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: 02/23/2024 – 02/29/2024

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
The team has been focused this week on preparing for the report and formatting our prior information into a format that is more compatible with a journal's requirements. We have also been focused on preparing for doing cell encapsulation assays and bioprinting gels late this week for epithelial cell culture next week.

Difficulties / Advice Requests
We ran into some troubles when deciding what to include in our report, how to format, and what we still need to accomplish. Specifically, we were unsure where to include aspects of bioprinting optimization, results or just a final set protocol in the methods section.

Current Protocols
Fabrication of non-cell laden pipette based hydrogels at two separate stiffnesses was accomplished by dissolving 50 mg of GelMA into 950 µL of PBS, adding 50 µL of LAP. 10 Hydrogels of 100µL 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. 0H 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 ℃, 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	link
					TOTAL:	**$1811.00**	

Next Week Team Goals

- Epithelial cell culture on bioprinted gels
- Cell encapsulation
- Order LIVE/DEAD stain

Next Week Individual Goals

- Carley
  - To conduct epithelial cell culture on bioprinted hydrogels
- Elijah
  - Get trials done on GelMA bioprinter and help team with settings optimization
  - Rheometry testing
- Caitriona
  - Additional GelMA bioprinting trials with the intent of providing at least 3 to Dianhua to attempt cell culture on
  - Update Lab Archives
- Will
  - Encapsulate fibroblasts in pipette-based hydrogels
  - Begin troubleshooting calcein imaging, determine imaging protocol

- Anuraag
  - Continue rheology as needed, help with fibroblast culture, and update lab notebook.
- Nick
  - Help out with fibroblast cell culture, bioprinting hydrogels, and hydrogel characterization as needed

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																	

Pipette-Based Hydrogel Characterization			X														
Bioprinted Hydrogel Protocol			X														
Consistent Bioprinted Hydrogel Fabrication							X										
Bioprinted Hydrogel Characterization																	
Fibroblast Encapsulation																	
Fibroblast Viability Testing																	
Epithelial Cell Culture w/ ALI																	
Deliverables																	
Progress Reports		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																	
Meetings																	
Client			X	X			X										
Advisor	X	X	X	X	X		X										
Website																	
Update	X	X	X	X	X	X	X										

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

Previous Week Goals and Accomplishments

- Team
    - Improve cell culture conditions for more growth/TLC
        - Yes! They are growing!

    - Continue to implement the field scientists' advice
        - Getting better, having more consistent prints, and being able to conduct rheology on some.
    - Order cell staining materials
        - Still need to do this!
    - Complete preliminary report
        - Completed!
- Carley
    - To continue improving print trials and the bioprinter
        - Yes, print session with caitriona went very well last time and we were able to find consistency
    - Discuss with the client cell adhesion with bioprinted gels
        - Yes, we talked with Dianhua about cell adhesion work for next week
- Elijah
    - Continue to optimize bioprinter settings and replicable structures
        - In Progress
    - Work on preliminary report and deliverables with the fellow team members
        - Completed
    - Perform rheometry testing when needed
        - Completed
- Caitriona
    - To continue optimizing bioprinter parameters and to produce at least 3 gels which can be given to Dianhua for the first round of cell seeding
        - Did not accomplish this goal this week due to preliminary article draft and exams. Pushed to goals for the upcoming week.
- Will
    - Continue to culture cells, until at a passage level reasonable for encapsulation
        - We now have enough cells to encapsulate at a reasonable quantity once the most recent passage grows out
- Anuraag
    - Change media for cells and passage level reasonable for encapsulation
        - Done Friday 02/23, 02/24
    - Perform rheometry testing when needed
        - Done Thurs 02/22
    - Work on preliminary report and deliverables with the fellow team members
        - Finished preliminary report and uploaded to team website. Website was also updated with the latest progress reports, PDS, etc.
- Nick
    - Help out with fibroblast cell culture and hydrogel characterization
        - Timing did not work out with my schedule, so I plan to help with bioprinting more hydrogels this weekend
    - Preliminary Report Sections
        - Graphical abstract, formatting, appendices, part of methods

Activities

Name(s)	Date	Activity	Time (hr)	Week Total (hr)	Sem. Total (hr)

Elijah and Anuraag	02/22/2024	Rheology	2	2	10.5
Anuraag	02/23/2024	Cell Culture/Media Change	1	1	2
Anuraag	02/24/2024	Cell Culture/ Media Change	1	2	3
Will	02/25/2024	Cell Culture/ Media Change	1	2	6
Carley	02/25/2024	Preliminary Report Template	1	1	1
Nick	02/25/2024	Preparing Templates/Organizing	0.5	0.5	3
	02/26/2024	Cell Culture	1		
Caitriona	2/26/2024	Lab Archives Research	3	3	3
Caitriona	2/27/2024	Preliminary Report Editing	1.5	1.5	1.5
Nick	02/27/2024	Lab Archives Research	1	1	3
Will, Elijah, Anuraag	02/27/2024	Cell Culture	1	3	6
	02/28/2024	Cell Culture	1		
Carley	02/29/2024	Report editing	2	2	2
Carley	2/29/2024	Lab archives research	.5	.5	.5
Everyone	02/29/2024	Progress Report	0.5	0.5	2