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

Client: Dr. Allan Brasier
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Team:
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Dates: 04/19/2024 – 04/26/2024

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 result 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 obtained the 2 week time points for the cell viability data. Besides that, the team spent their time completing the final poster, rehearsing their presentation, and beginning work on the final deliverables. This Friday is the poster presentation session, with final deliverables due next week.

Difficulties / Advice Requests
The team had no difficulties this week.

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**	

Timeline

Task	Jan	Feb					March				April				May	
	26	2	9	16	23	28	1	8	15	22	5	12	19	26	1	3
Project																
Pipette-Based Hydrogel Characterization			X													
Initial Bioprinted Hydrogel Protocol			X													
Final Bioprinted Hydrogel Protocol														X		
Consistent Bioprinted Hydrogel Fabrication														X		
Bioprinted Hydrogel Characterization														X		
Fibroblast Encapsulation									X							
Fibroblast Viability Testing												X				
Deliverables																
Progress Reports		X	X	X	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													X			
Final Poster														X		
Final Report																
Final Notebook																
Client Evaluation																
Meetings																
Client			X	X			X		X			X				
Advisor	X	X	X	X	X		X	X	X		X	X	X			
Website																
Update	X	X	X	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
  - Prepare Final Poster
    - Completed
  - Present at Poster Session 04/26/2024
    - Completed
  - Work on Final Report
    - In Progress
  - Analyze the LIVE/DEAD images with ImageJ to obtain cell viability data
    - Completed
- Carley
  - Final poster and paper
    - Completed and working on
  - 2 week live dead staining
    - Yes completed
- Elijah
  - Bioprinting Trials
    - Completed
  - Work on Final Poster + Final Report
    - Completed
- Caitríona
  - Bioprinting Trials
    - Completed the final round of bioprinting on Friday, planning to get some more gels made in the morning for the poster presentation
  - Final poster and report

        - Completed
- Will
    - 2 week live Dead
        - Completed
    - Work on final poster / report
        - Completed

- Anuraag
    - Work on final poster
        - Completed
    - Begin work on final report
        - In progress
    - Complete soft gel rheology
        - 4/20
- Nick
    - Help with cell viability data analysis
        - Did all ImageJ analysis
    - Work on Final Poster
        - Completed
    - Begin working on Final Report
        - In progress

Activities

Name(s)	Date	Activity	Time (hr)	Week Total (hr)	Sem. Total (hr)
Elijah	04/19/2024	Bioprinted Hydrogel Fabrication	3.5	3.5	24.0
Caitríona	04/19/2024	Bioprinted Hydrogel Fabrication	3.5	3.5	34.5
Anuraag	04/20/2024	Rheometry Testing of Hydrogels	1	1	12
Nick	04/20/2024	Preparing Templates/Organizing	0.5	0.5	6
Carley	04/19/2024	Final Poster	1	1	1
Nick	04/20/2024	Final Poster	1	1	1
Will	04/22/2024	LIVE/DEAD Staining and Imaging	3	3	18
Nick	04/22/2024	ImageJ Analysis	2	2	5
Elijah	04/22/2024	Final Poster	0.5	0.5	1.0
Nick	04/22/2024	Final Poster	1	2	2
Nick	04/23/2024	Final Poster	1	3	3
Carley	04/22/2024	Final Poster	1	1	2

Everyone	04/25/2024	Progress Report	0.5	0.5	6