Tissue Model of the Epithelial Mesenchymal Trophic Unit

DEPARTMENT OF
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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
- Our client, 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

- COPD is 3rd leading cause of death in the world [1]
  - In the US, over 120,000 people die yearly of COPD
- A tissue model would...
  - Contribute to more thorough understanding of chronic lung diseases
  - Aid in the development of individualized treatments/therapies

Figure 1: Graphical depiction of chronic lung diseases [1]

Background: Lung Extracellular Matrix

- Lung ECM
  - Collagen, elastin, laminin, and fibronectin [3]
- Function
  - Physical support, cell migration tract
  - Presents and stores growth factors [4]
- Fibroblasts
  - ECM protein production
  - Effector cell for injury repair [5]
- Collagen(I-IV and XVIII)
  - Provides tensile strength, regulates cell adhesion and directs tissue development [6]

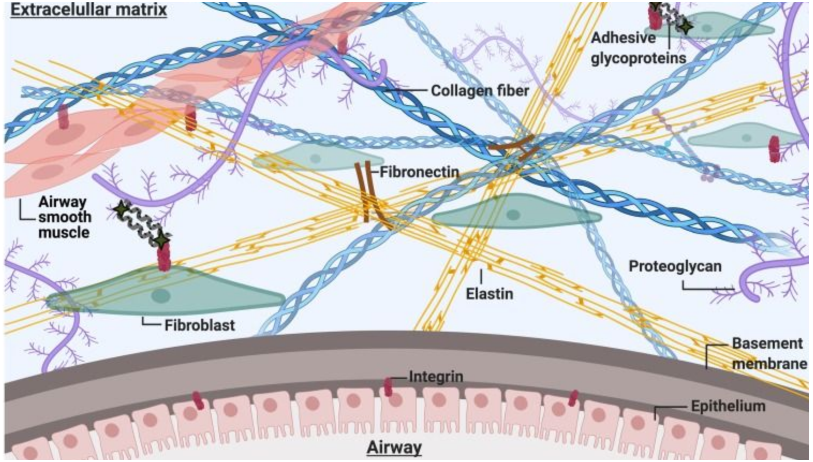

Figure 2: Lung ECM Diagram [7]

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 [8]

Hydrogels:

- Natural
  - Gelatin, Alginate, Collagen
  - Biodegradable, Natural adhesive properties
- Synthetic
  - PEG, PLA
  - Long lasting, replicable [9]

Figure 3: Cellink BioX Bioprinter [10]

Competing Designs

- 2D Models typically include layers of cells on top of polymer or glass dishes
    - Young's Modulus in 2-4 GPa range [11]
    - Negatively impacts gene expression
- 3D Models
    - Matrigel
        - Derived from Mouse Tumors
        - High variability in batches [12]
    - Human Lung ECM Hydrogels
    - Hyaluronic Acid (HA) Hydrogels
        - Incorporated in PEG hydrogels
        - Free Radical Toxicity

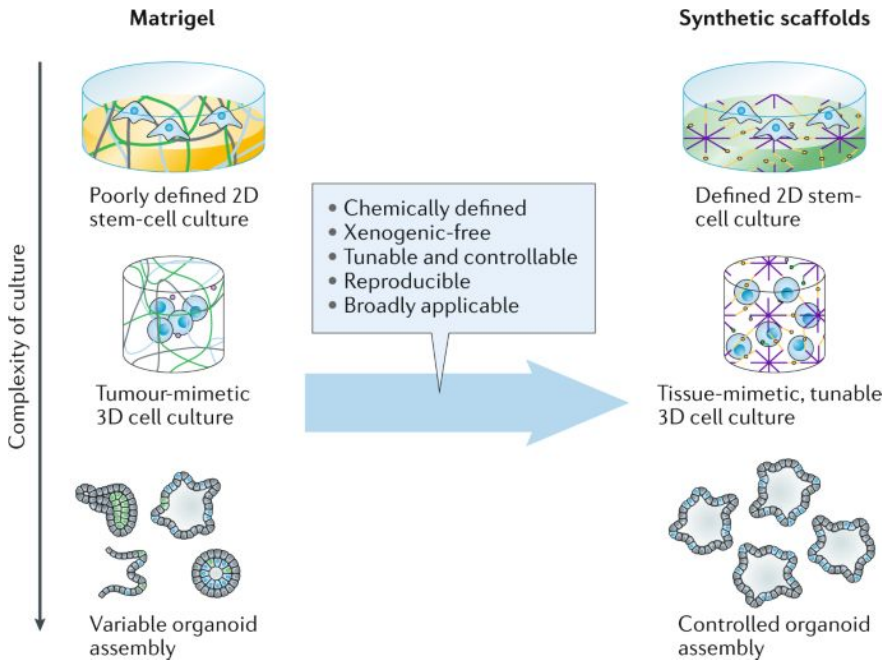

Figure 4: Competing Scaffold Design [12]

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
    - Pipette-based GelMA hydrogel for testing and comparison
    - Bioprinted GelMA hydrogel as final design
- Testing
    - Rheometry to determine Young's Modulus of pipette-based hydrogels
        - Varied setting time at 4 ℃ and UV-crosslinking time
    - LIVE/DEAD staining and gel imaging for preliminary cell viability testing
        - Staining results were unusable, need to identify new method of quantifying cell viability
    - Bioprinter Optimization
        - Changed equilibrium time, heating and printing temperatures, extrusion pressure, infill density, and UV time

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 5: Mechanical testing results for pipette-based hydrogel batches over course of semester..

Previous Semester

- Lessons Learned
  - Difficult to achieve replicability
    - Variations in mechanical properties between batches following the same conditions
  - Keep a careful eye on the shelf life of the GelMA bioink
  - Write up protocols before conducting experiments
    - Have a statistically significant number of samples ($n \geq 4$)
    - Have literature-based evidence to back up the protocols
  - Bioprinter parameters need to be adjusted more
    - UV time, extrusion pressure, and infill density especially

Semester Timeline

Task	Jan	Feb					March				April				May		
	26	2	9	16	23	28	1	8	15	22	5	12	19	26	1	3	10
Project Goals																	
Pipette-Based Hydrogel Characterization																	
Bioprinted Hydrogel Protocol																	
Consistent Bioprinted Hydrogel Fabrication																	
Bioprinted Hydrogel Characterization																	
Fibroblast Encapsulation																	
Fibroblast Viability Testing																	
Epithelial Cell Culture w/ ALI																	

Task	Jan	Feb					March				April				May		
	26	2	9	16	23	28	1	8	15	22	5	12	19	26	1	3	10
Deliverables																	
Progress Reports																	
Journal Selection																	
Preliminary Presentation																	
Preliminary Report																	
Preliminary Notebook																	
Show and Tell																	
Executive Summary Draft																	
Executive Summary																	
Final Poster																	
Final Report																	
Final Notebook																	
Client Evaluation																	

Budget

- $5,000 budget given Fall 2022
  - $679 spent Fall 2022: PEG hydrogel-related materials
  - $412 spent Spring 2023: GelMA hydrogel-related materials
  - $395 spent Fall 2023: Bioprinting-related materials
- Past materials mostly given to client due to project direction changing last semester
- $3,514 remaining
  - Client provided bioprinter and paraphernalia
  - Future purchases will primarily be more bioink cartridges and bioprinter paraphernalia
    - $325 for 3 x 3mL cartridges
    - $990 for 10 x 3mL cartridges
    - $85 for a 50-count pack of 22 gauge nozzles

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