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

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Team:
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Dates: 03/15/2024 – 03/21/2024

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 past week, the team has focused on analyzing LIVE/DEAD staining pictures with ImageJ and discussed plans for after spring break.

Difficulties / Advice Requests
Main difficulties have been due to shipping delays. Additionally, for the LIVE/DEAD staining protocol we ran into a problem where once we set the minimum area that we consider a stained cell it only counted the cells with that size even though it went to infinity. For example, if the smallest stained cell had an area of 130, the size we entered per the protocol's instructions was 130-Infinity but when we pressed okay to do the count it only counted 1 cell with that exact minimum area.

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	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 (After Spring Break)

- After spring break, we plan to conduct a round of pipette based gels that are both fibrotic and healthy stiffness with encapsulated cells and conduct live/dead staining to see if there is a difference in cell viability between the two. We will conduct ImageJ analysis on these. If GelMA ships as well, we plan to attempt epithelial cell culture again on bioprinted based gels.

Next Week Individual Goals (After Spring Break)

- Carley
  - Encapsulated cell pipette based gel construction
  - If GelMA arrived, bioprinting trial
- Elijah
  - Bioprint Hydrogels when GelMA arrives
  - Use Rheology to figure out stiffness values for varying UV times
- Caitríona
  - Bioprint Hydrogels
  - Help with pipette-gel cell encapsulation and fabrication. Need to develop protocol for a fibrotic model without use of the refrigerator.
- Will
  - Encapsulated cell pipette based gel construction
- Anuraag
  - Bioprint Hydrogels when GelMA arrives
  - Use Rheology to figure out stiffness values for varying UV times
- Nick
  - Bioprint trials

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							
Journal Selection		X															
Preliminary Presentation			X														
Preliminary Report						X											
Preliminary Notebook						X											
Show and Tell										X							
Executive Summary Draft																	
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								
Website																	
Update	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
  - Check in with shipping time for GelMA and cell viability kit
    -
  - Finalize our LIVE/DEAD staining protocol and attempt ImageJ analysis
    -
  - Show and Tell: provide advice to BME 301 teams
    - Will be done tomorrow, 03/22/2024
- Carley
  - Check in with Dianhua about shipping time info
    - Still hasn't arrived.
  - ImageJ analysis of cell viability images
    - Yes, protocol worked well until the final step.
- Elijah
  - Bioprint if GelMA order comes in
    - Still waiting on GelMA
  - Help with Live/Dead Staining
    - In Progress
- Caitríona
  - Bioprint trials if material arrives
    - Material has not arrived yet.
  - Help with cell encapsulation and staining
    - There was no cell encapsulation nor staining this week, but I was able to help with the ImageJ cell counting protocol run-through
- Will
  - Maintained cell culture
  - Developed protocols for coming back off break
- Anuraag
  - Help bioprint contingent on GelMA order
    - GelMA did not arrive
  - Help with Live/Dead Staining
    - Completed by Will
  - Perform Rheology as necessary
    - No gels made due to no GelMA
- Nick
  - Help out with fibroblast cell culture, bioprinting hydrogels, and hydrogel characterization as needed
    - I was unable to contribute much this week because I left partway through the week to go to Vanderbilt for my last BME Graduate Recruitment event. Now that I am done with these events, I will be able to fully contribute to the project

Activities

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

Nick	03/16/2024	Preparing Templates/Organizing	0.5	0.5	4.5
Will, Caitriona, Carley	03/20/2024	ImageJ analysis of LIVE/DEAD stain	1	1	1
Will	03/18,21/2024	Cell culture maintenance	3	3	
Everyone	03/14/2024	Progress Report	0.5	0.5	3.5