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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BPAG: Nick Herbst (nherbst2@wisc.edu)
Dates: 02/02/2024 – 02/08/2024

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
This week the team continued to run into difficulties with bioprinting hydrogels and characterizing pipette-based hydrogels. The pipette-based hydrogels once again decreased in mass after swelling, though rheometry revealed low inter-batch stiffness variation. For the bioprinted hydrogels, rheometry revealed drastic variability. Bioprinting the hydrogels was a challenge as well; the team had trouble printing even after troubleshooting the parameters. When the bioprinter did print, the gels were too big/malformed. The client will be connecting the team with a CELLINK field scientist.

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

- Get in contact with bioprinter field scientist
- 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
    - We'll likely need to consider where to obtain cells to use in the BME teaching lab for ease of assay execution
- Continue with bioprinting trials

Next Week Individual Goals

- Carley
    - Finalize protocols for bioprinted hydrogels at both healthy and fibrotic stiffness
        - Need to bring in field scientists with Dr. Brasier's 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
    - Attempt more possible GelMA bioprinting trials to familiarize myself with the instrument
    - Attempt trials with carrying UV time on the bioprinter to characterize their respective stiffness
    - Conduct research on hydrogel dehydration and determine possible causes
- Caitriona
    - One possible variable that was brought up by Dr. Brasier was that printing may be unsuccessful due to non-homogeneity in the cartridge. This might be reduced by an even longer heating time. My goal this week is to conduct a fabrication trial with at least one hour (ideally 1.5 hr) of heating prior to printing.
    - Attend a session of rheometry testing conducted by someone else on the team to get more comfortable with that element of the project.
- Will
    - Begin encapsulating cells in pippetted hydrogels
- Anuraag

    - Review literature to find out cause of gel shrinking post-swelling
    - Fabricate bioprinted hydrogels and perform rheometry as needed
    - Review literature with regard to altering UV times for more well characterized pipette-based gels
- Nick
    - Re-review literature on GelMA bioprinting troubleshooting
    - Help with characterization of fabricated hydrogels

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														
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														
Advisor	X	X	X														
Website																	
Update	X	X	X														

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

Previous Week Goals and Accomplishments

- Team
  - Choose a suitable journal for our final report
    - After discussion, the team chose *Biomaterials Science*
  - Complete Preliminary Presentation to present to advisor at 02/09/2024 advisor meeting
    - Slides were completed and sections were assigned
  - Establish protocols for bioprinting both healthy and fibrotic stiffness
    - See Lab Archives for protocol
  - Schedule a bioprinter viewing with our client
    - The demo meeting was scheduled and held 02/07/2024
  - Establish a set drying time for determining dry weight of the hydrogels that will be applied to all gels
    - Still being discussed
  - Begin to discuss cell culture being conducted on the hydrogels and encapsulation
    - Still being discussed
  - Begin to research cellular assays to be conducted
    - Still being discussed
- Carley
  - Meeting with Dr. Brasier involved me doing the interface commands with the bioprinter while he took notes and made some suggestions, Caitriona and Elijah mapped out what trials to conduct next and took notes on changes we made each time
    - The first 5+ print runs did not work (nothing extruded) then with the same parameters printed in the last 20 minutes of the meeting
  - Bioprinting gels on Monday with Caitriona yielded more success by determining a more beneficial pre-flow time, but still continued to have issues with consistency
    - Accomplishment is having things print but not successful as there are still many issues
- Elijah
  - Repeat trials of pipette based hydrogels for rheometry testing, perform rheometry testing when gels are made
    - Rheometry testing was performed on bioprinted GelMA hydrogels along with pipette-based hydrogels
  - Perform GelMA bioprinting and continue to familiarize myself with the process
    - Team meeting occurred this week, and I was able to take instructional notes and observe use of the bioprinter. I am learning very quickly and cannot wait to perform more trials.
- Caitriona
  - Find swelling ratio protocol to show to Professor Puccinelli
    - Did not achieve this goal as my focus was on the bioprinting trials, which ended up taking most of my time this week.
  - Make a trip into WIMR on Monday to fabricate bioprinted gels which can be tested on Tuesday

        - Fabrication trial was conducted on Monday. Carley and I were successful in achieving parameters that yielded gels, the most notable of these being the pre-flow delay time. However, when these parameters were followed again on Wednesday with Dr. Brasier, the results were inconsistent once again. Detailed notes on both of these sessions and parameters used can be found in LabArchives.
    - Continue to work with the Dr. Brasier to establish meeting times for the semester
        - Accomplished this goal late last week. Scheduled and participated in an in-person meeting with Dr. Brasier on Wednesday and scheduled bi-weekly Zoom meetings which start this coming Tuesday.
- Will
    - Fabricate bioprinted Gels for characterization under 37 °C conditions
        - Completed 2/5, 2/6
    - Potentially repeat pipetted gels, as the 37° storage conditions appear to be a factor in elastic modulus
        - Completed 2/5, 2/6, 2/7
- Anuraag
    - Fabricate bioprinted Gels for characterization under 37 C conditions
        - Completed Friday 02/02/224
    - Need to redo pipetted gels at 37 C for rheometric testing along
        - Not completed; addressed by Will
    - Meet with group to reorient the group for bioprinting
        - Completed Friday 02/02/224
- Nick
    - Re-review literature on cell viability of cells encapsulated in hydrogel
        - Found that most common is LIVE/DEAD staining, so I think we should try it again following a set protocol
    - Reorient self with bioprinter
        - Met with team 02/07/2024 to go over bioprinter use again and attempt some trials
    - Work on preliminary presentation
        - Updated our preliminary presentation slides from the fall to reflect current goals and past progress

Activities

Name(s)	Date	Activity	Time (hr)	Week Total (hr)	Sem. Total (hr)
Nick	02/02/2024	Preparing Templates/Organizing	0.5	0.5	2
Nick	02/02/2024	Preliminary Presentation Slides	1.5	1.5	1.5
Everyone	02/02/2024	Bioprinted Hydrogel Fabrication	2	2	2
Will	02/02/2024	Rheometry Testing of Hydrogels	1	1	1
Nick	02/03/2024	Lab Archives Research	1	1	2
Will	02/05/2024	Pipette-Based Hydrogel Fabrication	2	2	4.5

Carley	02/05/2024	Bioprinted Hydrogel Fabrication	3.5	3.5	3.5
Caitriona	02/05/2024	Bioprinted Hydrogel Fabrication	3.5	3.5	3.5
Caitriona	02/05/2024	Bioprinted gel incubator prep	0.75	0.75	0.75
Will	02/06/2024	Rheometry Testing of Hydrogels	1	2	2
Will	02/06/2024	Pipette-Based Hydrogel Fabrication	2	4	6.5
Elijah	02/06/2024	Rheometry Testing of Hydrogels	2	2	4
Carley, Elijah, Caitriona	02/07/2024	Client Bioprinter Demo	2.5	2.5	2.5
Will	02/07/2024	Rheometry Testing of Hydrogels	2	4	4
Elijah	02/08/2024	Rheometry Testing of Hydrogels	1	3	5
Everyone	02/08/2024	Progress Report	0.5	0.5	1