BME Design-Spring 2023 - ELIJAH DIEDERICH
Complete Notebook

PDF Version generated by

Nick Herbst

on

May 03, 2023 @09:26 PM CDT

Table of Contents

Team Contact Information

Nick Herbst - Mar 10, 2023, 2:39 PM CST

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Project Description

Nick Herbst - Apr 18, 2023, 9:13 PM CDT

Course Number: BME 301

Project Name: Tissue Model of The Epithelial Mesenchymal Trophic Unit

Short Name: Tissue Model

Problem statement: A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and 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 while having a uniform and replicable composition that allows for epithelial cell culture at an air-liquid-interface (ALI) so that his lab can study the effects of fibrosis on small-airway lung epithelial cells.

About the client: Dr. Allan Brasier is the Executive Director of the UW-Madison Institute for Clinical and Translational Research. His research focuses on the inflammation and its role in advancing pulmonary and cardiovascular disease.

Client Meeting 1

CARLEY SCHWARTZ - Feb 17, 2023, 8:29 AM CST

Title: client meeting 1

Date: 01-30-23

Content by: Carley and Elijah

Present: Self, Elijah, Client

Goals: To discuss PEG troubleshooting, future GelMA work, and client meetings in this semester

Content:

Key Points:

- Client meetings will be every other week and for this month it will be the 9th and the 27th on Thursdays at 11:30 am
- He prefers us to try and trouble shoot with PEG but likes the idea of GelMA as well
- Wants to do testing on a range of mechanical properties so we will have lower and higher compressive moduli of lung ECM (normal vs fibrotic)

Conclusions/action items:

- Need to assign roles for each person and delegate the research between everyone
- Need to attend Dr. Masters' lab to watch GelMA preparation
- See if we can troubleshoot PEG w any extra I2959 or LAP that Dr. Masters' lab has

Client Meeting 2

CARLEY SCHWARTZ - Feb 17, 2023, 8:30 AM CST

Title: Client Meeting 2

Date: 02-09-23

Content by: Self and Elijah

Present: Self, Elijah, Client

Goals: To discuss GelMA, future work he wants to conduct with the gels (cell encapsulation and culturing)

Content:

Key Points

- Wants to first encapsulate fibrinectin and collagen then move onto fibroblasts
- Wants to try collagen or fibronectin coating on any gels even if they do not have cells encapsulated
- Wants the gel to degrade with time to allow for fibroblast reconstruct the ECM but wants to place epithelium cells onto the gel surface right after construction
    - this is due to him wanting to have communication between the epithelial cells and the proteins within the gel for as long as possible

Conclusions/action items:

- Need to understand the degradation of GelMA further
- Need to apply these client requirements to our design matrix and search literature for how these design fit within it

Client Meeting 3

CARLEY SCHWARTZ - Feb 27, 2023, 9:57 AM CST

Title: Client Meeting 3

Date: 02-23-23

Content by: Carley Schwartz

Present: Elijah, Nick, Carley, and Client

Goals: to discuss GelMA and future testing (cell proliferation assays)

Content:

-GelMA wants to do some initial cell culturing on gels without the collagen or fibronectin incorporated

-wants to measure cell viability not cell proliferation

MTT assay

live dead assay - cytoflex [dissolve the hydrogel to cytometer the cells]

Conclusions/action items:

- Research the assays discussed
- Add these to future work/testing for presentation
- Look into length of gelma hydrogels cell culture timeline

Client Meeting 4

Nick Herbst - May 03, 2023, 7:47 PM CDT

Title: Client Meeting #4

Date: 3-9-23

Content by: Elijah Diederich

Present: Carley, Anuraag, Dr. Brasier

Goals: To see how first batch of gels performed (Cell adhesion etc....)

Content:

*****PDF with client meeting notes attached below*

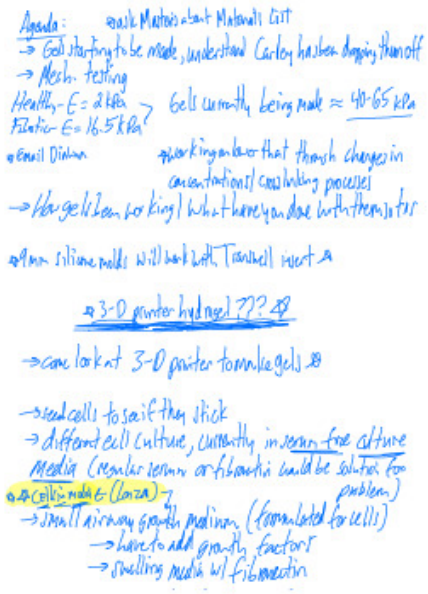
Conclusions/action items:

1. Email Dianhua with Material orders

2. Ask Dr. Masters about adhesion issue

3. Work on making gels with lower stiffness

Nick Herbst - May 03, 2023, 7:47 PM CDT



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Client_Meeting_4_2_.pdf (807 kB)

Client Meeting 5

Nick Herbst - May 03, 2023, 7:47 PM CDT

Title: Client Meeting #5

Date: 3-30-23

Content by: Elijah Diederich

Present: Carley, Anuraag, Dr. Brasier

Goals: To determine to-do items for last 3 weeks of project

Content:

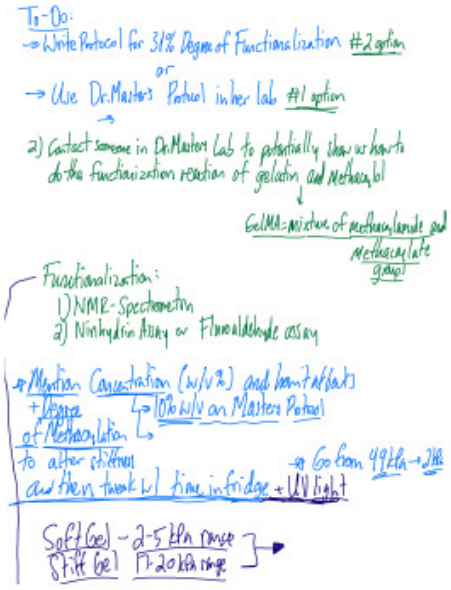
***** PDF with client meeting notes attached below*

Conclusions/action items:

1. Run GelMA reaction when materials arrive

2. Start prepping for final report/presentation

Nick Herbst - May 03, 2023, 7:47 PM CDT



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Client_Meeting_5.pdf (695 kB)

Client Meeting 6

Nick Herbst - May 03, 2023, 7:48 PM CDT

Title: Client Meeting #6

Date: 4-13-23

Content by: Elijah Diederich

Present: Anuraag, Dianhua

Goals: Discuss Normal kPa stiffness hydrogels and cell adhesion

Content:

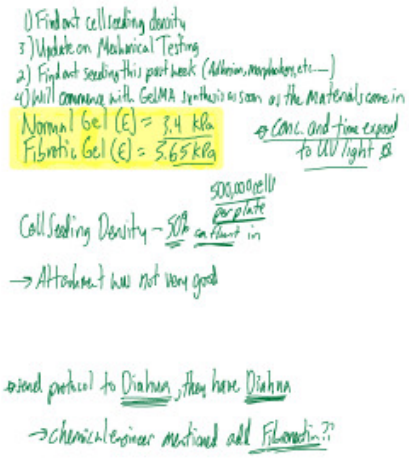
*****PDF with client meeting #6 attached below*

Conclusions/action items:

1. Talk to Dr. Masters about cell adhesion problem

2. Continue to prep for poster presentation

Nick Herbst - May 03, 2023, 7:48 PM CDT



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Client_Meeting_6.pdf (429 kB)

Advisor Meeting 1

WILLIAM ONUSCHECK - Feb 03, 2023, 2:03 PM CST

Title: Advisor Meeting 1

Date: 02/03/2023

Content by: William Onuscheck

Present: Dr. Masters, Carley, Elijah, Nick, Anuraag, and Will

Goals: To meet with Dr. Masters, establish where the team is at in the project, and discuss routes the team should explore moving forward.

Content:

Carley gave Dr. Masters a synopsis of the prior semester's progress and shortcomings. Namely, during the fabrication stage, it seems that the photoinitiator failed to work as expected, thus PEG hydrogels were never produced and the team opted to use gelatin as an alternative to stand in for testing. While meeting with the client (Dr. Braiser) over winter break, he expressed he would prefer for the team to continue troubleshooting PEG moving forward.

Dr. Masters gave her thoughts on how the team should move forward with the project:

Regarding PEG:

- Concern that the low molecular weight, 8-arm peg would be to short, yielding a denser mesh, yielding a stiffer than necessary matrix
- Issues with forming PEG hydrogels are common
- The tunability of PEG may be too overkill for the needs of the client
- Using PEG means no incorporation of fibers (collagen, fibronectin), and in modeling fibrosis, the incorporation of pathological presence of fibers is relevant
- The photoinitiator currently used by the team (I2959) is not great, better ones exist (2959, LAP), that the team could borrow from Dr. Master's lab

Regarding GelMA

- GelMA offers the same tunability for physical proprieties for the matrix
- Stiffness's of GelMA better align with goal ranges
- Addition of Collagen, Fibronectin fibers possible
- While batch to batch stiffnesses of GelMA have variability, once a good batch is made, it will produce highly replicable hydrogels
- Purchase of pre-characterized GelMA is possible
- If not purchasing pre-characterized GelMA, creation is relatively simple

Finally, in housekeeping, Dr. masters explained that as individuals, we could opt to have our LabArchives graded on a weekly basis or on a one time basis for the preliminary notebook check.

Conclusion: Moving forward, the team plans to spend some time further troubleshooting the PEG hydrogels begun last semester. The use of alternative photoinitiators and PEG molecular weights will be explored. The team will also explore the use of Gelatin Methacryloyl as an alternative design. The team will conduct a thorough literature review on GelMA, and weigh its prosand cons against those of PEG.

Action Items: Send clarifying questions to Dr. Masters, set up a time to observe GelMA formation process. Decide on whether notebook should be graded on a weekly basis or as a chunk.

Advisor Meeting 2

Nick Herbst - May 03, 2023, 7:49 PM CDT

Title: Advisor Meeting #2

Date: 2-17-2023

Content by: Elijah Diederich

Present: Group members

Goals: To inform Dr. Masters about the Week's progress and upcoming to-do list items

Content:

****PDF of Notes attached below****

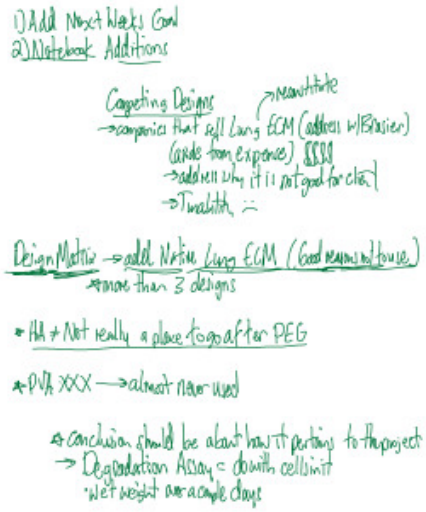
Notes taken: 2-10-2023

Conclusions/action items:

1. Look into degradation assay - wet weight over time period

2. Add native lung ECM to design matrix

Nick Herbst - May 03, 2023, 7:49 PM CDT



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Advisor_meeting_2.pdf (431 kB)

Advisor Meeting 3

Nick Herbst - May 03, 2023, 7:49 PM CDT

Title: Advisor Meeting #3

Date: 2-27-23

Content by: Elijah Diederich

Present: Group Members

Goals: To get feedback on previously submitted PDS and testing assays

Content:

****PDF w/ notes attached below****

Advisor Meeting occurred on 2-17-2023

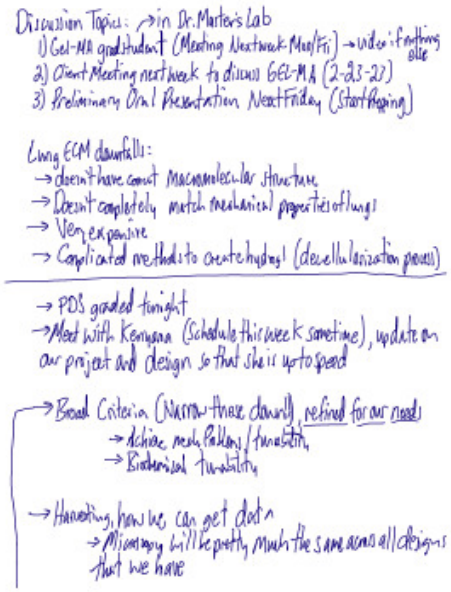
Conclusions/action items:

1. It's OK to have redundancy in the design criteria

2. Ask Brasier about cell proliferation assays that he runs

3. Make sure to wear pants to Lab

Nick Herbst - May 03, 2023, 7:49 PM CDT



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Advisor_Meeting_3.pdf (1.13 MB)

Advisor Meeting 4

Nick Herbst - May 03, 2023, 7:50 PM CDT

Title: Advisor Meeting #4

Date: 3-3-23

Content by: Elijah Diederich

Present: EMTU Team

Goals: To get feedback on preliminary presentation and discuss client meeting

Content:

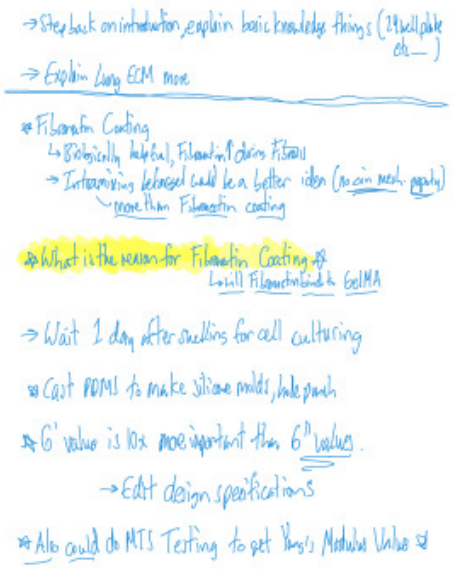
***** Advisor Meeting #4 notes attached below *

Conclusions/action items:

1. Edit Design Specs and take out viscoelastic modulus values

2. Discuss Fibronectin coating with client (Pros/Cons)

Nick Herbst - May 03, 2023, 7:50 PM CDT



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Advisor_Meeting_4.pdf (540 kB)

Advisor Meeting 6

Nick Herbst - May 03, 2023, 7:50 PM CDT

Title: Advisor Meeting #6

Date: 4-7-23

Content by: Elijah Diederich

Present: EMTU Team

Goals: Discuss Protocols and Materials for GelMA reaction

Content:

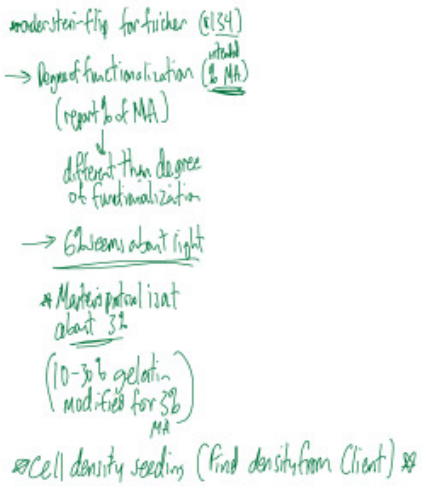
***** PDF of Advisor Meeting #6 notes attached below *

Conclusions/action items:

1. Do second round of material ordering

2. Find out cell density seeding from client

Nick Herbst - May 03, 2023, 7:50 PM CDT



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Advisor_Meeting_6.pdf (257 kB)

Advisor Meeting 7

Nick Herbst - May 03, 2023, 7:51 PM CDT

Title: Advisor Meeting #7

Date: 4-14-23

Content by: Elijah Diederich

Present: EMTU Team

Goals: To discuss most recent batch of low kPa stiffness gels

Content:

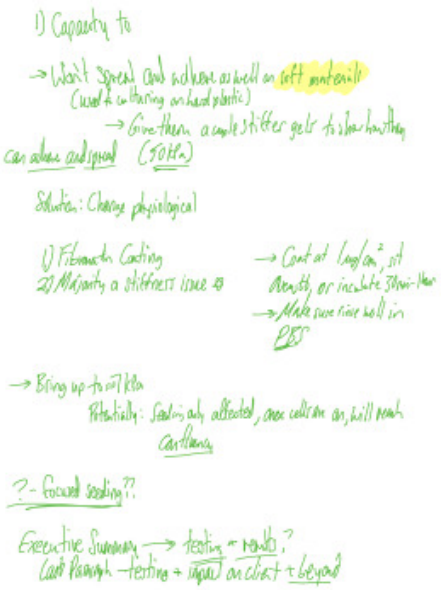
***** PDF of Advisor Meeting #7 Notes Below *

Conclusions/action items:

1. Make gel with high kPa stiffness to prove that soft materials have a harder time with cell adhesion

2. Edit Executive Summary

Nick Herbst - May 03, 2023, 7:51 PM CDT



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Advisor_meeting_7.pdf (492 kB)

Advisor Meeting 8

Nick Herbst - May 03, 2023, 7:51 PM CDT

Title: Advisor Meeting #8

Date: 4-14-23

Content by: Elijah Diederich

Present: EMTU Team

Goals: To discuss our latest round of gels and a potential fibronectin coating

Content:

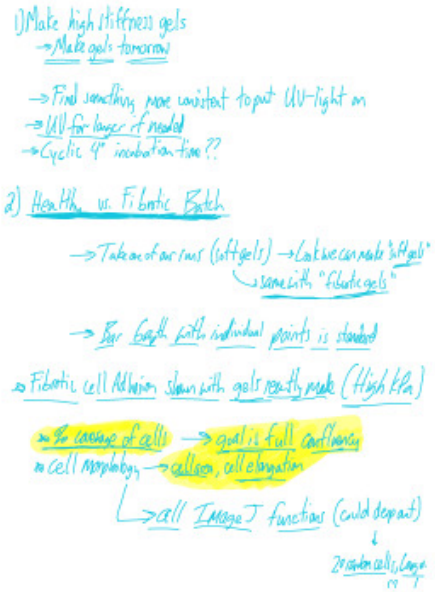
***** PDF of Advisor Meeting #8 Notes attached Below *

Conclusions/action items:

1. Get ready for presentation and final report

2. Look into ImageJ as a potential testing platform for confluency/cell morphology

Nick Herbst - May 03, 2023, 7:51 PM CDT



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Advisor_Meeting_8.pdf (771 kB)

02/10/2023 - PDS

Nick Herbst - Feb 10, 2023, 11:49 AM CST

Title: PDS

Date: 02/10/2023

Content by: Everyone

Present: Everyone

Goals: Establish specifications the design project

Content:

- See attachment for full PDS

Action items:

- Work on design matrix

Nick Herbst - Feb 10, 2023, 11:49 AM CST

Tissue Model of The Epithelial Mesenchymal Trophic Unit

BME Design

Date: February 10, 2023
BME 301

Product Design Specification

Client: Dr. Allan Brasier
Advisor: Dr. Kristyn Masters

Team Members:
Carley Schwartz cischwartz@wisc.edu (Co-Leader)
Elijah Diederich ediederich@wisc.edu (Co-Leader, BPAG)
Anurag Shreekanth Belavadi sbelavadi@wisc.edu (Communicator)
Will Orascheck orascheck@wisc.edu (BSAC)
Nick Herbst nherbst2@wisc.edu (BWIG)

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tissue_model_PDS.pdf (207 kB)

02/20/2023 - Design Matrix

Nick Herbst - Feb 20, 2023, 6:05 PM CST

Title: Design Matrix

Date: 02/20/2023

Content by: Everyone

Present: Everyone

Goals: Rank scaffold designs against a design matrix

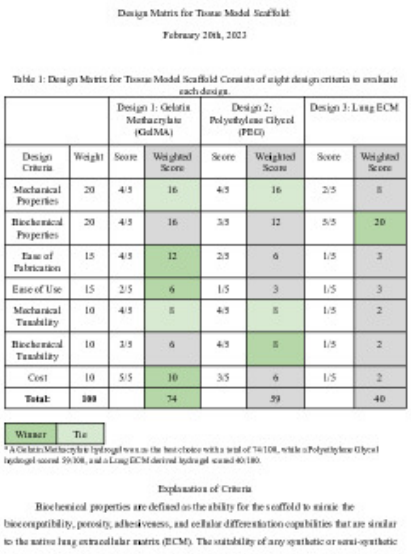
Content:

- We took our three designs (GelMA, PEG, and lung ECM) and ranked them in a design matrix
- Criteria
    - Mechanical Properties: E, G', and G'' match native lung ECM
    - Biochemical Properties: biocompatible, cell adhesive, and degradable
    - Ease of Fabrication: how easy it is to fabricate
    - Ease of Use: how easy it is for the client to reproduce and how easy it is for us to test
    - Mechanical Tunability: degree of altering the mechanical properties
    - Biochemical Tunability: degree of altering the biochemical properties
    - Cost: cost of materials and fabrication
- GelMA scored the highest, followed by PEG, and then lung ECM
    - GelMA stood out due to it's tunability and relatively easier fabrication
- See attachment for full design matrix with criteria explanations and ranking justifications

Action items:

- Work on preliminary presentation and preliminary report

Nick Herbst - Feb 20, 2023, 6:01 PM CST



Design Matrix for Tissue Model Scaffold

February 20th, 2023

Table 1: Design Matrix for Tissue Model Scaffold Consists of eight design criteria to evaluate each design.

		Design 1: Gelatin Methacrylate (GelMA)		Design 2: Polyethylene Glycol (PEG)		Design 3: Lung ECM	
Design Criteria	Weight	Score	Weighted Score	Score	Weighted Score	Score	Weighted Score
Mechanical Properties	20	4/5	16	4/5	16	2/5	8
Biochemical Properties	20	4/5	16	3/5	12	5/5	20
Ease of Fabrication	15	4/5	12	2/5	6	1/5	3
Ease of Use	15	2/5	6	1/5	3	1/5	3
Mechanical Tunability	10	4/5	8	4/5	8	1/5	2
Biochemical Tunability	10	3/5	6	4/5	8	1/5	2
Cost	10	5/5	10	3/5	6	1/5	2
Total:	**100**		74		59		40

Winner	Tie

* A Gelatin Methacrylate hydrogel was as the best choice with a total of 74/100, while a Polyethylene Glycol hydrogel scored 59/100, and a Lung ECM derived hydrogel scored 40/100.

Explanation of Criteria

Biochemical properties are defined as the ability for the scaffold to mimic the biocompatibility, porosity, adhesiveness, and cellular differentiation capabilities that are similar to the native lung extracellular matrix (ECM). The suitability of any synthetic or semi-synthetic

Download

Design_Matrix_1_.pdf (94.4 kB)

03/01/2023 - Revised PDS

Nick Herbst - Feb 27, 2023, 6:27 PM CST

Title: Revised PDS

Date: 03/01/2023

Content by: Everyone

Present: Everyone

Goals: Revise the PDS based on advisor feedback

Content:

- See attachment for full PDS

Action items:

- Work on preliminary deliverables

Nick Herbst - Mar 01, 2023, 8:15 PM CST

Tissue Model of The Epithelial Mesenchymal Trophic Unit

BME Design

Date: March 1, 2023
BME 301

Product Design Specification

Client: Dr. Allan Brasier
Advisor: Dr. Kristyn Masters

Team Members:
Carley Schwartz cischwartz@wisc.edu (Co-Leader)
Elijah Diederich ediederich@wisc.edu (Co-Leader, BPAG)
Anurag Shreekanth Belavadi abelavadi@wisc.edu (Communicator)
Will Ornscheck ornscheck@wisc.edu (BSAC)
Nick Herbst nherbst2@wisc.edu (BWIG)

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tissue_model-PDS_revised.pdf (217 kB)

04/17/2023 Entry - 03/24/2023 Completed - Show and Tell Session

Nick Herbst - Apr 17, 2023, 7:50 PM CDT

Title: Show and Tell Session

Date: 03/24/2023

Content by: Nick Herbst

Present: Nick, Carley, Anuraag, Will

Goals: Update peers on project status and get suggestions

Content:

- During the show and tell session, we told other design teams where we were at in the project and asked for suggestions regarding hydrogel formation
    - We are having issues with getting the gels out of the 24-well plates intact
- Suggestions:
    -

Conclusions/action items:

While some of the suggestions seem to be good ideas, our issues will most likely resolve once the silicone molds arrive.

02/26/2023 - Ongoing Materials List

CARLEY SCHWARTZ - Feb 27, 2023, 6:05 PM CST

Title: Materials List

Date: 02-26-23

Content by: Carley

Present: Whole Group

Goals: Table of Materials

Content:

Material	Price	Link	Product Number
Gelatin bloom:250 Type B	$36	https://www.sigmaaldrich.com/US/en/product/sigma/g9391	G9391-100G
Methacrylic anhydride	$56	https://www.sigmaaldrich.com/US/en/product/aldrich/276685	276685-100ML
LAP	$654	https://www.sigmaaldrich.com/US/en/product/aldrich/900889	900889-5G
silicone molds	$170	https://gracebio.com/products/hybridization-and-incubation/silicone-isolators-hybridization-and-incubation/search/	664201

- ISOELECTRIC POINT: Type A gelatin, produced using the acid process, has an IEP between 8 and 9. Alkaline-produced gelatin (type B) has an IEP of between 4.8 and 5.4. [need to see what type Masters uses but other literature used type b from bovine which is what is in the product chart]
    - https://pubs.acs.org/doi/full/10.1021/bm990017d this is the source that had a lot of protocols on GelMA synthesis
- BLOOM VALUE: Gel strength, also known as 'bloom' value, is a measure of the strength and stiffness of the gelatin, reflecting the average molecular weight of its constituents, and is usually between 30 and 300 bloom (< 150 is considered to be a low bloom, 150–220 a medium bloom, and 220–300 a high bloom).

Conclusions/action items:

- Talk to Dr. Masters about all the variables with order materials
    - specifically: type of collagen, sourced from, how much of each material, can we borrow silicon molds or should we purchase them?

04/17/2023 - Final Materials List

Nick Herbst - Apr 17, 2023, 7:59 PM CDT

Title: Final Materials List

Date: 04/17/2023

Content by: Nick

Present: Whole Group

Goals: Show materials that were ordered and used

Content:

Below are charts showing the materials that were ordered:

Material	Amount	Cost	ID
Gelatin	100mg	$54.00	G2500-100G
MAA	100mL	$56.00	276685-100ML
10x PBS	1L	$142.00	P5493-1L
Molds	5 (trial size)	$50.00	665201-S
LAP	500mg	$147.00	900889-1G
Dialysis Tubing	30.5 meters	$83.37	08-667A
Total			$532.37
Second Round			
Material	**Amount**	**Cost**	**ID**
18 gauge needle syringe	20	$9.99	LY-999
50mL steriflips	Case 12	$111.00	564-0020

Below is a chart of the items the team had the chance to use (limited list due to either time constraints or shipping delays):

Material	Cost	ID
GelMA	$0.00	NA
10x PBS (1L)	$142.00	P5493-1L
Molds (25ct)	$170.00	665201
LAP (500mg)	$147.00	900889-1G
Total	$459.00	

Conclusions/action items:

- Due to delays, we were not able to use the materials for methacrylating our own gelatin

02/27/2023 - GelMA Protocol from Shadowing (w/ Notes)

Nick Herbst - Feb 27, 2023, 6:38 PM CST

Title: GelMA Protocol from Shadowing (w/ Notes)

Date: 02/27/2023

Content by: Carley (notes) and Nick (entry)

Present: Carley, Elijah, and Will

Goals: Learn how to prepare GelMA hydrogels from a graduate student in Dr. Masters' lab

Content:

See attached image for GelMA protocol with annotations

Conclusions:

3 team members went to Dr. Masters' lab on 02/24/2023 to observe a graduate student, Ashley Scott, prepare GelMA hydrogels. Since she uses the gels for a different application than us, we will need to adjust her protocol. Additionally, she cultures cells on the GelMA hydrogels for 2 days while we are looking at 1 month, which will possibly need to be taken into consideration when adjusting the protocol.

Nick Herbst - Feb 27, 2023, 6:38 PM CST

Download

IMG_1757.HEIC (2.02 MB)

04/17/2023 - Gel Fabrication

Nick Herbst - Apr 17, 2023, 9:18 PM CDT

Title: Gel Fabrication

Date: 04/17/2023

Content by: Nick

Present: All

Goals: Describe the ongoing fabrication process/journey of the gels

Content:

- 03/10 & 03/11:
  - The fabrication protocol was followed in order to make the gels. However, they gels had to be made in a 24-well plate, which resulted in it being very hard to remove them after they set. In order to get them out intact, we had to make them very thick. This resulted in gels that had Young's moduli ranging from 40-60 kPa, which was drastically over-target.
- We were forced into a month-long break between fabrication of gels due to lack of photoinitiator and molds
  - We had to wait until those materials came in from our material order
- 04/09:
  - Using the molds results in much easier gel formation. However, the gels that were intended to be "fibrotic" were basically the same stiffness as the "normal" gels. The fridge time will be increased to try and hit the "fibrotic" target. The "normal" gels were around 3.6 kPa and the "fibrotic" were 3.8 kPa
- 04/16:
  - Once again, the gels that were intended to be "fibrotic" had similar stiffness to the "normal" gels. The "normal" gels were around 2.3 kPa and the "fibrotic" were 5.3 kPa. The cooling time is going to be *greatly* extended, and the UV time will be increased as well

Conclusions/action items:

We have still yet to make gels that are within our desired fibrotic ECM range when using the silicone molds. Since we got the molds, all gels have been 3-6 kPa. Updates will be made in the form of new entries attached to this one.

05/03/2023 Rheometry Testing Protocol

Nick Herbst - May 03, 2023, 6:01 PM CDT

Title: Rheometry Testing Protocol

Date: 05/03/2023

Content by: Nick and Will

Present: Will and Elijah

Goals: Describe a protocol for mechanical testing using a rheometer

Content:

Frequency Sweep Rheometry Protocol

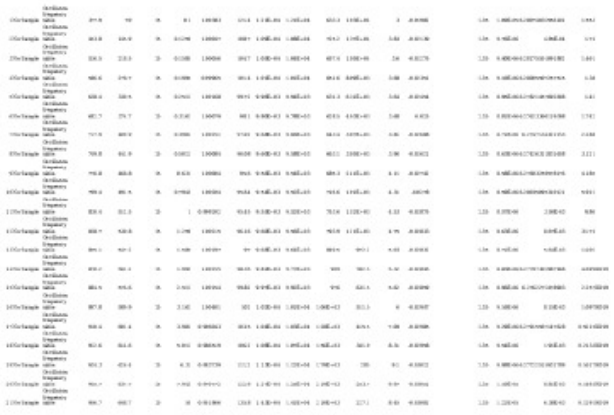
1. Once GelMA hydrogels have been formed and allowed to set and swell for approximately 12-24 hours, rheometry testing may be performed.
2. Carefully remove 3 hydrogels of each type; healthy lung ECM and fibrotic lung ECM, from 24 well cell culture plates, keeping the gels of the same type in the same weighing dish.
3. Once gels are in two separate weighing dishes, make your way over to the rheometer testing machine (Malvern Rheometer - Kinexus Ultra+)
4. Make sure that the bottom plate is locked on the rheometer by pushing the level, located on the front of the machine below the bottom parallel plate, all the way to the right
5. Open rSpace application on the computer and when prompted to select a certain test, select the 0035 test; Frequency Sweep Strain controlled.
6. When this specific test is selected, the user will then be prompted to enter a Gap value. This value will pertain to the thickness (mm) of the hydrogel being tested. Center the hydrogel on the bottom parallel plate. Measure the thickness (mm) of the hydrogel and enter the gap value. The upper plate will then move to this gap value.
7. Once making sure that the upper plate makes contact with the top of the hydrogel and the thickness is the correct value, enter values for various testing parameters such as room temperature, start frequency, end frequency, shear strain %, and samples per decade. In this specific test, the values were as follows: Start Frequency = 0.1 Hz, End Frequency = 10 Hz, Room Temperature = 25 ℃, shear strain = 1%, and 10 samples per decade.
8. Once the various testing parameters are entered, the user will then be able to start the test. A 5 minute calibration will be performed before the actual test begins. Once this calibration has been completed, the frequency sweep test will take approximately 10 minutes.
9. When the test is completed, the results table can be copied into an excel spreadsheet. Enter a gap value that is greater than the thickness of the hydrogel to remove the hydrogel from the machine. Clean upper and lower parallel plate surfaces with ethanol.
10. Repeat steps 5-9 for remaining hydrogels. In this specific test, 3 hydrogels of each type were tested for a total of 6 separate frequency sweeps.
11. When testing is completed, results can be interpreted in Microsoft Excel.

