylindrical structure
  - Tune UV exposure for fibrotic and healthy ECM stiffness
  - Plan for fibroblast encapsulation and hSAEC monolayer
    - 1×10^6 cells/mL
- Fabricate own GelMA if needed
  - Complete methacrylation, dialysis, and lyophilization process

References

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