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: 02/09/2024 – 02/15/2024

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 was able to meet with Dr.Brasier on 2-13-24. Progress was made on bioprinter issues as we were able to meet with a field scientist from BioX on 2-15-24. The team is working on constructing Bioprinted GelMA hydrogels.

Difficulties / Advice Requests
Some difficulties are the low throughput that this device has. We also are struggling with the correct extrusion pressure, temperature, and printing speed. Advice from the field scientist should help us with these struggles.

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	--
					TOTAL:	**$1486.00**	

Next Week Team Goals

- Improve the bioprinter trials with the advice from the field scientist in mind
    - Specifically use of the smaller nozzle and the specific temperature parameters
- Rheology of printed gels
- Need new cells that aren't dead

Next Week Individual Goals

- Carley
    - My goals are to use the advice from the field scientist meeting to print more successful trials
- Elijah
    - Optimize GelMA bioprinted gels with information now obtained during field scientist meeting
    - Perform rheology testing when needed
- Caitriona
    - Return to wimr on Friday and Monday to begin optimizing the new protocol/print parameters that we discussed with the representatives from Cellink
    - Attend a rheology testing session
- Will
    - Try bioprinting with 27G nozzle
- Anuraag
    - Join group to bioprint following suggestions from field scientist
    - Perform rheology when necessary
    - Passage cells as necessary
- Nick
    - Help out with fibroblast cell culture and hydrogel characterization
        - Due to midterms and a grad school recruitment weekend at Texas A&M, I am unsure how much time I'll actually have. After next week, my availability will go back to normal.

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													
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													
Website																	
Update	X	X	X	X													

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

Previous Week Goals and Accomplishments

- Team
    - Get in contact with bioprinter field scientist
        - Achieved: Meeting on 2-15-24

    - Finalize set drying time for determining dry weight of the hydrogels that will be applied to all gels
        -
    - Continue to discuss cell culture being conducted on the hydrogels and encapsulation
        -
    - Continue to research cellular assays to be conducted
        -
    - Continue with bioprinting trials
        - Bioprinting trials conducted on 2/14 and again on 2/15
- Carley
    - Finalize protocols for bioprinted hydrogels at both healthy and fibrotic stiffness
        - Getting there with new info from field scientist
    - Bring in field scientists with Dr. Brasier's help
        - Meeting was useful but still such low throughput
    - Discuss cell culture work to be done in BME teaching lab and devise a schedule on splitting responsibilities
        - Thawed cells but dead
- Elijah
    - Attempt more possible GelMA bioprinting trials to familiarize myself with the instrument
        - Achieved
    - Attempt trials with varying UV time on the bioprinter to characterize their respective stiffness
        - Achieved fibrotic range, still need to find UV time to achieve normal tissue stiffness
    - Conduct research on hydrogel dehydration and determine possible causes
        - Still working on this goal
- Caitriona
    - Conduct a fabrication trial with at least one hour (ideally 1.5 hr) of heating prior to printing.
        - Achieved on 2/14. Printer usage was better with the longer heating time, but ultimately was dispensing rather than extruding structures. Goal has been updated now to adjust protocol according to the advice we received from Cellink.
    - Attend a session of rheometry testing conducted by someone else on the team to get more comfortable with that element of the project
        - I was unable to achieve this due to my schedule conflicting with rheology testing sessions.
- Will
    - Begin encapsulating cells in pippetted hydrogels
        - Thawed cells died
- Anuraag
    - Review literature to find out cause of gel shrinking post-swelling
        - Completed and In Lab Archives
    - Review literature with regard to altering UV times for more well characterized pipette-based gels
        - Postponed; Need to try bioprinting trials following filed scientist suggestions
- Nick
    - Re-review literature on GelMA bioprinting troubleshooting
        - Decided to instead focus on writing up explicit protocol for LIVE/DEAD staining
    - Help with characterization of fabricated hydrogels
        - Was unable to do this due to a grad school recruitment weekend for UW-Madison

Activities

Name(s)	Date	Activity	Time (hr)	Week Total (hr)	Sem. Total (hr)
Nick	02/09/2024	Preparing Templates/Organizing	0.5	0.5	3
Will and Elijah	02/13/2024	Client Meeting	0.5	0.5	0.5
Will	02/14/2024	Cell Culture	2	2	2
Caitriona	02/14/2024	Bioprinted Hydrogel Fabrication	3	3	6.5
Will, Caitriona, Elijah, Carley	02/15/2024	Bioprinter Field Scientist Meeting	3	3	3
Elijah	02/09/2024	Rheometry Testing of Hydrogels	2.0	2.0	7
Everyone	02/15/2024	Progress Report	0.5	0.5	2