

# Tissue Model of The Epithelial Mesenchymal Trophic Unit

## BME 402: Tissue Model

Client: Dr. Allan Brasier

Advisor: Prof. Tracy Jane Puccinelli

Team:

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Dates: 03/01/2024 – 03/07/2024

### Problem statement

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and chronic obstructive pulmonary disease (COPD) can cause damage to 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 results in further disease and fibrosis. There are currently no tissue models that accurately recreate the lung extracellular matrix and its changes due to cell injury. Such a model would need to have tunable mechanical stiffness and porosity, as well as be cell adhesive and degradable. Dr. Brasier of the UW School of Medicine and Public Health requires a scaffold that meets these criteria to be fabricated with a bioprinter. The scaffold must have a uniform and replicable composition that allows for epithelial cell culture in an air-liquid-interface (ALI) so that his lab can study the effects of fibrosis on small-airway lung epithelial cells.

### Brief Status Update

This week the team made bioprinted hydrogels to give to Dianhua for epithelial cell seeding. According to him, the gels “mostly melted” and the cell confluency was unable to be photographed. The team also encapsulated fibroblasts in pipette-based hydrogels and did an initial LIVE/DEAD staining with the remaining dyes in the BME Teaching Lab.

### Difficulties / Advice Requests

This week the team ran into some difficulties with bioprinting hydrogels. During this round of printing, the first 2 printing attempts went perfectly, while the next >40 attempts resulted in the bioink extruding improperly. The filament “ripped” during extrusion which led to *very* thin and uneven prints.

### Current Protocols

Fabrication of non-cell laden pipette based hydrogels at two separate stiffnesses was accomplished by dissolving 50 mg of GelMA into 950  $\mu$ L of PBS, adding 50  $\mu$ L of LAP. 10 Hydrogels of 100 $\mu$ L were created at two different intended stiffnesses under 3, 5 min of setting in fridge at 4°C and 5, 5 minutes of UV crosslinking respectively. OH wet weights were recorded, and hydrogels were placed in a 24 well plate with 1 mL PBS added to each well at 37°C for further characterization.

After GelMA hydrogels had been allowed to set and swell for approximately 24 hours, 4-5 hydrogels of each type (healthy lung ECM and fibrotic lung ECM) were carefully removed and placed in separate weighing dishes. The Malvern Rheometer - Kinexus Ultra+ machine was then used, and the bottom plate was secured by pushing the lever, located on the front of the machine below the bottom parallel plate, all the way to the right. The rSpace application on the computer was opened, and the 0035 test (Frequency Sweep Strain controlled)

was selected. The gap value, representing the hydrogel thickness (mm), was entered, and the hydrogel was centered on the bottom parallel plate. Testing parameters, including start frequency, end frequency, room temperature, shear strain, and samples per decade, were inputted as follows: Start Frequency = 0.1 Hz, End Frequency = 10 Hz, Room Temperature = 25 °C, shear strain = 1%, and 10 samples per decade. The test was initiated, and a 5-minute calibration was performed before the 10-minute frequency sweep test commenced. Throughout the test, care was taken to ensure proper contact between the upper plate and the hydrogel.

Cell viability of encapsulated fibroblasts will be quantified via LIVE/DEAD staining. First, the media will be removed from cell-laden hydrogels and the gels will be washed twice with PBS. Then, 5µL of calcein and 20µL of ethidium will be added to 10mL of PBS to make the staining solution. 200µL of the staining solution will be added to each hydrogel, and the gels will be imaged with a fluorescence microscope.

## Materials and Expenses

Date	Item	Description	Vendor	#	Cost Each	Total Cost	Link
01/26/2024	Past Materials	All prior purchases (see FA23 final report)	–	–	\$1486	\$1486	--
02/19/2024	GelMA Bioink	3mL cartridges of GelMA Bioink	CELLINK	3	\$108.33	\$325	<a href="#">link</a>
03/01/2024	LIVE/DEAD Kit	LIVE/DEAD Cell Viability Kit	ThermoFisher	1	\$300	\$300	<a href="#">link</a>
<b>TOTAL:</b>						<b>\$1811.00</b>	

