BME Design-Fall 2022 - CARLEY SCHWARTZ
Complete Notebook

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SARA MOREHOUSE

on

Dec 14, 2022 @09:34 PM CST

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Team contact Information

ELIJAH DIEDERICH (ediederich@wisc.edu) - Sep 27, 2022, 7:55 PM CDT

Last Name	First Name	Role	E-mail	Phone	Office Room/Building
Murphy	William	Advisor	wlmurphy@wisc.edu	NA	M1077
Brasier	Allan	Client	abrasier@wisc.edu	NA	NA
Schwartz	Carley	Leader	cischwartz@wisc.edu	6306217554	NA
Treacy	Caitriona	Communicator	ctreacy2@wisc.edu	2625994130	N/A
Dierderich	Elijah	BSAC	ediederich@wisc.edu	920-517-9419	NA
Morehouse	Sara	BWIG	smorehouse2@wisc.edu	9202525749	NA
Martinez	Ana	BPAG	almartinez4@wisc.edu	2627511037	NA
Cao	Althys	BWIG	nvcao@wisc.edu	6164690243	NA

Project description

CARLEY SCHWARTZ - Sep 14, 2022, 3:30 PM CDT

Course Number:

Project Name: TISSUE MODEL OF THE EPITHELIAL MESENCHYMAL TROPHIC UNIT

Short Name: Tissue Model of the EMTU

Project description/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 include the following functions in combination; accurate sizing to the small airway, epithelial exposed on air interface, grown on extracellular matrix that has variation to its thickness and composition, and lies above normal lung fibroblasts.

About the client:

Dr. Allan Braiser is a part of ICTR which is the Institute for Clinical and Translational Research. He is also recognized for substantial research done on inflammation response and work within pulmonary and cardiovascular diseases.

Client Notes Meeting 1

CARLEY SCHWARTZ - Sep 25, 2022, 8:43 PM CDT

Running Notes

- Usual testing involves asthma and other lung diseases and how these affect the epithelium and adjacent fibroblasts

- Cells talk to one another and when there is injury the ECM and cell to cell communication will also cause changes in cell response

- synthetic 3D printed scaffold that can have bio- injections

- want ALI so epithelium will be polarized

- matrigel limited because its composition isn't for sure and causes variation

-plastic is high in tension and doesn't work well

-12 well plate, 10-100 microns deep at least

-needs to be biocompatible with fibronectin and collagen

-1 month of cell culturing for testing

Client Meeting Notes 10/20/22

SARA MOREHOUSE - Dec 05, 2022, 7:37 PM CST

Title: Client Meeting 10/20/22

Date: 10/20/22

Content by: Sara Morehouse

Present: Catriona Treacy, Elijah Diederich

Goals: To determine more specific needs of the client as far as budget, ALI model, and capabilities of the design.

Content:

- Molecules that can go through ECM?
    - Fibronectin
    - Integrin - receptor on cells, bound to cell membrane, sense extracellular environment, bind to fibronectin or collagen which triggers cellular signaling pathway that will modify cell/phenotype
    - Reduce to simplest
        - Fibronectin matrix → epithelial cells bind to fibronectin
- Incorporation of fibronectin and collagen?
    - Throughout the matrix - add before crosslinking
- Previously tried PLA-based matrix, epithelial cells did not stick very well
- Cost:
    - 5-10K budget
    - PEG is cheap
    - Other components may cost more
- 24 well plates provided
- ALI
    - Sterile cups that sit inside of 24-well plate, goes halfway down into well, medium inside, bottom of cells sit on it
    - Cannot get inserts because they are on backorder
- UV light not compatible with fibroblasts - will kill cells
    - Is there a way of crosslinking that will preserve cell compatibility
- Would like it to be degradable once cells grow and produce their own ECM
- Not sure what concentration of fibronectin would be needed? - get back to client
    - Normal fibronectin
    - Different types of fibronectin produced when cells are stressed
- How to test?
    - Do epithelial cells stick and “look happy”

Conclusions/action items: Discuss crosslinking methods with Dr. Murphy and do research into fibronectin concentrations.