Conclusions/action items:

Conduct mechanical testing by following this protocol

Batch 3 Gel 1

WILLIAM ONUSCHECK - May 02, 2023, 11:07 AM CDT

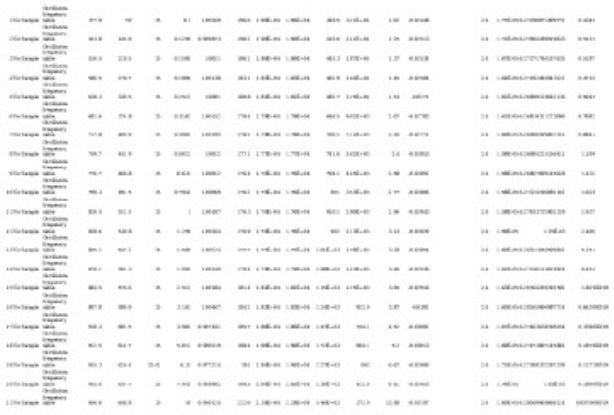


Download

Batch_3_Gel_1.xlsx (12.2 kB)

Batch 3 Gel 2

WILLIAM ONUSCHECK - May 02, 2023, 11:07 AM CDT



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Batch 3 Gel 3

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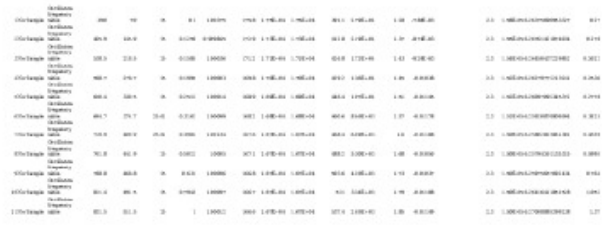

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Batch_3_gel_3.xlsx (10.5 kB)

Batch 4 Gel

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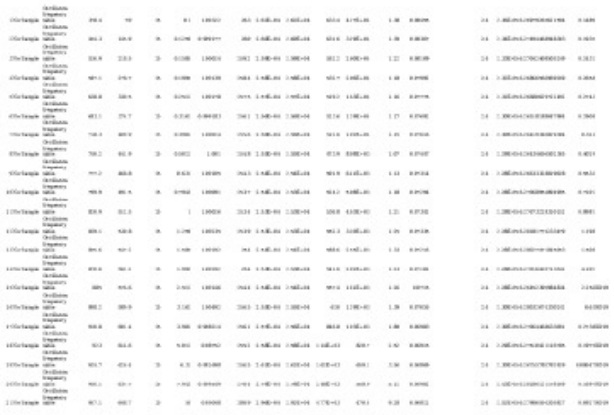

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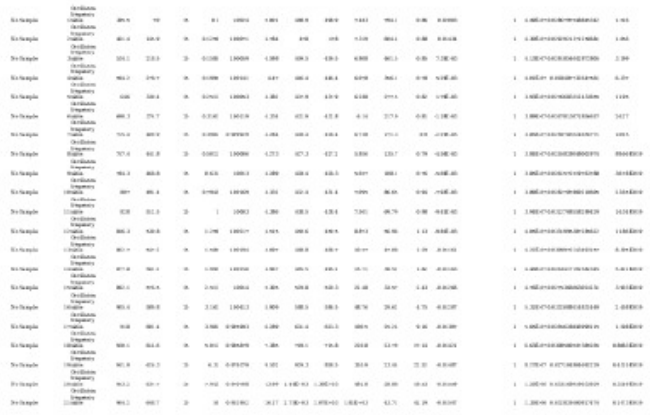
Batch_4_gel_1.xlsx (12.2 kB)

April 17th 1.1

WILLIAM ONUSCHECK - May 02, 2023, 11:09 AM CDT



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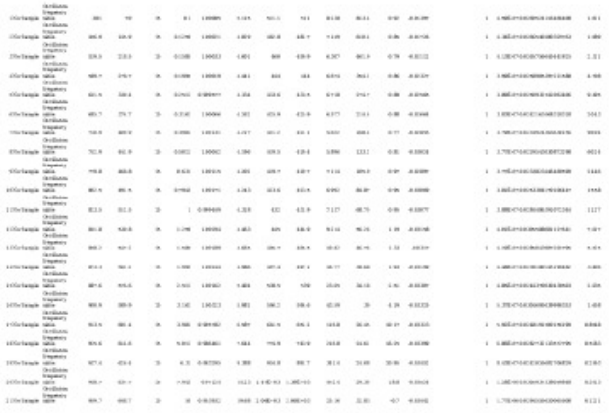
WHO_417_1.1.xlsx (12.3 kB)

April 17th 1.2

WILLIAM ONUSCHECK - May 02, 2023, 11:09 AM CDT



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April 17th 1.3

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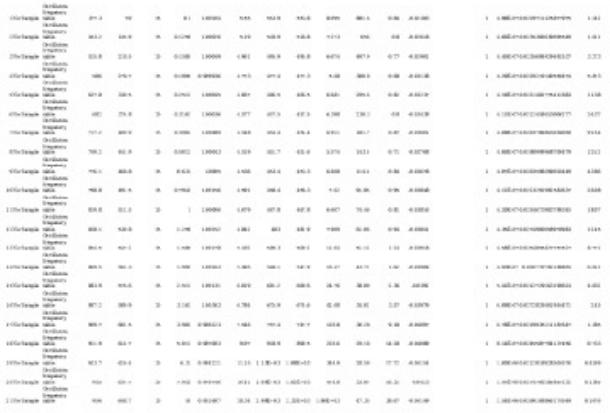

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April 17th 1.4

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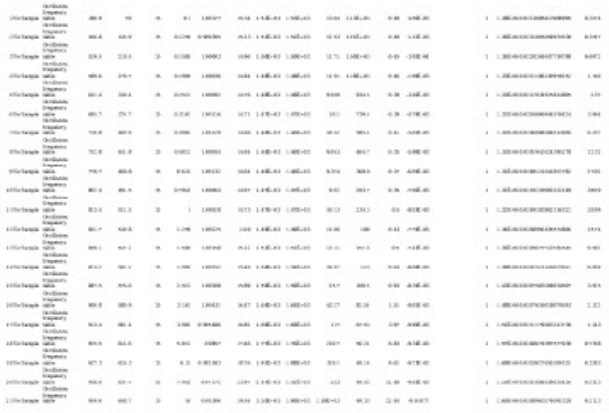

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April 17th 2.1

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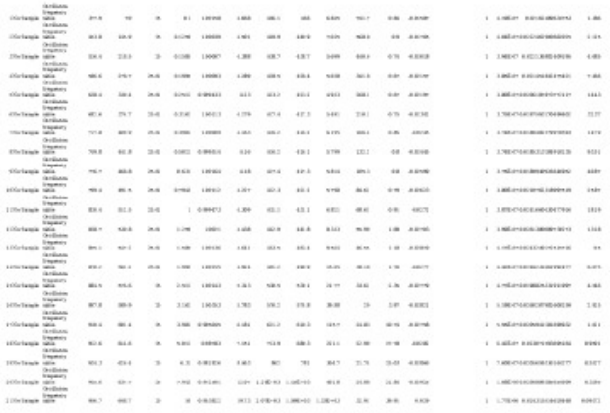

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April 17th 2.2

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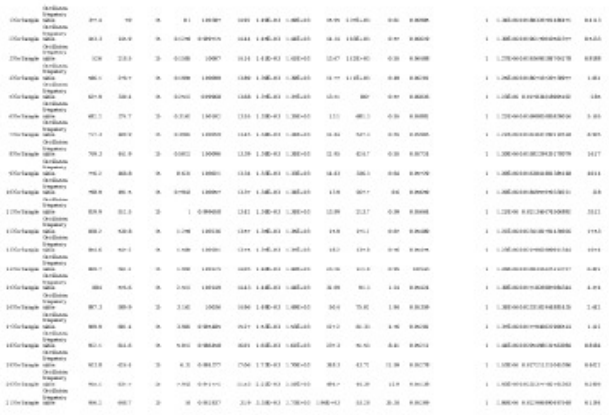

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April 17th 2.3

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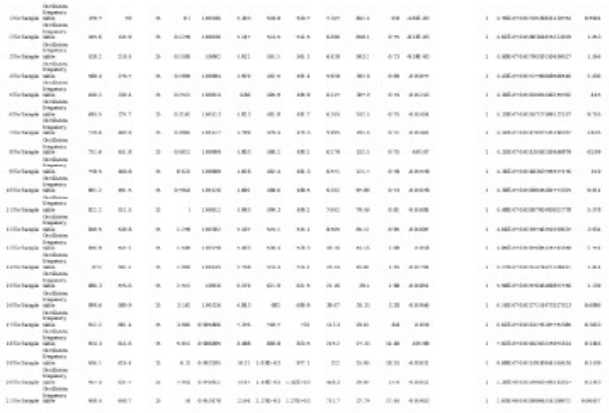

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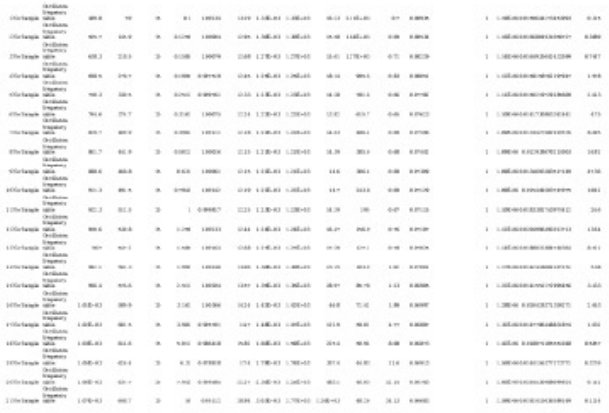
April 17th 3.1

WILLIAM ONUSCHECK - May 02, 2023, 11:11 AM CDT



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April 17th 3.2

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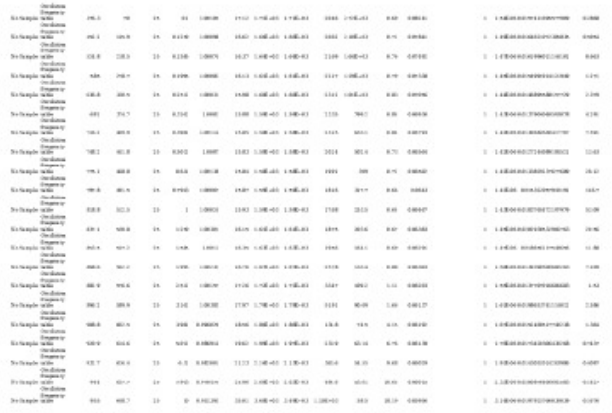

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April 17th 3.3

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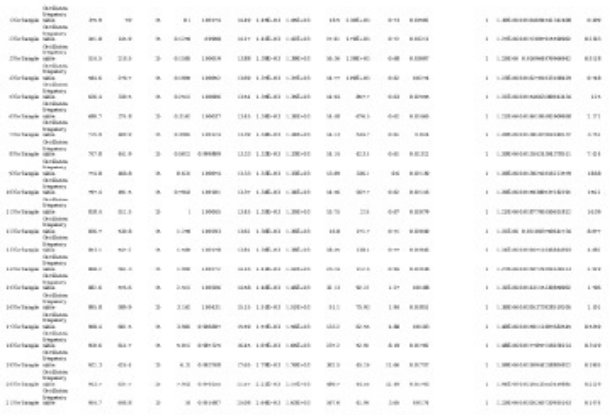

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April 17th 3.4

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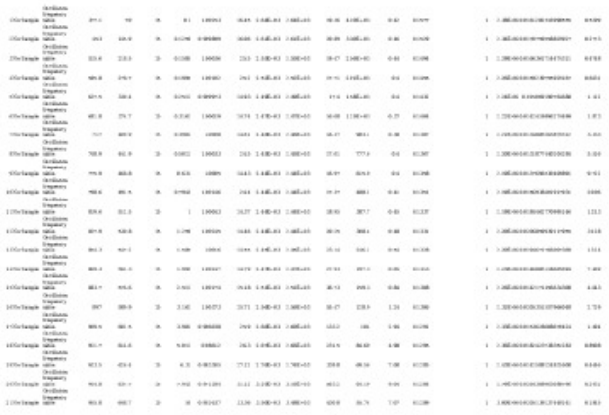

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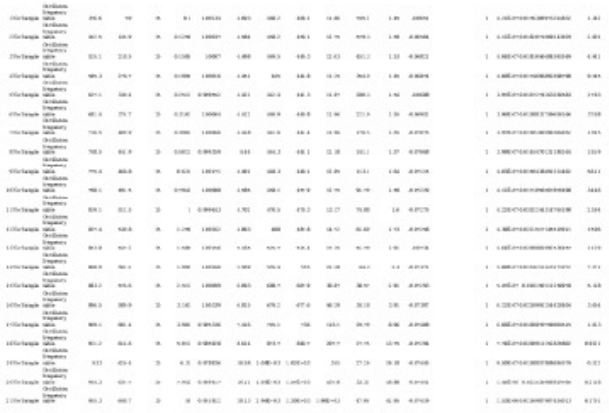
April 17th 4.1

WILLIAM ONUSCHECK - May 02, 2023, 11:12 AM CDT



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April 17th 4.2

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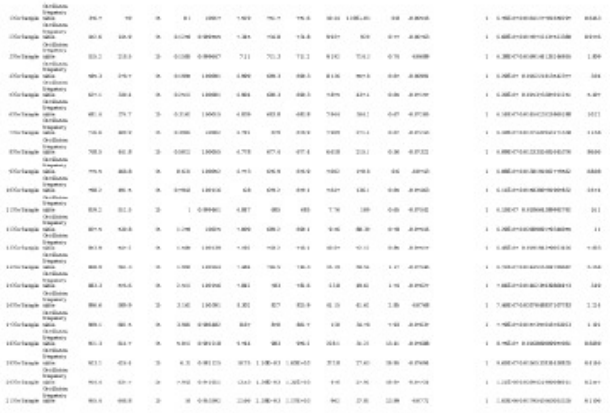

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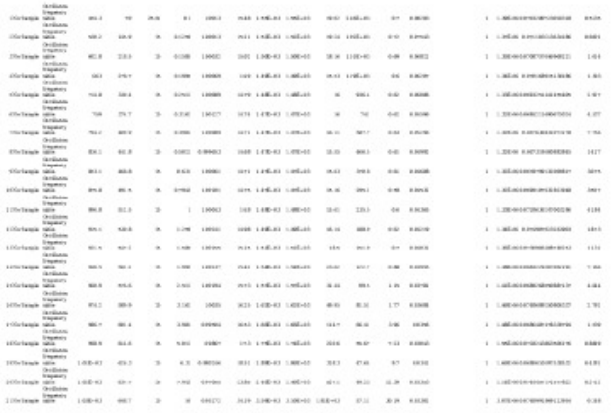
April 25th 1.1

WILLIAM ONUSCHECK - May 02, 2023, 11:12 AM CDT



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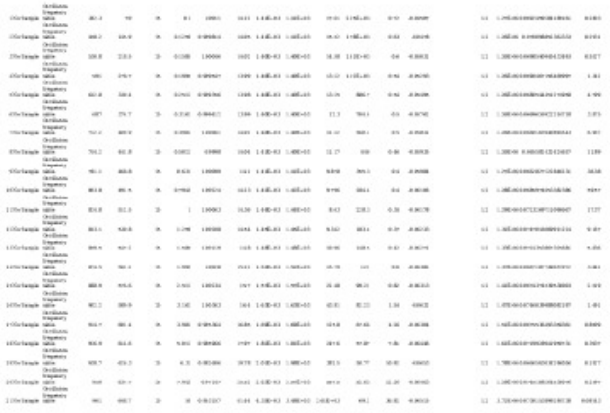
WHO_425_1.1.xlsx (12.1 kB)

April 25th 1.2

WILLIAM ONUSCHECK - May 02, 2023, 11:13 AM CDT



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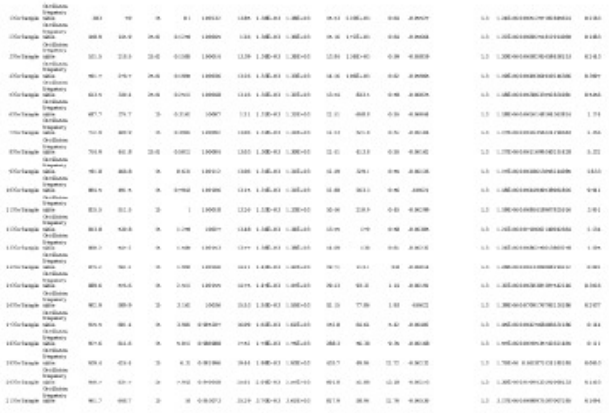
WHO_425_1.2.xlsx (12.1 kB)

April 25th 1.3

WILLIAM ONUSCHECK - May 02, 2023, 11:13 AM CDT



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WHO_425_1.3.xlsx (12.1 kB)

April 25th 1.4

WILLIAM ONUSCHECK - May 02, 2023, 11:13 AM CDT

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WHO_425_1.4.xlsx (11.9 kB)

05/03/2023 Mechanical Testing

Nick Herbst - May 03, 2023, 5:58 PM CDT

Title: Mechanical Testing

Date: 05/03/2023

Content by: Nick

Present: Elijah and Will

Goals: Find the Young's moduli of the hydrogels through rheometry testing

Content:

- All of the hydrogel batches were tested by some of the team throughout the semester by following the Rheometry Protocol found in the "Protocols" folder

- The rheometer gave G', which was approximated to G, and then E was found by this equation: $E = 2G(1+\nu)$, where nu is Poisson's ratio of 0.5.

- See the "Rheometer Data" subfolder in this folder for all raw data

Conclusions/action items:

This method of mechanical testing appears to be working well for this project. Compression testing was considered, but no one knew how to use the MTS machine and we could not get in contact with anyone to be trained.

02/24/2023 - Preliminary Presentation

Nick Herbst - Feb 20, 2023, 6:07 PM CST

Title: Preliminary Presentation

Date: 02/24/2023

Content by: Everyone

Present: Everyone

Goals: Present our design project progress thus far to our peers

Content:

- See attachment for full presentation slides

Action items:

- Work on preliminary report

Nick Herbst - Feb 23, 2023, 10:01 PM CST

Download

tissue_model-preliminary_presentation.pdf (953 kB)

03/01/2023 - Preliminary Report

Nick Herbst - Apr 25, 2023, 10:11 PM CDT

Title: Preliminary Report

Date: 03/01/2023

Content by: Everyone

Present: Everyone

Goals: Summarize our current design project process in a preliminary report

Content:

- See attachment for full report

Action items:

- Work on other preliminary deliverables

Nick Herbst - Mar 01, 2023, 8:15 PM CST

Tissue Model of the Epithelial Mesenchymal Trophic Unit

BME Design

Date: March 1, 2023
BME 301

Preliminary Report

Client: Dr. Allan Brasier
Advisors: Kristyn Masters

Team Members:
Carley Schwartz cschwartz@wisc.edu (Co-Leader)
Elijah Diederich ediederich@wisc.edu (Co-Leader, BPAG)
Anurag Shreekanth Belavadi sbelavadi@wisc.edu (Communicator)
Will Orascheck orascheck@wisc.edu (BSAC)
Nick Herbst nherbst2@wisc.edu (BWIG)

Download

tissue_model-prelim_report.pdf (276 kB)

04/28/2023 - Final Poster

Nick Herbst - Apr 25, 2023, 10:10 PM CDT

Title: Final Poster

Date: 02/24/2023

Content by: Everyone

Present: Everyone

Goals: Present our design project at the poster session

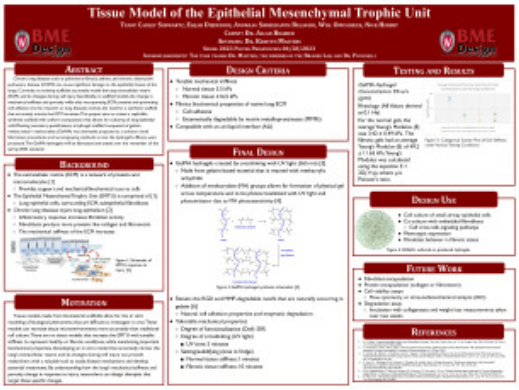
Content:

- See attachment for poster

Action items:

- Work on final deliverables

Nick Herbst - Apr 26, 2023, 7:52 PM CDT



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tissue_model-final_poster.pdf (556 kB)

05/03/2023 - Final Report

Nick Herbst - May 03, 2023, 9:25 PM CDT

Title: Final Report

Date: 05/03/2023

Content by: Everyone

Present: Everyone

Goals: Summarize our design project in a final report

Content:

- See attachment for full report

Action items:

- Work on final deliverables

Nick Herbst - May 03, 2023, 9:25 PM CDT

Tissue Model of the Epithelial Mesenchymal Trophic Unit

BME Design

Date: May 3, 2023
BME 301

Final Report

Client: Dr. Allan Brasier
Advisor: Dr. Kristyn Masters

Team Members:
Carley Schwartz cischwartz@wisc.edu (Co-Leader)
Elijah Diederich ediederich@wisc.edu (Co-Leader, BPAG)
Anurag Shreekanth Belavadi shreekanthbe@wisc.edu (Communicator)
Will Ornscheck ornscheck@wisc.edu (BSAC)
Nick Herbst nherbst2@wisc.edu (BWIG)

Download

tissue_model-final_report.pdf (4.72 MB)

03/27/2023 Entry- 02/02/2023 Completed- Progress Report Week 1

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:13 AM CDT

Title: Progress Report 1

Date: 02/02/2023

Content by: Entire Group

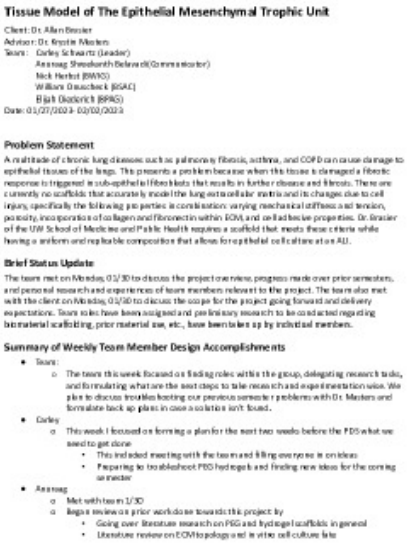
Present: Entire Group

Goals: Update client and project advisor with weekly progress the team makes towards the project with an emphasis on tasks completed, obstacles faced during the week, the team and individual goals for the following week, and an updated schedule of deadlines to be met.

Content: Attached

Conclusions/action items: Continue to update advisor and client on team progress every week and use feedback to improve week to week.

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:14 AM CDT



Tissue Model of The Epithelial Mesenchymal Trophic Unit

Client: Dr. Allen Bressler
Advisor: Dr. Kristin Masters
Team: Carley Schwartz (Leader)
Anuraag Shreekanth Belavadi(Communicator)
Nick Herbst (BWIG)
William Grauschack (BSAC)
Elijah Diedorich (BPAG)
Date: 01/27/2023- 02/02/2023

Problem Statement

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and 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 scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury, specifically the following properties in combination: varying mechanical stiffness and tension, porosity, incorporation of collagen and fibronectin within ECM, and cell adhesive properties. Dr. Brazier of the UW School of Medicine and Public Health requires a scaffold that meets these criteria while having a uniform and replicable composition that allows for epithelial cell culture at an ALI.

Brief Status Update

The team met on Monday, 01/30 to discuss the project overview, progress made over prior semesters, and personal research and experiences of team members relevant to the project. The team also met with the client on Monday, 01/30 to discuss the scope for the project going forward and delivery expectations. Team roles have been assigned and preliminary research to be conducted regarding biomaterial scaffolding, prior material use, etc., have been taken up by individual members.

Summary of Weekly Team Member Design Accomplishments

- Team:
  - The team this week focused on finding roles within the group, delegating research tasks, and formulating what are the next steps to take research and experimentation wise. We plan to discuss troubles hooting our previous semester problems with Dr. Masters and formulate back up plans in case a solution isn't found.
- Carley
  - This week I focused on forming a plan for the next two weeks before the PDS what we need to get done
    - This included meeting with the team and filling everyone in on ideas
    - Preparing to troubleshoot PEG hydrogels and finding new ideas for the coming semester
- Anuraag
  - Met with team 1/30
  - Began reviewing on prior work done towards this project by
    - Going over literature research on PEG and hydrogel scaffolds in general
    - Literature review on ECM topology and in vitro cell culture late

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Progress_Report_Week_1.pdf (67.4 kB)

03/27/2023 Entry- 02/09/2023 Completed- Progress Report Week 2

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:16 AM CDT

Title: Progress Report 2

Date: 02/09/2023

Content by: Entire Group

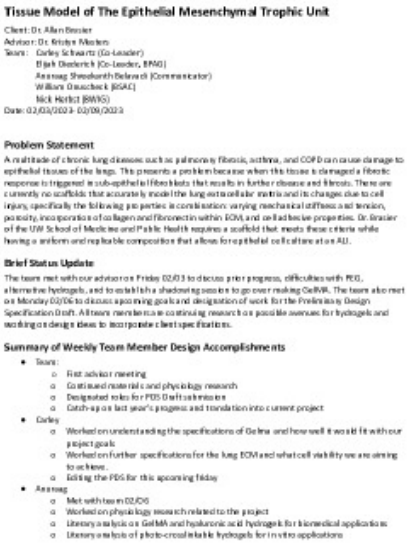
Present: Entire Group

Goals: Update client and project advisor with weekly progress the team makes towards the project with an emphasis on tasks completed, obstacles faced during the week, the team and individual goals for the following week, and an updated schedule of deadlines to be met.

Content: Attached

Conclusions/action items: Continue to update advisor and client on team progress every week and use feedback to improve week to week.

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:16 AM CDT



Tissue Model of The Epithelial Mesenchymal Trophic Unit

Client: Dr. Allen Bressler
Advisor: Dr. Kristyn Masters
Team: Carley Schwartz (Co-Leader)
Elijah Oesterlein (Co-Leader, BME)
Anuraag Shreekanth Belavadi (Communicator)
William Grauschack (BSAC)
Nick Herbst (BMES)
Date: 02/03/2023- 02/09/2023

Problem Statement

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and 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 scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury, specifically the following properties in combination: varying mechanical stiffness and tension, porosity, incorporation of collagen and fibronectin within ECM, and cell adhesive properties. Dr. Brasier of the UW School of Medicine and Public Health requires a scaffold that meets these criteria while having a uniform and replicable composition that allows for epithelial cell culture at an ALI.

Brief Status Update

The team met with our advisor on Friday 02/03 to discuss prior progress, difficulties with PEG, alternative hydrogels, and to establish a shadowing session to go over making GelMA. The team also met on Monday 02/06 to discuss upcoming goals and designation of work for the Preliminary Design Specification Draft. All team members are continuing research on possible avenues for hydrogels and working on design ideas to incorporate client specifications.

Summary of Weekly Team Member Design Accomplishments

- Team:
  - First advisor meeting
  - Continued material and physiology research
  - Designated roles for PDS Draft submission
  - Catch-up on last year's progress and translation into current project
- Carley
  - Worked on understanding the specifications of Gelma and how well it would fit with our project goals
  - Worked on further specifications for the lung ECM and what cell viability we are aiming to achieve.
  - Editing the PDS for this upcoming friday
- Anuraag
  - Met with team 02/06
  - Worked on physiology research related to the project
  - Literary analysis on GelMA and hyaluronic acid hydrogels for biomedical applications
  - Literary analysis of photo-crosslinkable hydrogels for in vitro applications

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Progress_Report_Week_2.pdf (65.7 kB)

03/27/2023 Entry- 02/16/2023 Completed- Progress Report Week 3

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:17 AM CDT

Title: Progress Report 3

Date: 02/16/2023

Content by: Entire Group

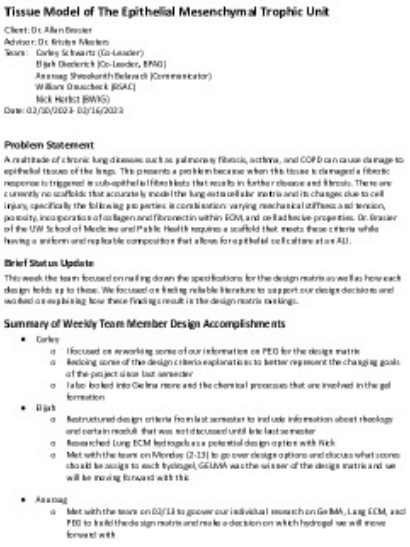
Present: Entire Group

Goals: Update client and project advisor with weekly progress the team makes towards the project with an emphasis on tasks completed, obstacles faced during the week, the team and individual goals for the following week, and an updated schedule of deadlines to be met.

Content: Attached

Conclusions/action items: Continue to update advisor and client on team progress every week and use feedback to improve week to week.

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:18 AM CDT



Tissue Model of The Epithelial Mesenchymal Trophic Unit

Client: Dr. Allen Bressler
Advisor: Dr. Kristyn Masters
Team: Corley Schwartz (Co-Leader)
Elijah Oesterle (Co-Leader, BME)
Anuraag Shreekanth Belavadi (Communicator)
William Osuscheck (BSAC)
Nick Herbst (BMES)
Date: 02/10/2023- 02/16/2023

Problem Statement

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and 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 scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury, specifically the following properties in combination: varying mechanical stiffness and tension, porosity, incorporation of collagen and fibronectin within ECM, and cell adhesive properties. Dr. Brazier of the UW School of Medicine and Public Health requires a scaffold that meets these criteria while having a uniform and replicable composition that allows for epithelial cell culture at an ALI.

Brief Status Update

This week the team focused on nailing down the specifications for the design matrix as well as how each design folds up to these. We focused on finding reliable literature to support our design decisions and worked on explaining how these findings result in the design matrix rankings.

Summary of Weekly Team Member Design Accomplishments

- Corley
  - I focused on reworking some of our information on PEG for the design matrix
  - Redoing some of the design criteria explanations to better represent the changing goals of the project since last semester
  - I also looked into Gelma more and the chemical processes that are involved in the gel formation
- Elijah
  - Restructured design criteria from last semester to include information about rheology and certain moduli that was not discussed until late last semester
  - Researched Lung ECM hydrogels as a potential design option with Nick
  - Met with the team on Monday (2-13) to go over design options and discuss what scores should be assign to each hydrogel, GELMA was the winner of the design matrix and we will be moving forward with this
- Anuraag
  - Met with the team on 02/13 to go over our individual research on GelMA, Lung ECM, and PEG to build the design matrix and make a decision on which hydrogel we will move forward with

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Progress_Report_Week_3.pdf (61.4 kB)

03/27/2023 Entry- 02/23/2023 Completed- Progress Report Week 4

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:21 AM CDT

Title: Progress Report 4

Date: 02/23/2023

Content by: Entire Group

Present: Entire Group

Goals: Update client and project advisor with weekly progress the team makes towards the project with an emphasis on tasks completed, obstacles faced during the week, the team and individual goals for the following week, and an updated schedule of deadlines to be met.

Content: Attached

Conclusions/action items: Continue to update advisor and client on team progress every week and use feedback to improve week to week.

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:21 AM CDT

Tissue Model of The Epithelial Mesenchymal Trophic Unit

Client: Dr. Allen Bressler
Advisor: Dr. Kristyn Masters
Team: Corley Schwartz (Co-Leader)
Elijah Oesterle (Co-Leader, BME)
Anuraag Shreekanth Belavadi (Communicator)
William Grauschuck (BSAC)
Nick Herbst (BMES)
Date: 02/17/2023- 02/23/2023

Problem Statement

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and 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 scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury, specifically the following properties in combination: varying mechanical stiffness and tension, porosity, incorporation of collagen and fibronectin within ECM, and cell adhesive properties. Dr. Brasier of the UW School of Medicine and Public Health requires a scaffold that meets these criteria while having a uniform and replicable composition that allows for epithelial cell culture in an ALI.

Brief Status Update

This week we focused on the preliminary design presentation and making necessary changes to our design matrix. These changes included adding both biochemical and mechanical tunability to our criteria as separate rankings than biochemical and mechanical properties. We also began to look at the preliminary report and prepare for going into shadow this Friday.

Summary of Weekly Team Member Design Accomplishments

- Corley
  - This week focused on reworking the design matrix and researching literature to back our rankings under this new criteria
  - Worked on my section of the preliminary presentation and rehearsing these slides
  - Made the preliminary report document and began delegating sections to rework and add
- Elijah
  - This week, I worked editing the design matrix after we met with Dr. Masters last Friday, criteria and explanations were more specifically defined
  - The team met on Monday for a group meeting and I worked on my slides for the preliminary oral presentation on Friday
  - Met with Dr. Bressler this morning to discuss updates on team progress and making of hydrogels in the upcoming week
- Anuraag
  - I worked on and edited the design matrix based on feedback from Dr. Masters

Download

Progress_Report_Week_4.pdf (59.2 kB)

03/27/2023 Entry- 03/02/2023 Completed- Progress Report Week 5

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:22 AM CDT

Title: Progress Report 5

Date: 03/02/2023

Content by: Entire Group

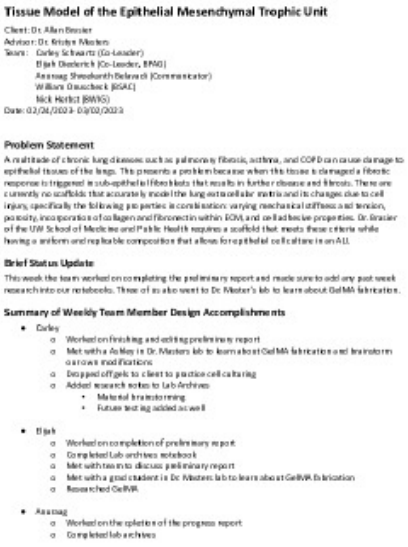
Present: Entire Group

Goals: Update client and project advisor with weekly progress the team makes towards the project with an emphasis on tasks completed, obstacles faced during the week, the team and individual goals for the following week, and an updated schedule of deadlines to be met.

Content: Attached

Conclusions/action items: Continue to update advisor and client on team progress every week and use feedback to improve week to week.

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:22 AM CDT



Tissue Model of the Epithelial Mesenchymal Trophic Unit

Client: Dr. Allen Bressler
Advisor: Dr. Kristyn Masters
Team: Carley Schwartz (Co-Leader)
Elijah Oesterle (Co-Leader, BME)
Anuraag Shreekanth Belavadi (Communicator)
William Grauschuck (BSAC)
Nick Herbst (BMES)
Date: 02/24/2023- 03/02/2023

Problem Statement

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and 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 scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury, specifically the following properties in combination: varying mechanical stiffness and tension, porosity, incorporation of collagen and fibronectin within ECM, and cell adhesive properties. Dr. Brasier of the UW School of Medicine and Public Health requires a scaffold that meets these criteria while having a uniform and replicable composition that allows for epithelial cell culture in an ALI.

Brief Status Update

This week the team worked on completing the preliminary report and made sure to add any past week research into our notebooks. Three of us also went to Dr. Master's lab to learn about GelMA fabrication.

Summary of Weekly Team Member Design Accomplishments

- Carley
  - Worked on finishing and editing preliminary report
  - Met with a Ashley in Dr. Masters lab to learn about GelMA fabrication and brainstorm our own modifications
  - Dropped off gels to client to practice cell culturing
  - Added research notes to Lab Archives
    - Material brainstorming
    - Future testing added as well
- Elijah
  - Worked on completion of preliminary report
  - Completed Lab archives notebook
  - Met with team to discuss preliminary report
  - Met with a grad student in Dr. Masters lab to learn about GelMA fabrication
  - Researched GelMA
- Anuraag
  - Worked on the updates of the progress report
  - Completed lab archives

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Progress_Report_Week_5.pdf (59.5 kB)

03/27/2023 Entry- 03/09/2023 Completed- Progress Report Week 6

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:23 AM CDT

Title: Progress Report 6

Date: 03/09/2023

Content by: Entire Group

Present: Entire Group

Goals: Update client and project advisor with weekly progress the team makes towards the project with an emphasis on tasks completed, obstacles faced during the week, the team and individual goals for the following week, and an updated schedule of deadlines to be met.

Content: Attached

Conclusions/action items: Continue to update advisor and client on team progress every week and use feedback to improve week to week.

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:23 AM CDT

Tissue Model of the Epithelial Mesenchymal Trophic Unit

Client: Dr. Allen Bressler
Advisor: Dr. Kristyn Masters
Team: Corley Schwartz (Co-Leader)
Elijah Diederich (Co-Leader, BME)
Anuraag Shreekanth Belavadi (Communicator)
William Osuschack (BSAC)
Nick Herbst (BMES)
Date: 03/03/2023- 03/09/2023

Problem Statement

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and 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 scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury, specifically the following properties in combination: varying mechanical stiffness and tension, porosity, incorporation of collagen and fibronectin within ECM, and cell adhesive properties. Dr. Brasier of the UW School of Medicine and Public Health requires a scaffold that meets these criteria while having a uniform and replicable composition that allows for epithelial cell culture in an ALI.

