Tissue Model of the Epithelial Mesenchymal Trophic Unit

DEPARTMENT OF

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UNIVERSITY OF WISCONSIN–MADISON

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Problem Statement

- Chronic lung diseases can cause damage to epithelial tissues of the lungs
  - Pulmonary fibrosis, asthma, and COPD
  - Damage causes the sub-epithelial fibroblasts to increase production
- Currently no scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury
  - Varying mechanical stiffness, porosity, incorporation of collagen and fibronectin, cell adhesive properties
- Dr. Brasier of the UW School of Medicine and Public Health requires such a scaffold that allows for lung epithelial cell culture in an ALI
  - Aim is to study cells in normal and fibrotic ECM conditions

Broader Impact

- Provide a more realistic and accurate 3D cell culture model for lung epithelial cells.
- Improvement to drug Discovery and Development
- Potential for Personalized Medicine
- Application in related fields
- Improve the quality of life for patients with chronic lung diseases.

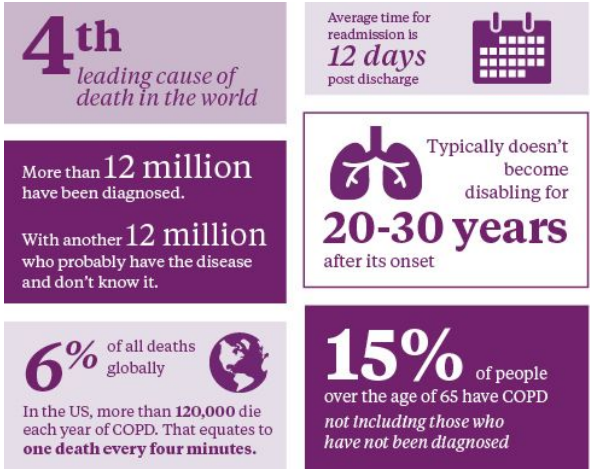

Figure 1: Prevalence of Chronic Lung Disease [1]

Background: Lung Extracellular Matrix

- Lung ECM
  - Collagen, elastin, laminin, and fibronectin [2]
- Function
  - Physical support, cell migration tract
  - Presents and stores growth factors [3]
- Fibroblasts
  - ECM protein production
  - Effector cell for injury repair [4]
- Collagen(I-IV and XVIII)
  - Provides tensile strength, regulates cell adhesion and directs tissue development [5]

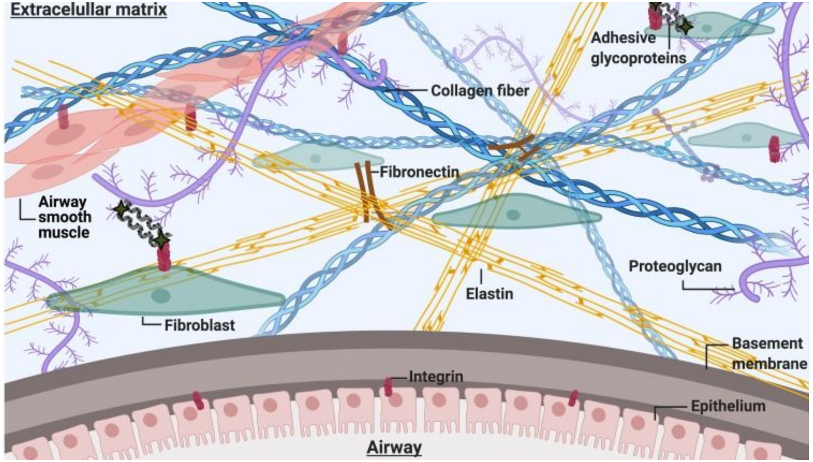

Figure 2: Lung ECM Diagram [6]

Background: Cell Culture

Bioprinting

- 3D tissue-like structures with precise spatial control
- BioInk - GelMa-Fibroblasts-Biomolecules

3D cell culture: Hydrogel

- More accurately mimic *in-vivo* ECM
- Allow cell-cell and cell-ECM interactions [7]

Hydrogels:

- Natural
  - Gelatin, Alginate, Collagen
  - Biodegradable, Natural adhesive properties
- Synthetic
  - PEG, PLA
  - Long lasting, replicable [8]

Figure 3: Cellink BioX Bioprinter [9]

Competing Designs

- 2D Models typically include layers of cells on top of polymer or glass dishes
  - Young's Modulus in 2-4 GPa range [10]
  - Negatively impacts gene expression
- 3D Models
  - Matrigel
    - Derived from Mouse Tumors
    - High variability in batches [11]
  - Human Lung ECM Hydrogels
  - Hyaluronic Acid (HA) Hydrogels
    - Incorporated in PEG hydrogels
    - Free Radical Toxicity