Advisor Meeting #1

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 11, 2022, 7:46 PM CDT

Title: Advisor Meeting #1 Notes

Date: 9-16-22

Content by: Elijah Diederich

Present: BME 300/200 Group

Goals: To meet with our advisor and develop a preliminary understanding of the semester design project

Content:

Picture of notes included below

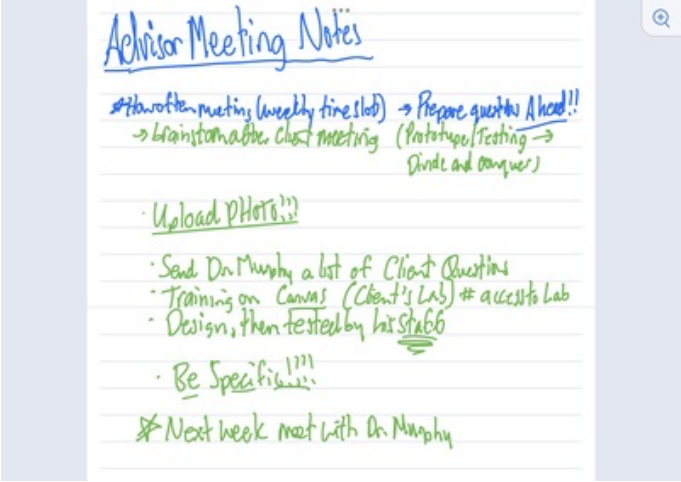
Conclusions/action items:

1. Send Dr. Murphy a list of client questions before meeting

2. Upload group photo

3. Research!!!!!!!!!!

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 11, 2022, 7:34 PM CDT



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Carley_Intro_Video_Notes_2_.png (294 kB) Notes taken from Advisor meeting #1

Advisor Meeting #2

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 11, 2022, 7:38 PM CDT

Title: Advisor Meeting #2

Date: 9-30-22

Content by: Elijah Diederich

Present: BME 300/200 EMTU Group

Goals: To introduce our design ideas and get insight on categories needed for design matrix

Content:

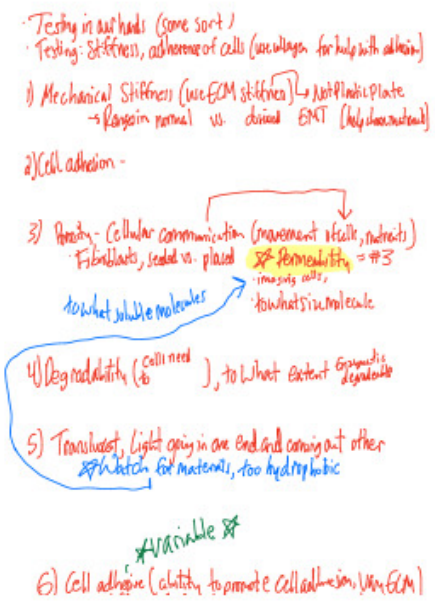
Picture of notes included below

Conclusions/action items:

1. Start presentation slides and email them to Dr. Murphy before Wednesday of next week

2. Research Alginate, PEG, Matrigel

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 11, 2022, 7:41 PM CDT



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Client_Meeting_2.pdf (1.6 MB)

Advisor Meeting 10/14/22

SARA MOREHOUSE - Dec 05, 2022, 7:33 PM CST

Title: Advisor Meeting 10/14/22

Date: 10/14/22

Content by: Sara Morehouse

Present: All

Goals: To update Dr. Murphy and to gain a better understanding of different paths that we could take when designing our model.

Content:

- PEGDA
- PEGMA
- PEGDMA
- PEG:
    - Long chain of monomers
    - Polyether
    - Cells will not attach, will be repelled
    - Proteins do not bind to PEG, cell will not bind
    - BLANK SLATE biomaterial
    - PEG + peptides (cell adhesion peptide), cell receptors can link to peptides
    - PRO: control cell adhesion
    - PRO: control PEG degradation (ECM remodeling)
    - Can create linkages by other types of peptides that can be cleaved by cells for cells to imbed
    - PRO: control PEG mechanical properties and porosity
    - By controlling initially properties of PEG chain (molecular weight)
        - Largest MW network will have greater porosity = weaker mechanical properties
        - Smaller MW network will be denser and stronger mechanical properties
- ***Need to figure out specifically what we need for mechanical properties, porosity, and cell interaction capabilities and degradability
- Deliver a range of conditions for making gels that will have the right mechanics, porosity, peptides, etc. (Cut out need for cell culture)
- Chain Growth Polymerization
    - 2-arm PEG (one chain), difunctionalized
    - Ex: PEG Diacrylate, Dimethacrylate (PEGDA, PEGMA)
    - Polymerizes by adding photoinitiator and UV light
    - Photoinitiator = I2959, then 365 UV light (long wave) (shorter wavelength will kill cells)
    - Polymerization of functional groups that form kinetic chains (PEG chains react at kinetic chain), makes a network