## Next Week Team Goals

- In the next week, the team will focus on attempting to conduct culturing epithelial cells on bioprinted gels and analyzing this week's encapsulated fibroblast gels

## Next Week Individual Goals

- Carley
  - Optimizing bioprinter
  - Re-attempt cell seeding
- Elijah
  - Continue to work on optimizing bioprinter and printing gels with similar structures
  - Rheometry testing as needed
- Caitríona
  - Perform a trial of bioprinting with the goal of producing thicker gels based on feedback received from the client after cell seeding this week.
  - Help with cell seeding as needed
- Will
  - Continue to troubleshoot the live dead imaging
- Anuraag
  - Rheometry testing as needed
  - Help out with fibroblast cell culture, bioprinting hydrogels, and hydrogel characterization as needed
- Nick
  - Help out with fibroblast cell culture, bioprinting hydrogels, and hydrogel characterization as needed
  - Client Meeting 03/12/2024

## Timeline

Task	Jan	Feb					March				April				May		
	26	2	9	16	23	28	1	8	15	22	5	12	19	26	1	3	10
<b>Project</b>																	
Pipette-Based Hydrogel Characterization			X														
Bioprinted Hydrogel Protocol			X														
Consistent Bioprinted Hydrogel Fabrication																	
Bioprinted Hydrogel Characterization																	
Fibroblast Encapsulation								X									
Fibroblast Viability Testing																	
Epithelial Cell Culture w/ ALI																	
<b>Deliverables</b>																	
Progress Reports		X	X	X	X		X	X									
Journal Selection		X															
Preliminary Presentation			X														
Preliminary Report						X											
Preliminary Notebook						X											
Show and Tell																	
Executive Summary Draft																	
Executive Summary																	
Final Poster																	
Final Report																	
Final Notebook																	
Client Evaluation																	
<b>Meetings</b>																	
Client			X	X			X										
Advisor	X	X	X	X	X		X	X									
<b>Website</b>																	
Update	X	X	X	X	X	X	X	X									

Filled boxes = projected timeline  
 X = task was worked on or completed

## Previous Week Goals and Accomplishments

- Team
  - Epithelial cell culture on bioprinted gels
    - Unsuccessful, gels “melted”
  - Cell encapsulation
    - Successfully completed at varying densities
  - Order LIVE/DEAD stain
    - Completed
- Carley
  - To conduct epithelial cell culture on bioprinted hydrogels
    - Gels were created, but epithelial cell culture did not work well
- Elijah
  - Get trials done on GelMA bioprinter and help team with settings optimization
    - Completed
  - Rheometry testing
    - In-Progress
- Caitríona
  - Additional GelMA bioprinting trials with the intent of providing at least 3 to Dianhua to attempt cell culture on
    - Achieved on 3/3
  - Update Lab Archives
    - Achieved, but there is more to update now.
- Will
  - Encapsulate fibroblasts in pipette-based hydrogels
    - Encapsulated fibroblasts in pipette-based hydrogels
  - Begin troubleshooting calcein imaging, determine imaging protocol
    - Performed bright field imaging, began troubleshooting live dead staining
- Anuraag
  - Continue rheology as needed, help with fibroblast culture, and update lab notebook.
    - Done Tue 03/05
- Nick
  - Help out with fibroblast cell culture, bioprinting, and hydrogel characterization
    - Bioprinting trials 03/03/2024

## Activities

Name(s)	Date	Activity	Time (hr)	Week Total (hr)	Sem. Total (hr)
Nick	03/01/2024	Preparing Templates/Organizing	0.5	0.5	3.5
Caitríona	03/03/2024	Bioprinted Hydrogel Fabrication	6	6	17
Elijah	03/03/2024	Bioprinted Hydrogel Fabrication	5	5	7
Nick	03/03/2024	Bioprinted Hydrogel Fabrication	4	4	4
Will	03/04/2024	Cell Encapsulation in Hydrogels	5	5	5

Carley	03/04/2024	Cell Encapsulation in Hydrogels	5	5	5
Anuraag	03/05/2024	Rheometry Testing of Hydrogels	1.5	1.5	6
Will	03/06/2024	LIVE/DEAD Imaging	2	2	2
Everyone	03/07/2024	Progress Report	0.5	0.5	2.5