Brief Status Update

This week we focused on GelMA formation and mechanical testing. We worked on running a few trails varying the cooling period and the overall extraction from 24 well plates. We also worked on completing our preliminary peer evaluations and reflection.

Summary of Weekly Team Member Design Accomplishments

- Corley
  - Worked on GelMA formation
  - Preliminary peer evals
  - Brainstorming cell culture methods
- Elijah
  - Formation of GelMA hydrogels
  - Peer evaluations
  - GelMA Rheology Testing
- Anuraag
  - Worked on forming GelMA
  - Preliminary Peer evaluations
  - Met with Dr. Bressler for updates on 03/09
- William
  - Preliminary peer evaluations and reflection
  - Attended gel formation session

Download

Progress_Report_Week_6.pdf (56.7 kB)

03/27/2023 Entry- 03/23/2023 Completed- Progress Report Week 8

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:24 AM CDT

Title: Progress Report 7 (Week 8)

Date: 03/23/2023

Content by: Entire Group

Present: Entire Group

Goals: Update client and project advisor with weekly progress the team makes towards the project with an emphasis on tasks completed, obstacles faced during the week, the team and individual goals for the following week, and an updated schedule of deadlines to be met.

Content: Attached

Conclusions/action items: Continue to update advisor and client on team progress every week and use feedback to improve week to week.

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:24 AM CDT

Tissue Model of the Epithelial Mesenchymal Trophic Unit

Client: Dr. Allen Bressler
Advisor: Dr. Kristyn Masters
Team: Corley Schwartz (Co-Leader)
Elijah Diederich (Co-Leader, BME)
Anuraag Shreekanth Belavadi (Communicator)
William Osuschack (BSAC)
Nick Herbst (BMES)
Date: 03/17/2023- 03/23/2023

Problem Statement

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and 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 scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury, specifically the following properties in combination: varying mechanical stiffness and tension, porosity, incorporation of collagen and fibronectin within ECM, and cell adhesive properties. Dr. Brasier of the UNM School of Medicine and Public Health requires a scaffold that meets these criteria while having a uniform and replicable composition that allows for epithelial cell culture in an ALI.

Brief Status Update

This week, we focused mainly on ordering materials and preparing for the show and tell presentation this Friday. We have also continued to focus on solutions to decreasing the stiffness of our GelMA hydrogels and will continue to perform rheometry testing to accurately measure our progress.

Summary of Weekly Team Member Design Accomplishments

- Corley
  - Prepared gels for show and tell
  - Research materials to order
- Elijah
  - Researched materials
- Anuraag
  - Research on GelMA synthesis and required materials
  - Prepared for show and tell questions
  - Attended team meeting on 03/20
- William
  - Prepared for show and tell
  - Discussed and edited materials list during team meeting (03/20)
  - Materials research
- Nick

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Progress_Report_Week_8.pdf (61.8 kB)

05/01/2023 Entry- 03/30/2023 Completed- Progress Report Week 9

ANURAAG SHREEKANTH BELAVADI - May 01, 2023, 1:50 PM CDT

Title: Progress Report 8 (Week 9)

Date: 03/30/2023

Content by: Entire Group

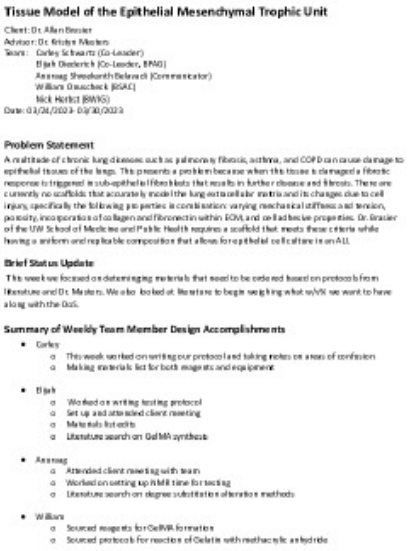
Present: Entire Group

Goals: Update client and project advisor with weekly progress the team makes towards the project with an emphasis on tasks completed, obstacles faced during the week, the team and individual goals for the following week, and an updated schedule of deadlines to be met.

Content: Attached

Conclusions/action items: Continue to update advisor and client on team progress every week and use feedback to improve week to week.

ANURAAG SHREEKANTH BELAVADI - May 01, 2023, 1:47 PM CDT



Tissue Model of the Epithelial Mesenchymal Trophic Unit

Client: Dr. Allen Bressler
Advisor: Dr. Kristyn Masters
Team: Corley Schwartz (Co-Leader)
Elijah Diederich (Co-Leader, BME)
Anuraag Shreekanth Belavadi (Communicator)
William Krauschack (BSAC)
Nick Herbst (BMES)
Date: 03/24/2023- 03/30/2023

Problem Statement

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and 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 scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury, specifically the following properties in combination: varying mechanical stiffness and tension, porosity, incorporation of collagen and fibronectin within ECM, and cell adhesive properties. Dr. Brasier of the UW School of Medicine and Public Health requires a scaffold that meets these criteria while having a uniform and replicable composition that allows for epithelial cell culture in an ALI.

Brief Status Update

This week we focused on determining materials that need to be ordered based on protocols from literature and Dr. Masters. We also looked at literature to begin weighing what w/VK we want to have along with the DoS.

Summary of Weekly Team Member Design Accomplishments

- Corley
  - This week worked on writing our protocol and taking notes on areas of confusion
  - Making materials list for both reagents and equipment
- Elijah
  - Worked on writing testing protocol
  - Set up and attended client meeting
  - Materials list edits
  - Literature search on GelMA synthesis
- Anuraag
  - Attended client meeting with team
  - Worked on setting up NMR time for testing
  - Literature search on degree substitution alternative methods
- William
  - Sourced reagents for GelMA formation
  - Sourced protocols for reaction of Gelatin with methacrylic anhydride

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Progress_Report_Week_9.pdf (64.4 kB)

05/01/2023 Entry- 04/06/2023 Progress Report Week 10

ANURAAG SHREEKANTH BELAVADI - May 01, 2023, 1:50 PM CDT

Title: Progress Report 9 (Week 10)

Date: 04/06/2023

Content by: Entire Group

Present: Entire Group

Goals: Update client and project advisor with weekly progress the team makes towards the project with an emphasis on tasks completed, obstacles faced during the week, the team and individual goals for the following week, and an updated schedule of deadlines to be met.

Content: Attached

Conclusions/action items: Continue to update advisor and client on team progress every week and use feedback to improve week to week.

ANURAAG SHREEKANTH BELAVADI - May 01, 2023, 1:50 PM CDT

Tissue Model of the Epithelial Mesenchymal Trophic Unit

Client: Dr. Allen Bressler
Advisor: Dr. Kristyn Masters
Team: Carley Schwartz (Co-Leader)
Elijah Oesterle (Co-Leader, BME)
Anurag Shreekanth Belavadi (Communicator)
William Grauschuck (BSAC)
Nick Herbst (BMES)
Date: 03/31/2023- 04/06/2023

Problem Statement

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and 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 scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury, specifically the following properties in combination: varying mechanical stiffness and tension, porosity, incorporation of collagen and fibronectin within ECM, and cell adhesive properties. Dr. Brazier of the UW School of Medicine and Public Health requires a scaffold that meets these criteria while having a uniform and replicable composition that allows for epithelial cell culture in an ALI.

Brief Status Update

This week the team worked on finalizing the materials to order as well as planning out our protocol for GelMA synthesis. We also looked into the MVR process after creating the GelMA.

Summary of Weekly Team Member Design Accomplishments

- Carley
  - This week focused on ordering materials
  - Planning out how dialysis process will work and liquid nitrogen
  - Thinking about DoE we will want
- Elijah
  - Met with Group on Monday
  - Completed section of the Executive summary
  - Continued to research GelMA functionalization and concentration on Young's Modulus
- Anurag
  - Met with group on Monday
  - Scheduled MVR time
  - Worked on executive summary draft
  -
- William
  - Completed executive summary sections

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Progress_Report_Week_10.pdf (64.8 kB)

05/01/2023 Entry- 04/13/2023 Progress Report Week 11

ANURAAG SHREEKANTH BELAVADI - May 01, 2023, 1:52 PM CDT

Title: Progress Report 10 (Week 11)

Date: 04/13/2023

Content by: Entire Group

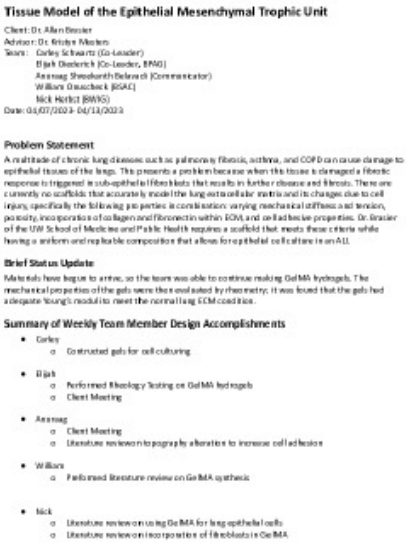
Present: Entire Group

Goals: Update client and project advisor with weekly progress the team makes towards the project with an emphasis on tasks completed, obstacles faced during the week, the team and individual goals for the following week, and an updated schedule of deadlines to be met.

Content: Attached

Conclusions/action items: Continue to update advisor and client on team progress every week and use feedback to improve week to week.

ANURAAG SHREEKANTH BELAVADI - May 01, 2023, 1:52 PM CDT



Tissue Model of the Epithelial Mesenchymal Trophic Unit

Client: Dr. Allen Bosler
Advisor: Dr. Kristyn Masters
Team: Corley Schwartz (Co-Leader)
Elijah Oesterich (Co-Leader, BME)
Anurag Shreekanth Belavadi (Communicator)
William Krauschack (BSAC)
Nick Herbst (BMES)
Date: 03/27/2023- 04/13/2023

Problem Statement

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and 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 scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury, specifically the following properties in combination: varying mechanical stiffness and tension, porosity, incorporation of collagen and fibronectin within ECM, and cell-adhesive properties. Dr. Brazier of the UW School of Medicine and Public Health requires a scaffold that meets these criteria while having a uniform and replicable composition that allows for epithelial cell culture in an ALI.

Brief Status Update

Materials have begun to arrive, so the team was able to continue making GelMA hydrogels. The mechanical properties of the gels were then evaluated by rheometry; it was found that the gels had adequate Young's moduli to meet the normal lung ECM conditions.

Summary of Weekly Team Member Design Accomplishments

- Corley
  - Constructed gels for cell culturing
- Elijah
  - Performed Rheology Testing on GelMA hydrogels
  - Client Meeting
- Anurag
  - Client Meeting
  - Literature review on topography alteration to increase cell adhesion
- William
  - Performed literature review on GelMA synthesis
- Nick
  - Literature review on using GelMA for lung epithelial cells
  - Literature review on incorporation of fibroblasts in GelMA

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Progress_Report_Week_11.pdf (63.2 kB)

05/01/2023 Entry- 04/20/2023 Progress Report Week 12

ANURAAG SHREEKANTH BELAVADI - May 01, 2023, 1:53 PM CDT

Title: Progress Report 11 (Week 12)

Date: 04/20/2023

Content by: Entire Group

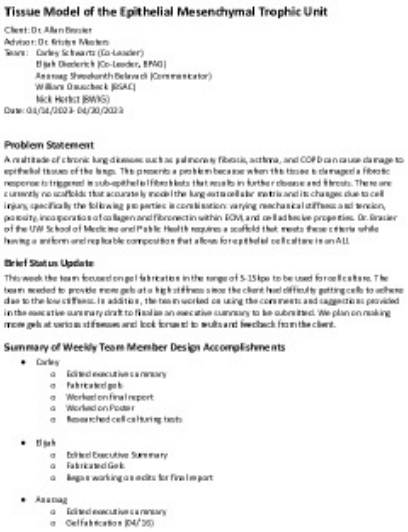
Present: Entire Group

Goals: Update client and project advisor with weekly progress the team makes towards the project with an emphasis on tasks completed, obstacles faced during the week, the team and individual goals for the following week, and an updated schedule of deadlines to be met.

Content: Attached

Conclusions/action items: Continue to update advisor and client on team progress every week and use feedback to improve week to week.

ANURAAG SHREEKANTH BELAVADI - May 01, 2023, 1:54 PM CDT



Tissue Model of the Epithelial Mesenchymal Trophic Unit

Client: Dr. Allen Bressler
Advisor: Dr. Kristyn Masters
Team: Carley Schwartz (Co-Leader)
Elijah Diederich (Co-Leader, BPAG)
Anuraag Shreekanth Belavadi (Communicator)
William Krauschack (BSAC)
Nick Herbst (BWIG)
Date: 03/14/2023- 04/20/2023

Problem Statement

A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and 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 scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury, specifically the following properties in combination: varying mechanical stiffness, surface tension, porosity, incorporation of collagen and fibronectin within ECM, and cell adhesive properties. Dr. Brasier of the UW School of Medicine and Public Health requires a scaffold that meets these criteria while having a uniform and replicable composition that allows for epithelial cell culture in an ALI.

Brief Status Update

This week the team focused on gel fabrication in the range of 5-15kpa to be used for cell culture. The team needed to provide more gels at a high stiffness since the client had difficulty getting cells to adhere due to the low stiffness. In addition, the team worked on using the comments and suggestions provided in the executive summary draft to finalize an executive summary to be submitted. We plan on making more gels at various stiffnesses and look forward to results and feedback from the client.

Summary of Weekly Team Member Design Accomplishments

- Carley
  - Edited executive summary
  - Fabricated gels
  - Worked on final report
  - Worked on Poster
  - Researched cell culturing tests
- Elijah
  - Edited Executive Summary
  - Fabricated Gels
  - Began working on edits for final report
- Anuraag
  - Edited executive summary
  - Cell fabrication (04/18)

Download

Progress_Report_Week_12.pdf (65.6 kB)

05/01/2023 Entry- 04/27/2023 Progress Report Week 13

ANURAAG SHREEKANTH BELAVADI - May 01, 2023, 1:55 PM CDT

Title: Progress Report 12 (Week 13)

Date: 04/27/2023

Content by: Entire Group

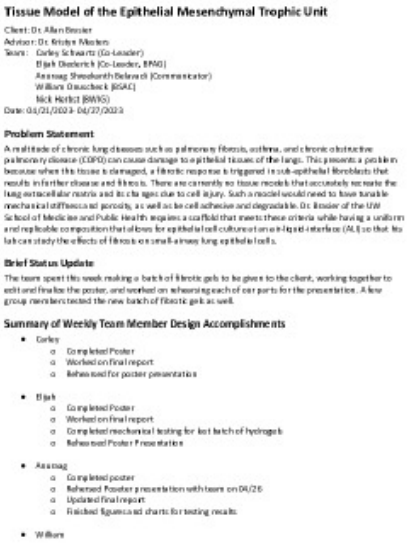
Present: Entire Group

Goals: Update client and project advisor with weekly progress the team makes towards the project with an emphasis on tasks completed, obstacles faced during the week, the team and individual goals for the following week, and an updated schedule of deadlines to be met.

Content: Attached

Conclusions/action items: Continue to update advisor and client on team progress every week and use feedback to improve week to week.

ANURAAG SHREEKANTH BELAVADI - May 01, 2023, 1:56 PM CDT



Tissue Model of the Epithelial Mesenchymal Trophic Unit

Client: Dr. Allen Bowler
Advisor: Dr. Kristyn Masters
Team: Corley Schwartz (Co-Leader)
Elijah Diederich (Co-Leader, BME)
Anuraag Shreekanth Belavadi (Communicator)
William Grauschuck (BSAC)
Nick Herbst (BMES)
Date: 04/21/2023- 04/27/2023

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 illness. 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. Bowler of the UW School of Medicine and Public Health requires a scaffold that meets these criteria while having a uniform and replicable composition that allows for epithelial cell culture at 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 spent this week making a batch of fibrotic gels to be given to the client, working together to edit and finalize the poster, and worked on rehearsing each of our parts for the presentation. A few group members tested the new batch of fibrotic gels as well.

Summary of Weekly Team Member Design Accomplishments

- Corley
  - Completed Poster
  - Worked on final report
  - Rehearsed for poster presentation
- Elijah
  - Completed Poster
  - Worked on final report
  - Completed mechanical testing for last batch of hydrogels
  - Rehearsed Poster Presentation
- Anuraag
  - Completed poster
  - Rehersed Poster presentation with team on 04/26
  - Updated final report
  - Finished figures and charts for testing results
- William

Download

Progress_Report_Week_13.pdf (64.2 kB)

2023/10/03-Gelatin Hydrogel

CARLEY SCHWARTZ - Feb 10, 2023, 1:22 PM CST

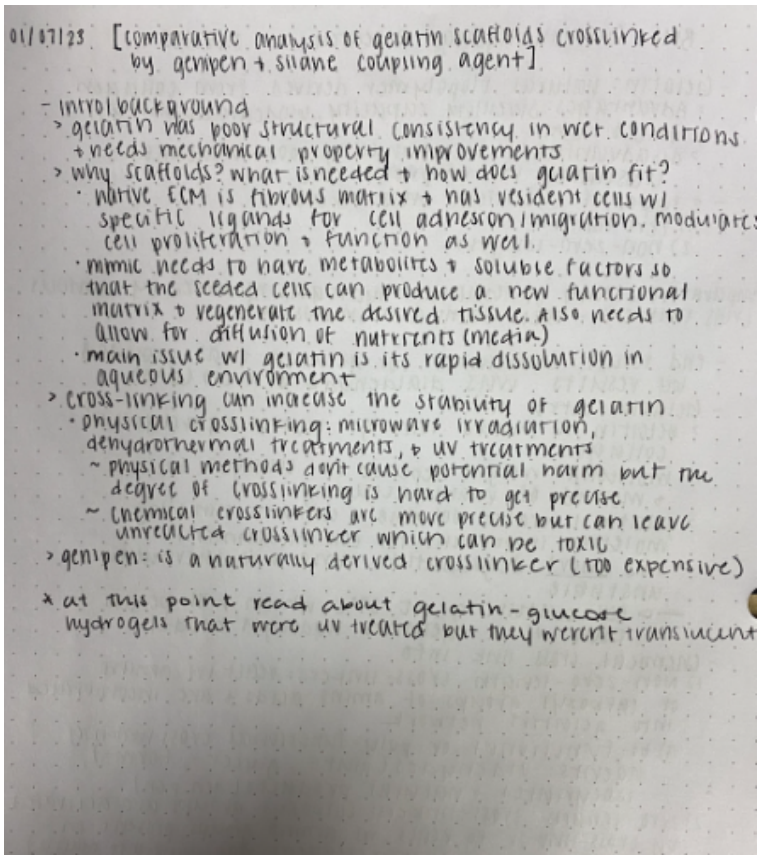
Title:

Date:

Content by:

Present: Self

Goals: To learn about gelatin hydrogels


01/07/23 [comparative analysis of gelatin scaffolds crosslinked by genipen + silane coupling agent]

- introl background
  - > gelatin has poor structural consistency in wet conditions + needs mechanical property improvements
  - > why scaffolds? what is needed + how does gelatin fit?
    - native ECM is fibrous matrix + has resident cells w/ specific ligands for cell adhesion/migration. modulates cell proliferation + function as well.
    - mimic needs to have metabolites + soluble factors so that the seeded cells can produce a new functional matrix + regenerate the desired tissue. Also needs to allow for diffusion of nutrients (media)
    - main issue w/ gelatin is its rapid dissolution in aqueous environment
  - > cross-linking can increase the stability of gelatin
    - physical crosslinking: microwave irradiation, dehydrothermal treatments, + UV treatments
      - ~ physical methods don't cause potential harm but the degree of crosslinking is hard to get precise
      - ~ chemical crosslinkers are more precise but can leave unreacted crosslinker which can be toxic
  - > genipen: is a naturally derived crosslinker (too expensive)

* at this point read about gelatin-glucose hydrogels that were uv treated but they weren't translucent

Conclusions/action items:

This article gave some details into how gelatin hydrogels have natural adhesive motifs for cells and are naturally degradable - this is much more beneficial than PEG

It also included how the crosslinking works for each of these gels which can include UV irridation (similar to PEG) or chemical crosslinking

2023/02/12-GelMA hydrogel research

CARLEY SCHWARTZ - Feb 10, 2023, 4:39 PM CST

Title: Gelatin-Methacryloyl (GelMA) Hydrogels with Defined Degree of Functionalization as a Versatile Toolkit for 3D Cell Culture and Extrusion Bioprinting

Date: 02-12-23

Content by: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6165498/

Present: self

Goals: to learn more about gelma

Content:

Gelatin-methacryloyl (GelMA) is a semi-synthetic hydrogel which consists of gelatin derivatized with methacrylamide and methacrylate groups. These hydrogels provide cells with an optimal biological environment (e.g., RGD motifs for adhesion) and can be quickly photo-crosslinked, which provides shape fidelity and stability at physiological temperature.

The hydrogel is obtained by the derivatization of gelatin with methacrylic anhydride, resulting in modification of lysine and hydroxyl residues with methacrylamide and methacrylate side groups

The GelMA hydrogel can thus provide an aqueous environment for cells and supports their adhesion, growth, and proliferation. In contrast to gelatin, however, the modification with methacryloyl side groups allows the GelMA molecule to undergo rapid polymerization in the presence of UV light and a photoinitiator (PI), resulting in covalent crosslinking through the creation of a methacryloyl backbone

However, cells proliferate and migrate better when not hindered by a dense polymer network. Cell spreading and long term survival, as well as remodeling of the construct, cannot be observed in such constructs

Type B material is derived from bovine skin and alkaline treatment. As a result, it has a more fragmented structure and chains of lower molecular weight (lower Bloom strength factor). This means that after derivatization, B materials will be softer than A materials of the same DoF. Type A gelatin is derived from porcine skin after acid treatment; it has a higher transparency than type B materials and can be obtained with a maximum Bloom factor of 300, also used in this work.

The pH of the reaction sinks constantly due to the production of methacrylic acid, requiring manual or automatic pH adjustment of the reaction back to 9, thus ensuring optimal reaction conditions which necessitate lower amounts of MAA and result in specific DoFs of the product.

Cells encapsulated in GelMA B70 with a 70% DoF also demonstrated spreading; however, it was less pronounced than the cell spreading observed in B50 and A50

In A100 hydrogels, only about 20% digestion (in 20 U/mL and 30 U/mL both) was measured with and without encapsulated cells after 3 h. A70 GelMA hydrogels with and without cells were fully digested after 6 h of incubation in 20 U/mL and 30 U/mL of collagenase. High DoF GelMA could also be completely digested in 30 U/mL collagenase after 5 h, but in 20 U/mL collagenase only after 8 h of incubation

Conclusions/action items:

GelMA is semi-synthetic, has natural adhesive (RGD motifs), is MMP degradable, is transparent, can be polymerized with UV light or photoinitiator, need to ask client about gelatin A or B, is very cheap, concentration can be varied to achieve desired stiffness, can be digested in a controllable manner

2023/15/02- GelMA degradation

CARLEY SCHWARTZ - Feb 17, 2023, 7:18 AM CST

Title: Gelatin methacryloyl and its hydrogels with an exceptional degree of controllability and batch-to-batch consistency -article name

Date: 02-15-23

Content by: https://www.nature.com/articles/s41598-019-42186-x

Present: self

Goals: to understand gelma degradation further

Content:

GelMA can be prepared through simple synthesis of gelatin with methacrylic anhydride (MAA), and its methacryloyl functionalization (or the degree of substitution (DS); the degree of methacryloylation (DM)) can be adjusted via a feed ratio of gelatin to MAA. The DS of GelMA is one of the main factors that can influence biophysiochemical properties of GelMA and its photocured hydrogels. Recently, GelMA has been commercially available through some vendors such as Sigma-Aldrich. Therefore, there is a wide interest in developing effective methods to prepare GelMA with high reproducibility and controllability in terms of composition and biophysiochemical properties.

In this study, two types of GelMA samples (target degrees of substitution (DS): DS = 100% and 60%) with five batches were synthesized with feeding mole ratios of MAA to amino groups of gelatin at 1.859:1 and 0.628:1, respectively. GelMA samples (DS = 100% and 60%) with different batches were labeled as DS100_1~5 and DS60_1~5.

There are many parameters involved in the reaction of gelatin and methacrylic anhydride (MAA) such as pH, temperature, reaction time, a gelatin concentration, a buffer system, a mole ratio of gelatin and MAA, and stirring speed. The crucial thing of GelMA synthesis is to maintain the pH of the reaction solution since the byproduct (methacrylic acid, MA) can decrease the pH of the solution during the reaction, hindering the forward reaction owing to the protonation of free amino groups.

Mechanical properties of GelMA hydrogels. Storage moduli of GelMA (DS100_1~5 and DS60_1~5) hydrogels at 20 (w/v)% were measured at 0.1% strain and 0.1–10 Hz at 37 °C. D100_1~5 and DS60_1~5 hydrogels exhibited storage moduli of around 30 kPa and 16 kPa, respectively.

Conclusions/action items:

This article helped gain a better understanding of how the GelMA procedure could work. It also explained how differing the degrees of substitution(DS) will influence the storage moduli by increasing with increased DS.

2023/17/02-GelMA synthesis and properties

CARLEY SCHWARTZ - Feb 17, 2023, 8:02 AM CST

Title:

Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels

Date: 02-17-24

Content by: Yue, K., Trujillo-de Santiago, G., Alvarez, M. M., Tamayol, A., Annabi, N., & Khademhosseini, A. (2015). Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. *Biomaterials*, *73*, 254-271. https://www.sciencedirect.com/science/article/pii/S014296121500719X

Present: Self

Goals: To learn more about how GelMA synthesis can affect its parameters.

Content:

GelMA undergoes photoinitiated radical polymerization (i.e. under UV light exposure with the presence of a photoinitiator) to form covalently crosslinked hydrogels. As the hydrolysis product of collagen, the major component of ECM in most tissues, gelatin contains many arginine-glycine-aspartic acid (RGD) sequences that promote cell attachment [19], as well as the target sequences of matrix metalloproteinase (MMP) that are suitable for cell remodeling [20].

When compared to collagen, the advantages of gelatin include better solubility and less antigenicity [21], [22]. The hydrolysis process also denatures the tertiary structure of collagen, reducing its structural variations due to different sources. A gelatin solution has, on its own, the unique property of gelation at low temperatures to form physically crosslinked hydrogels.

Specifically, the RGD motifs do not contain groups that will react with MA, which ensures the retention of good cell adhesive properties of GelMA [6], [19], [29]. Furthermore, the *in vitro* enzymatic degradation of GelMA hydrogels by type I and type II collagenases (also known as MMP-1 and MMP-8, respectively) proceeds at accelerated rates, indicating the existence of MMP-sensitive motifs in GelMA [30], [31]. This ensures that during the reaction process the much needed RGD and MMP sequences are retained in gelma

Photocrosslinking of the synthesized GelMA can be conducted using a water-soluble initiator under UV light. Common choices for photoinitiators include 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959) and lithium acylphosphinate salt (LAP). LAP works better because of its higher solubility in water than I2959 which speaking from experience I2959 wasn't the best.

We and others have also shown that the pore sizes in GelMA hydrogel can be tuned by changing the degree of methacryloyl substitution. For example, Chen et al. [9] synthetized GelMA hydrogels with different substitution degrees (49.8, 63.8, and 73.2%) using 1, 5, and 10 M MA solutions, respectively. The average pore size of the resulting GelMA hydrogels, as characterized by SEM after freeze drying, was 50 (49.8%), 30 (63.8%), and 25 μm (73.2%). This shows how differing the degree of substitution allows for different pore sizes. Specifically, the lower degree of substitution resulted in larger average pore sizes.

Chen et al. observed that the compressive modulus of a GelMA hydrogel was directly proportional to the degree of methacryloyl substitution (2.0 ± 0.18 kPa (49.8%), 3.2 ± 0.18 kPa (63.8%), and 4.5 ± 0.33 kPa (73.2%)) This shows examples of what % degree of substitution will yield particular compressive moduli.

The compressive modulus was also directly proportional to the GelMA mass/volume fraction. For example, Nichol et al. estimated compressive modulus values of 2.0, 10.0, and 22.0 kPa, respectively, for 5, 10, and 15% w/v GelMA (with a degree of substitution of 53.8%). This shows how the weight/volume of GelMA with a set degree of substitution will influence the compressive modulus values.

Nichol et al. reported that the swelling ratio decreased by increasing the degree of methacryloyl substitution and the GelMA mass fraction. Similarly, cell proliferation was inversely proportional to the GelMA mass fraction within the hydrogel. When the GelMA mass fraction is lower the cell proliferation was found to be higher.

For instance, cells can be suspended in GelMA prepolymer solutions and crosslinked upon exposure to UV light to form cell-laden 3D hydrogels. High cell viability (>80%) is generally observed in these photocrosslinked cell-laden GelMA hydrogels. In contrast to 2D cell culture, cells encapsulated in hydrogels should be able to remodel their surrounding environments for spreading and migration. This shows how the cells are capable of surviving the exposure to UV light for crosslinking and that cells encapsulated in this 3D gel will remodel and migrate in the environment

Conclusions/action items:

This source helped formulate ideas for the design matrix and understanding how GelMA fits within our specifications. I found specific use in the section confirming that not only are the RGD and MMP sequences retained but cell viability remains high in this gel after UV exposure.

03-27-23 GelMA cell viability

CARLEY SCHWARTZ - Feb 28, 2023, 11:20 AM CST

Title: cell viability info

Date: 03-27-23

Content by: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8309475/

Present: Carley

Goals: To learn more about cell viability

Content:

We encapsulated the osteoblast-like MG63 cells and human MSCs in HAp and Si-HAp composite hydrogels to assess the cytotoxicity of these photocrosslinkable hydrogels. The cell viability assay revealed that embedded cells were almost alive (green) in all groups after 14 days of cell culture

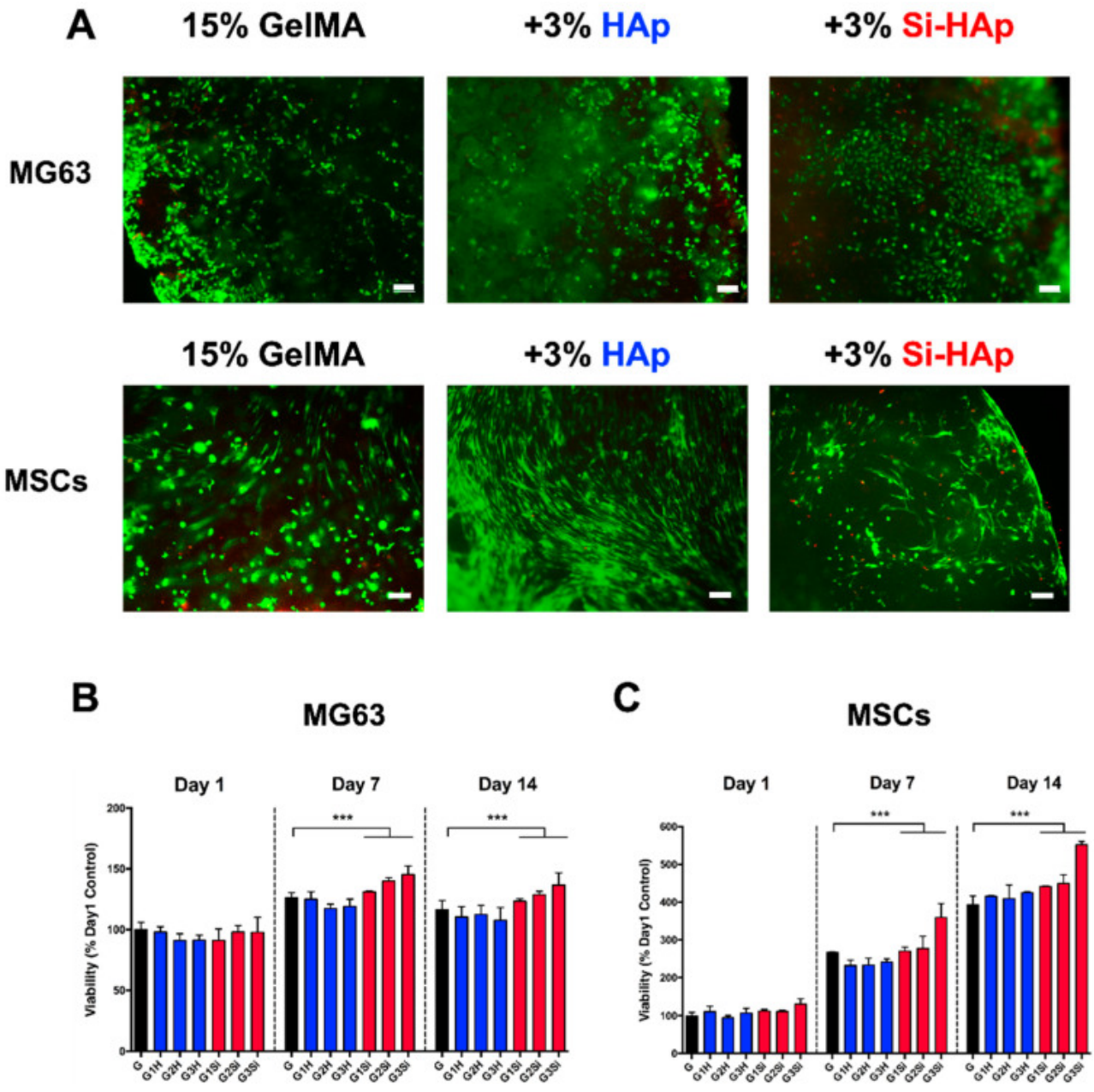


Figure 1 A shows there is still high cell viability after two weeks of cell culture which is good for our project - using 15% w/v gelma.

Conclusions/action items:

- Need to figure out if we can have mechanical properties we want and a slow enough degradation rate to allow for cell culture over multiple weeks

02-28-23 GelMA on a chip model

CARLEY SCHWARTZ - Feb 28, 2023, 11:25 AM CST

Title: GelMA long term cell culture research

Date: 02-28-23

Content by: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7698322/

Present: Carley

Goals: To find more designs with GelMA for long-term cell culture

Content:

Therefore, the microfluidic chip combined with GelMA hydrogels provided a 3-D environment for long-term cell culture and growth. In the latest studies, GelMA hydrogels were used as a part of the printed microchannels.

Lee et al. used 10 *w*/*v*% GelMA hydrogel as a semi-permeable physical barrier to control the molecular diffusion in a microfluidic co-culture device. For example, the larger pore size of GelMA resulted in an increased oxygen diffusion rate that promoted cell differentiation.

The inability to create large-scale tissue constructs containing micro-vascularized network channels and the lack of control over long-term cell survival remain unsolved.