    - Kinetic chains will be polyacrylate or polymethacrylate (small), most of the material will be the PEG chains
    - Can build in peptides - has to have cysteine peptide (Thiyl will react with PEG), cell adhesion peptide
- Step Growth Polymerization
    - Multi-armed PEG with functionality on all arms
    - Can cross link 4-arm PEG w 2-arm crosslinker, functionalized w something else
    - Speed depends on pH and pKa of groups
    - Won't work if want cells imbedded from the start before gelling
- Start with MW and UV light
- Reagent grade PEG(DA)
- Peptides are expensive - start with mechanics and porosity w MW, meet these specs
- Being able to vary tension is good (client wants a range)
- Porosity - figure out what size molecule needs to fit in (Dextran molecule for labeling and measuring, is cheap and can be similar in properties to molecules that client may want to be able to get inside) **- ask client what molecules**
- Concentration? Get one MW and vary concentration
- Don't worry about swelling right now
- Can do crosslinking inside of well plates as a mold
- Aluminum foil and refrigerate photoinitiator
- When you mix PEG and photoinitiator, not as important to keep that completely dark, just don't let it sit for extended period of time
- Talk to client about ALI
- How to test mechanical properties??
    - Elastic modulus - MTS testing

Conclusions/action items: Need to figure out specs for mechanical properties, porosity, cell interaction, and degradability.

Advisor Meeting 10/21/22

SARA MOREHOUSE - Dec 05, 2022, 7:39 PM CST

Title: Advisor Meeting Notes 10/21/22

Date: 10/21/22

Content by: Sara Morehouse

Present: All

Goals: Discuss crosslinking, go over model specs.

Content:

- No issues with UV light crosslinking and fibroblasts
    - Dependent on conditions - as long as use the right combination
    - Thiol-ene chemistry?
        - Use 4-arm PEG or 8-arm, functionalized w norbornene
        - Something else w thiol on the end (can be PEG or peptide to control cell degradability)
        - Add light and photoinitiator, norbornene reacts w thiol
        - Click chemistry
        - Dr. Murphy will send papers on this to us
            - Fibroblasts
            - Tumor modeling
    - Some MW of PEG w photocrosslinking are too dense that doesn’t allow for cell viability
- MW of PEG?
    - Match MW and concentration of PEG with desired mechanical properties
    - He can send papers on this
    - Might be able to do just one MW and then vary concentration
- Testing matrices to see if epithelial cells will stick?
    - Fibroblasts inside, epithelial cells on top
    - Need conditions to support epithelial cell attachment (need right kind of peptide)
    - Fibroblasts will use just about any cell adhesion peptide
    - Dr. Murphy will send info on epithelial cell attachment
- He can ask grad student to be consultant

Conclusions/action items: Read papers sent by Dr. Murphy to find specific amounts/concentrations needed to meet client's specifications.

Advisor Meeting 10/28/22

SARA MOREHOUSE - Dec 05, 2022, 7:44 PM CST

Title: Advisor Meeting 10/28/22

Date: 10/28/22

Content by: Sara Morehouse

Present: All

Goals: Go over questions involving degradable peptides, client requirements.

Content:

- What concentrations of collagen and fibronectin?
    - Client should know composition
    - Research
    - How much do we need to put in
- Collagen will affect stiffness - still have to stay in range
    - Peptides will not do what client wants - simplistic mimicking of collagen and fibronectin
    - Peptides are only for cell adhesion, not for everything that collagen and fibronectin do
    - Would need to test to figure out how this much collagen or this much fibronectin would affect mechanical properties
    - Peptides have no effect on mechanical properties
    - Proteins will be passively incorporated into hydrogel - not covalently bonded
    - Look into materials of the lung - typical concentrations of collagen and fibronectin in the lung?
- Criteria: (goal to mimic what fibroblasts have already produced)
    - Stiffness
    - Collagen and fibronectin concentration
        - Realistic? Cost? Solubility?
        - Does client want it for cell adhesion? Or other reasons too?
        - Fibronectin for cellular communication, stressed vs. unstressed
            - Fibronectin produced by cells when stressed or unstressed??
            - Fibroblast remodeling, add epithelial cells later
            - Naturally produced collagen and fibronectin
    - Fibroblasts embedded, epithelial cells at surface
    - ALI???
    - Transport?? (molecular)
- Fibroblasts inside gel (goal to have fibroblasts produce ECM)
    - Overtime those cells produce their own ECM, breaking down the PEG (remodeling environment)
    - Then, add epithelial cells to top so they are exposed to native ECM

    - Criteria
        - Stiffness
        - Degradability
        - Mechanism for cell adhesion (peptides)
        - Collagen and fibronectin concentrations irrelevant
    - Two different dithiols
        - PEG
        - degradable peptide
        - Control concentrations - more of peptide means more degradable
- Question: when will fibroblast incorporation happen??
    - Second model seems more like what client wants, consistent with papers Dr. Murphy already sent

Conclusions/action items: Figure out exact concentrations/amounts of peptides, fibroblasts, collagen, fibronectin needed.

