Tissue Model of the Epithelial Mesenchymal Trophic Unit

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Spring 2023 Poster Presentation 04/28/2023
Acknowledgements: The team thanks Dr. Masters, the members of the Brasier Lab, and Dr. Puccinelli

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. Currently, no existing scaffolds accurately model the lung extracellular matrix (ECM) and its changes during cell injury. Specifically, no scaffold models the change in mechanical stiffness and porosity while also incorporating ECM proteins and promoting cell adhesion. 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. Meeting necessary specifications, a hydrogel scaffold composed of gelatin methacryloyl / methacrylate (GelMA) was ultimately proposed as a solution. Initial fabrication procedures, and accompanying methods to test the hydrogel's efficacy were produced. The GelMA hydrogels will be fabricated and tested over the remainder of the spring 2023 semester.

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 comprised 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 like collagen and fibronectin
  - The mechanical stiffness of the ECM increases

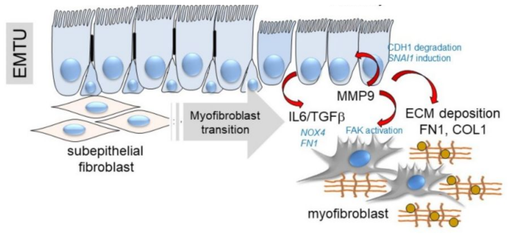


Figure 1: Schematic of EMTU response to injury [2].

Motivation

Tissue models made from biomaterial scaffolds allow for the *in vitro* modeling of biological phenomena that are difficult to investigate *in vivo*. These models can recreate tissue microenvironments more accurately than traditional cell culture. There are no tissue models that recreate the EMTU with tunable stiffness to represent healthy or fibrotic conditions, while maintaining important biochemical properties. Developing an in vitro model that accurately mimics the lung's extracellular matrix and its changes during cell injury can provide researchers with a valuable tool to study disease mechanisms and develop potential treatments. By understanding how the lung's mechanical stiffness and porosity change in response to injury, researchers can design therapies that target these specific changes.

Design Criteria

- Tunable mechanical stiffness
  - Normal tissue: 3.5 kPa
  - Fibrotic tissue: ≥16.5 kPa
- Mimics biochemical properties of native lung ECM
  - Cell adhesive
  - Enzymatically degradable by matrix metalloproteinases (MMPs)
- Compatible with an air-liquid interface (ALI)

Final Design

- GelMA hydrogels created by crosslinking with UV light (365 nm) [3]
  - Made from gelatin-based material that is reacted with methacrylic anhydride
  - Addition of methacrylate (MA) groups allows for formation of physical gel at low temperature and to be photocrosslinked with UV light and photoinitiator due to MA photosensitivity [4]

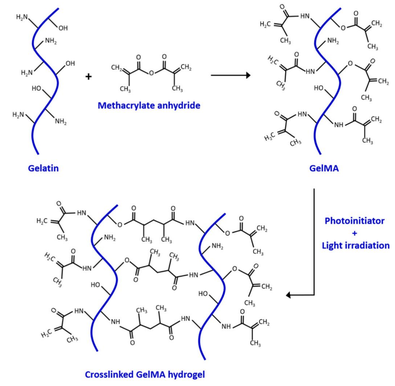


Figure 2: GelMA hydrogel synthesis schematics [5]

- Retains the RGD and MMP-degradable motifs that are naturally occurring in gelatin [6]
  - Natural cell adhesion properties and enzymatic degradation
- Tailorable mechanical properties
  - Degree of functionalization (Dof): 50%
  - Degree of crosslinking (UV light)
    - UV time: 5 minutes
  - Setting/solidifying (time in fridge)
    - Normal tissue stiffness: 1 minutes
    - Fibrotic tissue stiffness: 10 minutes

Testing and Results

GelMA Hydrogel Concentration: 5% w/v (g/ml)

Rheology: (All Values derived at 0.1 Hz)

For the normal gels, the average Young's Modulus (E) was 3.42 ± 0.49 kPa. The fibrotic gels had an average Young's Modulus (E) of 49.2 ± 11.65 kPa. Young's Modulus was calculated using the equation $E = 2G(1+\mu)$, where μ is Poisson's ratio.

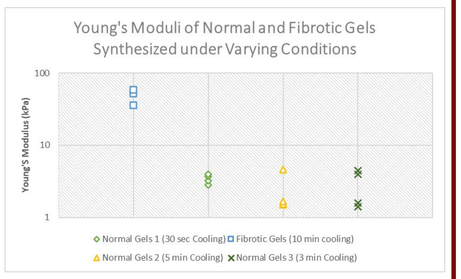
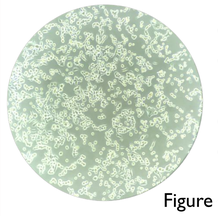


Figure 3: Categorical Scatter Plot of Gel Stiffness under Various Testing Conditions

Design Use



- Cell culture of small airway epithelial cells
- Co-culture with embedded fibroblasts
  - Cell cross talk, signaling pathways
- Phenotypic expression
- Fibroblast behavior in fibrotic states

Figure 4: HSAECs cultured on produced hydrogels

Future Work

- Fibroblast encapsulation
- Protein encapsulation (collagen or fibronectin)
- Cell viability assays
  - Flow cytometry or immunohistochemical analysis (IHC)
- Degradation assay
  - Incubation with collagenases and weight loss measurements taken over two weeks

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