Conclusions/action items:

- Need to consider that the longest the cell culture might last is only 2 weeks, can't find longer than that!

2023/02/11-Decellularized Lung Hydrogel

CARLEY SCHWARTZ - Feb 10, 2023, 5:11 PM

Title: Lung ECM Hydrogel Research

Date: 02-11-23

Content by:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8950628/#:~:text=The%20hydrophilic%20functionalities%20are%20responsible,water%20absorption%20than%20natural%20hydr

Present: self

Goals: To learn more about lung ECM hydrogels

Content:

The hydrophilic functionalities are responsible for holding a large amount of water, and the network chains' cross-linking allows them to retain water in their structure without being dissolved. Synthetic hydrogels have a higher capacity for water absorption than natural hydrogels. The amount of water in the hydrogel is determined by the polymer's properties the density of networking

Naturally derived hydrogels are particularly appealing because of their inherent biocompatibility, biodegradability and safety, including chitosan, alginate, hyaluronan, collagen and agarose, which are generally obtained from various renewable resources like animal, plant, algae, and microorganisms in the great world. Synthetic hydrogels possess tunable properties for facile fabrication of functional productions, which mainly contain polyethylene glycol (PEG)

Conclusions/action items: synthetic or semisynthetic hydrogels provide better water capacity as well as more tunable mechanical properties during fabrication.

03-11-23 Media Research

CARLEY SCHWARTZ - Mar 11, 2023, 2:50 PM CST

Title: Media Research

Date: 03-11-23

Content by: Carley

Present: Self

Goals: cell attachment hasn't been high on the first gels being used for cell culturing - why?

could this be due to them using a serum free media https://promocell.com/product/small-airway-epithelial-cell-growth-medium/

what role does fetal bovine serum found in many other cell cultures with GelMA have in cell attachment

Content: https://pubmed.ncbi.nlm.nih.gov/2412864/

- Fetal bovine serum (FBS) is a byproduct of harvesting cattle for the meatpacking industry—it’s used extensively by both academic and industrial researchers as a supplement to basal growth medium in cell culture applications.
- Bovine serum is a constituent of most media used for the culture of animal cells. The adhesion-promoting properties of serum are generally attributed to fibronectin, yet there have been frequent reports of other adhesion-promoting molecules in bovine serum
- Serum is added to culture medium at a concentration of 2-10% to provide attachment factors, nutrients, and hormones for mammalian cells, as well as to be a buffer against disruptions like pH changes and endotoxins. FBS has a high content of embryonic growth promoting factors like hormones, carrier proteins, and macromolecular proteins. It also has low levels of antibodies and other growth-inhibiting components.

Conclusions/action items:

03-11-23 Serum Starvation Research

CARLEY SCHWARTZ - Mar 11, 2023, 2:57 PM CST

Title: Serum Starvation

Date: 03-11-23

Content by: Carley

Present: Self

Goals: To understand how a serum free media might impact the cell adhesion

Content:

- Serum starvation is defined as growing cells in either serum-free, serum-reduced, or serum protein-free medium (Pirkmajer & Chibalin,), which has been used as a tool for molecular mechanism studies, such as autophagy, apoptosis and cellular stress response. Although serum starvation has been performed in hundreds of research studies, the impact of the condition is not well understood
    - Serum starvation reduces basal cellular activity
        - Does this mean that using some other type of ECM protein coating for basal activity will be beneficial? collagen?

Conclusions/action items:

Shows that serum starvation does have a decrease in basal cellular activity but not what this means for us with hydrogels?

03-11-23 Cell Culturing w Lung epithelial cells

CARLEY SCHWARTZ - Mar 11, 2023, 3:10 PM CST

Title: airway cell culturing

Date: 03-11-23

Content by: Carley

Present: Self

Goals: To learn more about airway cell culturing

Content: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6040659/

- Airway basal stem cells are the progenitor cells within the airway that exhibit the capacity to self-renew and give rise to multiple types of differentiated airway epithelial cells.
- Airway disease modeling and drug discovery have benefited greatly from the development and use of primary airway epithelial cultures grown on permeable transwell filters at air-liquid interface (ALI).
    - This model has several advantages over immortalized cell lines in that the primary epithelial cells can differentiate into an airway mucosa that features multiple epithelial cell types including ciliated, serosal, and basal cells and their arrangement is quite reflective of in vivo cellular organization. Primary ALI models exhibit functional micro-physiological processes including beating cilia and the ability to secrete mucus, features which are notably absent in the cell line-derived epithelial monolayer.
        - Epithelial basal cell culture no longer relies upon co-culture of primary epithelial cells with mitotically inactive fibroblast feeder layers, a technique established in the 1970’s to enhance epithelial cell proliferation by improving the capacity of cultured cells to escape senescence

Conclusions/action items:

Gained a better understanding of the small airway cell culturing specifics but not sure on application of this to hydrogel.

04-18-23 Cell culturing

CARLEY SCHWARTZ - Apr 18, 2023, 9:21 AM CDT

Title: Cell culturing on hydrogel

Date: 04-18-23

Content by: https://www.biorxiv.org/content/10.1101/2022.09.21.508886v1.full

Present: carley

Goals: to understand what types of cell would be ideal to find on the hydrogel and at what concentrations

Content:

- We also quantified individual ECM components: collagen (338.8 ± 116.5μg/mL), elastin (241.7 ± 111.9μg/mL), laminin (9.80 ± 2.48μg/mL), fibronectin (70.4 ± 21.1μg/mL), sulfated glycosaminoglycans (sGAGs) (195.5 ± 103.4μg/mL) and the major non-sulfated GAG, hyaluronic acid (8.2 ± 4.3μg/mL)
- With an increase in stiffness, there was a corresponding decrease in porosity of the hydrogel as measured by SEM imaging and quantification with average pore sizes of: 56.45±14.65μm (Soft), 20.47±2.19μm (Medium), and 15.20±1.82μm (Stiff)
- fabricated OTEs with hydrogels without either fibroblasts (FB) or lung sECM, with each individually incorporated into the hydrogel, and with both fibroblasts and lung sECM. The OTEs were fabricated with a density of 250,000 fibroblasts/OTE and a lung sECM protein concentration of 2mg/mL to evaluate the feasibility of the OTE model.
- A critical characteristic of ALI cultures is the epithelial barrier that functions to maintain an air-liquid interface and epithelial functionality. The standard assay to assess epithelial integrity and the corresponding polarization is measurement of the trans-epithelial electrical resistance (TEER). Epithelial integrity and barrier function was assessed by TEER and corresponding brightfield whole mount imaging to assess confluency of the epithelial monolayer
    - Epithelial detachment and holes in the HBE monolayer could be observed in all OTE cultures that did not include both sECM and lung fibroblasts except for the FB+ only group in the soft hydrogel group
    - Qualitatively, the cultures that were unable to maintain a confluent monolayer had HBE cells that either formed aggregates or spread out on the surface in contrast to their typical cobblestone phenotype This might be what ours are doing right now or at least on the softer gels
    - TEER measurements supported the image quantification results with the cultures that did not include both FB and sECM dropping to near baseline resistance values at the end of their culture periods
        - In contrast, OTEs with both FB and sECM had final TEER values more than 7X their baseline values indicating quality epithelial integrity and polarization compared to the other groups ($p<0.0001$). shows that FB incorporation would help-future evidence
        - The soft hydrogel with only FB (Group 2) did show a 4X increase in TEER from baseline but did not develop resistance values as large as the group with both FB and sECM ($p<0.0001$).
        - An important feature that was noted during the culture period of the OTEs was that none of the hydrogel models contracted from the edges resulting in the loss of the air-liquid interface and barrier function (**Fig. 3A**) typically seen with hydrogels with embedded fibroblasts. this could be a concern
- quantification of their capability to differentiate and HBE phenotype characterization was performed via histological, multispectral immunohistochemical and RNA sequencing transcriptomic analysis. First, we performed H&E staining on the histologically processed cultures collected at the end of ALI culture and compared them to human lower and upper airway tissue samples

Conclusions/action items:

This paper can be very helpful in the future and might help with troubleshooting any issues later on

GelMA protocol article

CARLEY SCHWARTZ - Mar 27, 2023, 10:15 AM CDT

PROTOCOL

Functionalization, preparation and use of cell-laden gelatin methacryloyl–based hydrogels as modular tissue culture platforms

Download

nprot.2016.037.pdf (2.23 MB)

CARLEY SCHWARTZ - Mar 27, 2023, 5:57 PM CDT

Notes on this protocol and materials

- Gelatin from porcine skin, type A, gel strength 300 bloom = store it at room temperature (RT) at 23–25 °C)
  - Other Materials: Methacrylic anhydride, PBS, NaHCO3, I2959 (we will use LAP), hyaluronic acid, liquid nitrogen
- Equipment
  - Round bottom flask and stir bar
  - freeze dryer
  - sterile 50 mL centrifuge tubes with vented caps (.2 um pore size)
  - sterile syringes (50 mL volume)
  - syringe filter units or disposable filters with polyethersulfone (PES) membrane (0.2 um pore size)
  - Mold
  - Untreated microscope glass slides
- Procedure
  - Some notes: GelMA light sensitive so may need to wrap dialysis setup and centrifuge tubes in aluminum foil

1. Soak gelatin to a final concentration of 10% (wt/volume) in PBS at RT in a round bottom flask with a stir bar (stir moderately for 10-60 min for gelatin dissolution) -In their lab, they had reaction volume of 300 mL with 30 g gelatin and needed an overhead stir bar bc such a large reaction volume
2. While stirring moderately heat the reaction mixture (and keep at) 50 C until gelatin fully dissolved and solution become clear
3. While stirring vigorously, slowly add .6 g of MAA (very viscous liquid) per 1 g of dissolved gelatin for a high degree of methacryloyl functionalization(75% DoF) and continue stirring vigorously for 60 min - solution will turn opaque if mixed sufficiently (can be ran for 3 hr but will alter the GelMA functionalization) (if not stirring adequately there will be phase separation and need to use glass pipettes with MAA because it can react w plastic) for low DoF use 0.06 g of MAA per 1 g of gelatin (31% DoF)
4. After reaction period transfer the solution into 50 mL tubes and remove unreacted MAA by centrifuging at 3,500g for 3 min at RT
5. Separate GelMA supernatant into a large (200-500 ml) glass beaker and discard the pellet
6. Dilute the supernatant solution with two volumes of PBS ?? confused
7. Transfer solution to dialysis membrane and dialyze at 40 C against a large volume of PBS for 5-7 days in chemical safety fume hood, change water at least once daily (dialysis is completed when the gelma solution appears clear and the when the odor of the residual MAA is no longer noticeable
8. Adjust pH of gelMA solution to 7.4 using 1 M NaHCO3
9. In biosafety cabinet, filter-sterilize the GelMA solution using .2 um syringe filter units or disposable vacuum filtration units with PES membrane
10. Divide the gelma solution into 50 ml tubes and snap freeze them in liquid nitrogen
11. Transfer all aliquots to the freeze fryer without allowing the solutions to thaw and lyophilized them until the gelma is fully dehydrated which takes 4-7 days (need to be sealed with vented screw top caps or press-fitted with .2 um syringe filter units before lyophilization which will be switched to fitted caps after this step)
12. Store lyophilized gelma away from light and at -20 C

PBS Recipe (if needed)

CARLEY SCHWARTZ - Mar 27, 2023, 11:28 AM CDT

PBS (Phosphate Buffered Saline) (1X, pH 7.4) Preparation and Recipe

PBS is an isotonic buffer frequently used in biological applications, such as washing cells, transportation of tissues, and dilutions. PBS closely mimics the pH, osmolarity, and ion concentrations of the human body. Since it is nontoxic to cells, it is extensively used for cell container rinsing and other preparations that might leave a residue. It is simple to prepare and has good shelf life, but will precipitate in the presence of zinc ions.

To prepare 1 L of PBS (Phosphate Buffered Saline) (1X, pH 7.4):

Change the value in the textbox above to scale the recipe volume

Table 1. Required components

Component	Amount	Concentration
Sodium chloride (mw: 58.44 g/mol)	8 g	0.137 M
Potassium Chloride (mw: 74.55 g/mol)	0.2 g	0.0027 M
Sodium Phosphate Dibasic (mw: 141.96 g/mol)	1.44 g	0.01 M
Potassium Phosphate Monobasic (mw: 136.09 g/mol)	0.245 g	0.0018 M

1. Prepare 800 mL of distilled water in a suitable container.
2. Add 8 g of Sodium chloride to the solution.
3. Add 0.2 g of Potassium Chloride to the solution.
4. Add 1.44 g of Sodium Phosphate Dibasic to the solution.
5. Add 0.245 g of Potassium Phosphate Monobasic to the solution.
6. Adjust solution to desired pH (typically pH = 7.4).
7. Add distilled water until the volume is 1 L.

Download

Screen_Shot_2023-03-27_at_11.27.40_AM.png (359 kB)

CARLEY SCHWARTZ - Mar 27, 2023, 11:28 AM CDT

https://www.aatbio.com/resources/buffer-preparations-and-recipes/pbs-phosphate-buffered-saline

In case we want to make our own PBS and this table is interactive so it will allow you to alter the volume being prepared.

03-27-23 Materials Research/Procedure

CARLEY SCHWARTZ - Mar 27, 2023, 10:14 AM CDT

Title: GelMA procedure research

Date: 03-27-23

Content by: Carley

Present: Self

Goals: to learn about needed materials for gelma production

Content: https://www-ncbi-nlm-nih-gov.ezproxy.library.wisc.edu/pmc/articles/PMC5556948/#R35

- Briefly, type-A porcine skin gelatin was dissolved at 10% w/v in phosphate buffered saline (PBS) at 50°C. While both type A and type B gelatin can be methacrylated, gelMA synthesized with type A is more common in the field and better characterized.
- Methacrylic anhydride (MA) was added to the gelatin solution using a peristaltic pump at a rate of 200 μL/min under aggressive stirring.

Conclusions/action items:

Need to make sure that we have all the necessary components and a genuine understanding of the protocol and what to do when we run into errors.

04-02-23 Master's Protocol

CARLEY SCHWARTZ - Apr 03, 2023, 10:25 AM CDT

Title:

Date: 04-02-23

Content by: Carley

Present: Self

Goals: To understand the GelMA protocol and necessary materials

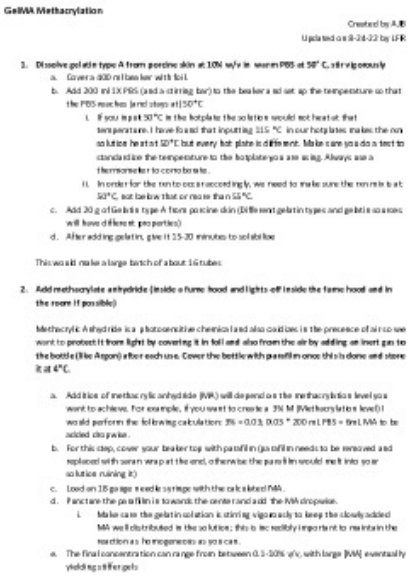
Content:

- **Need to understand better how the dialysis tubing is clipped in place while inside fume hood?**
- **Flash freezing? Do you just place into the liquid nitrogen and wait until it evaporated then remove them and place into freezer?**

Conclusions/action items:

Need to ask questions about liquid nitrogen process

CARLEY SCHWARTZ - Apr 03, 2023, 10:22 AM CDT



GelMA Methacrylation

Created by AJB
Updated on 8-24-22 by LFR

1. **Dissolve gelatin type A from porcine skin at 10% w/v in warm PBS at 50° C, stir vigorously**
   a. Cover a 400 ml beaker with foil.
   b. Add 200 ml 1X PBS (and a stirring bar) to the beaker and set up the temperature so that the PBS reaches (and stays at) 50°C
      i. If you input 50 °C in the hotplate the solution would not heat at that temperature. I have found that inputting 115 °C in our hotplates makes the GelMA solution heat at 50°C but every hot plate is different. Make sure you do a test to standardize the temperature to the hotplate you are using. Always use a thermometer to corroborate.
      ii. In order for the GelMA to occur accordingly, we need to make sure the reaction is at 50°C, not below that or more than 55°C.
   c. Add 20 g of Gelatin type A from porcine skin (Different gelatin types and gelatin sources will have different properties)
   d. After adding gelatin, give it 15-30 minutes to solubilize

   This would make a large batch of about 16 tubes

2. **Add methacrylate anhydride (inside a fume hood and lights off inside the fume hood and in the room if possible)**

   Methacrylic Anhydride is a photosensitive chemical and also oxidizes in the presence of air so we want to **protect it from light by covering it in foil and also from the air by adding an inert gas to the bottle (like Argon) after each use. Cover the bottle with parafilm once this is done and store it at 4°C.**

   a. Addition of methacrylic anhydride (MA) will depend on the methacrylation level you want to achieve. For example, if you want to create a 3% M (Methacrylation level) I would perform the following calculations: 3% = 0.03, 0.03 * 200 ml PBS = 6ml MA to be added dropwise.
   b. For this step, cover your beaker top with parafilm (paraffin needs to be removed and replaced with saran wrap at the end, otherwise the paraffin would melt into your solution ruining it)
   c. Load an 18 gauge needle syringe with the calculated MA.
   d. Puncture the parafilm in towards the center and add the MA dropwise.
      i. Make sure the gelatin solution is stirring vigorously to keep the slowly added MA well distributed in the solution; this is incredibly important to maintain the reaction as homogeneous as you can.
   e. The final concentration can range from between 0.1-10% v/v, with large [MA] eventually yielding stiffer gels

Download

General_GelMA_Methacrylation_Protocol_24_.pdf (80.2 kB)

04-18-2023 Cell Viability and Degradation Assays

CARLEY SCHWARTZ - Apr 18, 2023, 9:01 AM CDT

Title: testing research

Date: 04-18-23

Content by: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3267487/

Present: Carley Schwartz

Goals: To learn more about testing ideas after cell culturing

Content:

EMT is a biological process whereby polarized epithelial cells undergo morphological changes to assume a mesenchymal phenotype. EMT has been characterised by the switch from epithelial (E-cadherin) to mesenchymal (N-cadherin) calcium-ion-dependent adhesion accompanied by the gain of other markers such as α-SMA. During the process of EMT cells become more motile, invasive and gain resistance to apoptosis

Several transcription and growth factors have been implicated in the pathogenesis of IPF and are recognised drivers of EMT. The transcription factor Twist, (not normally expressed in healthy human adult lung) inhibits proliferation and differentiation of cells and is proposed to drive EMT. Overexpression of Twist results in increased N-cadherin which in turn leads to a decrease in E-cadherin. Hypoxia or mechanical stresses are known to induce Twist expression

Cell proliferation associated antigen Ki-67 and cell cycle regulator p16INK4A were also evaluated to examine wound remodelling mechanisms via cell division.

In all control lung samples, E-cadherin expression was evident in the cell membrane of ATII cells and bronchiolar epithelium (mean expression score 4.21, 33-75%) . In IPF, increased E-cadherin expression was observed in cell membranes of hyperplastic ATII cells present in areas of interstitial fibrosis and overlying the fibroblastic foci (mean expression score 5, >75%) . E-cadherin expression was absent within fibroblastic foci

Antigen Ki-67 expression was negligible in the nuclei of control lung ATII cells (mean expression score 0.21, <1%) compared to the IPF group where expression was significantly elevated (mean expression score 1.76, 1% -10%) $p \leq 0.05$

p16INK4A expression was absent or negligible in ATII cells of control samples (mean expression score 0.52, <1%) . In IPF tissue samples cytoplasmic p16INK4A expression was observed (mean expression score 4.61, >75%) in ATII cells directly overlying fibroblastic foci

Overview: N-cadherin expression within ATII cells had a strong positive correlation with disease activity (Pearson correlation co-efficient 0.557). Ki-67 expression in ATII cells had a moderate negative correlation (Pearson correlation co-efficient -0.366). Within the fibroblastic foci we demonstrated moderate positive correlations for Twist (Pearson correlation co-efficient 0.402), Ki-67 (0.32) and Collagen I (Pearson correlation coefficient 0.468).

Conclusions/action items:

Honestly not sure how useful this article was. Discussed studying the expression of EMT transformation markers which could be useful to see if there are a higher amount of markers on stiffer gels vs not as stiff ones to assess how well they are replicating their environment.

04-18-23 Cell Viability

CARLEY SCHWARTZ - Apr 18, 2023, 9:08 AM CDT

Title: Cell viability assays

Date: 04-18-23

Content by: https://www.cellsignal.com/science-resources/cell-viability-and-survival#:~:text=Cell%20proliferation%20assays%20are%20performed,immunofluorescence%20and%20high%20content%20imaging.

Present: Carley

Goals: To learn more about cell viability assays and how that might fit into hydrogels

Content:

Cell viability is a measure of the proportion of live, healthy cells within a population. Cell viability assays are used to determine the overall health of cells, optimize culture or experimental conditions, and to measure cell survival following treatment with compounds, such as during a drug screen.

Typically, cell viability assays provide a readout of cell health through measurement of metabolic activity, ATP content, or cell proliferation. Cell viability can also be assessed using cell toxicity assays that provide a readout on markers of cell death, such as a loss of membrane integrity.

Cell proliferation assays are performed using standard methods, including enzyme-linked immunosorbent assay (ELISA), flow cytometry, immunofluorescence and high content imaging. Would all of these require breakdown of matrix and separation of cells to measure bc flow cytometry would?

Detection of proliferation proteins

Dividing cells have high expression of cell cycle proteins compared to quiescent or senescent cells, thus the level of cell cycle-specific proteins can be measured as a readout of cell proliferation. Some common proliferation proteins include proliferating cell nuclear antigen (PCNA), Ki67, and Phospho-histone H3, which can be detected using western blot (WB), IF, IHC, flow cytometry, and ELISA. Other paper also discussed doing IHC with ones particular to normal vs fibrotic cell state, could we use that to look at both cell viability and affect of mechanical stress at the same time?

Cell Viability: ATP measurement assays quantify ATP content to determine the number of viable, metabolically active cells in a sample. These assays are performed in multiwell plates with a colorimetric, fluorometric, or luminescent readout of a metabolic activity requiring ATP, where substrate generation is proportional to the number of healthy cells with active mitochondria.

Conclusions/action items: Might want to consider both flow cytometry after matrix breakdown to count cells but also could look into IHC.

02-10-23 GelMA Testing Brain Storm - Degradation testing

CARLEY SCHWARTZ - Feb 28, 2023, 11:44 AM CST

Title: testing ideas

Date: 02-10-23 [Hi I reopened this on the 28th to add more to it but I'm not sure how to save it without changing the date]

Content by: https://iopscience.iop.org/article/10.1088/1748-605X/ac1e9d/pdf (source for #1 degradation assay) and https://www.frontiersin.org/articles/10.3389/frsfm.2022.1101680/full (source #2 degradation assay)

Used two articles to cross references processes and determine some of the different factors we need to consider.

Present: Carley

Goals: to start thinking about GelMA test besides rheology and how they might work

Content:

#1 Degradation assay A polytetrafluoroethylene (PTFE) chamber with a diameter of 10 mm and a height of 6 mm with 471 µl volume was filled with sterilized warm GelMA 10 wt% solution containing LAP as a photoinitiator and crosslinked for 30 s using a handheld UV-lamp (395–400 nm; 80–150 mcd) (EFL41UV UV, Perel, Gavere, Belgium). The chambers were then incubated in DPBS and DPBS containing 1.75 µg ml−1 collagenase (Sigma-Aldrich) in a humidified atmosphere (37 ◦C, 95% relative humidity, 5% CO2) with a change of media three times a week. The weight of the chambers containing GelMA was taken over 14 d, where the empty chamber weight was subtracted and the degradation calculated via the mass loss.

In this article they constructed their hydrogels and then incubated them with PBS containing 1.75 ug/mL of collagenase and changed the media three times a week. The weight of the chambers were taken over 14 days. We could place each hydrogel in a petri dish and subtract the petri dish weight which will give us the mass loss.

Accelerated enzyme degradation study

ICC and bulk hydrogels made of GelMA (30 w/v%) were tested for enzymatic degradation in 2 mg/mL of collagenase type Ⅱ (125 CDU/mg solid) in Hank's Balanced Salt Solution (HBSS), containing 3 mM CaCl2. Surface morphology of the GelMA ICC hydrogels and GelMA hydrogels was observed by optical microscopy. For each degradation time point, gross images were taken, and mass loss of GelMA ICC and GelMA bulk hydrogels was simultaneously measured. The initial swollen weight (Wi) of each hydrogel sample (n = 5) was measured, and then each hydrogel sample was put into a 2 mg/mL collagenase type Ⅱ solution and was incubated at 37°C. At each degradation time point, each sample was taken out and washed with HBSS solution 3 times, and the excess surface water was removed using Kim-wipes, and the degraded weight (Wd) of each sample was recorded again.

In this one they also took the weight of the samples but didn't specify a time range. They also had higher %w/v hydrogels and used significantly more collagenase.

Conclusions/action items:

- Need to consider ordering collagenases or where we could obtain some for testing
  - We will need to devise what control groups we need, how many samples, will we test them in an ALI with the collagenase and media from the bottom or completely submerge? much to consider - talk to Dr. Masters
  - Need to consider based on out %w/v gelma and the size of the gels which much collagenase to use and maybe what type?

02-24-23 Shadowing Question and Notes

CARLEY SCHWARTZ - Feb 27, 2023, 6:09 PM CST

Download

IMG_1758.HEIC (1.97 MB)

CARLEY SCHWARTZ - Feb 27, 2023, 6:09 PM CST

Download

IMG_1757.HEIC (2.02 MB)

03-07-2023 WARF lecture

CARLEY SCHWARTZ - Mar 07, 2023, 5:01 PM CST

Title: WARF Lecture Notes

Content:

- Speakers: Justin Anderson and Janine Burmania
- WARF: technology transfer, patenting and licensing
    - one of the first to patent innovations from campuses and patent them to industry
    - governed by an independent board of trustees
- Key piece of intellectual property is a patent
- 3 billion dollars total that WARF has given back to the university
- You can have a patent on a method of doing something
- Could have a patent on a machine or a device
    - composition of matter, improvements on an existing process of method or a trade secret
        - Trade secret can only be protected so long as its a secret
- Grace period is a year to file for a patent after your first public disclosure
- withholding information -> not enabling -> not public disclosure
- Examiner: Is this in nature? is it a mathematical concept? is it new?
- WARF doesn't draft its own patent applications and use outside counsel
- What is WARFs track record? lots of success in EGG and medical devices/imaging and pharmaceuticals
- Market: contacting companies? Start up company? research project?
- Third party vendors that can push more globally to do some targeted marketing and identify companies
- Patent is valid and enforceable for 20 years from date filed

Conclusions/action items:

While we aren't creating a device if a novel approach to hydrogel creation for the lung ECM is used this would be under the classification of patentable?

Tong Lecture Notes

CARLEY SCHWARTZ - May 03, 2023, 9:00 PM CDT

Speaker: Jinger Zeng- founder of a startup

She discussed the interrelationship between entrepreneurship and engineering. She works as a product manager to design the contests and promote new ideas for the hackster.io internet company. She also created a drone to win a grant for sky works but also discussed the hardships she faced with failures that occur during the design process.

2023/30/01-Client Meeting 1 Overview

CARLEY SCHWARTZ - Feb 17, 2023, 8:17 AM CST

Title: client meeting 1

Date: 01-30-23

Content by: Carley and Elijah

Present: Self, Elijah, Client

Goals: To discuss PEG troubleshooting, future GelMA work, and client meetings in this semester

Content:

Key Points:

- Client meetings will be every other week and for this month it will be the 9th and the 27th on Thursdays at 11:30 am
- He prefers us to try and trouble shoot with PEG but likes the idea of GelMA as well
- Wants to do testing on a range of mechanical properties so we will have lower and higher compressive moduli of lung ECM (normal vs fibrotic)

Conclusions/action items:

- Need to assign roles for each person and delegate the research between everyone
- Need to attend Dr. Masters' lab to watch GelMA preparation
- See if we can troubleshoot PEG w any extra I2959 or LAP that Dr. Masters' lab has

2023/9/02-Client Meeting 2 Overview

CARLEY SCHWARTZ - Feb 17, 2023, 8:28 AM CST

Title: Client Meeting 2

Date: 02-09-23

Content by: Self and Elijah

Present: Self, Elijah, Client

Goals: To discuss GelMA, future work he wants to conduct with the gels (cell encapsulation and culturing)

Content:

Key Points

- Wants to first encapsulate fibrinectin and collagen then move onto fibroblasts
- Wants to try collagen or fibronectin coating on any gels even if they do not have cells encapsulated
- Wants the gel to degrade with time to allow for fibroblast reconstruct the ECM but wants to place epithelium cells onto the gel surface right after construction
    - this is due to him wanting to have communication between the epithelial cells and the proteins within the gel for as long as possible

Conclusions/action items:

- Need to understand the degradation of GelMA further
- Need to apply these client requirements to our design matrix and search literature for how these design fit within it

Fibroblast Spreading and Focal Adhesion

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 28, 2023, 5:06 PM CST

Title: The combined influence of viscoelastic and adhesive cues on Fibroblast spreading and Focal Adhesion Organization

Date: 2-28-2022

Content by: Elijah Diederich

Present: Myself

Goals: To understand the importance of including viscoelasticity in hydrogels

Content:

****PDF w/ notes attached below****

Citation: E. Hui, L. Moretti, T. H. Barker, and S. R. Caliari, “The combined influence of viscoelastic and adhesive cues on fibroblast spreading and focal adhesion organization,” *Cellular and molecular bioengineering*, 02-Jun-2021. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8548477/. [Accessed: 28-Feb-2023].

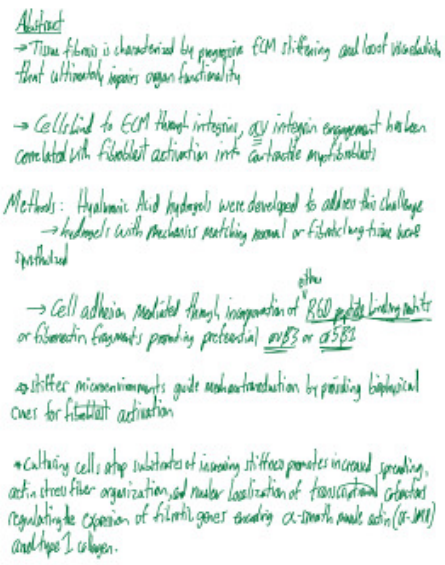
Conclusions/action items:

1. Research more on Gel-MA mechanical properties and G', G" values specifically

2. Ask Dr. Masters about "Supramolecular" guest-host interactions to introduce viscous characteristics in hydrogel system.

3. Research cell shape importance and check with team members on Cell profiler equation.

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 28, 2023, 5:07 PM CST



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The_combined_influence_of_viscoelastic_and_adhesive_cues_on_Fibroblast_spreading_and_Focal_Adhesion_Organization.pdf (2.75 MB)

Lung Extracellular Matrix and Fibroblast function

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 28, 2023, 9:06 PM CST

Title: Lung Extracellular Matrix and Fibroblast Function

Date: 2-28-2023

Content by: Elijah Diederich

Present: Myself

Goals: To review and reinforce base knowledge of fibroblasts in healthy and fibrotic lung tissue

Content:

PDF w/ notes attached below

Citation: E. S. White, “Lung extracellular matrix and fibroblast function,” *Annals of the American Thoracic Society*, 01-Mar-2015. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4430981/. [Accessed: 28-Feb-2023].

Conclusions/action items:

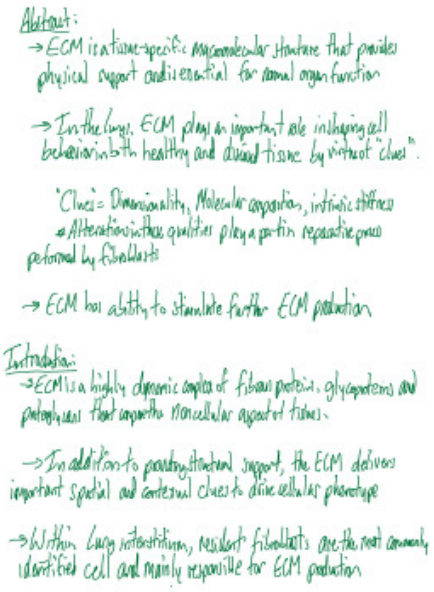
1. Phenotypic behavior of fibroblasts depends on the position of the cell in space

2. Tissue culture plastic has E= 2-4 GPa which is what client is currently using

a. This high of a Young's Modulus has detrimental impact on fibroblast phenotype

3. ECM within a physiologic range of stiffness is capable of reversing the activated myofibroblast phenotype.

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 28, 2023, 9:08 PM CST



Download

Lung_ECM_and_Fibroblast_Function.pdf (1.63 MB)

Nanofibrillar Cellulose Hydrogel (NFC)

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 09, 2023, 9:22 PM CST

Title: Nanofibrillar Cellulose Hydrogel (NFC)

Date: 2-2-2023

Content by: Elijah Diederich

Present: Me

Goals: To explore alternative options besides PEG hydrogels that could work for our EMTU lung matrix

Content:

- Carley Schwartz (Co-leader) shared this article with me as a potential alternative option to PEG

Citation: M. Bhattacharya, M. Malinen, P. Lauren, Y.-R. Lou, S. Kuisma, L. Kanninen, M. Lille, A. Corlu, C. Guguen-Guilluizo, M. Yliperttula, A. Urtti, A. Laukkanen, and O. Ikkala, “Nanofibrillar cellulose hydrogel promotes three-dimensional liver cell culture,” *Journal of Controlled Release*, 06-Jul-2012. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0168365912005391?via%3Dihub. [Accessed: 10-Feb-2023].

