TISSUE MODEL OF THE EPITHELIAL MESENCHYMAL TROPHIC UNIT

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BME Design

Abstract

Chronic lung diseases such as pulmonary fibrosis, asthma, and chronic obstructive pulmonary disease (COPD) can cause significant damage to the epithelial tissues of the lungs. This presents a problem because when this tissue is damaged, a fibrotic response is triggered in sub-epithelial fibroblasts that result in further disease and fibrosis. Currently, no scaffolds exist that accurately model the lung ECM and its changes due to cell injury. Specifically, incorporating the following in combination: varying mechanical stiffness and tension, incorporation collagen and fibronectin into the ECM, and cell adhesive properties [1]. As the research on lung diseases evolves, the need for a synthetic scaffold that accurately mimics the ECM increases. This project aims to create a replicable synthetic scaffold with uniform composition that allows for culturing of lung epithelial cells. A synthetic material of polyethylene glycol seeded with collagen and fibronectin was decided to be a viable option for the design with another alternative being a geometrically and practically simpler material: gelatin. In this design iteration, gelatin hydrogels were fabricated and tested within the BME teaching lab at UW-Madison.

Problem Definition

- Client Dr. Brasier of UW School of Medicine and Public Health needs scaffold with easily-modifiable concentrations to mimic a range of stiffnesses
- Commercial models (Matrigel) have batch to batch variations that do not suitably model the composition of the small airway ECM
- Hydrogel must contain fibronectin and collagen at accurate concentrations to mimic environment for fibroblast and epithelial cell culture
- Synthetic model must have cell adhesion properties
- A degradable model to allow for matrix remodeling by fibroblasts to produce a natural ECM and allow epithelial cell culture
- Must allow for epithelial cell culture at an air-liquid interface (ALI) to accurately model small airway tissue

Lung ECM Background

- Lung ECM Function
  - Physical support, Cell migration track, Injury repair [2]
- Fibroblasts
  - ECM protein production [3]
  - Effector cell for injury repair [4]
  - Fibronectin, maintains and directs tissue organization
- Collagen
  - Secreted by myofibroblasts
  - Provides tensile strength, regulates cell adhesion [5]
  - Directs tissue development
- RGD Peptide Sequence
  - principal integrin-binding domain present within ECM proteins such as fibronectin, vitronectin, fibrinogen

Air-liquid-Interface (ALI)

- Consists of the basal surface and apical surface [6]

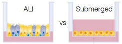

Figure 1: ALI vs. Submerged model

Design Specifications

- The primary design goal is to mimic the conditions of the small airway ECM:
  - Mechanical stiffness (Young's Modulus of 0.44 - 7.5 kPa) [7]
  - Epithelial Cell Adhesion
  - Viscoelastic properties - Storage Modulus (G' = 2.12 ± 0.61 kPa) & Loss Modulus (G'' = 0.212 ± .061 kPa) [8]
- 3D scaffold must support the epithelial cells used for experimentation
- It should be reasonable the future iterations of the model can take the form of an ALI and incorporate new proteins such as fibronectin and collagen into the matrix such as needed

Final Designs

PEG-DT & PEG-NB Hydrogel

In this model, polyethylene glycol synthetic materials are cross linked via photopolymerization with UV light allowing for fibroblasts to be encapsulated within the gel [9]. This model allows for very precise selection of stiffness based on PEG-NB and PEG-DT concentration detailed in the table below. Synthetic hydrogels such as PEG do not have any natural adhesive properties [10]. As a result, RGD sequences are used to create binding sites with the selected concentration below. Lastly, to allow for fibroblast reconstruction of the ECM, fibroblasts are added at concentrations of 100,000-200,000 cells/mL [11] and MMP peptides are incorporated at set concentrations to allow for the synthetic matrix degradation timeline to match that of reconstruction.

