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: 03/08/2024 – 03/14/2024

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 has used the remaining cartridges of GelMA resulting in a cease using the bioprinter. The team has been working to improve our LIVE/DEAD staining, specifically, Will recently captured some images of encapsulated fibroblasts that do not have high levels of background!

Difficulties / Advice Requests
Our main difficulty is the shipping of supplies halting our work on the bioprinter. We would also like to discuss our recent images of encapsulated cells.

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

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

- Check in with shipping time for GelMA and cell viability kit - hopefully arrive by the time we are back from spring break.
- Finalize our LIVE/DEAD staining protocol
    - Attempt ImageJ analysis
- Show and Tell: provide advice to BME 301 teams

Next Week Individual Goals

- Carley
    - Check in with Dianhua about shipping time info
    - ImageJ analysis of cell viability images
- Elijah
    - Bioprint if GelMA order comes in
    - Help with Live/Dead Staining
- Caitríona
    - Bioprint trials if material arrives
    - Help with cell encapsulation and staining
- Will
    - Use successfully verified protocol to perform Live/Dead staining on properly seeded
        - Encapsulate more cells in more gels
        - Sans fridge time
- Anuraag
    - Help bioprint contingent on GelMA order
    - Help with Live/Dead Staining
    - Perform Rheology as necessary
- Nick
    - Help out with fibroblast cell culture, bioprinting hydrogels, and hydrogel characterization as needed

- I will be leaving on Wednesday to go to a BME graduate recruitment event at Vanderbilt University, so my availability will be limited next week

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								
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		X								

Advisor	X	X	X	X	X		X	X	X								
Website																	
Update	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
  - Attempting to conduct culturing epithelial cells on bioprinted gels
    - Epithelial cell culture was done on three bioprinted gels, but gels dissolved in media solution in the incubator, making it difficult to discern gel boundaries.
  - Analyzing last week's encapsulated fibroblast gels
    - On 3/13, live-dead staining was troubleshooted, and the first successful images were obtained. Further analysis of these images is required for quantification of encapsulation results.
- Carley
  - Optimizing bioprinter
    - Working towards it but no GelMA
  - Re-attempt cell seeding
    - No, we have run out of GelMA
- Elijah
  - Continue to work on optimizing bioprinter and printing gels with similar structures
    - In Progress: Ran out of GelMA
  - Rheometry testing as needed
    - In Progress
- 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
    - Accomplished on 3/8, stiffness values have been entered in the drive.
  - Help with cell seeding as needed
    - Did not accomplish this as I was unavailable when team cell seeding occurred.
- Will
  - Continue to troubleshoot the LIVE/DEAD imaging
    - Successfully verified modified protocol for LIVE/DEAD imaging.
- Anuraag
  - Rheometry testing as needed
    - Done 03/13
  - Help out with fibroblast cell culture, bioprinting hydrogels, and hydrogel characterization as needed
    - Did not accomplish this as I was unavailable when team cell seeding occurred.
- Nick
  - Help out with fibroblast cell culture, bioprinting, and hydrogel characterization
    - Bioprinting trials 03/08/2024, got 4 good gels, swelled them in media, and put them in incubator for later rheology
  - More Lab Archives research on LIVE/DEAD staining protocol
    - Found information on troubleshooting our issues as well as a protocol for image analysis
    - Rewrote LIVE/DEAD protocol and wrote Cell Viability Image Analysis protocol
  - Client Meeting

- Done 03/12/2024

Activities

Name(s)	Date	Activity	Time (hr)	Week Total (hr)	Sem. Total (hr)
Caitríona	03/03/2024	Bioprinted Hydrogel Fabrication	3	3	20
Nick	03/03/2024	Bioprinted Hydrogel Fabrication	2.5	2.5	6.5
Elijah	03/03/2024	Bioprinted Hydrogel Fabrication	1.5	1.5	8.5
Nick	03/08/2024	Preparing Templates/Organizing	0.5	0.5	4
Nick	03/08/2024	Lab Archives Research	2	2	5
Nick, Elijah, and Anuraag	03/12/2024	Client Meeting	0.5	0.5	1.5
Nick	03/12/2024	Protocol Writing	1	1	2
Will	03/12/2024	Cell Encapsulation	3	3	8
Caitríona	03/13/2024	Lab Archives Research	0.5	0.5	3.5
Anuraag	03/14/2024	Gel Rheology	1	1	7
Will	03/13/2024	LIVE/DEAD Imaging	3	3	5
Everyone	03/14/2024	Progress Report	0.5	0.5	3