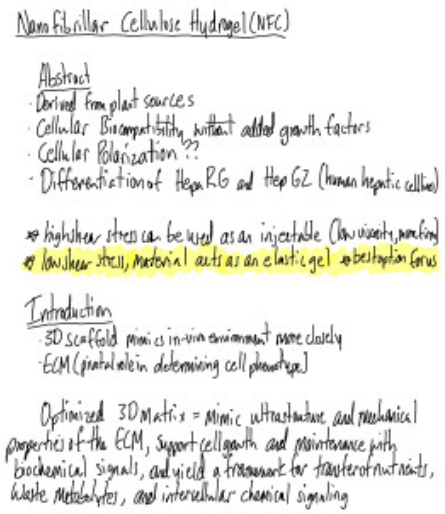
PDF attached below with notes

Conclusions/action items:

1. Research ECM Viscoelastic Properties

2. Set up weekly meetings with client

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 09, 2023, 9:28 PM CST


Nanofibrillar Cellulose Hydrogel (NFC)

Abstract
- Derived from plant sources
- Cellular Biocompatibility without added growth factors
- Cellular Polarization ??
- Differentiation of HepaRG and HepG2 (human hepatic cell line)

* high shear stress can be used as an injectable (low viscosity, printing)
* low shear stress, material acts as an elastic gel * best option for us

Introduction
- 3D scaffold mimics in-vivo environment more closely
- ECM (giant role in determining cell phenotype)

Optimized 3D matrix = mimic ultrastructure and mechanical properties of the ECM, support cell growth and maintenance with biochemical signals, and yield a framework for transfer of nutrients, waste metabolites, and intercellular chemical signaling

Download

Nanofibrillar_cellulose_hydrogel_promotes_3D_liver_cell_culture.pdf (4.71 MB)

Hydrogels derived from Decellularized Lung Tissue Supports Cholangiocyte Organoids

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 27, 2023, 3:41 PM CST

Title: Lung ECM Hydrogels from Decellularized Liver Tissue

Date: 2-13-2023

Content by: Elijah Diederich

Present: Myself

Goals: To research Lung ECM hydrogels as a potential competing design

Content:

Citation: J. Willemse, G. van Tienderen, E. Van Hengel, I. Schurink, D. Van der Ven, and Y. Kan, “Hydrogels derived from decellularized liver tissue support the growth and differentiation of cholangiocyte organoids,” *Biomaterials*, 24-Mar-2022. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0142961222001120?via=ihub. [Accessed: 12-Feb-2023].

Notes taken: 2-13-23

Conclusions Updated: 2-27-23

****PDF notes attached below****

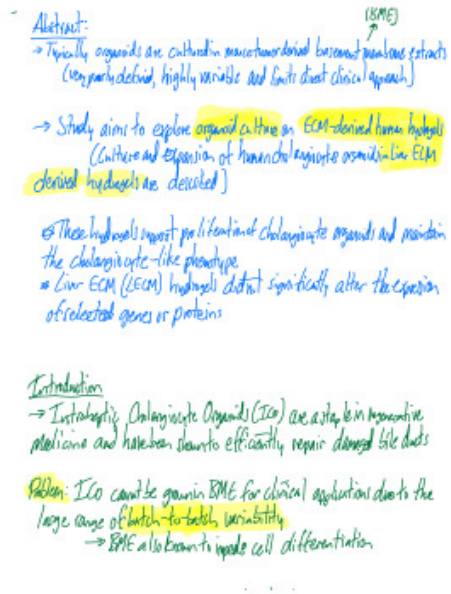
Conclusions/action items:

1. Tuning of Lung ECM hydrogels to match in-vivo ECM is very challenging

2. Lower cell proliferation compared to other commercial products

3. Very expensive and hard to obtain

4. Hard to create (Check materials and methods decellularization process)



Download

Hydrogels_derived_from_decellularized_liver_tissue_supports_cholangiocyte_organoids_.pdf (2.85 MB)

Human Lung ECM hydrogels - Stiffness + Viscoelasticity

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 09, 2023, 9:08 PM CST

Title: Human Lung ECM Hydrogels resemble the stiffness and viscoelasticity of native lung tissue

Date: 2-4-2023

Content by: Elijah Diederich

Present: Me

Goals: To gain insight on hydrogels and their ability to accurately mimic native and diseased lung tissue

Content:

PDF of notes attached below

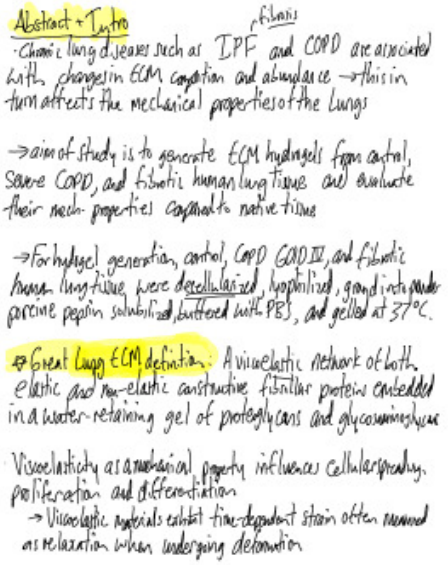
Citation: R. H. J. de Hilster, P. K. Sharma, M. R. Jonker, E. S. White, E. A. Gercama, M. Roobeek, W. Timens, M. C. Harmsen, M. N. Hylkema, and J. K. Burgess, “Human lung extracellular matrix hydrogels resemble the stiffness and viscoelasticity of native lung tissue,” *American journal of physiology. Lung cellular and molecular physiology*, 01-Apr-2020. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7191637/. [Accessed: 10-Feb-2023].

Conclusions/action items:

1. Prepare for Client meeting 2-9

2. Start working on PDS assignments

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 09, 2023, 9:08 PM CST



Download

Human_lung_ECM_hydrogels_resemble_the_stiffness_and_viscoelasticity_of_native_lung_tissue.pdf (1.46 MB)

Client Meeting #1

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 09, 2023, 9:35 PM CST

Title: Client Meeting #1

Date: 1-30-2023

Content by: Elijah Diederich

Present: Carley Schwartz and I

Goals: To meet with Dr. Brasier and discuss the semester design plan

Content:

PDF included below of notes taken during client meeting

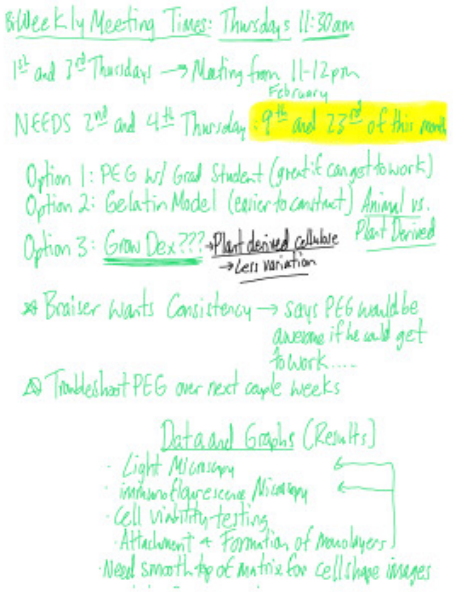
Conclusions/action items:

1. Talk to BME 430 professor about MMP concentrations in hydrogel

2. Assign specific research sections for upcoming report

3. Get everyone caught up to speed on PEG

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 09, 2023, 9:36 PM CST



Download

Client_Meeting_1_.pdf (1.68 MB)

Client Meeting #2

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 09, 2023, 9:38 PM CST

Title: Client Meeting #2

Date: 2-9-2023

Content by: Elijah Diederich

Present: Carley Schwartz and I

Goals: To lock design specifications and pitch GELMA as alternative option to PEG

Content:

*****PDF attached below with notes taken during client meeting #2*

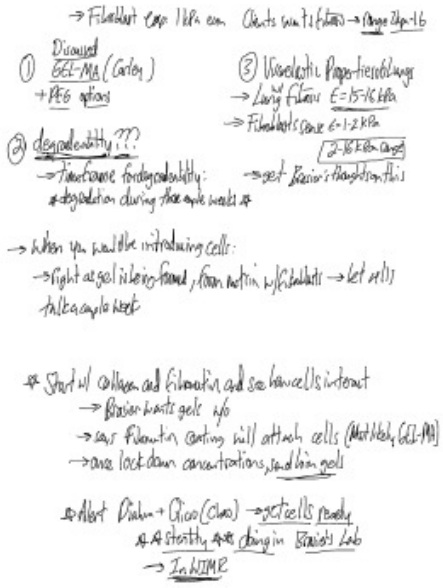
Conclusions/action items:

1. Start to get prepared for design matrix

2. Research more on GELMA

3. Client meeting tomorrow 2-10

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 09, 2023, 9:40 PM CST



Download

Client_meeting_2_2_.pdf (575 kB)

Client Meeting #3

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 27, 2023, 4:07 PM CST

Title: Client Meeting #3

Date: 2-23-23

Content by: Elijah Diederich

Present: Carly Schwartz, Nick Herbst, Myself

Goals: To inform client of progress and determine certain assays

Content:

PDF of Client Meeting #3 Notes

Notes taken 2-23-23

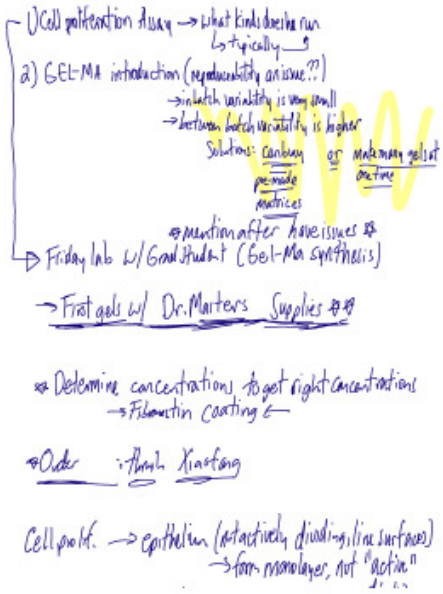
Conclusions/action items:

1. More worried about Cell viability assay vs. Cell proliferation (Cells not actively dividing, form monolayer)

2. Cell viability assays (Live-Dead assay or MTT assay)

3. At end, would like to use dissolve matrix and isolate individual cells if possible (Flow Cytometry mentioned) --> Ask Masters

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 27, 2023, 4:06 PM CST



Download

Client_Meeting_3.pdf (973 kB)

Client Meeting #4

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 11:55 AM CDT

Title: Client Meeting #4

Date: 3-9-23

Content by: Elijah Diederich

Present: Carley, Anuraag, Dr. Brasier

Goals: To see how first batch of gels performed (Cell adhesion etc....)

Content:

*****PDF with client meeting notes attached below*

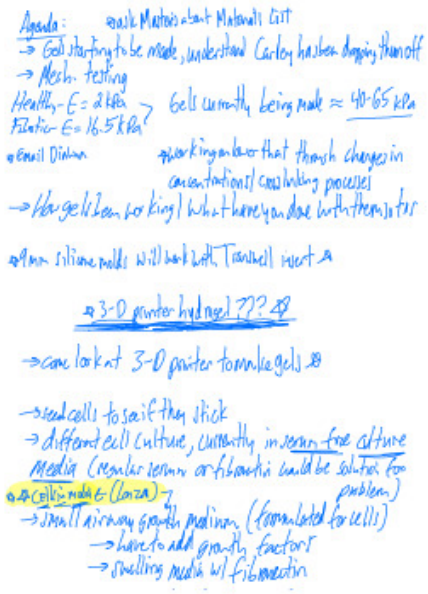
Conclusions/action items:

1. Email Dianhua with Material orders

2. Ask Dr. Masters about adhesion issue

3. Work on making gels with lower stiffness

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 11:38 AM CDT



Download

Client_Meeting_4_2_.pdf (807 kB)

Client Meeting #5

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 11:54 AM CDT

Title: Client Meeting #5

Date: 3-30-23

Content by: Elijah Diederich

Present: Carley, Anuraag, Dr. Brasier

Goals: To determine to-do items for last 3 weeks of project

Content:

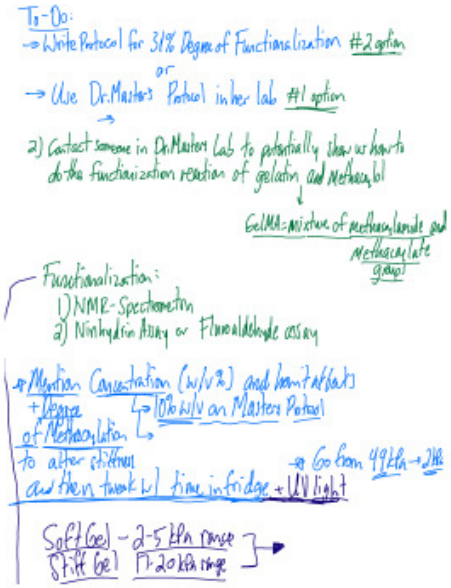
***** PDF with client meeting notes attached below*

Conclusions/action items:

1. Run GelMA reaction when materials arrive

2. Start prepping for final report/presentation

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 11:47 AM CDT



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Client_Meeting_5.pdf (695 kB)

Client Meeting #6

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 11:54 AM CDT

Title: Client Meeting #6

Date: 4-13-23

Content by: Elijah Diederich

Present: Anuraag, Dianhua

Goals: Discuss Normal kPa stiffness hydrogels and cell adhesion

Content:

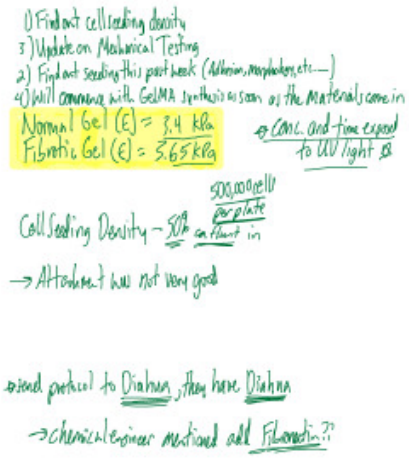
*****PDF with client meeting #6 attached below*

Conclusions/action items:

1. Talk to Dr. Masters about cell adhesion problem

2. Continue to prep for poster presentation

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 11:54 AM CDT



Download

Client_Meeting_6.pdf (429 kB)

Advisor Meeting #1

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 09, 2023, 10:01 PM CST

Title: Advisor Meeting #1

Date: 2-3-2023

Content by: Elijah Diederich

Present: All group members

Goals: Meet advisor and introduce group members

Content:

PDF attached below with notes taken during advisor meeting

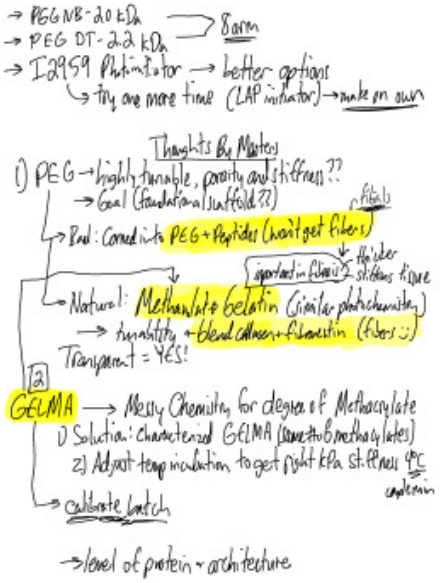
Conclusions/action items:

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 17, 2023, 7:24 PM CST

Conclusion/action items:

1. Look into Gel-MA as a potential alternate design option (will form fibrils compared to PEG which won't)
2. Gel-MA is very tunable + blend collagen and fibronectin
3. Think about changing to 4 intermediate notebook grades vs. 1 midterm notebook grade check

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 17, 2023, 7:25 PM CST



Download

Advisor_meeting_1_1_.pdf (951 kB)

Advisor Meeting #2

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 27, 2023, 4:10 PM CST

Title: Advisor Meeting #2

Date: 2-17-2023

Content by: Elijah Diederich

Present: Group members

Goals: To inform Dr. Masters about the Week's progress and upcoming to-do list items

Content:

****PDF of Notes attached below****

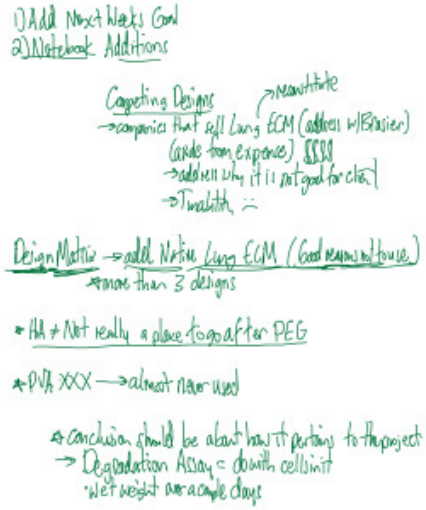
Notes taken: 2-10-2023

Conclusions/action items:

1. Look into degradation assay - wet weight over time period

2. Add native lung ECM to design matrix

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 27, 2023, 4:11 PM CST



Download

Advisor_meeting_2.pdf (431 kB)

Advisor Meeting #3

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 27, 2023, 4:07 PM CST

Title: Advisor Meeting #3

Date: 2-27-23

Content by: Elijah Diederich

Present: Group Members

Goals: To get feedback on previously submitted PDS and testing assays

Content:

****PDF w/ notes attached below****

Advisor Meeting occurred on 2-17-2023

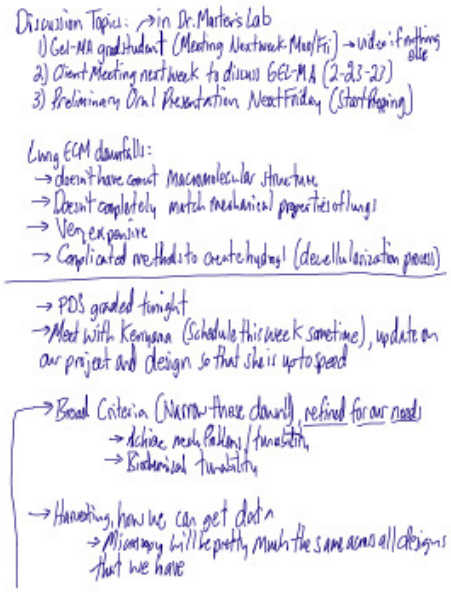
Conclusions/action items:

1. It's OK to have redundancy in the design criteria

2. Ask Brasier about cell proliferation assays that he runs

3. Make sure to wear pants to Lab

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 27, 2023, 3:53 PM CST



Download

Advisor_Meeting_3.pdf (1.13 MB)

Advisor Meeting #4

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 11:59 AM CDT

Title: Advisor Meeting #4

Date: 3-3-23

Content by: Elijah Diederich

Present: EMTU Team

Goals: To get feedback on preliminary presentation and discuss client meeting

Content:

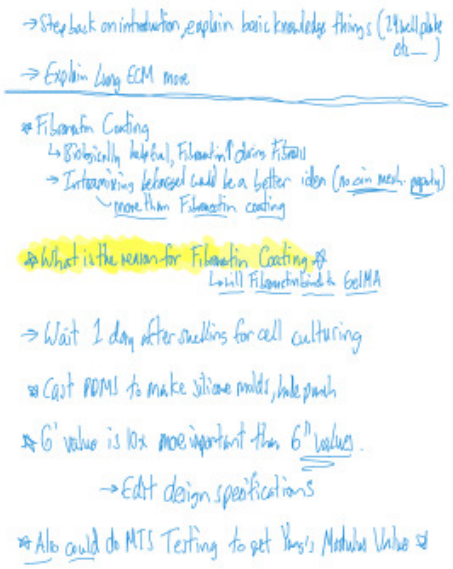
***** Advisor Meeting #4 notes attached below *

Conclusions/action items:

1. Edit Design Specs and take out viscoelastic modulus values

2. Discuss Fibronectin coating with client (Pros/Cons)

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 12:00 PM CDT



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Advisor_Meeting_4.pdf (540 kB)

Advisor Meeting #6

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 12:03 PM CDT

Title: Advisor Meeting #6

Date: 4-7-23

Content by: Elijah Diederich

Present: EMTU Team

Goals: Discuss Protocols and Materials for GelMA reaction

Content:

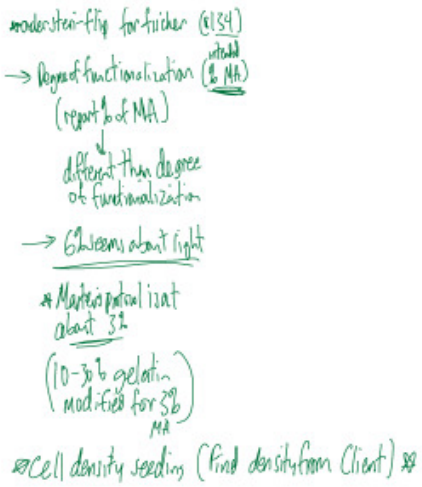
***** PDF of Advisor Meeting #6 notes attached below *

Conclusions/action items:

1. Do second round of material ordering

2. Find out cell density seeding from client

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 12:04 PM CDT



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Advisor_Meeting_6.pdf (257 kB)

Advisor Meeting #7

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 12:07 PM CDT

Title: Advisor Meeting #7

Date: 4-14-23

Content by: Elijah Diederich

Present: EMTU Team

Goals: To discuss most recent batch of low kPa stiffness gels

Content:

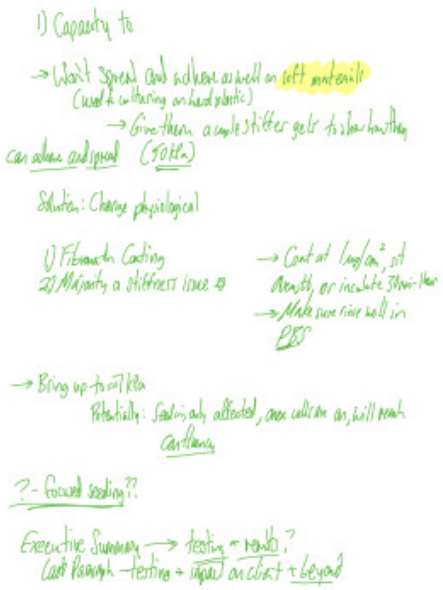
***** PDF of Advisor Meeting #7 Notes Below *

Conclusions/action items:

1. Make gel with high kPa stiffness to prove that soft materials have a harder time with cell adhesion

2. Edit Executive Summary

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 12:08 PM CDT



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Advisor_meeting_7.pdf (492 kB)

Advisor Meeting #8

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 12:11 PM CDT

Title: Advisor Meeting #8

Date: 4-14-23

Content by: Elijah Diederich

Present: EMTU Team

Goals: To discuss our latest round of gels and a potential fibronectin coating

Content:

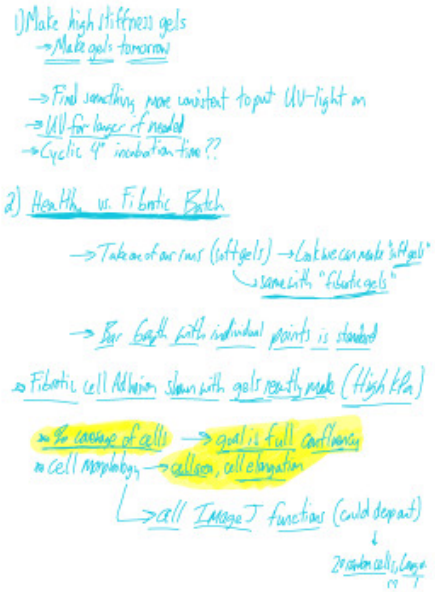
***** PDF of Advisor Meeting #8 Notes attached Below *

Conclusions/action items:

1. Get ready for presentation and final report

2. Look into ImageJ as a potential testing platform for confluency/cell morphology

ELIJAH DIEDERICH (ediederich@wisc.edu) - May 03, 2023, 12:11 PM CDT



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Advisor_Meeting_8.pdf (771 kB)

Gelatin Methacrylate Hydrogel for Tissue Engineering Applications

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 27, 2023, 9:09 PM CST

Title: Gel-MA hydrogel for Tissue Engineering Applications

Date: 2-27-2023

Content by: Elijah Diederich

Present: Myself

Goals: To inform myself on the capabilities of Gel-MA as the team continues forward with this design

Content:

PDF w/ notes attached below

Citation: S. Bupphathong, C.-H. Lin, H.-Y. Tao, P.-F. Chung, W. Huang, and C. Quiroz, “Gelatin methacrylate hydrogel for tissue engineering applications-a review on Material Modifications,” *Pharmaceuticals (Basel, Switzerland)*, 29-Jan-2022. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/35215284/. [Accessed: 27-Feb-2023].

Conclusions/action items:

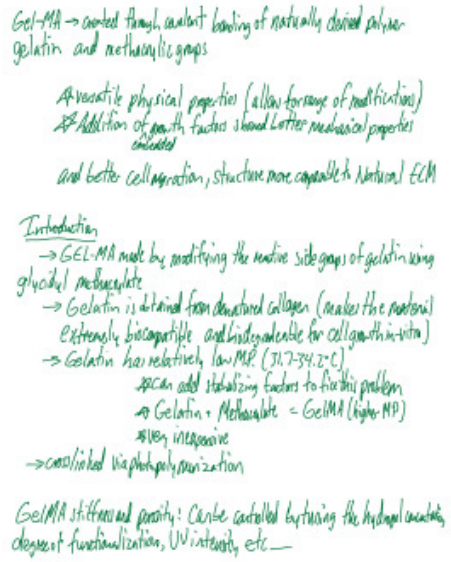
1. Gelatin is formed from denatured collagen which means this gel will be both biocompatible and biodegradable

2. Gel-MA has versatile physical properties that can be tuned

3. Gel-MA stiffness and porosity can be controlled by tuning the hydrogel concentration, degree of functionalization, UV intensity etc...

4. Can polymerize Gel-MA with polymers such as Alginate to increase properties and tier the design to its intended use

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 27, 2023, 9:12 PM CST



Download

Gelatin_Methacrylate_Hydrogel_for_Tissue_Engineering_Applications.pdf (2.15 MB)

GelMA-Collagen 3D printed Hydrogel

ELIJAH DIEDERICH (ediederich@wisc.edu) - Mar 01, 2023, 3:37 PM CST

Title: 3D-Printed Hybrid Collagen/GelMA Hydrogels for Tissue Engineering Applications

Date: 3-1-2023

Content by: Elijah Diederich

Present: Myself

Goals: To learn about collagen influences in a GelMA hydrogel and possible concentrations of collagen in a hydrogel

Content:

PDF w/ notes attached below

Citation: Nagaraj, Anushree, et al. “3D-Printed Hybrid Collagen/GELMA Hydrogels for Tissue Engineering Applications.” *Biology*, U.S. National Library of Medicine, 25 Oct. 2022, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9687496/.

Conclusions/action items:

1. Continue to research articles on collagen concentration for further design considerations

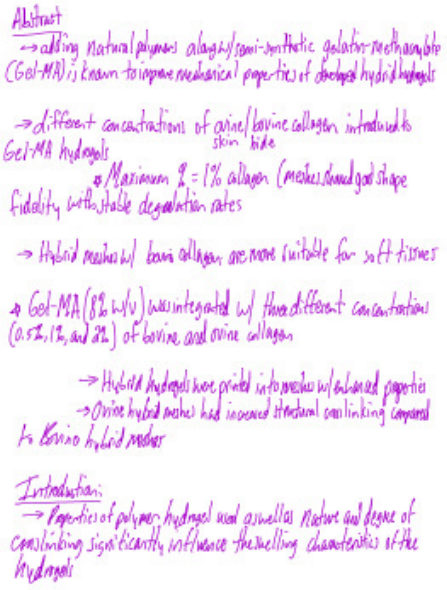
2. Maximum 1% collagen concentration to ensure constant degradation

3. 4 days until matrix was completely degraded

4. Bovine collagen best choice for soft tissue engineering applications

5. Degree of Methacrylation equation in notes

ELIJAH DIEDERICH (ediederich@wisc.edu) - Mar 01, 2023, 3:37 PM CST



Download

3D-Printed_Hybrid_Collagen-GelMA_Hydrogels_for_Tissue_Engineering_Applications.pdf (2.12 MB)

WARF Presentation - 3/15/2019

ELIJAH DIEDERICH (ediederich@wisc.edu) - Mar 19, 2023, 2:53 PM CDT

Title: WARF Presentation

Date: 3-19-2023

Content by: Elijah Diederich

Present: Myself

Goals: To gain introductory knowledge of the WARF on the UW-Madison campus

Content:

I think that the design that I am currently working on in BME 301 could have intellectual property in the field of research.

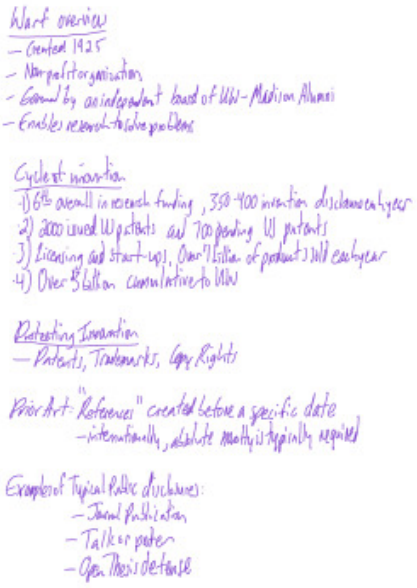
PDF of notes attached below

Conclusions/action items:

1. Get gels ready for show-n-tell this week

2. Research cells/serums

ELIJAH DIEDERICH (ediederich@wisc.edu) - Mar 19, 2023, 2:54 PM CDT



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WARF_Presentation.pdf (1.31 MB)

Ashley Scott Gel-MA Training

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 27, 2023, 9:20 PM CST

Title: Gel-MA Training

Date: 2-24-23

Content by: Elijah Diederich

Present: Carley, Will, and I

Goals: Learning how to form Gel-MA hydrogels from grad student

Content:

PDF w/ Notes from meeting attached below

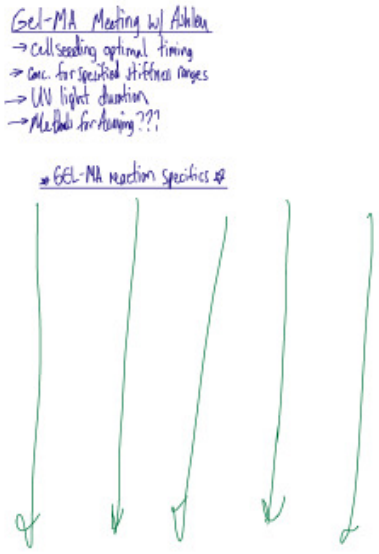
Team was also given a basic protocol on steps for forming these gels

Conclusions/action items:

1. Reach out to Dr. Brasier's team to figure out when to deliver first batch of gels

2. Get in lab and make some GELS!!!

ELIJAH DIEDERICH (ediederich@wisc.edu) - Feb 27, 2023, 9:21 PM CST



Download

Grad_Student_Meeting_2_.pdf (865 kB)

Synthesis, Properties, and Biomedical Applications of GelMA hydrogels

ELIJAH DIEDERICH (ediederich@wisc.edu) - Mar 28, 2023, 6:55 PM CDT

Title: Synthesis, Properties, and Biomedical Applications of Gel-MA hydrogels

Date: 3-27-2023

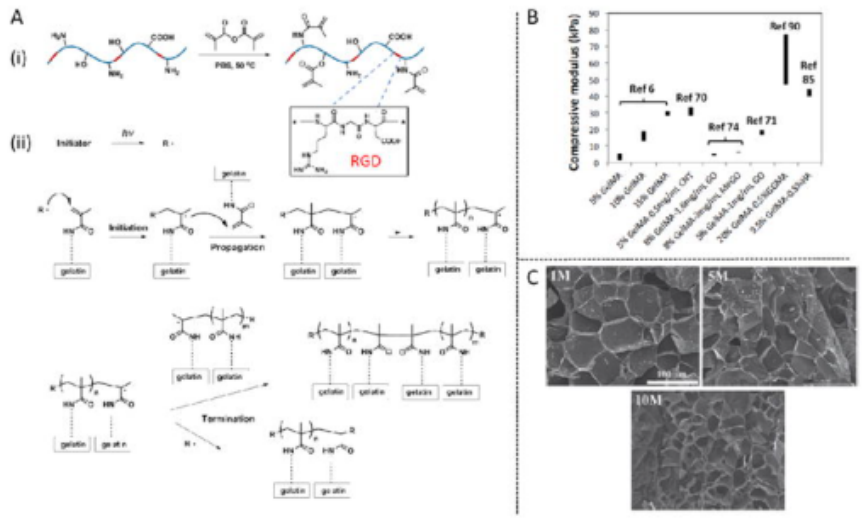
Content by: Elijah Diederich

Present: Myself

Goals: To better understand the synthesis of Gel-MA so that the process can be completed by our group

Content:

Citation: K. Yue, G. Trujillo-de Santiago, M. M. Alvarez, A. Tamayol, N. Annabi, and A. Khademhosseini, “Synthesis, properties, and biomedical applications of gelatin methacryloyl (gelma) hydrogels,” *Biomaterials*, 01-Dec-2015. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4610009/. [Accessed: 27-Mar-2023].



Synthesis and characterization of GelMA hydrogels. (A) Scheme for preparation of photocrosslinked GelMA hydrogel. (i) Reaction of gelatin and methacrylic anhydride for grafting of methacryloyl substitution groups. The modification occurs at primary amine and hydroxyl groups. The RGD domains are illustrated as red segments along the GelMA chains, and their chemical structure is depicted within the inset. (ii) Representative reactions during the photocrosslinking of GelMA to form hydrogel networks. Free radicals are generated from photoinitiators, which initiate the chain polymerization of the methacryloyl substitutions. Propagation occurs between methacryloyl groups located on the same chain and on different chains. Termination occurs between two propagating chains or between one propagating chain and a second radical. Chain transfers and many other minor reactions are not shown, for clarity. (B) The compressive modulus reported by several studies on GelMA hydrogels [6, 70, 71, 74, 85, 90]. (C) SEM images of GelMA hydrogels, showing the effect of the degree of methacryloyl substitution on the pore sizes of GelMA hydrogels. Adapted from Chen *et al.* [9], with permission from Wiley, copyright 2012.

Figure 1 in article shows a great description of the reaction and GelMA from a chemical standpoint

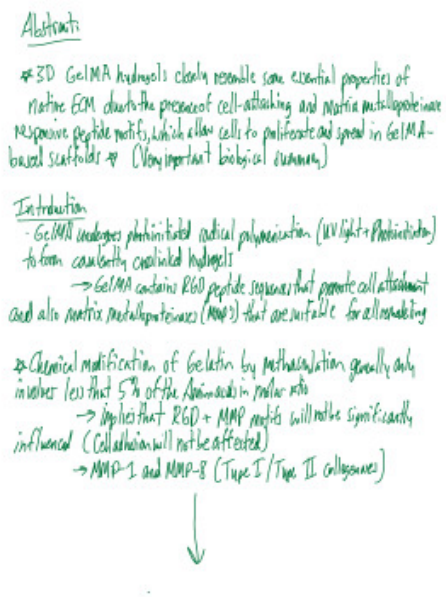
**********PDF w/ Notes attached below**********

Conclusions/action items:

1. **Continue to look at protocol's for Gel-MA**

2. **Schedule Client Meeting for 3-30-23**

ELIJAH DIEDERICH (ediederich@wisc.edu) - Mar 28, 2023, 6:58 PM CDT



Download

Synthesis_Properties_and_Biomedical_Applications_of_GelMA_Hydrogels.pdf (2.07 MB)

Functionalization, Preparation, and use of Gel-MA hydrogels as Tissue Culture Platforms

ELIJAH DIEDERICH (ediederich@wisc.edu) - Mar 28, 2023, 8:35 PM CDT

Title: Functionalization, Preparation and use of Gel-MA hydrogels as tissue culture platforms

Date: 3-28-2023

Content by: Elijah Diederich

Present: Myself

Goals: To better understand the synthesis and preparation of Gel-MA

Content:

Citation: D. Loessner, D. Hutmacher, A. Khademhosseini, F. Melchels, T. Klein, P. Levett, K. Yue, L. Martine, E. Kaemmerer, and C. Meinert, “Functionalization, preparation and use of cell-laden gelatin methacryloyl-based hydrogels as modular tissue culture platforms,” *Nature protocols*, 17-Mar-2016. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/26985572/. [Accessed: 29-Mar-2023].