RGD sequence mM	2.6	3.0	3.4	4.0	4.6	5.0
uL of RGD	9.6	11.1	12.6	14.8	17	18.5

Table 1: RGD Sequence Concentrations [13]

Hydrogel Stiffness	PBS (uL)	PEG-DT (uL)	PEG-NB (uL)	I2959 (uL)
2 kPa	122.4	13.8	61.9	49.5
4 kPa	100.7	23.1	74.3	49.5
5 kPa	68.2	30.8	99	49.5
6 kPa	118.8	99	29.7	49.5

Table 2: PEG-DT & PEG NB Concentrations[14]

Gelatin Hydrogel

While working through the design process, the crosslinking process for PEG hydrogels proved difficult. One of the main requirements the client wanted was a replicable and easy to fabricate design. From these challenges, a secondary design was created with gelatin. This gelatin hydrogel can be created with controlled stiffness based on powder concentration used that fit within the necessary range for the client. Additionally, gelatin hydrogels are translucent, biocompatible/non-toxic to fibroblasts, and has a replicable fabrication process [12].

Type of Gelatin	Storage Modulus (G')
3.15 A	13.07 kPa
3.15 B	10.23 kPa
3.15 C	13.94 kPa
2.36 A	6.29 kPa
2.36 B	5.31 kPa
2.36 C	6.78 kPa

Table 3: Constructed Gelatin Storage Modulus

Testing Protocols

Rheology Testing

Storage, Loss and Young's Modulus' were obtained using the Malvern Kinexus Ultra+, Test 0035 - Frequency Sweep, Strain Controlled. 3 gels of each concentration (3.15% and 2.36%) were tested using 1% strain at 25℃. The frequency range was set between 0.1-10 Hz. The thickness of hydrogels varied between 3.45-4 mm.

Translucency Testing

This test is to assure gels are translucent enough for imaging purposes.

Shine a light at the bottom of the gel. If light can be observed going through the gel and out of its surface, the gel passes the translucency test.

Diffusion Testing

Mold aluminum mesh over top of petri dish. Measure mass of mesh. Place gel in center, measure mass of mesh and gel. Add 5 mL of DMEM to dish, place mesh back onto dish with gel at air-liquid interface. Refrigerate at 4°C. At specific time points (1 hr, 23 hrs), remove mesh from dish, pat bottom dry, measure mass of mesh and gel, take picture. Replace mesh and gel and refrigerate.

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Testing Results

Gel concentrations: 3.15% & 2.36% w/v.

Rheology (All values derived at 0.1 Hz)

For 3.15% gels, the average G' value was 12.41 ± 1.94 kPa, and the average G'' value was 0.246 ± 0.04 kPa. For 2.36% gels, the average G' value was 6.125 ± 0.747 kPa, and the average G'' value was 0.112 ± 0.018 kPa. The Young's Modulus was obtained using the equation [$E=2G(1+\mu)$] where μ=Poisson's ratio). The average Young's Modulus of the 3.15% gel was 37.24 ± 5.82 kPa. The average Young's Modulus of the 2.36% gel was 18.375 ± 2.24 kPa.

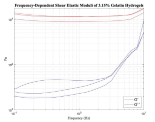

Figure 2: Frequency-Dependent Shear Elastic Moduli of 3.15% Gel

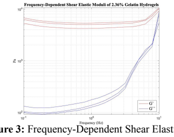

Figure 3: Frequency-Dependent Shear Elastic Moduli of 2.36% Gel

Translucency

All gels passed the translucency test.

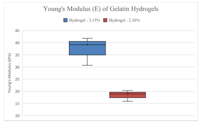

Figure 4: Box and Whisker plot of Young's Modulus of 3.15% and 2.36% gels

Figure 5: Gel during translucency testing

Diffusion

Mass decreased over time for all gels when media was diffused through ALI. Gels with the lower concentration have a steeper mass decrease. However, as indicated by gradual darkening of the pink shade of gels in Figure 6, media was clearly absorbed by gels. Decrease in mass may be due to natural degradation of the gel.

Results indicate media would be able to diffuse through a hydrogel to supply nutrients to fibroblasts and epithelial cells.

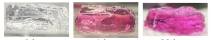

Figure 6: Gels at different time stamps

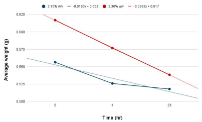

Figure 7: Average weight of different gel concentrations over time

Future Project Development

- Perform translucency, rheology, and diffusion testing on PEG-DT and PEG-NB hydrogels
- Incorporate (backordered) transwell insert requested by client for ALI
- Incorporate appropriate fibroblast concentrations into hydrogels
- Incorporate MMP peptides to facilitate fibroblasts' ECM degradation
- Incorporate epithelial cells into hydrogels for cell culture
- Utilize models for imaging

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