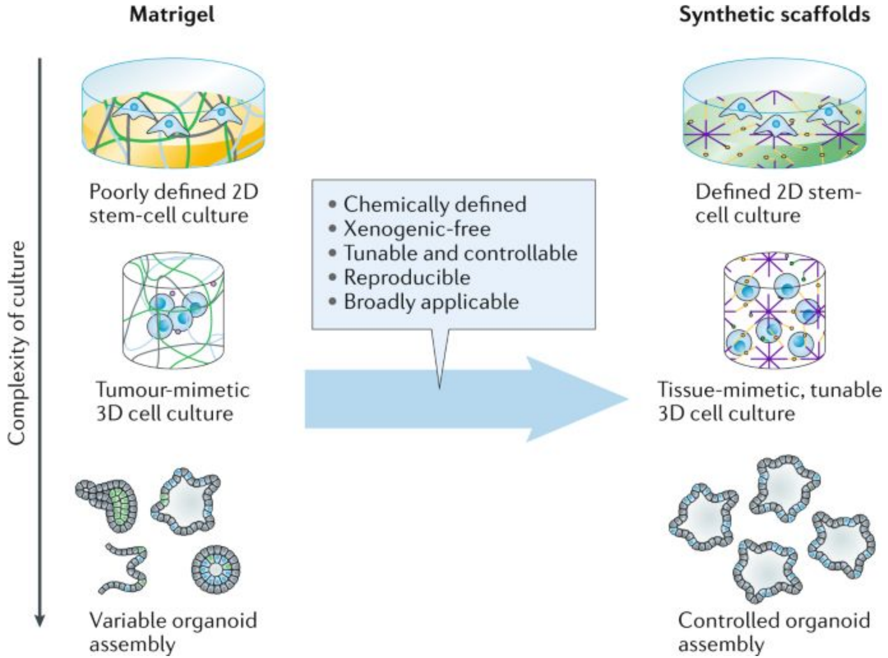

Figure 4: Competing Scaffold Design [12]

Key Design Criteria

- Biochemical Properties
  - Supports cell adhesion and capable of fibroblast encapsulation
  - Porosity
    - Transportation of media through hydrogel
  - Degradation to allow for ECM remodeling
- Mechanical Properties
  - Two types of hydrogels produced: healthy and fibrotic tissue states
    - Healthy Young's Modulus: 2-5 kPa
    - Fibrotic Young's Modulus: ≥16.5 kPa
- Replicability/Ease of fabrication
  - Simple protocol that is easy to follow
  - Accessible materials
    - Pre-characterized Cellink GelMA for easier fabrication

Previous Semester

- Final Design
  - GelMA (50% DOF) hydrogel created by pipetting solution into molds and photocrosslinking with 365 nm wavelength
- Testing
  - Mechanical properties: Rheometry to determine Young's Modulus
    - Lower stiffness reached: 4.08 +/- 0.56 kPa average
    - Higher stiffness reached: 24.2 +/- 9.2 kPa average
  - Cell adhesion: Imaging of hydrogel surface and cell counting
- Lessons Learned
  - Difficult to achieve replicability
    - Variations in mechanical properties between batches following the same protocol
  - GelMA promotes cell viability
    - At lower stiffness there was significantly less cell attachment
      - Incubating in cell media rather than PBS for 24 hours aids in attachment

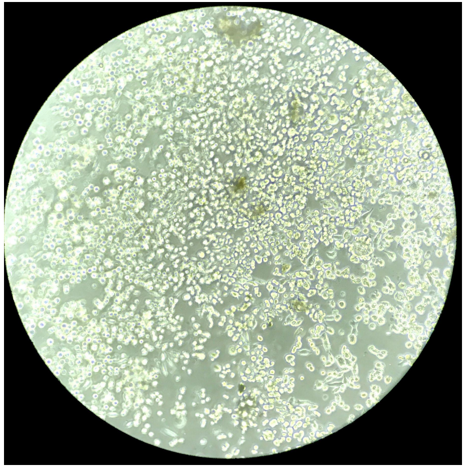

Figure 5: Image of previous semester cell culture on hydrogel

October Goals

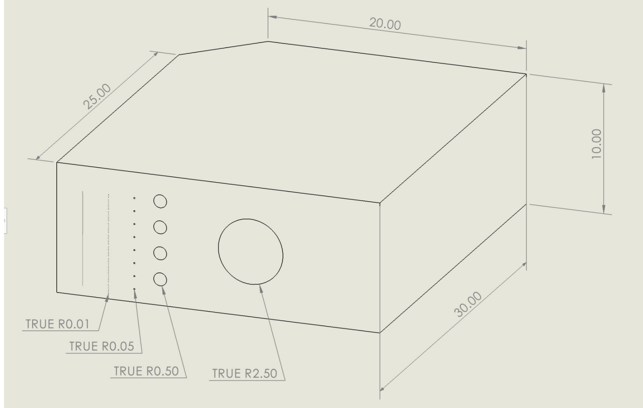

Figure 6: Test part to determine printer resolution for different materials or print parameters (mm)

6th
- Spatial parameter exploration
- Printer Resolution
- Minimum Ø

13th
- Grow out hSAECs and 3T3 Fibroblasts

20th
- UV crosslink / stiffness curve determination

27th
- GelMA Cytotoxicity / cell viability assays

November Goals

3rd
- Design of bulk architecture to support fibroblasts

10th
- Encapsulation of fibroblasts in GelMA

17th
- Design of surface topology to support epithelial cells

24th
- Coating of hSAECs onto gels

December Goals

1st

- Optimize printer settings to get healthy and fibrotic stiffness scaffolds

8th

- Poster Presentation

13th

- Final Deliverables

Budget

- $5,000 budget given Fall 2022
  - $679 spent Fall 2022: PEG hydrogel-related materials
  - $412 spent Spring 2023: GelMA hydrogel-related materials
- Past materials mostly given to client due to project direction changing
- $3,909 remaining
  - Client provided bioprinter, GelMA bioink, and paraphernalia
  - Potential future purchases
    - Additional GelMA bioink cartridges
    - UV light

Acknowledgements

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