Tissue Model of The Epithelial Mesenchymal Trophic Unit

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Problem Statement

- Chronic lung diseases can cause damage to epithelial tissues of the lungs
- Tissue damage triggers fibrotic response in sub-epithelial fibroblasts that results in further disease and fibrosis
- Currently no scaffolds that accurately model the lung extracellular matrix (ECM) and its changes due to cell injury, specifically the following properties in combination:
  - varying mechanical stiffness and tension
  - porosity
  - incorporation of collagen and fibronectin within ECM
  - cell adhesive properties
- Dr. Brasier of the UW SMPH requires scaffold that meets criteria while having a uniform and replicable composition to allow for epithelial cell culture at air-liquid interface

Lung Extracellular Matrix Background

- Lung ECM
  - Basement membranes vs. Interstitial Spaces [1]
- Function
  - Physical support, Cell migration track, Injury repair [2]
- Fibroblasts
  - ECM protein production
  - Effector cell for injury repair [3]
  - Activated by the stimulation of cytokines (TGF-β1, PGDF, and FGF2)
  - Fibronectin, maintains and directs tissue organization
- Collagen(I-IV and XVIII)
  - Secreted by Myofibroblasts [4]
  - Provide tensile strength, regulate cell adhesion, direct tissue development [5]

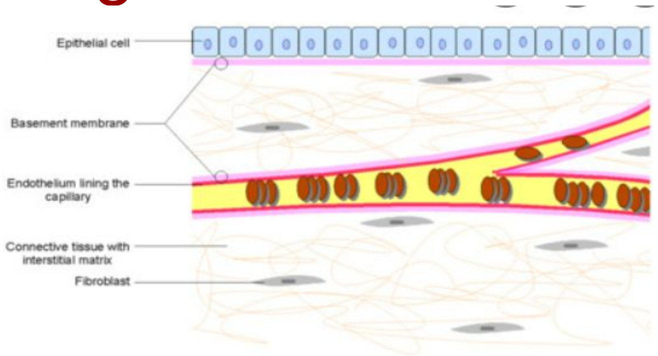

Figure 1: ECM Diagram

Cell Culture Scaffold Background

3D cell culture:

- Mimic *in-vivo* ECM better
- Allow cell-cell and cell-ECM interactions [6]

Air-liquid-interface (ALI):

- Basal surface (bottom): liquid culture medium
- Apical surface (epithelial cells): air [7]

Natural vs synthetic scaffolds:

- Natural: biodegradable but unreliable
- Synthetic: longer life, replicable, known composition [8]

  → Semi-synthetic

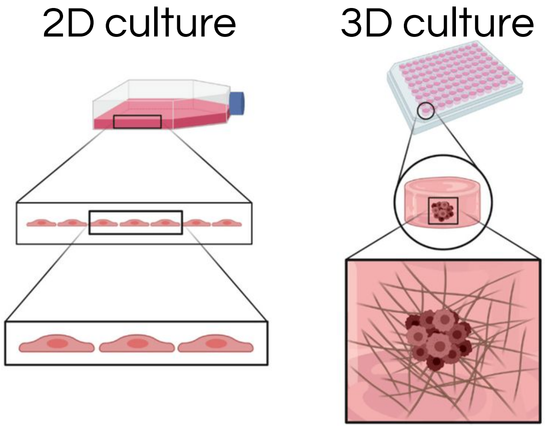

Figure 2: 2D vs 3D culture

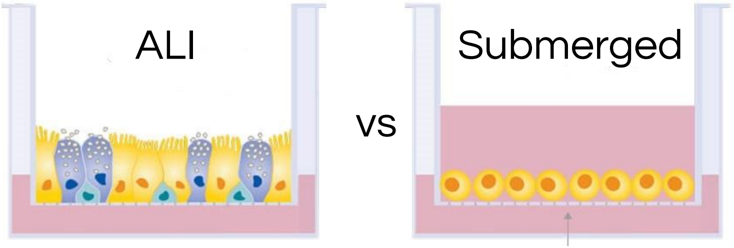

Figure 3: ALI vs submerged model

Design Criteria and Specifications

- Primary design goal is to mimic the small airway extracellular matrix (ECM)
  - Mechanical stiffness (0.44-7.5 kPa) [9]
  - Epithelial cell adhesion
  - Permeability to water soluble molecules
- Model must include and air-liquid interface (ALI)
- Mechanical and biochemical properties of the 3D scaffold must be compatible with lung epithelial cells used for experimentation
- The ECM should be able to incorporate different proteins such as fibronectin and collagen

