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: 01/26/2024 - 02/01/2024

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 focused mainly on repeating prior pipette based protocols to reach relevant numbers in both the fibrotic and healthy stiffness groups. The team encountered some hiccups with the rheometer but since then gel formation and rheology has been running smoothly. The team attempted bioprinting on three separate occasions with multiple trials in each and continued to run into the same issues. The meeting with Dr. Brasier and the bioprinter highlighted current protocols being created but also showed many of the difficulties the team has been encountering. Lastly, in lab archives there is an up to date and detailed bioprinting protocol.

Difficulties / Advice Requests
The team found some literature that suggests a time-based air-drying duration is sufficient to characterize swelling in hydrogels (Bittner et al.). However, other articles have used lyophilization as their protocol for measuring dry weight (Hydrogel Characterization - swelling section). We would like to discuss with Professor Puccinelli to determine what the proper time duration should be for drying and where would be the best storage place.
Our first round of rheology testing for the semester did not yield favorable values for the stiffness of the pipette-based hydrogels fabricated on Wednesday. Values for both the fibrotic and healthy tissue gels ranged between 50 and 200 Pa, far lower than what is required by the design specification as well as what has been achieved in previous semesters. Wet weight measurements taken at t=0 and t=24 hr after fabrication revealed that weight decreased after 24 hours, another unexpected result. Unsure of where these discrepancies originated in the fabrication protocol, another round of fabrication is necessary. The only change that was made to the protocol from previous semesters that was successful for the fabrication of pipette-based gels was the use of the incubator in order to conduct rheology testing at body temperature.

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.

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

- Choose a suitable journal for our final report
- Complete Preliminary Presentation to present to advisor at 02/09/2024 advisor meeting
- Establish protocols for bioprinting both healthy and fibrotic stiffness
- Get in contact with field scientist
- Establish a set drying time for determining dry weight of the hydrogels that will be applied to all gels
- Begin to discuss cell culture being conducted on the hydrogels and encapsulation
- Begin to research cellular assays to be conducted
    - We'll likely need to consider where to obtain cells to use in the BME teaching lab for ease of assay execution

Next Week Individual Goals

- Carley
    - Finalize protocols for bioprinted hydrogels at both healthy and fibrotic stiffness
        - Need to bring in field scientists with Dr. Brasiers help, we are reaching a point where progress is not being made regardless of trial changes and attempts
    - Discuss cell culture work to be done in BME teaching lab and devise a schedule on splitting responsibilities
- Elijah
    - Repeat trials of pipette based hydrogels for rheometry testing, perform rheometry testing when gels are made
    - Perform GelMA bioprinting and continue to familiarize myself with the process
- Caitriona

    - Find swelling ratio protocol to show to Professor Puccinelli
    - Make a trip into WIMR on Monday to fabricate bioprinted gels which can be tested on Tuesday
    - Continue to work with the Dr. Brasier to establish meeting times for the semester
- Will
    - Fabricate bioprinted Gels for characterization under 37 °C conditions
    - Potentially repeat pipetted gels, as the 37° storage conditions appear to be a factor in elastic modulus
- Anuraag
    - Fabricate bioprinted Gels for characterization under 37 C conditions
    - Need to redo pipetted gels at 37 C for rheometric testing along
    - Meet with group to reorient the group for bioprinting
- Nick
    - Re-review literature on cell viability of cells encapsulated in hydrogel
    - Reorient self with bioprinter
    - Conduct bioprinting trials

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 Goals																	
Pipette-Based Hydrogel Characterization																	
Bioprinted Hydrogel Protocol																	
Consistent Bioprinted Hydrogel Fabrication																	
Bioprinted Hydrogel Characterization																	
Fibroblast Encapsulation																	
Fibroblast Viability Testing																	
Epithelial Cell Culture w/ ALI																	
Deliverables																	
Progress Reports		X															
Journal Selection																	
Preliminary Presentation																	
Preliminary Report																	
Preliminary Notebook																	
Show and Tell																	

Executive Summary Draft																	
Executive Summary																	
Final Poster																	
Final Report																	
Final Notebook																	
Client Evaluation																	
Meetings																	
Client																	
Advisor	X	X															
Website																	
Update	X	X															

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

Previous Week Goals and Accomplishments

- Team
  - Bioprinted hydrogels - lack consistency still
    - Rheometry to be conducted
  - Pipette based gels created and rheology went successfully
  - Meeting with Dr. Brasier
- Carley
  - Meeting with Dr. Brasier involved me doing the interface commands with the bioprinter while he took notes and made some suggestions
    - The first 5+ print runs did not work zzzz
- Elijah
  - Perform rheometry testing on GelMA hydrogels fabricated by other team members
    - Completed on 1/31/2024
  - Research swelling ratio and hydrogel drying methods to determine if gels can be air-dried
    - Completed on 1/30/2024
- Caitriona
  - Establish meeting times with the team, the client, and advisor to be maintained throughout the semester
    - The team has established a check-in time to take place every Monday afternoon to map out the following week. Meeting times with the client are currently being finalized as we compare team availability with Dr. Brasier. Weekly advisor meetings will take place on Fridays at 12:05.
  - Find and save relevant protocols in literature for the current steps in fabrication
    - Started reading about swelling ratios, need to dedicate more time to this goal and document in the coming days.
- Will
  - Fabricate pipette based hydrogels for the purpose of characterize swelling ratio, and rheometry under 37°C conditions
    - Completed on Tuesday, January 30th
- Anuraag
  - Fabricate pipette based hydrogels for the purpose of characterize swelling ratio, and rheometry under 37°C conditions

        - Completed on Tuesday, January 30th
    - Perform rheometry testing on GelMA hydrogels fabricated by other team members
        - Completed on 1/31/2024
- Nick
    - Review literature on drying hydrogels for obtaining dry weight for swelling ratio characterization
        - Found evidence to support my claim that the hydrogels can be air-dried
    - Meet with team to figure out plan for first couple of weeks
        - As a team we decided on meeting times for the first few weeks (time will change soon)
        - Discussed potential methods for obtaining dry weight of hydrogels

Activities

Name	Date	Activity	Time (hr)	Week Total (hr)	Sem. Total (hr)
Nick	01/26/2024	Preparing Templates/Organizing	1.5	1.5	1.5
Nick	01/26/2024	Lab Archives Research	1	1	1
Everyone	1/29/2024	Team Check-In	0.5	0.5	0.5
Will	01/30/2024	Pipette-Based Hydrogel Fabrication	2.5	2.5	2.5
Anuraag	01/30/2024	Pipette-Based Hydrogel Fabrication	2.5	2.5	2.5
Carley	01/30/2024	Pipette-Based Hydrogel Fabrication	1	1	1
Caitriona	01/30/2024	Lab Archives Research	0.5	0.5	0.5
Elijah	01/30/2024	Lab Archives Research	1.5	1.5	1.5
Elijah	01/31/2024	Rheometry Testing of Hydrogels	2.0	2.0	2.0
Anuraag	01/31/2024	Rheometry Testing of Hydrogels	2.0	2.0	2.0
Everyone	02/01/2024	Progress Report	0.5	0.5	0.5