EQUIPMENT GelMA functionalization, dialysis and lyophilization

1. Round-bottom flask with a magnetic stir bar CRITICAL Use a stir bar of sufficient size with a powerful stirrer to ensure good dispersion of methacrylic anhydride. Alternatively, an overhead stirrer with propeller agitator can be used instead of a magnetic stir bar.

2. Freeze-dryer

3. 50-ml centrifuge tubes with vented caps (0.2 µm pore size)—for example, Corning 50-ml mini bioreactor (Sigma-Aldrich, cat. no. CLS431720)

4. Sterile syringes (50-ml volume)

5. Syringe filter units or disposable vacuum filtration units with polyethersulfone (PES) membrane (0.2-µm pore size)—for example, Nalgene Rapid-Flow sterile disposable filters (Thermo Scientific, cat. no. 595-3320)

6. Dialysis membrane with a 12-kDa MWCO

7. CL-1000 UV cross-linker (UVP; or similar) with 365-nm wavelength tubes

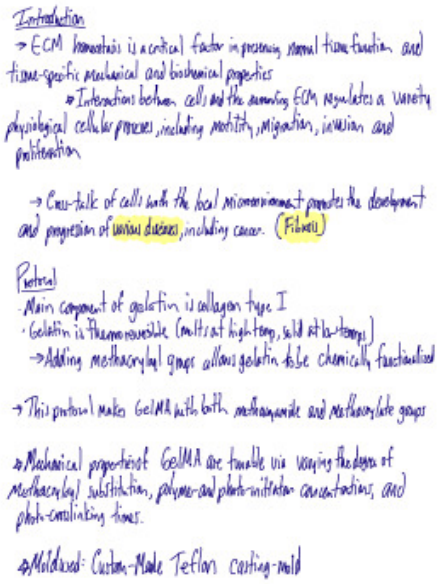
**********PDF w/ notes attached below**********

Conclusions/action items:

1. Continue to research protocols

2. Write protocol

3. Schedule client meeting



Download

Functionalization_Preparation_and_use_of_cell-laden_GelMA_hydrogels_as_tissue_culture_platforms.pdf (1.25 MB)

02/13/2023 Degradation and Swelling in GelMA-alginate hydrogels

ANURAAG SHREEKANTH BELAVADI - Feb 13, 2023, 4:41 PM CST

Title: Development of 3D bioprinted GelMA-alginate hydrogels with tunable mechanical properties

Date: 02/13/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Understand the mechanical properties of GelMA and its degree of tunability, specifically looking a degradation and swelling of GelMA hydrogels, to be applied in material selection and further hydrogel DEVICE.

Content:

- For optimum printing, gelMA-alginate concentrations should be between 10 and 15% w/v and the polymer ratio and concentration modulate the rheological and compressive moduli of hydrogels.
- Sheep adipose derived stem cells were included in these formulations and cell viability was >75% in all hydrogels.
- In general, in optimizing a material's printability, governed by its physical properties (e.g. viscosity/stiffness), non-linear viscoelasticity, etc.), the cell viability decreased. It has been shown that hydrogel viscosity directly affect shear stress, which is the main cause for loss of cell viability
- For the degradation and swelling assays, the samples were incubated in Phosphate Buffer Saline (PBS) (pH 7.4) at 37 °C over time and their changes in mass were determined at selected time points using a weight scale. Hydrogels discs were obtained with a diameter of 4 mm and a thickness of 2 mm. After washing the hydrogels with PBS, the degradation and swelling were tested.
- 3 mL of PBS @ the samples were added and placed the vials in an incubator at 37 °C. Inspections and supplementations of fresh buffer took place every day. Buffer was removed at 1 day, 7 days and 14 days and the samples were dried using a freeze-dryer. The dried samples were reweighed (Wd) and the degradation percentage (D) was calculated using
  - $D = [(W_o - W_d)/100]W_o$
- Hydrogels discs were added into pre-weighed triplicate glass vials and reweighed (initial weight of hydrogel, Wi). Then, 3 mL of PBS were added to the samples, and placed the vials in an incubator. Buffer was changed every day and vials reweighed at 1 h, 3 h, 6 h, 1 day, 3 days, 7 days and 14 days. The swelling ratio (S) was calculated as defined in Eq.
  - $S = (W_s - W_i)/W_i$
  - Gels with the lowest polymer concentration (i.e., G5A5) showed a swelling ratio of approximately 1.7 within 6 h of incubation. The degradation of G5A5 gels was zero and 30% after 24 h and 55% at 14 days. G5A7 gels swell in the first day to a ratio of about 2.5 while no degradation was observed at this time. By increasing the alginate concentration (G5A5 compared to G5A7), the degradation rate was slower, but the mass loss at 14 days was similar.

Download

02_13_2023_Degradation_and_Swelling_in_GelMA-alginate_hydrogels.docx (79.8 kB)

02/22/23 In Vitro and in vivo analysis of visible light crosslinkable gelatin methacryloyl (GelMA) hydrogels

ANURAAG SHREEKANTH BELAVADI - Feb 22, 2023, 2:11 PM CST

Title: In vitro and in vivo analysis of visible light crosslinkable gelatin methacryloyl (GelMA) hydrogels

Date: 02/22/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Understand the mechanical and biophysical properties of GelMA and their ability to support cell growth and differentiation and their potential for use in tissue integration

Content:

- The article presents a study on the use of visible light cross-linkable gelatin methacryloyl (GelMA) hydrogels for tissue engineering applications.
- The researchers used a visible light crosslinking method to create the GelMA hydrogels. This method uses a photosensitizer that is activated by visible light to initiate the crosslinking reaction, which allows for greater control over the crosslinking process and reduces the risk of damage to cells and tissues.
- The GelMA hydrogels were evaluated for their mechanical and biological properties. The researchers measured the swelling behavior and degradation of the hydrogels, as well as their ability to support cell growth and differentiation.
- The GelMA hydrogels were found to have mechanical properties similar to those of natural tissues, with a high degree of elasticity and a modulus that varied depending on the degree of cross-linking.
- The researchers also found that the GelMA hydrogels supported the growth and differentiation of different types of cells, including bone marrow-derived stem cells and chondrocytes. The hydrogels showed high cell viability and proliferation rates, and the cells were able to differentiate into the desired cell types.
- In vivo experiments were conducted using a mouse model. The GelMA hydrogels were implanted into the mice, and their ability to support tissue regeneration and integration with surrounding tissues was evaluated.
- The results of the in vivo experiments showed that the GelMA hydrogels were able to support tissue regeneration and integration, with no signs of inflammation or rejection. The hydrogels were also found to be biocompatible and biodegradable, with no significant adverse effects observed.
- The study suggests that visible light crosslinkable GelMA hydrogels have potential for use in a wide range of tissue engineering applications, including cartilage and bone regeneration, as well as drug delivery and wound healing. The use of visible light for crosslinking also makes the method more accessible and cost-effective than other methods that require UV light or chemical crosslinking agents.

Conclusions/action items:

- GelMA is a promising hydrogel material for tissue engineering applications, with high cell viability and proliferation rates, and the ability to support cell growth and differentiation.
- The GelMA hydrogels created using a visible light crosslinking method had mechanical properties similar to those of natural tissues, with a high degree of elasticity and a modulus that varied depending on the degree of crosslinking.
- The GelMA hydrogels were found to be biocompatible and biodegradable, with no significant adverse effects observed in in vivo experiments.
- The use of visible light for crosslinking GelMA hydrogels provides greater control over the crosslinking process and reduces the risk of damage to cells and tissues, making the method more accessible and cost-effective than other methods that require UV light or chemical crosslinking agents.
- GelMA hydrogels have potential for use in a wide range of tissue engineering applications, including cartilage and bone regeneration, as well as drug delivery and wound healing.

Citation:

I. Noshadi, S. Hong, K. E. Sullivan, E. Shirzaei Sani, R. Portillo-Lara, A. Tamayol, S. R. Shin, A. E. Gao, W. L. Stoppel, L. D. Black III, A. Khademhosseini, and N. Annabi, “In vitro and in vivo analysis of visible light crosslinkable gelatin methacryloyl (gelma) hydrogels,” *Biomaterials Science*, vol. 5, no. 10, pp. 2093–2105, Jul. 2017.

02/15/23 Human lung extracellular matrix hydrogels resemble the stiffness and viscoelasticity of native lung tissue

ANURAAG SHREEKANTH BELAVADI - Feb 22, 2023, 2:28 PM CST

Title: Human lung extracellular matrix hydrogels resemble the stiffness and viscoelasticity of native lung tissue

Date: 02/15/2022

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: To learn about the development of lung extracellular matrix (ECM) hydrogels as a potential biomaterial for use in lung tissue engineering applications. Also, learning more about the decellularization process, how lung ECM hydrogels are developed, and how aspects and characteristics of lung ECM scaffolds can be applied and tuned to in a different hydrogel.

Content:

- The article presents a study on the development of human lung extracellular matrix (ECM) hydrogels as a potential biomaterial for use in lung tissue engineering applications.
- The researchers used a decellularization process to remove the cells from human lung tissue, leaving behind the ECM. They then used this ECM to create hydrogels using a crosslinking method.
- The mechanical properties of the ECM hydrogels were evaluated using atomic force microscopy and rheological testing. The stiffness and viscoelasticity of the hydrogels were compared to those of native lung tissue.
- The results of the mechanical testing showed that the ECM hydrogels had stiffness and viscoelastic properties similar to those of native lung tissue. The hydrogels were able to withstand mechanical deformation and exhibited both elastic and viscous behavior, which are important properties for the function of lung tissue.
- The researchers also evaluated the biocompatibility of the ECM hydrogels by seeding them with human lung epithelial cells. The cells were able to adhere and proliferate on the hydrogels, indicating that the hydrogels were able to support cell growth and differentiation.
- The ECM hydrogels were further tested for their ability to support the growth and differentiation of human lung organoids. The organoids were able to attach and grow on the hydrogels, and showed evidence of differentiation into lung epithelial cells.
- The study suggests that human lung ECM hydrogels have potential for use in lung tissue engineering applications. The hydrogels have mechanical properties similar to those of native lung tissue, and are biocompatible and able to support cell growth and differentiation. The use of human lung ECM as the source material for the hydrogels also makes them a promising candidate for use in personalized medicine applications.

Conclusions/action items:

The use of human lung ECM as the source material for hydrogels makes them a promising candidate for use in personalized medicine applications, as well as in lung tissue engineering applications. Further research is needed to fully evaluate the potential of lung ECM hydrogels for use in lung tissue regeneration and repair, and to optimize the decellularization and crosslinking methods used to create the hydrogels. Further research is also necessary to figure out if the use of lung ECM hydrogels is even possible for the team within the scope of our project, especially the decellularization processes.

Citation:

R. H. de Hilster, P. K. Sharma, M. R. Jonker, E. S. White, E. A. Gercama, M. Roobeek, W. Timens, M. C. Harmsen, M. N. Hylkema, and J. K. Burgess, “Human lung extracellular matrix hydrogels resemble the stiffness and viscoelasticity of native lung tissue,” *American Journal of Physiology-Lung Cellular and Molecular Physiology*, vol. 318, no. 4, Mar. 2020.

02/22/2023 Synthesis and Characterization of Tunable Poly(Ethylene Glycol): Gelatin Methacrylate Composite Hydrogels

ANURAAG SHREEKANTH BELAVADI - Feb 22, 2023, 2:52 PM CST

Title: Synthesis and Characterization of Tunable Poly(Ethylene Glycol): Gelatin Methacrylate Composite Hydrogels

Date: 02/22/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Compare the tunability, biochemical, and mechanical properties of PEG and gelMA in order to evaluate each hydrogel to be incorporated into the project and for Design Matrix evaluation.

Content:

- The article presents a study on the development and characterization of composite hydrogels made from poly(ethylene glycol) (PEG) and gelatin methacrylate (GelMA).
- The researchers used a photopolymerization process to create the hydrogels, which involved crosslinking the PEG and GelMA monomers using ultraviolet light.
- The mechanical properties of the hydrogels were evaluated using compression testing, which showed that the stiffness of the hydrogels could be tuned by adjusting the concentration of GelMA in the composite.
- The researchers also investigated the swelling properties of the hydrogels and found that the swelling ratio increased with increasing PEG concentration.
- The biocompatibility of the hydrogels was evaluated by seeding them with human dermal fibroblasts. The cells were able to adhere and proliferate on the hydrogels, indicating that they were biocompatible and able to support cell growth.
- The researchers also investigated the potential of the hydrogels for drug delivery applications by testing their ability to release a model drug (rhodamine B) over time. The results showed that the release rate of the drug could be controlled by adjusting the concentration of PEG in the composite.
- The composite hydrogels were further evaluated for their potential use in tissue engineering applications by testing their ability to support the growth and differentiation of mouse myoblast cells. The cells were able to adhere and differentiate into myotubes on the hydrogels, indicating that they have potential for use in muscle tissue engineering applications.
- The study suggests that composite hydrogels made from PEG and GelMA have potential for use in a variety of biomedical applications, including tissue engineering and drug delivery. The ability to tune the mechanical and swelling properties of the hydrogels, as well as their biocompatibility and drug release properties, make them a promising candidate for use in personalized medicine applications.

Conclusions made from testing composite hydrogels made from PEG and GelMA:

- Can be synthesized using a photopolymerization process to create crosslinked networks of the PEG and GelMA monomers.
- Have mechanical properties that can be tuned by adjusting the concentration of GelMA in the composite, and swelling properties that can be controlled by adjusting the concentration of PEG.
- Are biocompatible and able to support cell growth, including the growth and differentiation of myoblast cells.
- Have the potential for use in drug delivery applications, as the release rate of model drugs can be controlled by adjusting the concentration of PEG in the composite.
- Are a promising candidate for use in tissue engineering applications, including personalized medicine applications, due to their ability to be customized for specific applications.

Conclusions/action items:

Both GelMA and PEG hydrogels are versatile in their biomedical applications and mechanical tunability. From the study, the need for photocrosslinking and the introduction of degradation proteins requires more work for PEG as compared to GelMA. However, PEG can be tuned more accurately to the team’s requirements. Further research is required to make a design decision.

Citation:

C. B. Hutson, J. W. Nichol, H. Aubin, H. Bae, S. Yamanlar, S. Al-Haque, S. T. Koshy, and A. Khademhosseini, “Synthesis and characterization of tunable poly(ethylene glycol): Gelatin methacrylate composite hydrogels,” *Tissue Engineering Part A*, vol. 17, no. 13-14, pp. 1713–1723, Apr. 2011.

03/27/2023 GelMA Synthesis Process and Purpose

ANURAAG SHREEKANTH BELAVADI - Mar 27, 2023, 11:38 AM CDT

Title: Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels

Date: 03/27/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Understand why we are performing the steps we are, to synthesize GelMA. I also need to know what materials should be ordered, materials the team should incorporate that weren't used by the researchers in the article, and how we can save costs.

Content:

The GelMA synthesis process involves several steps, as follows:

1.
2. Extraction of gelatin: The first step involves the extraction of gelatin from animal-derived collagen. This is typically done by acid hydrolysis, where the collagen is treated with acid to break down the protein into smaller molecules. The resulting gelatin is then purified through filtration and concentration.
    a. The team should/will be using Type B, bovine gelatin
3. Methacrylation of gelatin: The second step involves the addition of methacrylic anhydride to the gelatin solution. Methacrylic anhydride is a reactive molecule that can attach to the amino groups of the gelatin molecules, thereby creating methacrylated gelatin (GelMA). The reaction is typically carried out under controlled pH and temperature conditions, to ensure that the reaction proceeds smoothly and without side reactions.
4. Purification of GelMA: After the methacrylation reaction is complete, the GelMA solution is typically purified through dialysis. Dialysis involves placing the GelMA solution in a semipermeable membrane that allows small molecules to pass through, while retaining larger molecules such as GelMA. This process helps to remove any unreacted methacrylic anhydride and other impurities from the GelMA solution.
5. Crosslinking of GelMA: The final step in the GelMA synthesis process is crosslinking, which involves the formation of covalent bonds between GelMA molecules. This is typically achieved through exposure to ultraviolet (UV) light or a chemical crosslinking agent. Crosslinking helps to stabilize the GelMA hydrogel and to control its mechanical properties.

Conclusions/action items:

The GelMA synthesis process is a complex and multi-step process that requires careful attention to detail and precise control of reaction conditions. However, the resulting GelMA hydrogel is a versatile and biocompatible material. Further research on the bloom strength of the gelatin used is needed. In addition, lyophilization is not extensively mentioned. This needs further research as well.

05/02/2023 Entry- 04/13/2023 Cross-evaluation of stiffness measurement methods for hydrogels

ANURAAG SHREEKANTH BELAVADI - May 02, 2023, 1:37 PM CDT

Title: Cross-evaluation of stiffness measurement methods for hydrogels

Date: 04/13/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Find optimal methods for hydrogel testing.

Content:

- The article discusses the comparison of various methods used to measure the stiffness of hydrogels.
- Traditional methods such as compression testing and rheology have limitations and may not accurately represent the mechanical behavior of hydrogels.
- Recently, several new methods have been developed, including atomic force microscopy (AFM), magnetic twisting cytometry (MTC), and Brillouin microscopy (BM).
- The study compares the accuracy, precision, and practicality of these new methods in measuring hydrogel stiffness.
- The results show that each method has its advantages and disadvantages, and the choice of method should depend on the specific properties of the hydrogel and the experimental setup.
- AFM is highly accurate and versatile, but requires expensive equipment and expertise in sample preparation.
- MTC is simple and fast, but may not be suitable for very soft hydrogels or those with low cellularity.
- BM can provide high-resolution maps of stiffness distribution, but is relatively slow and requires specialized equipment.

Citation:

Wang, Y., Chen, Y., Liu, X., Ramakrishna, S., & Zhang, Y. (2019). Cross-evaluation of stiffness measurement methods for hydrogels. Materials Science and Engineering: C, 103, 109774. https://doi.org/10.1016/j.msec.2019.109774

Conclusions/action items:

There are multiple methods for us to test stiffness. The team will try rheometry testing first and will consider other alternative methods if needed.

05/01/2023 Entry- 03/29/2023 Stiffness modification of photopolymerizable gelatin-methacrylate hydrogels influences

ANURAAG SHREEKANTH BELAVADI - May 02, 2023, 12:59 PM CDT

Title: Stiffness modification of photopolymerizable gelatin-methacrylate hydrogels influences endothelial differentiation of human mesenchymal stem cells

Date: 03/29/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: To understand the effects and possible applications of crosslink duration and its influence on cultured cells in a 3D scaffold

Content:

- Used human adipose-derived MSCs and a photocrosslinkable GelMA hydrogel system, which can be modified in stiffness, swelling, and degradation properties.
- They demonstrated that the stiffness of the GelMA hydrogel influenced the chondrogenic differentiation of MSCs and ultimately the cartilage regeneration.
- The study shows that soft GelMA hydrogels promote the expression of chondrogenic markers and lead to better cartilage formation compared to stiffer GelMA hydrogels.
- The authors also found that MSCs in soft GelMA hydrogels expressed higher levels of TGF-β3, which is known to enhance chondrogenic differentiation.
- In vivo experiments in a rabbit model showed that the GelMA hydrogel encapsulated MSCs improved the quality of cartilage repair.
- The study uses three different stiffness variations of GelMA hydrogels (0.5 kPa, 2 kPa, and 8 kPa) to investigate the effect on hMSC differentiation.
- The results indicate that the stiffness of the GelMA hydrogels had a significant impact on the differentiation of hMSCs into endothelial cells.
- The authors found that cells cultured on the 8 kPa hydrogel exhibited the highest expression of endothelial markers, while cells cultured on the 0.5 kPa hydrogel exhibited the lowest expression.
- The study also found that hMSCs cultured on the 8 kPa hydrogel had the highest level of alignment and elongation, which are characteristics of endothelial cells.
- The study concludes that the stiffness of the GelMA hydrogels can modulate the differentiation of hMSCs into endothelial cells and highlights the importance of mechanical signals in stem cell differentiation.
-

Citation:

S. Schrader, J. Schmieder, S. Giselbrecht, S. Lutzki, A. Pilz, E. Schenke-Layland and R. R. Netz, "Stiffness modification of photopolymerizable gelatin-methacrylate hydrogels influences endothelial differentiation of human mesenchymal stem cells," Journal of Tissue Engineering and Regenerative Medicine, vol. 14, no. 5, pp. 752-763, May 2020, doi: 10.1002/term.2745.

Conclusions/action items: Low-stiffness gels, in general, have lower cell adhesion capabilities as cells are not able to elongate and align at the same level as cells cultured at higher stiffnesses. To help the client achieve higher confluency, gels at a higher stiffness must be made.

05/02/2023 Entry- 04/03/2023 Permeability mapping of gelatin methacryloyl hydrogels

ANURAAG SHREEKANTH BELAVADI - May 02, 2023, 1:00 PM CDT

Title: Permeability mapping of gelatin methacryloyl hydrogels

Date: 04/03/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: To understand and measure crosslink-density and tuned mechanical properties and their influence on GelMA performance as an effective cell-culture media.

Content:

- GelMA hydrogels have the potential to mimic the extracellular matrix of tissues and promote cell growth and differentiation.
- The study focuses on the development of a method for mapping the permeability of GelMA hydrogels, which is important for understanding nutrient and oxygen transport within the hydrogel and its effect on cell behavior.
- The method involves the use of a fluorescent dye and confocal microscopy to measure the diffusion of the dye within the hydrogel.
- The study found that the permeability of GelMA hydrogels can be modulated by changing the degree of crosslinking and the concentration of GelMA.
- The researchers also demonstrated the potential of the permeability mapping method for evaluating the effectiveness of different crosslinking agents and identifying regions of the hydrogel with different permeability.
- The permeability mapping method developed in the study can provide valuable information for designing and optimizing GelMA hydrogels for specific tissue engineering applications.
- The study also highlights the importance of understanding the relationship between the degree of crosslinking, GelMA concentration, and permeability in GelMA hydrogels.
- Increasing the degree of crosslinking or GelMA concentration resulted in a decrease in permeability.

Citation:

S. Schrader, S. Lutzki, J. Schmieder, M. Schulze, E. Schenke-Layland, and R. R. Netz, "Permeability mapping of gelatin methacryloyl hydrogels," Materials Today Communications, vol. 26, pp. 101959, Mar. 2021, doi: 10.1016/j.mtcomm.2020.101959.

Conclusions/action items:

Since the concentration of GelMA cannot be changed till materials are ordered, the swelling rate and permeability of the gels can be improved by testing the degree of crosslinking the team can achieve with LAP. These results may provide info on how to better tune stiffness without sacrificing gel permeability.

05/02/2023 Entry- 04/03/2023 Systematic optimization of visible-light-induced crosslinking conditions

ANURAAG SHREEKANTH BELAVADI - May 02, 2023, 1:00 PM CDT

Title: Systematic optimization of visible-light-induced crosslinking conditions of gelatin methacryloyl (GelMA)

Date: 04/03/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Find a specific and replicable protocol for GelMA synthesis to eliminate batch/batch error and to streamline the testing process.

Content:

- The article describes a study on optimizing the conditions for crosslinking GelMA, a hydrogel used in tissue engineering, using visible light as the initiator.
- The researchers used a statistical approach called the design of experiments (DOE) to systematically study the effects of various parameters on the crosslinking reaction.
- The parameters studied included the type and concentration of the initiator, light intensity, exposure time, and temperature.
- The DOE analysis helped identify the optimal conditions for GelMA crosslinking, which resulted in improved mechanical properties and cell viability compared to suboptimal conditions.
- The researchers also investigated the effect of crosslinking conditions on the release of a model drug from the GelMA hydrogel.
- The optimized conditions identified in this study can aid in the development of GelMA-based hydrogels for various tissue engineering applications.

Citation:

Sharifi, S., Sharifi, H., Akbari, A. et al. Systematic optimization of visible-light-induced crosslinking conditions of gelatin methacryloyl (GelMA). Sci Rep 11, 23276 (2021). https://doi.org/10.1038/s41598-021-02830-x

Conclusions/action items:

The team needs to employ a systematic approach to test each contributing factor that influences the mechanical properties of our gel like time spent under UV, type of photoinitiator, time in the refrigerator, etc.

05/02/2013 Entry- 04/17/2023 Triggering Cell Adhesion, Migration or Shape Change with a Dynamic Surface Coating

ANURAAG SHREEKANTH BELAVADI - May 02, 2023, 1:37 PM CDT

Title: Triggering Cell Adhesion, Migration or Shape Change with a Dynamic Surface Coating

Date: 04/17/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Find methods to improve cell adhesion.

Content:

- The article highlights the importance of surface chemistry in controlling cell behavior, and describes the development of the dynamic surface coating as a novel approach to achieving this control.
- The cleavable linker used in the coating is designed to be responsive to specific signals, allowing precise control over the timing and location of ligand release.
- The article demonstrates the versatility of the coating by using different ligands to trigger different cellular responses, such as cell migration, spreading, or contraction.
- The coating is shown to be effective in controlling the behavior of different cell types, including fibroblasts and neurons, suggesting its potential for a wide range of applications.
- The article discusses potential challenges and future directions for the use of a dynamic surface coating, including optimization of ligand release kinetics and scaling up the coating for larger tissue engineering applications.

Citation:

M. Mrksich and G. M. Whitesides, "Triggering Cell Adhesion, Migration or Shape Change with a Dynamic Surface Coating," Angew. Chem. Int. Ed., vol. 37, no. 6, pp. 769-772, 1998

Conclusions/action items:

The use of dynamic patterning is beyond the scope of this project at the time. The team should continue focusing on adjusting stiffness to address the client’s issues.

05/02/2023 Entry- 04/22/2023 Controlling the Surface Chemistry of a Hydrogel for Spatially Defined Cell Adhesion

ANURAAG SHREEKANTH BELAVADI - May 02, 2023, 1:38 PM CDT

Title: Controlling the Surface Chemistry of a Hydrogel for Spatially Defined Cell Adhesion

Date: 04/22/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Find methods to improve cell adhesion.

Content:

- The article describes a method for creating a hydrogel material that can control cell adhesion in specific spatial locations.
- The hydrogel is made from a mixture of polyethylene glycol diacrylate (PEG-DA) and a photoinitiator, which can be crosslinked using UV light to form a 3D network.
- To modify the surface chemistry of the hydrogel, a photocleavable molecule is added that can be selectively cleaved using UV light. This leaves behind an unmodified surface in the cleaved regions.
- By selectively exposing the hydrogel to UV light through a mask, the surface can be patterned with regions that either promote or inhibit cell adhesion.
- The hydrogel is then tested using fibroblast cells, which adhere only to the patterned regions of the hydrogel.
- The researchers found that the patterned hydrogel can be used to create spatially defined patterns of cells on a substrate.
- This approach could be useful for tissue engineering, as it allows for the creation of 3D structures with defined cell patterns.
- The article describes how the researchers optimized the hydrogel synthesis and patterning conditions to achieve the desired results.
- The researchers also demonstrate that the approach can be used to create hydrogel surfaces with multiple cell-adhesive and non-adhesive regions, allowing for complex cell patterning.

Citation:

Ito, Y., Hasuda, H., & Kamihira, M. (2004). Controlling the surface chemistry of a hydrogel for spatially defined cell adhesion. Biomaterials, 25(12), 1875-1881.

Conclusions/action items:

The use of UV light to increase cell adhesion is already performed as UV light is required for cross-linking. However, finding methods of micro-patterning or topographical alterations may be useful for future work.

02/01/2023 Training Documentation

ANURAAG SHREEKANTH BELAVADI - Feb 01, 2023, 3:35 PM CST

2/1/23, 3:34 PM Training Information Lookup Tool

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This certifies that Anuraag Shreekanth Belavadi has completed training for the following course(s):

Course	Assignment	Completion	Expiration
Biosafety Required Training	Biosafety Required Training Quiz	10/18/2021	10/18/2026
Chemical Safety: The OSHA Lab Standard	Final Quiz	10/25/2021	

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2/3/2023 Air Liquid Interfaces

WILLIAM ONUSCHECK - Feb 03, 2023, 11:47 AM CST

Title: Air Liquid Interfaces

Date: Feb 3, 2023

Content by: William Onuscheck

Present: William Onuscheck

Goals: In catching up with the group, review the term "Air Liquid Interface" as used in the prior semester's final report.

Content:

Consider the human tracheobronchial epithelium: dominated by ciliated, secretory, or basal cells. Tight junctions maintain epithelial integrity as well as its function as a physical barrier. Secreted mucus traps irritants, pathogens, etc and is transported via ciliary action. Secreted protective mediators (antimicrobial peptides, inflammatory mediators, etc) form chemical and immunological barriers against irritants, pathogens. The culture of primary human bronchial epithelial cells has demonstrated that to differentiate into a cell with a pseudostratified mucociliary phenotype, then the apical side of the cells must be exposed to air. Considering this, culture of primary human bronchial epithelial cells in an “air liquid interface” - where a pore membrane is used as the basal side of the culture chamber, allowing only the basal side to be nourished, but allowing the apical side to be exposed to air - results in a diverse composition of epithelial cells (secretory, ciliate, basal etc.)

Search Term: Google: "Air liquid Interface Cell Culture"

Citation: “Air-liquid interface culture for respiratory research,” STEMCELL Technologies, Jul-2019. [Online]. Available: https://www.stemcell.com/air-liquid-interface-culture-respiratory-research-lp.html. [Accessed: 03-Feb-2023].

Conclusions/action items: Consider the application of ALIs to the project, communicate with the client about their significance. If our goal is to first culture fibroblasts prior to epithelial cells, do we need to be immediately concerned with ALIs?

2/3/2023 3D Extracellular Matrix Mimics

WILLIAM ONUSCHECK - Feb 03, 2023, 11:47 AM CST

Title: 3D Extracellular Matrix mimics

Date: Feb 3, 2023

Content by: William Onuscheck

Present: William Onuscheck

Goals: In catching up with the group, literature on 3D ECM mimics.

Content:

Conventional 2D models of an ECM have been the standard microenvironment in study, however oftentimes these 2D models fail to mimic a majority of interactions, or even functions that, as the interactions present in a 3D microenvironment are lacking (especially relevant is the lack of development of certain cellular phenotypes). A 3D microenvironment can be developed in vitro by cell aggregates, or suspension of cells in hydrogels composed of ECM proteins. Environmental factors contribute to the change of behavior of cells in 3D culture vs 2D monolayers. Both mechanical and biomolecular changes to the ECM are detectable by cell receptors, which ultimately affect gene expression.

Consider the ECM not only for structural, but functional roles. Consider the influence of the ECM on the cells, but also the influence of cells upon the ECM. Matrisome as a blanket term for proteins, glycoproteins, and proteoglycans of the ECM. Other ECM associated proteins are mucins, lectins, semaphorins, and plexins. Collagens compose ~30% of ECM constituency, not just structural, but have function in cell signaling. Fibronectin and laminin are relevant non collagenous glycoproteins, where FNs dictate stem cells adhesion, cell fate and cell−ECM interaction, and LMs are a major component of the basement membrane, as well as dictate structural organization. Proteoglycans and Glycosaminoglycans bind to water and provide hydration of ECM and therefore compressive resistance, "Biglycan is important for collagen integrity, and functional structure, and cell-ECM interaction."

An important role of ECM function is to provide an adhesive and structured substrate for integrins and other adhesive cell receptors to bind to. Bioresponsive molecules of the ECM can also influence cell adhesion, and also cell differentiation. Cells take an active role in local microenvironmental regulation of the ECM, in turn, the stiffness and molecular composition of the ECM regulates some aspects of cell behavior.

Consider receptor based cell-ECM communication. Most commonly facilitated by integrin-ligand bonding (with a ligand of ECM constituency), regulate "adhesion events" as well as stress transmission, bidirectional signaling, viability and differentiation. The article also offers a description of non integrin receptor bonding functions, summarized with the table below:

Consider what is important for differentiation, consider cross referencing with what is present in bronchial ECM,

Table 1. Representative Receptor Involved in Cell−ECM Communication

ECM receptors	ECM interactors	cellular functions
integrins	FN, LM, collagen, soluble galectins, and several matrix glycoproteins	cell adhesion, regulation of stress transmission and bidirectional signaling, and angiogenesis
discoidin domain receptors (DD1 and DD2)	different fibrillar collagen types	embryo development, cell migration, cell survival, proliferation and differentiation, and remodeling of extracellular matrices
syndecans	collagens, FN and TSP, βFGF, VEGF, βTGF, and PDGF	growth-factor receptor, activation, cell-adhesion, cell−cell communication, cell proliferation, differentiation, and adhesion and migration.
dystroglycan	LM, agrin, and perlecan in basement membranes and neurexins transmembrane	cell development, basement membrane formation, epithelial morphogenesis, membrane stability, cell polarization, and adhesion and migration
lectins	integrins, FN, LM, TSP and VN, and other glycoproteins and GAGs	cell adhesion and migration and cell growth, apoptosis, and differentiation
CD44	GAGs	cellular motility and cell−cell and cell−ECM adhesions
CD36	collagen	fatty acid uptake, cell adhesion, and angiogenesis

Consider tissue engineering as a 2 pronged use of a proper stem cell and the proper 3D microenvironment or ECM mimic such that the proper tissue differentiation occurs. Requisite bioactive motifs induce proper differentiation.

Composition, stiffness and topological structure of the ECM scaffold effect cell-ECM interaction

Use of natural biopolymers allows suspended cells to take advantage of signaling motifs already found in the matrix. Chemoselective ligations can be used "to obtain a controlled cross-linking process during cell encapsulation without affecting the cell viability". Consider the maintenance of conditions for viable cell culture (pH, temperature).

Adhesion, differentiation, and proliferation are resultant from the interaction of cell receptor to receptor, or receptor to ECM

Bioresponsive polymers mimic the mechanical, biophysical, and adaptive properties of the ECM by the establishment of direct interactions with cells.

Search Term: Pubmed: "3D In-Vivo Cell Culture ECM"

Citation: J. Nicolas, S. Magli, L. Rabbachin, S. Sampaolesi, F. Nicotra, and L. Russo, "3D extracellular matrix mimics: Fundamental concepts and role of materials chemistry to influence Stem Cell Fate," *Biomacromolecules*, vol. 21, no. 6, pp. 1968–1994, 2020.

