Tissue Model of The Epithelial Mesenchymal Trophic Unit
BME 402: Tissue Model

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Team:
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Dates: 03/22/2024 – 04/04/2024

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
Since another delay with the GelMA bioink ordering process was missed and thus the shipment has not gone out, the team was unable to continue their GelMA bioprinting trials. Additionally, the 3T3 fibroblasts being cultured in the ECB Teaching Lab needed maintenance this week. These 2 factors meant that the team could not do much regarding wet lab activities. Therefore, the team focused this week on writing the Executive Summary draft for the BME Design awards.

Difficulties / Advice Requests
The team's main difficulty continues to be delays in the shipping of GelMA bioink, which has halted work with the bioprinter.

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 3-5 times with PBS for 5 minutes. Then, optimized volumes of calcein AM and ethidium homodimer-1 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 after they incubate at room temperature for 30 minutes. The images will then be analyzed with ImageJ software to get the percentage of live cells.

Materials and Expenses

Date Ordered	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
03/01/2024	LIVE/DEAD Kit	LIVE/DEAD Cell Viability Kit	ThermoFisher	1	$300	$300	link
					TOTAL:	**$1811.00**	

Next Week Team Goals

- Finalize and submit Executive Summary
- Encapsulate fibroblasts in pipette-based hydrogels
- Conduct LIVE/DEAD staining and imaging
- Analyze the images with ImageJ to obtain cell viability data

Next Week Individual Goals

- Carley
    - Pipette-based encapsulation experiments
    - ImageJ analysis
    - GelMA printing
- Elijah
    - Pipette-based Gel Experiments
    - GelMA bioprinting trials
- Caitríona
    - GelMA delivery came in 4/3, goal is to resume bioprinting trials on Friday. I also hope to do more trials early next week.
    - Cell viability testing and ImageJ analysis
- Will
    - Pipette-based encapsulation experiments
    - Live Dead Staining
- Anuraag
    - Pipette-based Gel Experiments
    - GelMA bioprinting trials
    - Finalize Executive Summary

- Nick
    - Client Meeting
    - Finalize Executive Summary
    - Help with cell viability testing and data analysis

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														
Initial Bioprinted Hydrogel Protocol			X														
Final Bioprinted Hydrogel Protocol																	
Consistent Bioprinted Hydrogel Fabrication																	
Bioprinted Hydrogel Characterization																	
Fibroblast Encapsulation									X								
Fibroblast Viability Testing																	
Epithelial Cell Culture																	
Deliverables																	
Progress Reports		X	X	X	X		X	X	X	X	X						
Journal Selection		X															
Preliminary Presentation			X														
Preliminary Report						X											
Preliminary Notebook						X											
Show and Tell										X							
Executive Summary Draft											X						
Executive Summary																	
Final Poster																	
Final Report																	
Final Notebook																	
Client Evaluation																	

Meetings																	
Client			X	X			X		X								
Advisor	X	X	X	X	X		X	X	X		X						
Website																	
Update	X	X	X	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
  - Contact Dianhua to ask for status on bioink shipment
    - On 04/01/2024, Dianhua said that there was another email about another delay in the ordering process, and that he has reached out to check on the status
    - On 04/03/2024 We were informed that there is now GelMA
  - Executive Summary Draft
    - The team worked together to write this
    - The team will be going for the BME Design Excellence Award
- Carley
  - Worked on executive summary
  - Now have GelMA so can begin printing
- Elijah
  - Print Hydrogels
    - In progress, just became notified that GelMA has arrived
  - Rheometry
    - In progress: After gels are printed, rheometry will be performed
- Caitríona
  - Bioprint Hydrogels
    - Could not accomplish this week due to delayed delivery of GelMA.
  - Help with pipette-gel cell encapsulation and fabrication. Need to develop protocol for a fibrotic model without use of the refrigerator.
    - Schedule is being made to run experiments continuously in the next 7 days.
- Will
  - Maintained cell culture
- Anuraag
  - Finished executive summary draft
- Nick
  - Executive Summary Draft
    - Worked on portions of the draft

Activities

Name(s)	Date	Activity	Time (hr)	Week Total (hr)	Sem. Total (hr)
Will	04/01/2024	Cell Culture	1.5	2.5	10.5
Nick	04/01/2024	Preparing Templates/Organizing	0.5	0.5	4.5

Will	04/02/2024	Cell Culture	1	2.5	11.5
Carley	04/02/2024	Executive Summary	1	1	1
Nick	04/03/2024	Executive Summary	1	1	1
Nick	04/03/2024	Cell Culture	1	1	1
Caitríona	04/03/2024	Executive Summary	0.5	0.5	0.5
Caitríona	04/03/2024	Executive Summary	0.5	1	1
Anuraag	04/03/2024	Executive Summary	0.5	0.5	0.5
Everyone	04/04/2024	Progress Report	0.5	0.5	4.5