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

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Team:
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Dates: 04/05/2024 – 04/11/2024

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 continues to make headway in all areas of the project as they have resumed bioprinted this week. Gels were able to be bioprinted during multiple points during this week and the team has been able to become much more efficient during printing sessions. LIVE/DEAD cell analysis also kicked off this week with Will Onuscheck leading the way. 24 hour images were taken. Further ImageJ analysis of these images will be performed in the following weeks.

Difficulties / Advice Requests
On the bioprinting side of things, the team continues to struggle with optimizing the structure of the printed hydrogels. With limited time remaining in the semester, the team will be focusing on structure in bioprinting sessions to determine multiple protocols that will lead to maximum replicability.

On the LIVE/DEAD imaging side of things, the team found it difficult to get *completely* clear/crisp images because even if some cells are brought into focus there will always be other cells that are out-of-plane and thus make parts of the images blurry. This is unavoidable since the hydrogel is 3D and thus there will always be multiple planes in one image.

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

- Conduct more bioprinting trials
- Conduct additional LIVE/DEAD imaging timepoints
- Analyze the images with ImageJ to obtain cell viability data

Next Week Individual Goals

- Carley
  - Final cell staining session next week
  - ImageJ analysis
- Elijah
  - Characterize bioprinted GelMA hydrogels with rheometry to determine stiffness for UV times
  - Help out with ImageJ of LIVE/DEAD analysis performed this week
- Caitríona
  - Bioprinting fabrication of healthy tissue stiffness
  - ImageJ analysis
- Will
  - 1 week, 2 week LIVE/DEAD staining
  - Maintain scaffold culutres

- Anuraag
    - Continue with rheometry when needed
    - Help with imaging and/orimageJ analysis
    - Work on final Executive Summary
- Nick
    - 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												X					
Epithelial Cell Culture																	
Deliverables																	
Progress Reports		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																	
Final Poster																	
Final Report																	
Final Notebook																	

Client Evaluation																	
Meetings																	
Client			X	X			X		X			X					
Advisor	X	X	X	X	X		X	X	X		X	X					
Website																	
Update	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
  - Encapsulate fibroblasts in pipette-based hydrogels
    - Done
  - Conduct LIVE/DEAD staining and imaging
    - 24hr time point done
  - Analyze the images with ImageJ to obtain cell viability data
    - In progress
- Carley
  - Pipette-based encapsulation experiments
    - In progress
  - ImageJ analysis
    - Soon to be conducted
  - GelMA printing
    - Just resumed this week
- Elijah
  - Pipette-based Gel Experiments
    - In progress; still finding time to help team out
  - GelMA bioprinting trials
    - Achieved: GelMA cartridges came in and was able to print two times this week
- Caitríona
  - Resume bioprinting trials on Friday
    - Successfully bioprinted both Friday and Wednesday, stiffnesses were variable and too high, need to optimize parameters for healthy tissue in general.
  - Cell viability testing and ImageJ analysis
    - Not available during the times when others on the team did this, focused more on bioprinting trials.
- Will
  - Fabricated cell laden scaffolds Monday
  - 24 Hour Live dead staining Tuesday
  - 72 Hour Live Dead staining Thursday
  - Maintained cell culture

- Anuraag
  - Completed rhometry on Monday 04/08 and Thursday 04/11
  - Lab archives updated
  - Updated team website
- Nick
  - Lab Archives Research

        - Did literature search to see if LIVE/DEAD is an endpoint or if you can continue to culture cells and reimage later
    - Help with cell viability testing and data analysis
        - Did 24hr LIVE/DEAD staining and imaging on 04/09/2024

Activities

Name(s)	Date	Activity	Time (hr)	Week Total (hr)	Sem. Total (hr)
Elijah	04/05/2024	Bioprinted Hydrogel Fabrication	3.0	3.0	14.0
Caitríona	04/05/2024	Bioprinted Hydrogel Fabrication	3.0	3.0	23.0
Elijah	04/05/2024	Outreach Writing	1.0	1.0	1.0
Nick	04/07/2024	Preparing Templates/Organizing	0.5	0.5	5
Anuraag	04/08/2024	Rheometry Testing of Hydrogels	1	1	8
Anuraag	04/08/2024	Outreach Essay	0.5	0.5	0.5
Will	04/08/2024	Cell Encapsulation	4		
Carley	04/08/2024	Cell Encapsulation	3.5		
Nick	04/09/2024	Lab Archives Research	1	1	6
Nick	04/09/2024	LIVE/DEAD Staining and Imaging	3	3	3
Will	04/09/2024	LIVE/DEAD Staining and Imaging	3	3	
Carley	04/09/2024	LIVE/DEAD Staining and Imaging	2	2	
Elijah	04/10/2024	Bioprinted Hydrogel Fabrication	2.5	5.5	14.0
Caitríona	04/10/2024	Bioprinted Hydrogel Fabrication	2.5	5.5	25.5
Anuraag	04/11/2024	Rheometry Testing of Hydrogels	2	3	10
Will	4/11/2024	Cell Culture Maintenance	2	2	
Everyone	04/11/2024	Progress Report	0.5	0.5	5