Conclusions/action items: Consider what is important for differentiation, consider cross referencing with what is present in bronchial ECM,

2/3/2023 PEG

WILLIAM ONUSCHECK - Feb 03, 2023, 11:49 AM CST

Title: PEG

Date: Feb 3, 2023

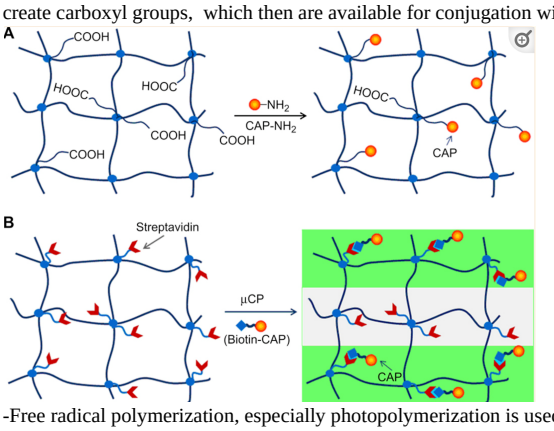
Content by: William Onuscheck

Present: William Onuscheck

Goals: In catching up with the group, review literature on Polyethylene Glycol as polymeric hydrogel.

Content:

-Lack of cell adhesion with unmodified polymer (bad, increase)
-Photopolymerization to modify (any photopolymerization must be done prior to culturing/seeding as UV light kills cells (see client meeting notes))
-Lack of degradation from unmodified polymer (bad) (methods of degradation may require UV light, unacceptable for our purposes) find different method of polymer degradation
  -Consider a method of enzymatic degradation?
-Variable / easily manipulated mechanical properties (good)
-Linear structure vs. branched PEG
-For enhanced hydrolytic degradation consider incorporating
  -polyester
  -disulfide
  -acetal
  -polypropylene fumarate
-Read *Spotlight on hydrogels, Hydrogel Cell Culture*
- Consider control of ligand density and spatial distribution to modulate specific cellular responses
-Type I (mot common) and type II collagen as providing tensile strength to the ECM
-Type IV collagen a network forming
-Elastin as providing elasticity to the ECM
-Fibronectin and laminin as attachment proteins in the ECM
-Consider the self assembly of the ECM
- Integration of PLA and PGA into PEG has been used to increase the hydrolytic degradation of the scaffold, but this degradation is not cell mediated (bad) (but also useful to increase cell adhesion)
-Integration of cell adhesive peptide, enzyme sensitive peptide,
-Integration of bioactive peptide sequences derived from ECM proteins (LN, FN, collagen) into the PEG structure to increase the bioactivity of the PEG scaffold
    -Use of multivalent reactive groups to accomplish the “tethering” of these proteins (acrylate, amine, thiol, azide, maleimide and biotin/strepavidin)
-Adhesion has to be cell specific??
-Determine Cell-adhesive peptides (CAPs) that are specific to epithelial mesenchymal trophic unit
-Determine enzyme sensitive peptides to insert
-Would growth factors be a relevant modification to the PEG?
-Would biofunctionalized groups be a relevant modification? (Matrix-protein binding, Immuno-isolating, Nitric Oxide Bearing)
-Matrix protein binding PEG gels respond not only to signals from cell receptors but also via protein deposition and organization
-Immuno isolating seems more relevant to *in situ* scaffolds
- Nitric oxide bearing scaffolds produce NO (seems more relevant for anti inflammatory *in situ*)
-Methods for bioactive modifications
    -Post grafting is the process of making PEG hydrogels, given the lack of polymerizable functional groups following, acrylic acid is copolymerized with PEGDA to create carboxyl groups, which then are available for conjugation with the amine groups of peptides or proteins



-Free radical polymerization, especially photopolymerization is used to incorporate bioactive molecules into PEG networks

- Copolymerization of peptide monoacrylates, generally CAPs to promote the spreading of fibroblast. The use of a PEG spacer can allow for free movement of the peptide within the biological environment.
-Copolymerization with peptide diacrylates, allows for control that the random distribution of RGD peptides incorporated by monoacrylate copolymerization, takes advantage of the C=C bonds of
-Thiol-acrylate photopolymerization utilizes thiol bearing RGD peptides to photopolymerize with PEGDA micheal addition
-Click chemistry
-Enzymatic formation
-Photoregulation irrelevant from UV constraint?

-PEG HYDROGELS CONTAINING RGD PEPTIDES ACHIEVE SIGNIFICANTLY IMPROVED CELL ATTACHMENT, AND PROLIFERATION THAN CELLS CULTURED IN CONTROL HYDROGELS

Search Term: Google: "Air liquid Interface Cell Culture"

Citation: J. Zhu, “Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering,” *Biomaterials*, vol. 31, no. 17, pp. 4639–4656, 2010.

Conclusions/action items: In yellow.

2/3/2023 More ECM Notes

WILLIAM ONUSCHECK - Feb 03, 2023, 11:57 AM CST

Title: More ECM Notes

Date: Feb 3, 2023

Content by: William Onuscheck

Present: William Onuscheck

Goals: In catching up with the group, review ECM physiology

Content:

-The stiffness of an organ or a tissue is determined by the stiffness of the ECM

-The stiffness of the ECM has been shown to have regulatory effects on cell function

-Migration of cells from soft to stiff areas, but cannot move in the reverse gradient (ere on the side of softer tissue / lower Young's Modulus??)

-Consider the Young's modulus ranges as soft: 0.1–1 kPa; medium: 8–17 kPa; and stiff: 25–40 kPa

-Mesenchymal stem cells cultured on these ranges showed preference to differentiate into neurons; myoblasts; and osteoblasts respectively

-The topology of the substrate of the ECM also has implications for cellular differentiation

-Look further into the topology of the ECM and differentiation / feasibility of modifying the topology of a PEG scaffold

Search Term: Google: "Air liquid Interface Cell Culture"

Citation: T. Hoshiba and T. Yamaoka, "Chapter 1. extracellular matrix scaffolds for tissue engineering and Biological Research," *Decellularized Extracellular Matrix*, pp. 1–14, 2019.

Conclusions/action items: See yellow

2/3/2023 Even More ECM Notes - Copy

WILLIAM ONUSCHECK - Feb 03, 2023, 12:02 PM CST

Title: More ECM Notes (Topology)

Date: Feb 3, 2023

Content by: William Onuscheck

Present: William Onuscheck

Goals: In catching up with the group, review ECM physiology

Content:

-Too large a pore size limits the movement of cells, nutrients etc, to large a pore size leads to a decrease in surface area, limiting cell adhesion
-Adhesion is initially driven by surface area, but shifts to be driven by infiltration and migration after 48H
-Shape, (granted) in a 2D scaffold, has an effect on mesenchymal stem cells
-3D ECMs produce cell aggregates or tissue, whereas 2D results in monolayers
-Higher growth rates on higher fractal dimensions, more complex, continuously irregular structure
-Stiffness as influencing cell behavior
-Read T.Su, Y. Liu, H. He, J. Li, Y. Lv,L.Zhang,Y. Sun,C.Hu,ACSMacroLett.2016,5,1217–1221.
-Stiffness affecting migratio

Search Term: Pubmed: "ECM composition"

Citation: T. Wang, S. S. Nanda, G. C. Papaefthymiou, and D. K. Yi, “Mechanophysical cues in extracellular matrix regulation of cell behavior,” *ChemBioChem*, vol. 21, no. 9, pp. 1254–1264, 2020.

Conclusions/action items: See yellow

2/3/2023 More PEG notes - Copy

WILLIAM ONUSCHECK - Feb 03, 2023, 12:05 PM CST

Title: More PEG notes

Date: Feb 3, 2023

Content by: William Onuscheck

Present: William Onuscheck

Goals: In catching up with the group, review literature on Polyethylene Glycol as polymeric hydrogel.

Content:

-Spheroids of cells in vitro occur when there is a lack of attachment points in the environment, so they adhere to one another in aggregate
-Integration of ECM components such as laminin or cell adhesion peptide motifs (ie RGD peptide) allows for attachment and migration of the cells to and within the material
-Mention of hyaluronic acid as a scaffold itself
-SPACC
-Uses HA-PEG hydrogel
-Variation of viscoelastic properties based on concentration of HA-PEG

Search Term: Google: "Air liquid Interface Cell Culture"

Citation: J. Christoffersson, C. Aronsson, M. Jury, R. Selegård, D. Aili, and C.-F. Mandenius, “Fabrication of modular hyaluronan-peg hydrogels to support 3D cultures of hepatocytes in a perfused liver-on-a-chip device,” *Biofabrication*, vol. 11, no. 1, p. 015013, 2018.

Conclusions/action items: In yellow.

2/3/2023 Viscoelasticity characterization

WILLIAM ONUSCHECK - Feb 03, 2023, 12:10 PM CST

Title: More PEG notes

Date: Feb 3, 2023

Content by: William Onuscheck

Present: William Onuscheck

Goals: In catching up with the group, reviewing viscoelastic characterization

Content:

-Comparison of hookian vs newtonian deformation properties in a material
-Maxwell vs Kelvin model via consideration of viscous deformation and elastic deformation as in series vs in parallel respectively
-Rheological behavior of a material can be characterized as a function of temperature, time, strain/stress amplitude, and frequency
-A phase shift between stress and strain is explained in viscoelastic materials by the delay between instant elastic deformation and the slowed viscous dashpot deformation
-Storage modulus (G') as a measure of energy stored in a material
-Loss modulus (G'') as a measure of energy dissipated into material
-Cell type matching to hydrogel important?

Search Term: Google: "Viscoelastic properties"

Citation: "Dynamic testing," *Dynamic Mechanical Analysis*, 1999.

Conclusions/action items: Learn testing set up.

2/9/2023 GelMA notes

WILLIAM ONUSCHECK - Feb 27, 2023, 6:14 PM CST

Title: GelMA Notes

Date: February 9th, 2023

Content by: William Onuscheck

Present:

Goals: Following meeting with Dr. Masters, methacrylated gelatin has become a material of interest for the team. The focus of this literature review will be to characterize its viscoelastic properties, cell adhesion, biocompatibility, degradability / ECM reconstruction.

Content:

Gelatin Methacrylate Hydrogel for Tissue Engineering Applications—A Review on Material Modifications

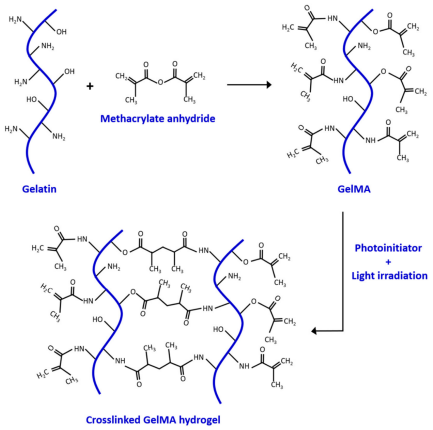
-Formation of Gelatin Methacrylate is via covalent bonding of naturally derived polymeric gelatin and methacrylic groups.

-Tunable

-Gelatin is obtained from denatured collagen making it biocompatible and biodegradable (read *Gelatin-polysaccharide composite scaffolds for 3D cell culture and tissue engineering: Towards natural therapeutics* for degradation info??)

-Low melting temperature (31.7-34.2 °C), to simulate in-vivo conditions, other materials must be introduced (in-vivo at 37 °C)

-Crosslinking of GelMA yields hydrogel structure with use of photoinitiator + light



-Stiffness, porosity tunable by gelatin concentration, functionalization, UV intensity, supplementation

-Consider modifications via growth factors, their mimics, biopolymers, nanoparticles

The instructive extracellular matrix of the lung: basic composition and alterations in chronic lung disease

-The interstitial ECM of alveoli is composed mainly of a meshwork of type I, III collagen, as well as elastin

- Elastin is crossed linked to form “inner core” of elastic fibers, outer fiber formed by 10 - 15nm microfibrils.

-Native lung tissue has the an elastic modulus of 0.44 to 7.5 kPa

-Fibrotic lung tissue has an average elastic modulus of 16.25 kPa

Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels

-GelMA has metalloproteinase sensitive motifs within its structure → degradability

-GelMA also has naturally occurring RGD sequences

-More soluble than collagen, with less antigenicity as well

-RGD sequences that occur naturally in gelatin are not functionalized by methacrylic groups

-Use of collagenases (MMP-1 MMP-8) can be used to degrade crosslinked GelMA at increased rate

-Synthesis via

  -Direct reaction of Gelatin and methacrylic groups in a phosphate buffer at 50 °C

    -Degree of methacryloyl substitution controlled via concentration

  -Crosslinking via UV light and photoinitiator

-Elastic modulus of GelMA tunable via concentrations, UV light exposure, directly proportional to degree of methacryloyl substitution (read *Functional Human VascularNetwork Generated in Photocrosslinkable Gelatin Methacrylate Hydrogels.* for details)

-Also proportional to the mass / volume of GelMA in hydrogel

Gelatin-polysaccharide composite scaffolds for 3D cell culture and tissue engineering: Towards natural therapeutics

-An opacity issue may arise from the introduction of some additives (protein-carbon interactions)

Conclusions/action items:

Citations:

S. Bupphathong, C. Quiroz, W. Huang, P.-F. Chung, H.-Y. Tao, and C.-H. Lin, “Gelatin methacrylate hydrogel for tissue engineering applications—a review on Material Modifications,” *Pharmaceuticals*, vol. 15, no. 2, p. 171, 2022.

S. Afewerki, A. Sheikhi, S. Kannan, S. Ahadian, and A. Khademhosseini, “Gelatin-polysaccharide composite scaffolds for 3D cell culture and Tissue Engineering: Towards Natural Therapeutics,” *Bioengineering & Translational Medicine*, vol. 4, no. 1, pp. 96–115, 2018.

G. Burgstaller, B. Oehrle, M. Gerckens, E. S. White, H. B. Schiller, and O. Eickelberg, “The instructive extracellular matrix of the lung: Basic composition and alterations in chronic lung disease,” *European Respiratory Journal*, vol. 50, no. 1, p. 1601805, 2017.

K. Yue, G. Trujillo-de Santiago, M. M. Alvarez, A. Tamayol, N. Annabi, and A. Khademhosseini, “Synthesis, properties, and biomedical applications of gelatin methacryloyl (gelma) hydrogels,” *Biomaterials*, vol. 73, pp. 254–271, 2015.

S. Afewerki, A. Sheikhi, S. Kannan, S. Ahadian, and A. Khademhosseini, “Gelatin-polysaccharide composite scaffolds for 3D cell culture and Tissue Engineering: Towards Natural Therapeutics,” *Bioengineering & Translational Medicine*, vol. 4, no. 1, pp. 96–115, 2018.

Search Term: Pubmed: GelMA for tissue engineering

02/05/2023 EMTU Background Research

Nick Herbst - Feb 05, 2023, 5:14 PM CST

Title: EMTU Background Research

Date: 02/05/2023

Content by: Nick Herbst

Goals: Read one of the client's prior publications to gain a better understanding of the epithelial mesenchymal trophic unit

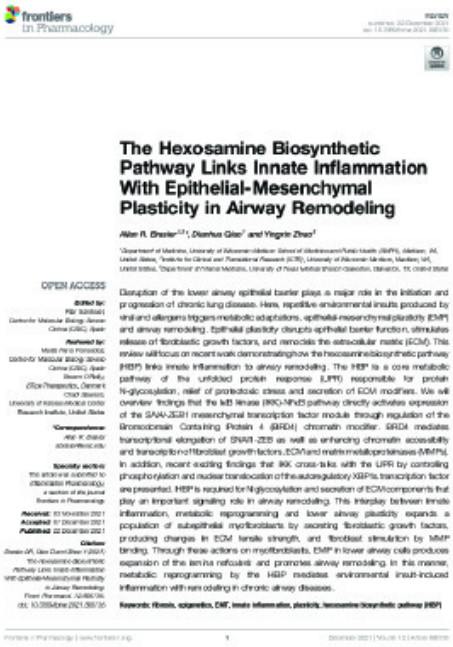
Source: A. R. Brasier, D. Qiao, and Y. Zhao, “The hexosamine biosynthetic pathway links innate inflammation with epithelial-mesenchymal plasticity in airway remodeling,” Frontiers in Pharmacology, vol. 12, Dec. 2021.

Content:

- The paper discusses how the HBP is involved in remodeling the EMTU's ECM during inflammation
    - I just focused on the background information regarding the EMTU
- The EMTU is in the "transition zone" in the respiratory system
    - area between bronchioles and alveoli
    - epithelial ---> mesenchymal transition
- The EMTU is a basement membrane
    - contains collagen (I & III), fibronectin, epithelial cells, and **fibroblasts**
    - in respiratory inflammation, the EMTU is affected by and the fibroblasts increase their activity
        - this fibrosis remodels the lung ECM and makes it stiffer
- See attachment for the full journal article

Conclusions:

In addition to reading over the notes taken by the members of the prior semester's team, I read this article to learn more about the EMTU, which is what we are modeling our synthetic scaffold off of. I now have a better understanding of what the EMTU is, which gives me a better idea of what our goal is for this project. We need to make a synthetic scaffold that has mechanical properties similar to the EMTU (and can be changed to reflect the changing EMTU ECM stiffness caused by fibrosis) so that the client can culture lung epithelial cells on it and investigate fibrosis/stiffness effects on the lung epithelium.



frontiers in Pharmacology

The Hexosamine Biosynthetic Pathway Links Innate Inflammation With Epithelial-Mesenchymal Plasticity in Airway Remodeling

Alan R. Brasier[1,2]†, Dianhua Qiao[2] and Yingxin Zhao[1]

OPEN ACCESS

Disruption of the lower airway epithelial barrier plays a major role in the initiation and progression of chronic lung diseases. Here, repetitive environmental insults produced by viral and allergens triggers metabolic adaptations, epithelial-mesenchymal plasticity (EMP) and airway remodeling. Epithelial plasticity disrupts epithelial barrier function, stimulates release of fibroblastic growth factors, and remodels the extracellular matrix (ECM). This review will focus on recent work demonstrating how the hexosamine biosynthetic pathway (HBP) links innate inflammation to airway remodeling. The HBP is a core metabolic pathway of the unfolded protein response (UPR) responsible for protein N-glycosylation, relief of proteotoxic stress and secretion of ECM modifiers. We will overview findings that the IκB kinase (IKK)-NFκB pathway directly activates expression of the SNAI-ZEB1 mesenchymal transcription factor module through regulation of the Bromodomain Containing Protein 4 (BRD4) chromatin modifier. BRD4 mediates transcriptional elongation of SNAI-ZEB as well as enhancing chromatin accessibility and transcription of fibroblast growth factors, ECM and matrix metalloproteinases (MMPs). In addition, recent exciting findings that IKK cross-talks with the UPR by controlling phosphorylation and nuclear translocation of the autoregulatory XBP1s transcription factor are presented. HBP is required for N-glycosylation and secretion of ECM components that play an important signaling role in airway remodeling. This interplay between innate inflammation, metabolic reprogramming and lower airway plasticity expands a population of subepithelial myofibroblasts by secreting fibroblastic growth factors, producing changes in ECM tensile strength, and fibroblast stimulation by MMP binding. Through these actions on myofibroblasts, EMP in lower airway cells produces expansion of the lamina reticularis and promotes airway remodeling. In this manner, metabolic reprogramming by the HBP mediates environmental insult-induced inflammation with remodeling in chronic airway diseases.

Keywords: fibrosis, epigenetics, EMT, innate inflammation, plasticity, hexosamine biosynthetic pathway (HBP)

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fphar-12-808735.pdf (2.73 MB)

02/05/2023 GelMA Hydrogel

Nick Herbst - Feb 05, 2023, 5:55 PM

Title: GelMa Hydrogel

Date: 02/05/2023

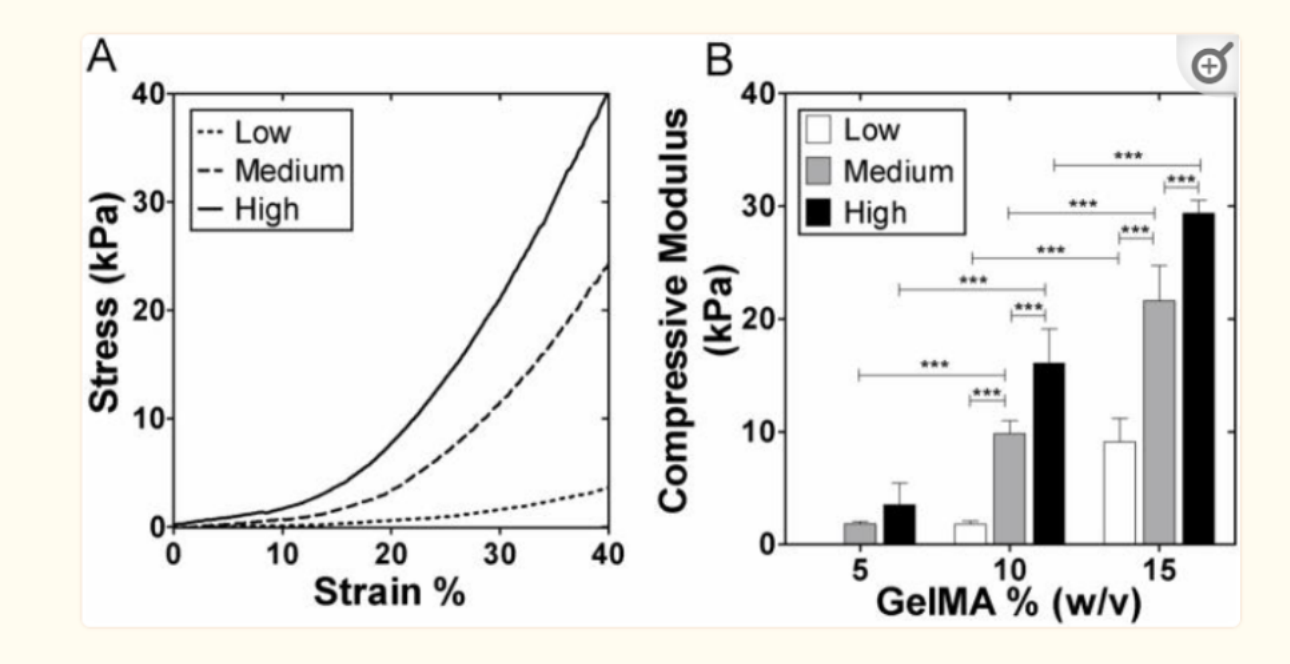
Content by: Nick Herbst

Goals: Gain a better understanding of methacrylated gelatin hydrogels

Source: J. W. Nichol, S. T. Koshy, H. Bae, C. M. Hwang, S. Yamanlar, and A. Khademhosseini, “Cell-laden microengineered gelatin methacrylate hydrogels,” Biomaterials, vol. 31, no. 21, pp. 5536–5544, 2010.

Content:

- This article assess the ability of GelMA hydrogels to be used for tissue engineering scaffold
- The hydrogels are made by adding tunable amounts of methacrylic anhydride to gelatin
  - a photoinitiator (I2959) is added and the gel is crosslinked with UV light
  - the degree off crosslinking is directly related to the
- degree of methacrylation was determined by Habeeb assay and confirmed by ^{1}H-NMR (looking at amine groups)
- human umbilical vein endothelial cells were cultured on the GelMA to prove its cell adhesion abilities and cell viability
  - gelatin has RGD sequences
  - 2D and 3D cell adhesion was successful and cells didn't die when cultured on the GelMA
- mechanical testing was done on 5%, 10%, and 15% GelMA gels with varying degrees of methacrylation
  - below is figure from the article



- See attachment for the full journal article

Conclusions:

While our current design is a PEG hydrogel with added RGD and MMPs, we are having issues gelling the PEG so a new approach is needed. After researching GelMA, I see it as a highly viab hydrogel for this project since it is translucent, has very tunable mechanical properties, is cell adhesive, and degradable/remodelable by MMPs.

Nick Herbst - Feb 05, 2023, 5:41 PM CST

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Published as: Biomaterials. 2010 July ; 31(21): 5536–5544. doi:10.1016/j.biomaterials.2010.03.064.

Cell-laden microengineered gelatin methacrylate hydrogels

Jason W. Nichol[1,2,†], Sandeep Koshy[1,2,3,†], Hojae Bae[1,2,†], Chang Mo Hwang[1,2], Seda Yamanlar[1,2], and Ali Khademhosseini[1,2,*]

[1] Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 65 Landsdowne Street, Cambridge, MA 02139, USA

[2] Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[3] Department of Chemical Engineering, University of Waterloo, Waterloo, ON, N2L 3G1, Canada

Abstract

The cellular microenvironment plays an integral role in improving the function of microengineered tissues. Control of the microarchitecture in engineered tissues can be achieved through photopatterning of cell-laden hydrogels. However, despite high pattern fidelity of photopolymerizable hydrogels, many such materials are not cell-responsive and have limited biodegradability. Here we demonstrate gelatin methacrylate (GelMA) as an inexpensive, cell-responsive hydrogel platform for creating cell-laden microtissues and microfluidic devices. Cells readily bound to, proliferated, elongated and migrated both when seeded on micropatterned GelMA substrates as well as when encapsulated in microfabricated GelMA hydrogels. The hydration and mechanical properties of GelMA were demonstrated to be tunable for various applications through modification to the methacrylation degree and gel concentration. Pattern fidelity and resolution of GelMA was high and it could be patterned to create perfusable microfluidic channels. Furthermore, GelMA microparticles could be used to create cellular microparticles for in vitro cell studies or 3D microtissue fabrication. These data suggest that GelMA hydrogels could be useful for creating complex, cell-responsive microtissues, such as endothelialized microvasculature, or for other applications that requires cell-responsive microengineered hydrogels.

Keywords

tissue engineering; hydrogel; gelatin; photopolymerization; microprinting

Introduction

The cellular microenvironment plays a critical role in controlling cell behavior and function [1]. Recent work has been directed towards controlling the microenvironment to investigate morphologies by mediated cell behaviors such as cell shape [2,3], cell-cell contacts, and signaling [4,5]. As specific microarchitectural features of the cell niche and the micromechanical environment have been demonstrated to be vital to controlling cell

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† Jason W. Nichol, Sandeep Koshy, and Hojae Bae contributed equally to this work.

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02/10/2023 GelMA Tunability

Nick Herbst - Feb 10, 2023, 6:43 PM CST

Title: GelMA Tunability

Date: 02/10/2023

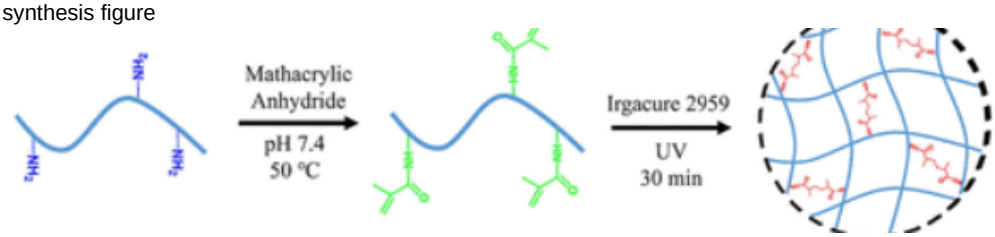
Content by: Nick Herbst

Goals: Gain a better understanding of how GelMA hydrogels are prepared and how the properties of the gel can be adjusted

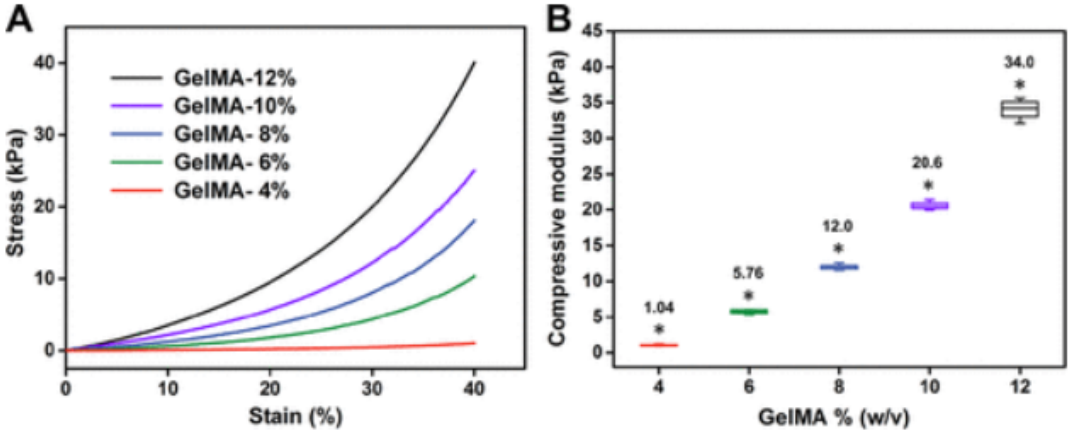
Source: Y. Sun, R. Deng, X. Ren, K. Zhang, and J. Li, “2D gelatin methacrylate hydrogels with tunable stiffness for investigating cell behaviors,” ACS Applied Bio Materials, vol. 2, no. 1, pp. 570–576, Dec. 2018.

Content:

- The authors made a GelMA hydrogel and used it as a 2D cell culture platform to investigate the effects of stiffness on cell morphology and gene expression
  - cell morphology and gene expression change with environment stiffness via mechanotransduction from integrins "sensing" surroundings
    - stiffer environment = cells spread out and are more spindly than round
  - GelMA was used because it is like collagen (bioactive sites, biocompatible, and degradable) and the stiffness can be tuned by the degree of methacrylation or concentration of GelMA
- GelMA synthesis figure



  - NMR was used to confirm addition of methacrylate groups to gelatin
- Mechanical Tunability
  - the authors tuned their hydrogel by altering GelMA concentration during gelation
  - different % w/v GelMA concentrations were used

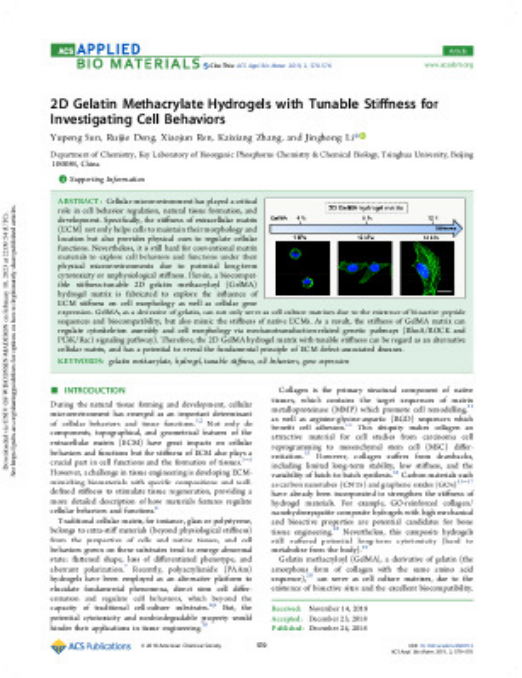


- See attachment for full article

Conclusions:

We still need to find/create a GelMA synthesis protocol, but the basic steps are shown in the figure from the paper. We have discussed that we can alter the stiffness of our hydrogel by changing the amount of methacrylic anhydride we use, but in this paper the authors altered their stiffness by changing the concentration of GelMA in the hydrogel. It looks like a hydrogel with a concentration of 4% GelMA is the closest to what we are aiming for. We will likely need to make a lot of different samples with varying MA concentrations and/or varying GelMA concentrations to find the exact hydrogel we need.

Nick Herbst - Feb 10, 2023, 4:10 PM CST



ACS APPLIED BIO MATERIALS

2D Gelatin Methacrylate Hydrogels with Tunable Stiffness for Investigating Cell Behaviors

Yupeng Sun, Ruijie Deng, Xiaojun Ren, Kaixiang Zhang, and Jinghong Li

Department of Chemistry, Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Tsinghua University, Beijing 100084, China

Supporting Information

ABSTRACT: Cellular microenvironment has played a critical role in cell behavior regulation, natural tissue formation, and development. Specifically, the stiffness of extracellular matrix (ECM) not only helps cells to maintain their morphology and location but also provides physical cues to regulate cellular functions. Nevertheless, it is still hard for conventional matrix materials to explore cell behaviors and functions under their physical microenvironments due to potential long-term cytotoxicity or unphysiological stiffness. Herein, a biocompatible stiffness-tunable 2D gelatin methacryloyl (GelMA) hydrogel matrix is fabricated to explore the influence of ECM stiffness on cell morphology as well as cellular gene expression. GelMA, as a derivative of gelatin, can not only serve as cell culture matrices due to the existence of bioactive peptide sequences and biocompatibility, but also mimic the stiffness of native ECMs. As a result, the stiffness of GelMA matrix can regulate cytoskeleton assembly and cell morphology via mechanotransduction-related genetic pathways [Rho/ROCK and PI3K/Rac1 signaling pathway]. Therefore, the 2D GelMA hydrogel matrix with tunable stiffness can be regard as an alternative cellular matrix, and has a potential to reveal the fundamental principle of ECM defect-associated diseases.

KEYWORDS: gelatin methacrylate, hydrogel, tunable stiffness, cell behaviors, gene expression

INTRODUCTION

During the natural tissue forming and development, cellular microenvironment has emerged as an important determinant of cellular behaviors and tissue functions.[1,2] Not only do components, topographical, and geometrical features of the extracellular matrix (ECM) have great impacts on cellular behaviors and functions but the stiffness of ECM also plays a crucial part in cell functions and the formation of tissues.[3,4] However, a challenge in tissue engineering is developing ECM-mimicking biomaterials with specific compositions and well-defined stiffness to stimulate tissue regeneration, providing a more detailed description of how materials features regulate cellular behaviors and functions.[5]

Traditional cellular matrix, for instance, glass or polystyrene, belongs to extra-stiff materials (beyond physiological stiffness) from the properties of cells and native tissues, and cell behaviors grown on these substrates tend to emerge abnormal state: flattened shape, loss of differentiated phenotype, and aberrant polarization.[6] Recently, polyacrylamide (PAAm) hydrogels have been employed as an alternative platform to elucidate fundamental phenomena, direct stem cell differentiation and regulate cell behaviors, which beyond the capacity of traditional cell culture substrates.[6,7] But, the potential cytotoxicity and nonbiodegradable property would hinder their applications in tissue engineering.[8]

Collagen is the primary structural component of native tissues, which contains the target sequences of matrix metalloproteinase (MMP) which promote cell remodeling,[9] as well as arginine-glycine-aspartic [RGD] sequences which benefit cell adhesion.[10] This disparity makes collagen an attractive material for cell studies from carcinoma cell reprogramming to mesenchymal stem cell (MSC) differentiation.[11] However, collagen suffers from drawbacks, including limited long-term stability, low stiffness, and the variability of batch-to-batch synthesis.[12] Carbon materials such as carbon nanotubes (CNTs) and graphene oxides (GOs)[13−17] have already been incorporated to strengthen the stiffness of hydrogel materials. For example, GO-reinforced collagen/methacrylated polyethylene composite hydrogels with high mechanical and bioactive properties are potential candidates for bone tissue engineering.[18] Nevertheless, this composite hydrogels still endured potential long-term cytotoxicity [hard to metabolize from the body].[19]

Gelatin methacryloyl (GelMA), a derivative of gelatin (the amorphous form of collagen with the same amino acid sequence),[20] can serve as cell culture matrices, due to the existence of bioactive sites and the excellent biocompatibility.

