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: 02/16/2024 – 02/22/2024

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
We have made improvements on the bioprinter by having a flatter print and were able to print with more consistency. We also have been able to have some more cell culture growth with the same TLC.

Difficulties / Advice Requests
Need to email our client about the cell staining we want to use and why.

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 twice with PBS. Then, 5µL of calcein and 20µL of ethidium 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.

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
					TOTAL:	**$1811.00**	

Next Week Team Goals

- Improve cell culture conditions for more growth/TLC
- Continue to implement the field scientists advice
- Order cell staining materials

Next Week Individual Goals

- Carley
  - To continue improving print trials and the bioprinter
  - Discuss with the client cell adhesion with bioprinted gels
- Elijah
  - Continue to optimize bioprinter settings and replicable structures
  - Work on preliminary report and deliverables with the fellow team members
  - Perform rheometry testing when needed
- Caitriona
  - To continue optimizing bioprinter parameters and to produce at least 3 gels which can be given to Dianhua for the first round of cell seeding.
- Will
  - Continue to culture cells, until at a passage level reasonable for encapsulation
- Anuraag
  - Change media for cells and passage level reasonable for encapsulation
  - Perform rheometry testing when needed
  - Work on preliminary report and deliverables with the fellow team members
- Nick
  - Help out with fibroblast cell culture and hydrogel characterization
    - Now that my graduate school recruitment visits are over for the time being, I will be able to contribute more to the project

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														
Bioprinted Hydrogel Protocol			X														
Consistent Bioprinted Hydrogel Fabrication																	
Bioprinted Hydrogel Characterization																	
Fibroblast Encapsulation																	
Fibroblast Viability Testing																	
Epithelial Cell Culture w/ ALI																	
Deliverables																	
Progress Reports		X	X	X	X												
Journal Selection		X															
Preliminary Presentation			X														
Preliminary Report																	
Preliminary Notebook																	
Show and Tell																	
Executive Summary Draft																	
Executive Summary																	
Final Poster																	
Final Report																	
Final Notebook																	
Client Evaluation																	
Meetings																	
Client			X	X													
Advisor	X	X	X	X	X												
Website																	
Update	X	X	X	X	X												

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

Previous Week Goals and Accomplishments

- Team
    - Improve the bioprinter trials with the advice from the field scientist in mind (Specifically use of the smaller nozzle and the specific temperature parameters)

        - Yes we are still working on this, but it's getting better
    - Rheology of printed gels
        - Achieved!
    - Maintain viable cell culture
        - With some TLC, we will get there
- Carley
    - Use the advice from the field scientist meeting to print more successful trials
        - I feel that there is a lot of progress being made towards this, but with a bit more work in the coming week
- Elijah
    - Optimize GelMA bioprinted gels with information now obtained during field scientist meeting
        - Still optimizing, continuing to make progress
    - Perform rheology testing when needed
        - Ongoing
- Caitriona
    - Return to WIMR on Friday and Monday to begin optimizing the new protocol/print parameters that we discussed with the representatives from CELLINK
        - These prints were accomplished this week. As has been alluded to by other team members, work still needs to be done to further this optimization
    - Attend a rheology testing session
        - Again, there were no sessions scheduled that I could attend with my class schedule.
- Will
    - Begin cell culture in ECB
        - Began
- Anuraag
    - Join group to bioprint following suggestions from field scientist
        - Going to work with bioprinter Friday 02/23
    - Perform rheology when necessary
        - Performing rheology on bioprinted gels Thursday 02/22
    - Passage cells as necessary
        - Changing media and passaging as necessary 02/23, 02/24, and 02/25
- Nick
    - Help out with fibroblast cell culture and hydrogel characterization
        - Due to midterms UW-Madison's grad school recruitment events, I was unable to dedicate time to contributing to the project goals (After my Texas A&M BME Recruitment events this week, my availability will go back to normal)

Activities

Name(s)	Date	Activity	Time (hr)	Week Total (hr)	Sem. Total (hr)
Will	02/16/2024	Cell Culture	1	1	3
Elijah	02/16/2024	Bioprinted Hydrogel Fabrication	2.0	2.0	2.0
Caitriona	02/16/2024	Bioprinted Hydrogel Fabrication	2.0	2.0	8.5
Will	02/17/2024	Cell Culture	1	2	3

Anuraag	02/19/2024	Cell Culture	1	1	1
Nick	02/20/2024	Preparing Templates/Organizing	0.5	0.5	2.5
Carley	02/21/2024	Bioprinted Hydrogel Fabrication	2.5	2.5	6
Caitriona	02/21/2024	Bioprinted Hydrogel Fabrication	2.5	4.5	11
Will	02/21/2024	Cell Culture	1	3	4
Will	02/22/2024	Cell Culture	1	4	5
Elijah	02/22/2024	Rheometry Testing of Hydrogels	1.5	1.5	8.5
Everyone	02/22/2024	Progress Report	0.5	0.5	1.5