Competing Designs

- 2D Models typically include layers of cells on top of polymer or glass dishes
  - Stiffness of these models range from 2-4 GPa. Stiffness of the human lung ranges from .44-7.5 kPa [9]
- 3D Models
  - Matrigel: Co-culture model using ECM protein gel. ECM environment is more similar to in vivo than 2D, but there exists a lot of variation between batches [10]

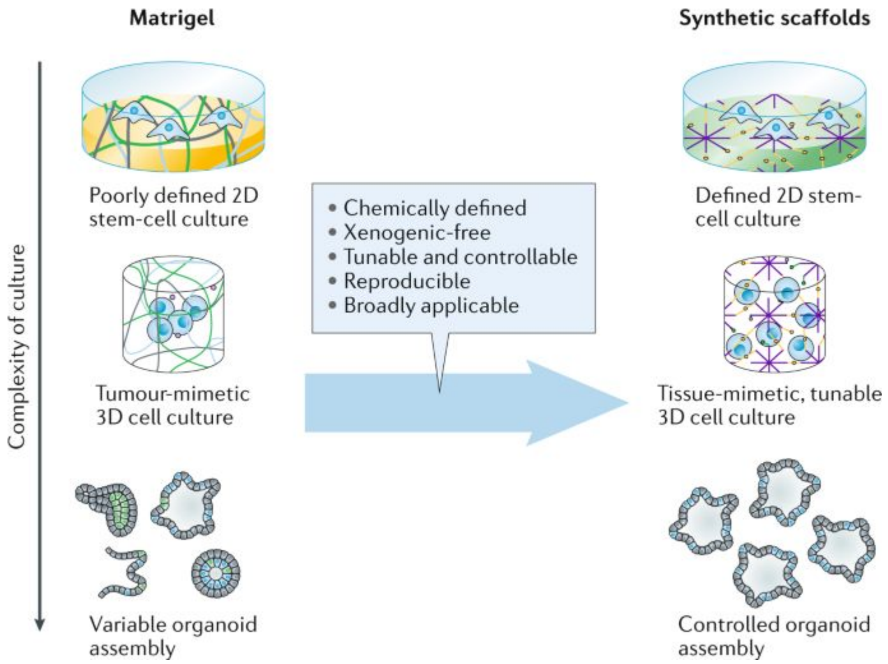

Figure 4: Competing Scaffold Design

Preliminary Scaffold Designs

- Lung Young's Modulus from .44-7.5 kPa [9]
- GelMa and PEG Young's Modulus have a range of 5-300 kPa, altered by % concentration [11]
- Alginate ranges from 9-1000 kPa [12]

<u>Gelatin Methacrylate (GelMA)</u>

- Natural adhesive properties
- Variation during reaction of formation[13]
- Young's Modulus: 6.23+/-.64 kPa (7%)

<u>Polyethylene Glycol (PEG)</u>

- Biocompatible with no natural adhesive properties [14]
- PEG-Collagen scaffold has shown high cell viability (hMSCs) [15]
- Young's Modulus: 6.56+/-.18 kPa (5%) [16]

<u>Alginate</u>

- Variation in structure due to it being extracted from brown seaweed [17]
- Counteractions for these variations too expensive
- Possible contaminants

Scaffold Design Matrix

		Design 1: Gelatin Methacrylate (GelMA)		Design 2: Polyethylene Glycol (PEG)		Design 3: Alginate	
Design Criteria	Weight	Score	Weighted Score	Score	Weighted Score	Score	Weighted Score
Biochemical Properties	30	4/5	**24**	3.5/5	21	3.5/5	21
Mechanical Properties	25	4/5	20	5/5	**25**	3/5	15
Ease of Fabrication	20	3/5	12	4/5	**16**	2/5	12
Ease of Use	15	5/5	**15**	5/5	**15**	3/5	9
Cost	10	4/5	**8**	3/5	6	2/5	4
Total:	**100**		**79**		**83**		**61**

Future Work

Testing

- Mechanical Stiffness
  - Range within native EMT unit
- Porosity/Permeability
  - In presence of hydrophilic molecules
- Translucency
  - Required by 3D scaffold to observe cells under microscope
- Cell Adhesiveness
  - Ability for incorporated cells to adhere to ECM scaffold
- Degradability
  - Balance between ease of cells entering scaffold and premature scaffold breakdown

Testing Upon Model's Success

- Client will:
  - Perform cell culture
  - Use scaffold for imaging
  - Incorporate fibroblasts into scaffold

Acknowledgements

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