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Published: December 24, 2018

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02/20/2023 Lung Extracellular Matrix Background Research

Nick Herbst - Feb 20, 2023, 6:51 PM CST

Title: Lung Extracellular Matrix Background Research

Date: 02/20/2023

Content by: Nick Herbst

Goals: Understand the composition of the lung's ECM

Source: E. S. White, “Lung extracellular matrix and fibroblast function,” Annals of the American Thoracic Society, Mar-2015.

Content:

- ECM gives structure and provides cues to cells
    - increased stiffness of ECM leads to increased fibroblast activity
        - positive feedback loop
- ECM of lung is split into 2 domains
    - basement membrane
        - thin layer under endothelial and epithelial cell layers
    - interstitial space
        - where the fibroblasts are located
- Composition
    - collagen, elastin, fibronectin, laminin, GAGs, PGs, MMPs, fibroblasts
- Lung ECM has an elastic modulus between 0.44 and 7.5 kPa
    - heterogenous due to varying tissues in the region (ex: alveoli vs bronchial)
    - The fibroblasts in the lung ECM experience an elastic modulus of ~1kPa
        - This is what we are aiming for our scaffold
- See attachment for the full journal article

Conclusions:

Since we are trying to create a scaffold that mimics the mechanical properties of the lung ECM and has appropriate biochemical properties, it is important to understand what native lung ECM is made of.

Nick Herbst - Feb 20, 2023, 5:36 PM CST

ROGER S. MITCHELL LECTURE

Lung Extracellular Matrix and Fibroblast Function

Eric S. White

Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan

Abstract

Extracellular matrix (ECM) is a tissue-specific macromolecular structure that provides physical support to tissues and is essential for normal organ function. In the lung, ECM plays an active role in shaping cell behavior both in health and disease by virtue of the constituent clues it imparts to cells. Qualities including tissue elasticity, molecular composition, and intrinsic stiffness all promote normal function of the lung ECM. Alterations in composition and/or modulation of stiffness of the finely tuned or diseased lung ECM microenvironment plays a part in reparative processes performed by fibroblasts. Under conditions of remodeling or in disease states, inhomogeneous stiffening (or softening) of the pathologic ECM may both precede modifications in cell behavior and be a result of disease progression. The ability of ECM to stimulate further ECM production by fibroblasts and drive disease progression has potentially significant implications for mesenchymal stromal cell–based therapies in the setting of pulmonary ECM stiffness or composition, the therapeutic intent of progenitor cells may be subverted. Taken together, current data suggest that lung ECM actively contributes to health and disease; thus, mediators of cell-ECM signaling or factors that influence ECM stiffness may represent viable therapeutic targets in many lung disorders.

Keywords: extracellular matrix; fibroblasts; disease progression; cell shape; cellular mechanotransduction

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Supported by National Institutes of Health grants UO1 HL111090 and RO1 HL108565.

Author Contributions: E.S.W. is solely responsible for the content of this manuscript.

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DOI: 10.1513/AnnalsATS.201406-240MG

Internet address: www.atsjournals.org

Extracellular matrix (ECM) is a highly dynamic complex of fibrous proteins, glycoproteins, and proteoglycans that comprises the noncellular aspect of tissues and varies in composition according to its tissue localization and physiological circumstances. In addition to providing structural integrity, ECM delivers important spatial and contextual cues to drive cellular phenotypes. The ECM in the lung is typically restricted to two basic compartments: basement membranes and the interstitial space. Basement membranes are thin, specialized layers of ECM found under all epithelial and endothelial cell layers, whereas interstitial spaces form the parenchyma of the lung (1). Within the lung interstitium, resident fibroblasts are the most commonly identified cell and are mainly responsible for ECM production; they also serve as effector cells during injury repair.

The term "matrisome" has been introduced to describe the various fibrillar proteins, glycoproteins, proteoglycans, and their associated modifying molecules (e.g., metalloproteases, matricellular proteins) that compose the ECM of tissues (2). Recently, the matrisome of both rodent (3) and human lungs (4) has been characterized. Not surprisingly, qualitative differences between rodent and human lung ECM are observed, although the bulk of matrisome constituents are conserved between the two species. Importantly, both studies make clear that the extracellular lung parenchyma is not solely composed of collagen, elastin, glycosaminoglycans, and basement membrane; laminin, as has been traditionally thought.

The approach used to identify the matrisome, including removal of all cellular and nuclear material followed by digestion of resultant matrix and application of validated mass spectrometry technology, allows for the identification of previously unrecognized ECM components in the lung, thus paving the way for potentially new areas of study in cell–matrix interactions. It is worthwhile mentioning that not all methods of decellularizing the lung are necessarily equivalent; variable loss of proteins, growth factors, and matrix-associated molecules occur depending on the detergents used, the pressures applied, and the length of the process (5–7). Aside from rodent and human lungs and rodent cardiac tissue (3), the matrisome of other organs in either human or experimental animals has not yet been defined.

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02/27/2023 GelMA Chemistry

Nick Herbst - Feb 27, 2023, 8:13 PM CST

Title: GelMA Chemistry

Date: 02/27/2023

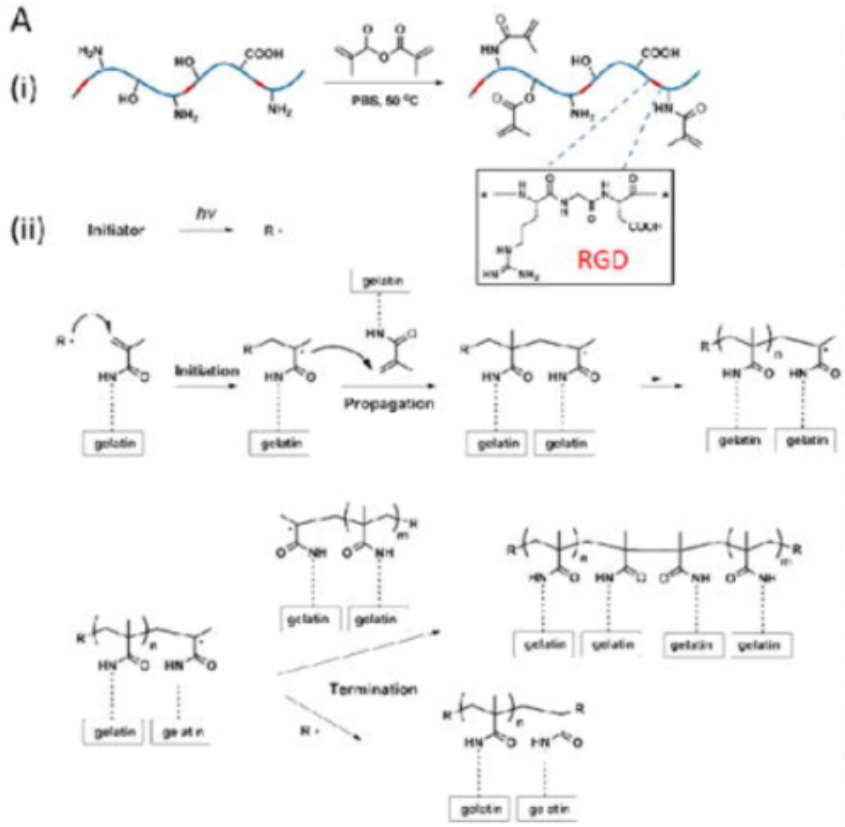
Content by: Nick Herbst

Goals: Better understand GelMA's chemistry

Source: K. Yue, G. Trujillo-de Santiago, M. M. Alvarez, A. Tamayol, N. Annabi, and A. Khademhosseini, “Synthesis, properties, and biomedical applications of gelatin methacryloyl (gelma) hydrogels,” Biomaterials, vol. 73, pp. 254–271, Aug. 2015.

Content:

- This journal article focuses on the
  - I will only be reviewing the synthesis of GelMA with an emphasis on the methacrylation reaction
- Methacrylation reaction is gelatin and methacrylic anhydride in PBS (pH = 7.4) at 50 ºC
  - Tunability = adjust amount of MA added to reaction mixture
  - pH at 7.4 enhances amine and hydroxyl reactivity
    - amines will protonate in a lower pH which makes them non-productive
- The reaction is a two phase rxn
  - organic is added to aqueous
  - rate of MA addition and rate of mixing affects dispersion which affects degree of MA substitution
- Methacryloyl substitution occurs at amine or hydroxyl groups
  - amine or hydroxyl on gelatin reacts with carbonyl on methyl anhydride
- GelMA is crosslinked by a water-soluble photoinitiator
  - I-2959 is common
    - this is what the prior semester used for PEG
  - lithium acylphosphinate salt (LAP) is what we plan on using now
    - higher solubility than I-2959 which means it will be easier to prepare the photoinitiator
  - With UV light, photoinitiator becomes a radical, which creates a methacryloyl radical on gelatin, which propagates to make more methacryloyl radicals, which then react to crosslink gelatin
- Below is a figure from the article that illustrates the methacrylation of gelatin and the crosslinking of GelMA (no way to fix low-quality figure, this is how it was presented in the article)



- See attachment for the full journal article

Conclusions:

I knew that GelMA was methacrylated gelatin, but I never fully understood the methacrylation reaction. Combining my knowledge from organic chemistry and BME 545 with the information from this article, I now understand GelMA's chemistry, which can potentially help me as we try to alter the degree of methacrylation to achieve a stiffer hydrogel scaffold.

Nick Herbst - Feb 27, 2023, 6:54 PM CST


HHS Public Access

Author manuscript

Biomaterials. Author manuscript; available in PMC 2016 December 01.

Published in final edited form as:

Biomaterials. 2015 December ; 73: 254–271. doi:10.1016/j.biomaterials.2015.08.045.

Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels

Kan Yue[1,2,*], Grissel Trujillo-de Santiago[1,2,3,*], Mario Moisés Alvarez[1,2,3], Ali Tamayol[1,2], Nasim Annabi[1,2,4,5,**], and Ali Khademhosseini[1,2,4,6,**]

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[6]Department of Physics, King Abdulaziz University, Jeddah 21569, Saudi Arabia

Abstract

Gelatin methacryloyl (GelMA) hydrogels have been widely used for various biomedical applications due to their suitable biological properties and tunable physical characteristics. These three-dimensional (3D) GelMA hydrogels closely resemble some essential properties of native extracellular matrix (ECM) due to the presence of cell attaching and matrix metalloproteinase responsive peptide motifs, which allow cells to proliferate and spread in GelMA-based scaffolds. GelMA is also versatile from a processing perspective. It crosslinks when exposed to light irradiation to form hydrogels with tunable mechanical properties which mimic the native ECM. It can also be microfabricated using different methodologies including micromolding, photomolding, bioprinting, self-assembly, and microfluidic techniques to generate constructs with controlled architectures. Hybrid hydrogel systems can also be formed by mixing GelMA with nanoparticles such as carbon nanotubes and graphene oxide, and other polymers to form networks with desired combined properties and characteristics for specific biological applications. Recent research has demonstrated the proficiency of GelMA-based hydrogels in a wide range of applications including engineering of bone, cartilage, cardiac, and vascular tissues, among others. Other applications of GelMA hydrogels, besides tissue engineering, include fundamental single-single cell research, cell signaling, drug and gene delivery, and bio-sensing.

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02/27/2023 GelMA Rheological Properties

Nick Herbst - Feb 27, 2023, 9:10 PM CST

Title: GelMA Rheological Properties

Date: 02/27/2023

Content by: Nick Herbst

Goals: Better understand the rheological properties (G' and G") of GelMA.

Source: A. I. Van Den Bulcke, B. Bogdanov, N. De Rooze, E. H. Schacht, M. Cornelissen, and H. Berghmans, “Structural and rheological properties of methacrylamide modified gelatin hydrogels,” Biomacromolecules, vol. 1, no. 1, pp. 31–38, Feb. 2000.

Content:

- The authors of this article studied the rheological properties of GelMA
    - rheology deals with deformation and flow of matter
    - G' = storage modulus (Pa)
        - elastic behavior
        - stored deformation energy
    - G" = loss modulus (Pa)
        - viscous behavior
        - deformation energy lost through internal friction
- GelMA vs Gelatin
    - G' increases when gelatin is methacrylated
    - G" increases when gelatin is methacrylated
- Temperature Dependence
    - G' decreases very slightly as temperature increases
    - G" increases as temperature increases
- Degree of Crosslinking
    - G' increases as crosslinking increases
- See attachment for the full journal article

Conclusions:

I knew that we would be doing frequency sweep rheology on our hydrogels to get G' and G" to calculate E, but I have had trouble understanding exactly what G' and G" were. After reading this article, I have a better understanding of these properties. I now see how changing the degree of GelMA crosslinking will affect G'/G". I knew that increasing crosslinking would increase E, and know I see that increasing crosslinking would increase G' and G".

Structural and Rheological Properties of Methacrylamide Modified Gelatin Hydrogels

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Received December 27, 1999; Revised Manuscript Received January 19, 2000

Dynamic shear oscillation measurements at small strain were used to characterize the viscoelastic properties and related differences in the molecular structure of hydrogels based on gelatin methacrylamide. Gelatin was derivatized with methacrylamide side groups and was subsequently cross-linked by radical polymerization via photoinitiation. The light treatment of methacrylamide gelatin solutions resulted in the production of hydrogel films with high storage modulus (G′). Mechanical spectra and thermal scanning rheology of the obtained hydrogels are described. The temperature scan of the network below and above melting point of gelatin allowed us to identify the respective contributions of chemical and physical cross-linkage to the hydrogel elastic modulus. The results indicate that the rheological properties of the gelatin-based hydrogels can be controlled by the degree of substitution, polymer concentration, initiator concentration, and UV irradiation conditions.

1. Introduction

Gelatin is a proteinaceous material obtained by hydrolytic degradation of naturally occurring collagen.[1,2] It derives in particular from the fundamental molecular unit of collagen, a triple helical structure, the tropocollagen rod. Gelatins are soluble in warm water (>40 °C), but on cooling thermoreversible transparent gels are normally formed.[3] In fact, gel formation obtained by cooling gelatin aqueous solutions is accompanied by some characteristic changes which have been ascribed to a partial regain of the collagen triple-helix structure. Over the years much work has been devoted to the study of gel formation by biopolymers such as gelatin.[4−11] The structures and mechanisms of the formation of the gel networks involved, and their mechanical properties are now well understood.

Because of its unique gelling properties, gelatin is an attractive candidate as starting material for preparing hydrogels. Hydrogels are materials which, when placed in excess water, are able to swell and retain large volumes of water in its swollen three-dimensional structure without dissolution. Many materials of this type are considered to be biocompatible and a wide range of biomedical applications has been described. Among these are contact lenses,[12] artificial tendons,[13] matrices for tissue engineering,[14] and drug delivery systems.[15] Nevertheless, there is still a need to develop nontoxic biodegradable hydrogels for specific biomedical applications, e.g., wound treatment.

As a biomaterial, gelatin displays several advantages:[16] it is a natural polymer that has not shown antigenicity, it is completely resorbable in vivo and its physicochemical properties can be suitably modulated. Furthermore, due to the large number of functional side groups, gelatin readily undergoes chemical cross-linking, which is very important for its use as a biomaterial, e.g., as a drug delivery system or wound dressing.

For many applications there is a need for chemically cross-linked or so-call ed hardened gelatin materials. Since gelatin gels have a relatively low melting point, they are not stable at body temperature. Therefore, it is imperative to stabilize these gels by establishing chemical cross-links between the protein chains. A variety of hardening procedures are described in the literature.[6,17] Chemical cross-linking typically utilizes bifunctional reagents such as glutaraldehyde[18,19] and diisocyanates,[20] as well as carbodiimides,[21] polyepoxy compounds,[22] and acyl azides.[23] Glutaraldehyde is by far the most widely used agent, due to its high efficiency to stabilize collagen-based biomaterials and despite local cytotoxicity[24] and calcification of long-term implants.[25]

In our previous studies,[26] gelatin hydrogels were developed by making use of a polymeric cross-linker, dextran dialdehyde. The hydrogels were found to be biocompatible and biodegradable.[27] The rheological properties[28,29] and release characteristics[30] were studied in detail. It was shown that chemical aging of the gelatin−dextran dialdehyde hydrogel occurred, influencing the release patterns as a function of

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10.1021/bm990017d CCC: $19.00
Published on Web 05/18/2000

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Nick Herbst - Feb 27, 2023, 8:55 PM CST

Title: Understanding G' and G"

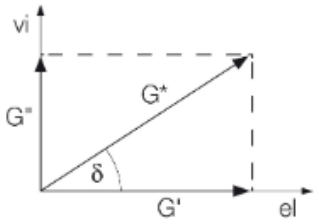
Date: 02/27/2023

Content by: Nick Herbst

Goals: Better understand the math behind G' and G".

Source: “Basics of rheology,” Anton Paar. [Online]. Available: https://wiki.anton-paar.com/us-en/basics-of-rheology/#oscilliation-tests-and-viscoelasticity. [Accessed: 27-Feb-2023]..

Content:

- The storage and loss moduli (G' and G") are vector components of the complex shear modulus G*
    - 

- G* = sqrt(G'^2 + G"^2)
- G* is like G, the shear modulus
    - G = shear stress / shear strain = τ / γ

Conclusions:

I believe I now fully understand how we will be calculating the elastic modulus after we do rheology testing. Now that I know how to get G from G' and G", I can use my knowledge from prior EMA courses to get E. I know that E = 2G (1 + ν) where ν is Poisson's ratio. That means we need to identify Poisson's ratio for GelMA.

04/18/2023 GelMA + Lung Epithelial Cells

Nick Herbst - Apr 17, 2023, 9:43 PM CDT

Title: GelMA + Lung Epithelial Cells

Date: 04/18/2023

Content by: Nick Herbst

Goals: Look at an example of GelMA hydrogels being used in conjunction with lung epithelial cells

Source: D. Huang, T. Liu, J. Liao, S. Maharjan, X. Xie, M. Pérez, I. Anaya, S. Wang, A. Tirado Mayer, Z. Kang, W. Kong, V. L. Mainardi, C. E. Garciamendez-Mijares, G. García Martínez, M. Moretti, W. Zhang, Z. Gu, A. M. Ghaemmaghami, and Y. S. Zhang, “Reversed-engineered human alveolar lung-on-a-chip model,” Proceedings of the National Academy of Sciences, vol. 118, no. 19, May 2021.

Content:

- The authors made an alveolar lung-on-a-chip system that consists of a porous GelMA hydrogel connected to an ALI & pump system.
  - I will be focusing on the GelMA + cell portion of the system
- They cultured human alveolar epithelial cells on GelMA with a Young's modulus of ~6 kPa
  - GelMA was double crosslinked
    - photocrosslinking with I2959 followed by crosslinking with mTG
  - Swelling ratio is smaller for double crosslinked gels compared to just photocrosslinked gels.
  - Gels were given pores by gelling them around alginate beads and then removing said beads
- The GelMA hydrogel allowed for great cell adhesion and proliferation
- See attachment for the full journal article

Conclusions:

This paper shows another application of using GelMA with lung cells. I found it beneficial to see a similar set up to our project from a different perspective. At this point in time, we are producing GelMA gels with E's between 2 and 5 kPa. A member of the client's lab is noticing that the ~3 kPa gels aren't getting good cell adhesion. This paper shows that you can get good lung epithelial cell adhesion on a lower stiffness GelMA gel. We likely need to raise the stiffness just a bit higher.

Nick Herbst - Apr 17, 2023, 7:37 PM CDT


Reversed-engineered human alveolar lung-on-a-chip model

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pnas.202016146.pdf (2.05 MB)

02/10/2023 Lung ECM Hydrogel

Nick Herbst - Feb 10, 2023, 7:15 PM CST

Title: Lung ECM Hydrogel

Date: 02/10/2023

Content by: Nick Herbst

Goals: Learn about hydrogels made from lung ECM and determine why there is a need for an alternative

Source: R. H. de Hilster, P. K. Sharma, M. R. Jonker, E. S. White, E. A. Gercama, M. Roobeek, W. Timens, M. C. Harmsen, M. N. Hylkema, and J. K. Burgess, “Human lung extracellular matrix hydrogels resemble the stiffness and viscoelasticity of native lung tissue,” American Journal of Physiology-Lung Cellular and Molecular Physiology, vol. 318, no. 4, Apr. 2020.

Content:

- The authors made human lung ECM-based hydrogels derived from normal tissue, COPD tissue, and fibrotic tissue to evaluate the stiffness and viscoelastic properties of the gels
- lung ECM is viscoelastic
    - basically a matrix of fibrillar proteins in a proteoglycan water gel
- the lung ECM hydrogels were made by decellularizing lung tissue with detergent, freeze-drying the resulting ECM solution, solubilizing the solution via proteolytic enzymes (such as pepsin) for 72 hr, and then neutralizing and heating the solution to get spontaneous gelation
    - the ECM hydrogels will have same composition as native ECM *but not the same architecture*
- Mechanical properties were measured by stress-relaxation compression tests and comparing the gels' properties to the tissues they came from
    - the stiffness (Young's modulus) of the gels was **reduced** compared to their tissues
        - However, the tissues were heterogeneous while the gels were more homogeneous
        - normal lung
            - 3.7 +/- 1.3 kPa tissue vs. 1.1 +/- 0.2 kPa hydrogel
        - COPD lung
            - 2.9 +/- 0.8 kPa tissue vs. 1.5 +/- 0.4 kPa hydrogel
        - fibrotic lung
            - 18.9 +/- 11.1 kPa tissue vs. 6.8 +/- 2.8 kPa hydrogel
    - relaxation and other viscoelastic properties of the hydrogels did not mimic their respective tissues
- See attachment for full article

Conclusions:

While it is possible to make hydrogels out of lung ECM, it is not practical for our purposes. Yes, the hydrogel would have the appropriate biochemical properties (degradability and adhesion), but the mechanical properties are not properly reflected. Furthermore, the hydrogel synthesis would not be feasible for us due to the need for many detergents and enzymes to decellularize and solubilize the lung tissue.

Nick Herbst - Feb 10, 2023, 3:53 PM CST

Human lung extracellular matrix hydrogels resemble the stiffness and viscoelasticity of native lung tissue

Abstract

Chronic lung diseases such as idiopathic pulmonary fibrosis (IPF) and chronic obstructive pulmonary disease (COPD) are associated with changes in extracellular matrix (ECM) composition and abundance affecting the mechanical properties of the lung. This study aimed to generate ECM hydrogels from control, severe COPD (Global Initiative for Chronic Obstructive Lung Disease (GOLD) IV), and fibrotic human lung tissue and evaluate whether their stiffness and viscoelastic properties were reflective of native tissue. For hydrogel generation, control, COPD GOLD IV, and fibrotic human lung tissues were decellularized, lyophilized, ground into powder, porcine pepsin solubilized, buffered with PBS, and gelled at 37°C. Rheological properties from tissues and hydrogels were assessed with a low-load compression tester measuring the stiffness and viscoelastic properties in terms of a generalized Maxwell model representing phases of viscoelastic relaxation. The ECM hydrogels had a greater stress relaxation than tissues. ECM hydrogels required three Maxwell elements with slightly faster relaxation times (τ) than that of native tissue, which required four elements. The relative importance (R_i) of the first Maxwell element contributed the most in ECM hydrogels, whereas for tissue the contribution was spread over all four elements. IPF tissue had a longer-lasting fourth element with a higher R_i than the other tissues, and IPF ECM hydrogels did require a fourth Maxwell element, in contrast to all other ECM hydrogels. This study shows that hydrogels composed of native human lung ECM can be generated. Stiffness of ECM hydrogels resembled that of whole tissue, while viscoelasticity differed.

Keywords: COPD, extracellular matrix, hydrogel, IPF, rheology

INTRODUCTION

Chronic respiratory diseases are a prominent cause of morbidity and mortality worldwide, with chronic obstructive pulmonary disease (COPD) being the third leading cause of death in the United States. Chronic lung diseases, such as COPD and idiopathic pulmonary fibrosis (IPF), are characterized by extensive changes in the extracellular matrix (ECM), the three-dimensional

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Human_lung_extracellular_matrix_hydrogels_resemble_the_stiffness_and_viscoelasticity_of_native_lung_tissue_-_PMC.pdf (1.14 MB)

02/20/2023 GelMA Hydrogel Scaffold

Nick Herbst - Feb 20, 2023, 6:20 PM CST

Title: GelMA Hydrogel Scaffold

Date: 02/20/2023

Content by: Nick Herbst

Present: Nick Herbst

Goals: Summarize current design idea and how it was thought of

Content:

- Last semester the team attempted to make a PEG hydrogel scaffold
    - Could not get the solution to gel
- The team had considered GelMA before, but after I learned about GelMA in my BME 545 class, I proposed that we revisit it
- GelMA has cell adhesion sequences and MMP-degradable sequences in it already
    - We would not need to add them like in the case of PEG
- GelMA has highly tunable mechanical properties
    - 3-fold tunability to adjust degree of crosslinking
        - Adjust amount of methacrylation
        - Adjust concentration of GelMA in the gel
        - Adjust the amount of time exposed to UV light
    - Can adjust these parameters to get the scaffold to the stiffness of normal lung ECM or to the stiffness of fibrotic lung ECM
- Fibroblasts can be encapsulated in the hydrogel scaffold since the UV light used to crosslink is not germicidal/cytotoxic
- GelMA is translucent so the epithelial cells cultured on it can be imaged properly
- See Research Notes section for more information on GelMA as well as sources

Action items:

- work on preliminary deliverables
- do additional research into specifics of GelMA fabrication, degradation, and mechanical properties

03/29/2023 Gelatin Methacrylation Protocol

Nick Herbst - Mar 30, 2023, 10:04 AM CDT

Title: Gelatin Methacrylation Protocol

Date: 03/29/2023

Content by: Nick Herbst

Goals: Read protocol article that details the process of synthesizing GelMA in order to understand *exactly* what materials and equipment we will need for fabrication

Source: D. Loessner, C. Meinert, E. Kaemmerer, L. C. Martine, K. Yue, P. A. Levett, T. J. Klein, F. P. Melchels, A. Khademhosseini, and D. W. Hutmacher, “Functionalization, preparation and use of cell-laden gelatin methacryloyl–based hydrogels as modular tissue culture platforms,” Nature Protocols, vol. 11, no. 4, pp. 727–746, 2016.

Content:

- The authors of this protocol characterized a GelMA hydrogel for use in tissue culture
  - In this notebook entry, I will only focus on the methacrylation of gelatin
- Materials
  - gelatin
    - porcine skin, type A, 300 bloom
  - methacrylic anhydride
    - can cause skin, eye, and respiratory irritation so proper protection
  - PBS
    - pH 7.4
  - $NaHCO_3$
  - I-2959
  - liquid nitrogen
    - need proper protection
- Equipment
  - PPE
  - round-bottom flask (RBF)
  - 200-500 mL beaker
  - magnetic stir bar
  - magnetic stirrer + hot plate
  - glass pipettes
  - 50 mL centrifuge tubes
  - centrifuge
  - 12-kDa MWCO dialysis tubing
  - pH probe
  - micropipette + tips
  - 0.2 µm syringe filter + syringe OR disposable vacuum filtration unit w/ PES membrane + vacuum pump
  - liquid nitrogen gloves
  - freeze-dryer
  - peristaltic pump
- Procedure (1-2 weeks)
  - Notes
    - perform in fume hood, wear gloves/lab coat/googles
    - GelMA is photosensitive so keep stuff with GelMA in the dark, cover dialysis setup in aluminum foil
    - Amount of methacrylic anhydride added can be varied to affect degree of methacrylation
    - Dialysis is complete when GelMA solution is clear and/or when methacrylic anhydride odor is gone

  1. Soak gelatin in PBS at a concentration of (10% w/v) in an RBF w/ stir bar by stirring moderately
  2. Dissolve gelatin fully in the mixture by heating to 50 ºC and stirring until solution becomes clear
  3. Use a glass pipette to add methacrylic anhydride (0.6 g per 1 g gelatin for 75% functionalization or 0.06 g per 1 g gelatin for 31% functionalization) while stirring vigorously for 1-3 hr until solution is homogenous and opaque
  4. Transfer solution to 50 mL centrifuge tubes and remove excess methacrylic anhydride by centrifuging the solution at 3500g for 3 min at 25 ºC and then decanting the GelMA supernatant into a glass beaker while discarding the methacrylic anhydride pellet
  5. Dilute the GelMA supernatant with 2 volumes of 40 ºC PBS

6. Transfer solution to dialysis tubing and dialyze at 40 ºC against a large volume of PBS for 5-7 days with the PBS changed daily
7. After dialysis, transfer GelMA solution to a beaker and use 1 M $NaHCO_3$ to adjust pH to 7.4
8. In a biosafety cabinet, filter and sterilze the GelMA solution with either a vacuum filter or a syringe filter
9. Divide sterile GelMA solution into aliquots in 50 mL tubes and snap-freese them with liquid nitrogen
10. Right after snap-freezing, add vented screw-top caps to the tubes and transfer aliquots to freeze-dryer to lyophilize until dehydrated (≥1 week)
11. Exchange vented caps for normal tube caps and store GelMA in a cool environment

- See attachment for the full protocol

Conclusions

This protocol details exactly what we would need to do in order to methacrylate our own gelatin instead of using the leftover batch we obtained from Dr. Masters. The majority of the team believes that making our own GelMA is crucial. I see the benefits since we would have more control over the properties of the GelMA, and we would be able to give the client a more thorough hydrogel protocol. However, since this is a very time-consuming process that we have never done before and it requires a lot of equipment we don't have experience in using (mainly the dialysis setup), I believe we should revisit using the GelMA from Dr. Masters.

Once the LAP, PBS, and gel molds arrive from our material order, I want to try and make gels. While we wouldn't have the degree of tunability from methacrylation, we would still have the degrees of tunability from the GelMA w/v concentration, the cooling time, and the UV exposure time.

Nick Herbst - Mar 29, 2023, 2:15 PM CDT

PROTOCOL

Functionalization, preparation and use of cell-laden gelatin methacryloyl–based hydrogels as modular tissue culture platforms

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nprot.2016.037.pdf (2.23 MB)

02/05/2023 Prior Completed Trainings

Nick Herbst - Feb 05, 2023, 3:24 PM CST

Title: Prior Completed Trainings

Date: 02/05/2023

Content by: Nick Herbst

Present: Nick Herbst

Goals: Provide proof of training that was completed in prior semesters

Content:

WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

This certifies that Nicholas Herbst has completed training for the following course(s):

Course	Assignment	Completion	Expiration
Biosafety Required Training	Biosafety Required Training Quiz	8/21/2020	8/21/2025
Chemical Safety: The OSHA Lab Standard	Final Quiz	1/13/2022	
Responsible Conduct of Research	RCR Certification	9/7/2020	

Data Last Imported: 09/18/2022 08:30 PM

You have the following permits and upgrades:

Name	Date
Green Permit	01/29/2022
Lab Orientation	09/26/2020
Red Permit	01/26/2022
Laser 1	10/06/2020

Conclusions/action items:

- I have completed all necessary training for use of the TEAM Lab and the Teaching Lab

- I can get additional qualifications for the TEAM Lab/Makerspace if I want/need to

03/10/2023 WARF Presentation

Nick Herbst - Mar 10, 2023, 3:21 PM CST

Title: WARF Presentation

Date: 03/10/2023

Content by: Nick Herbst

Present: Nick Herbst

Goals: Watch the WARF lecture recording and take notes on intellectual property

Content:

- WARF is a nonprofit organization that supports/manages the UW-Madison research community
    - helps research "get off campus"
- Intellectual Property (IP): **Patents**, Copyrights, Tradmarks
    - Patents
        - machines, dives, compounds, processes, method improvements
    - Copyrights
        - literary works, webpages, software
    - Trademarks
        - words and phrases, colors, sound, pictures, logos
    - Trade Secret
        - ideas that are completely secret, not handled by WARF
    - CATEGORIES AREN'T MUTUALLY EXCLUSIVE
- Cycle of Innovation: UW Research ---> IP Protection ---> Licensing and Startups ---> Funding to Support Research
- Researchers sent WARF an Invention Disclosure Report (IDR)
- There is no global patent system, need patents for each region
    - WARF helps researchers get US patents
- Patent offices evaluate idea against the novelty and non-obviousness of prior art (by the inventor or another person)
    - prior art by the inventor is only considered after 1 year of idea conception in the US
    - internationally has no grace period, ideas need absolute novelty
    - Patentability Reqs: eligible, useful, enabled, novel, non-obvious
        - to prove not novel, there needs to be a SINGLE reference that describes what is proposed (can't use multiple to Frankenstein a counter)
        - non-obviousness is hard req to meet because multiple reference *can* be combined to make a counter
- Public disclosure of invention that signals start of US grace period
    - journal publications, talks at conference, poster presentations, non-confidential seminars, open thesis defenses, dissertations, online descriptions
    - leave out key details when telling others to avoid enabling disclosure
- US patent process takes 3-5 years and costs around $30,000
- Licensing takes a lot longer, establish contract with a big company to let them use the invention so they can develop and commercialize it and give money back

Conclusions:

I am unsure if our design has IP considerations since GelMA is a widely used hydrogel. I guess our GelMA scaffold fabrication protocol could potentially be considered for a patents if we manage to get the *specifics* down in order to get the desired healthy and diseased state stiffnesses of lung ECM, though we would likely run into issues with non-obviousness. Since patenting is so time intensive and we likely wouldn't get a patent, we won't apply for a patent.

2014/11/03-Entry guidelines

John Puccinelli - Sep 05, 2016, 1:18 PM CDT

Use this as a guide for every entry

- Every text entry of your notebook should have the **bold titles** below.
- Every page/entry should be **named starting with the date** of the entry's first creation/activity, subsequent material from future dates can be added later.

You can create a copy of the blank template by first opening the desired folder, clicking on "New", selecting "Copy Existing Page...", and then select "2014/11/03-Template")

Title: Descriptive title (i.e. Client Meeting)

Date: 9/5/2016

Content by: The one person who wrote the content

Present: Names of those present if more than just you (not necessary for individual work)

Goals: Establish clear goals for all text entries (meetings, individual work, etc.).

Content:

Contains clear and organized notes (also includes any references used)

Conclusions/action items:

Recap only the most significant findings and/or action items resulting from the entry.

2014/11/03-Template

John Puccinelli - Nov 03, 2014, 3:20 PM CST

Title:

Date:

Content by:

Present:

Goals:

Content:

Conclusions/action items: