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: 04/12/2024 – 04/18/2024

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 focused on bioprinting with the new round of GelMA, in particular batch to batch variation was lower. We also focused on multiple rounds of staining and are beginning to do imageJ analysis. We attempted to start epithelial cell culture on bioprinted gels but were unable to secure epithelial cells.

Difficulties / Advice Requests
The team is continuing to get better on optimizing the bioprinter settings but reproducibility has still been a struggling factor. The team will be able to determine a set of protocols through their many trials of bioprinting to supply the client with the best protocols possible for success. Also we were unable to acquire epithelial cells from the Brasier lab to put on the final poster presentation. The IMAGEJ analysis of the pipette based gels with encapsulated fibroblasts will be the bulk of the cell images on the final poster.

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

- Prepare Final Poster
- Present at Poster Session 04/26/2024
- Work on Final Report
- Analyze the LIVE/DEAD images with ImageJ to obtain cell viability data

Next Week Individual Goals

- Carley
  - ImageJ analysis
  - Final poster and paper
  - 2 week live dead staining
- Elijah
  - IMAGEJ Analysis of gels
  - Work on Final Poster + Final Project
- Caitríona
  - Bioprinting Trials
  - ImageJ analysis
  - Final poster and report
- Will
  - 2 week live Dead
  - Work on final poster / report
  - Continue maintaining cell culture
- Anuraag
  - Work on final poster

    - Begin work on final report
    - Complete soft gel rheology
- Nick
    - Help with cell viability data analysis
    - Work on Final Poster
    - Begin working on Final Report

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	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																	
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				

Filled boxes = projected timeline
X = task was worked on or completed

Previous Week Goals and Accomplishments

- Team
  - Conduct more bioprinting trials
    - Yes, many rounds with lower variability
  - Conduct additional LIVE/DEAD imaging timepoints
    - Yes, only the two week round is left
  - Analyze the images with ImageJ to obtain cell viability data
    - In the process
  - Finalize Executive Summary
    - Edits were made based on feedback and values will be implemented soon
- Carley
  - Cell staining session next week
    - Yes, Will and I completed the week 1 live/dead staining
  - ImageJ analysis
    - Starting to this week
- Elijah
  - Characterize bioprinted GelMA hydrogels with rheometry to determine stiffness for UV times
    - Gels were printed, stiffness measurements need to be obtained
  - Help out with ImageJ of LIVE/DEAD analysis performed this week
    - In Progress, Analysis will start 04/19/2024
- Caitríona
  - Bioprinting fabrication of healthy tissue stiffness
    - Conducted two bioprinting trials, stiffness values were variable and too high to be within the healthy stiffness range.
- Will
  - 1 week LIVE/DEAD staining
  - Maintain cell culture
  - Image J
- Anuraag
  - Continue with rheometry when needed
    - 04/18
  - Help with imaging and/orimageJ analysis
    - N/A
  - Work on final Executive Summary
    - Executive summary is completed
- Nick
  - Work on final Executive Summary
    - Completed
  - Help with cell viability testing and data analysis

- In progress

Activities

Name(s)	Date	Activity	Time (hr)	Week Total (hr)	Sem. Total (hr)
Elijah	04/12/2024	Bioprinted Hydrogel Fabrication	3.5	3.5	17.5
Caitríona	04/12/2024	Bioprinted Hydrogel Fabrication	3.5	3.5	29
Will	04/15/2024	LIVE/DEAD Staining and Imaging	3	3	14
Carley	04/15/2024	LIVE/DEAD Staining and Imaging	2	2	11
Nick	04/16/2024	Preparing Templates/Organizing	0.5	0.5	5.5
Carley	04/16/2024	Executive Summary	1	1	
Nick	04/16/2024	Executive Summary	0.5	0.5	1.5
Caitríona	04/17/2024	Bioprinted Hydrogel Fabrication	3.0	6.5	31
Elijah	04/17/2024	Bioprinted Hydrogel Fabrication	3.0	6.0	20.5
Anuraag	04/18/2024	Bioprinted Gel Rheology	1	1	11
Nick	04/18/2024	ImageJ Analysis	3	3	3
Everyone	04/11/2024	Progress Report	0.5	0.5	5.5