Advisor Meeting 11/11/22

SARA MOREHOUSE - Dec 05, 2022, 7:46 PM CST

Title: Advisor Meeting 11/11/22

Date: 11/11/22

Content by: Sara Morehouse

Present: All

Goals: Go over ALI concerns, parameters.

Content:

- Gel parameters - stiffness range, degradability over what time frame?, cell adhesion (RGD peptide)
- Surface parameters - epithelial cell adhesion, epithelial cell viability, proliferation (find literature that supports idea of these cell types will adhere to surface - Peptides, growth factors in medium?
- Need a reason for doing submerged culture
- What fibroblast cell type - has it been cultured in PEG before
- Transport - test with labeled dextran, similar molecular weight to another molecule
    - Transport assay/measurement
- Peptides won’t affect stiffness, transport (stay below threshold)

Conclusions/action items: Continue reasearching parameters.

Advisor Meeting 11/18/22

SARA MOREHOUSE - Dec 05, 2022, 7:48 PM CST

Title: Advisor Meeting 11/18/22

Date: 11/18/22

Content by: Sara Morehouse

Present: All

Goals: Go over progress with ordering materials and future steps.

Content:

- Reproducibility spec
- RGD peptides won't have any impact on properties of gel other than cell adhesion
    - Expensive, weren't doing testing with cells, wouldn't affect stiffness,etc. outcomes (need papers to back this up. Need to know ranges of peptides that will be incorporated)
- MMP peptides
    - Time periods?
    - Fibroblasts embedded in degradable gels, how did they behave? Based on this, give ___ peptide range needed.
    - Can increase or decrease range based on client's thoughts
    - Target amount of degradability? Collagen-like matrix?
- Plan testing, performing testing, write testing protocols.
    - Stiffness
    - Translucency
    - Media being taken in by gel

Conclusions/action items: Need protocols for fibroblast concentrations, MMP peptides, RGD peptides, translucency testing

Advisor Meeting 12/2/22

SARA MOREHOUSE - Dec 05, 2022, 7:50 PM CST

Title: Advisor Meeting 12/2/22

Date: 12/2/22

Content by: Sara Morehouse

Present: All

Goals: To go over progress and difficulties with fabrication, go over progress with researching and writing protocols and testing.

Content:

- Parameters, rationale for parameters
- Testing would not reflect parameters, but is proof of concept → we can test these qualities, gelatin is a phantom material
- Could gelatin be used for the actual model??
- Translucent, allow for transport of media, known to maintain viability of fibroblasts, can vary mechanical properties, shown to allow for epithelial cell culture

Conclusions/action items: Finish up protocols for testing, conduct testing, fabricate a prototype or "phantom" model to be used at presentation, work on presentation and final report.

Design Ideas/components

SARA MOREHOUSE - Dec 14, 2022, 4:08 PM CST

Title: Design Outline and Components

Date: 11/8/2022

Content by: Sara Morehouse

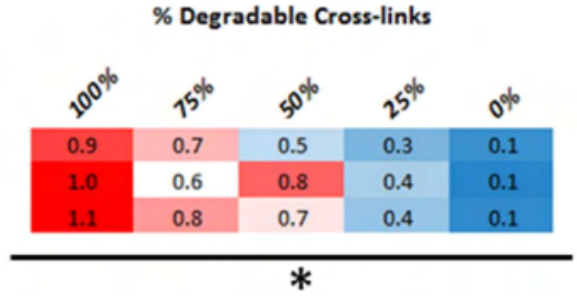
Present: Catriona Treacy

Goals: To clearly lay out our plans for our design and to figure out the exact concentrations we need.

Content:

Hydrogel components:

- PEG-NB
  - 8arm-PEG20K-Norbornene, tripentaerythritol core
- Poly(ethylene glycol) dithiol 3,400
- MMP peptide?
  - Synthesis and characterization of MMP degradable and maleimide cross-linked PEG hydrogels for tissue engineering scaffolds - ScienceDirect (wisc.edu)
- Table from Matt Parlato Dissertation - information on concentrations/ratios of crosslinkers and PEG
- Section from Dissertation talking about degradability, PEG concentrations, and use of MMP peptides vs PEGDT
  - “Regardless of PEGNB wt% or hydrogel cross-linking, hydrogels cross-linked with a peptide susceptible to MMP degradation (hereafter referred to as the MMP degradable peptide) allowed more CAC invasion than did hydrogels cross-linked with a non-degradable PEG-dithiol cross-linker”
  - Decreasing PEG wt% promotes higher levels of cell invasion (3% vs 4-10%)
  - Replacing MMP degradable peptide crosslinkers with PEG-dithiol crosslinkers reduces cell invasion (dosage-dependent)
  - Best combination for highest level of cell invasion is 3 wt% PEGNB with 100% MMP degradable peptide cross-linker



  - Red = most cell invasion and blue = least cell invasion (heat map)
- RGD peptides

Cross linking:

- Irgacure 2959-Photoinitiator

Conclusions/action items:

Need to determine if we will be using both MMP degradable peptide cross-linkers and PEGDT cross-linkers (and if so what percentages), or if we will just be using one or the other.

Final Design

SARA MOREHOUSE - Dec 14, 2022, 7:13 PM CST

Title: Final Design

Date: 12/14/22

Content by: All

Goals: Lay out clear description of final design and components.

Content:

In this model, polyethylene glycol synthetic materials are cross linked via photopolymerization with UV light. This allows for fibroblasts to be encapsulated within the gel [19]. This model allows for very precise selection of stiffness based on PEG-NB and PEG-DT concentrations detailed in the protocol table. Synthetic hydrogels such as PEG do not have any natural adhesive properties. As a result, RGD sequences are used to create binding sites with the selected concentration below. Lastly, to allow for fibroblast reconstruction of the ECM, fibroblasts are added at concentrations of 100,000-200,000 cells/mL and MMP peptides are incorporated at a concentration of 3% (weight/volume) when degradation by enzymes at 1 nM follows to allow for the synthetic matrix degradation timeline to match that of reconstruction.

PEG-NB used:

- 8arm-PEG20K-Norbornene, tripentaerythritol core

PEG-DT used:

- Poly(ethylene glycol) dithiol, 3,400

Photoinitiator:

- Irgacure 2959-Photoinitiator

Conclusions/action items: Overall, this entry is just to essentially summarize all components of our final design in one clear spot.

Materials and Expenses

SARA MOREHOUSE - Dec 11, 2022, 8:56 PM CST

Title: Materials and Expenses

Product	Price	Link
PEG-NB 8arm-PEG20K-Norbornene, tripentaerythritol core Material Number: JKA10037-1G 20kDa	$575.00 1 Gram	https://www.sigmaaldrich.com/US/en/product/aldrich/jka10037
Poly(ethylene glycol) dithiol 3,400 Material Number: 704539-1G	$407.00 1 Gram	https://www.sigmaaldrich.com/US/en/substance/polyethyleneglycoldithiol1234598765
Irgacure 2959-Photoinitiator Material Number: 5200-5GM	$110 5 Grams	https://www.cellink.com/product/irgacure-2959-photoinitiator/
24 Well Plates	Provided by Client	
PBS	Provided by Team Lab	
Peptides	Not yet needed	

PEG DT-NB Formation Protocol

CARLEY SCHWARTZ - Dec 06, 2022, 6:14 PM CST

Hydrogel Stiffness	PBS (uL)	PEG-DT (uL)	PEG-NB (uL)	I2959 (uL)
2 kPa	122.4	13.8	61.9	49.5
4 kPa	100.7	23.1	74.3	49.5
5 kPa	68.2	30.8	99	49.5
6 kPa	118.8	99	29.7	49.5

Protocol Overview

1. When taking out thaw aliquots on ice
2. Make sure to vortex everything before adding and in between each step
3. Add calculated amount if PBS
    a. For all of the below additions go slow, super viscous so need to make sure no air bubbles
4. Add calculated crosslinker to solution
5. Add calculated PEG-NB to solution
6. Add photoinitiator last
7. Leave to set in UV light for 5 minutes
    a. Before setting the gels give the UV light 5 minutes to warm up
8. After the gels are set need to be submerged in PBS
    a. This is to allow for swelling, 24 hours needed

[for all of the below protocols use 1x PBS and make sure to vortex between steps]

Forming Aliquots

PEG-DT

1. For our experiment, we will striving for 200 mM crosslinker aliquots (400 mM more accurately because DI-thiol)
    a. This means that we will add 1 gram of crosslinker into 1.47 mL of PBS
    b. This is based on calculations on separate document
2. To form this we will weigh out 1 gram of PEG-DT {halved it bc only 1 gram total in vial}
3. Then we will add this to a 15 mL conical tube with 1.47 mL of PBS
4. Vortex the solution
5. Leave on ice to till clear (roughly 30 minutes)

PEG-NB

1. For this PEG it is the same exact process as above just with 300 mg of PEG into 800 uL of PBS

Photo-Initiator [CANNOT BE EXPOSED TO LIGHT-TINFOIL]

1. We want to have a .5% (wt/v) of I2959 this means that we will need 5mg/ mL
    a. This means that we will add 20 mL of PBS with 100 mg of I2959
2. Place 20 mL of PBS in a glass bottle
3. Place on heated platform at 50C
4. Add 100 mg of 12959 to the bottle (wrapped in aluminum foil)
5. Let dissolve on the heated platform at 50C and mixing for over an hour
6. Store in Eppendorf tubes at -20C

Theoretical RGD Concentrations

CARLEY SCHWARTZ - Dec 06, 2022, 6:15 PM CST

RGD sequence mM	2.6	3.0	3.4	4.0	4.6	5.0
uL of RGD	9.6	11.1	12.6	14.8	17	18

Diffusion Testing

Althys Cao - Dec 14, 2022, 2:21 PM CST

Title: Hydrogel Diffusion Testing

Date: 11/28/22

Content by: Sara Morehouse, Ana Martinez, Althys Cao

Present: Sara Morehouse, Ana Martinez, Althys Cao

Goals: To establish protocols for testing diffusion of media through our hydrogel.

Content:

Idea 1: Measure diffusion by mass

- Measure mass of hydrogels before experiment, use inverted microscope with camera (BME Lab) to image gel prior to diffusion
- Set gel mold on top of media so only bottom is touching
    - Option 1: Could use forceps/tweezers to manually hold gel mold on
    - Option 2: Could get some kind of mesh sheet to set on top of dish that holds the media, then set the gel (mold) on top of mesh so that the media can absorb through (kind of like ALI but without transwell insert)
- After certain time increments (15 min, 30 min, 1 hour), measure mass of gels to compute mass of media diffused through the gel in certain amount of time
- At each time increment, use microscope to image gel for a visual reference of media diffusion through gels
    - For imaging, we may need to use a dye (alternative to labeled dextrans) in order for the media to show in the gel. While DMEM contains phenol red, a pH indicator, this may not be dark enough to appear on the microscope image. Alternatively, possible dyes could include a simple food coloring such as Blue No. 1.

Idea 2: Measure diffusion via labeled dextrans

- Labeled dextrans - hydrophilic polysaccharides used to monitor cell division, movement of live cells, and hydrodynamic properties of the cytoplasmic matrix
- Can be added to media to track the movement of a specific molecule (of a specific size) from the media into the gels
    - Because we are just monitoring diffusion of the media in general, this method might not be as appropriate

Media Used for Testing:

- DMEM, 5 mL

Steps:

1. Prior to experiment, image each gel with camera.
2. Mold sheet of thin aluminum wire mesh over top of petri dish with concave shape to mimic a transwell insert. Remove and measure mass of mesh wire form.
3. Place gel in center of mesh and measure mass of mesh and gel.
4. Fill petri dish with 5 mL of DMEM and place mesh back onto petri dish with gel on top so that the bottom of gel is in contact with the media but the top is exposed to air.
5. Leave gel in place for specified time increment (1 hour, 23 hours) inside of refrigerator at 4°C.

6. After each increment, remove mesh from petri dish.
    1. Pat bottom of mesh dry with paper towel to remove excess media.
    2. Measure mass of mesh with gel still on top.
    3. Take picture of gel to document diffusion of pink-colored media.
    4. Replace mesh and gel back onto petri dish and refrigerate.
7. Using subtraction, determine masses of gels at each time interval.
8. Use images taken throughout time increments to display a visual representation of media diffusion into gels

Conclusions/action items:

We will be measuring diffusion via mass of the hydrogel using the steps outlined above.

Note: Based on the contrasting results seen via the decrease in the gelatin hydrogels' masses and the gradual, significant darkening of the gels as the test proceeded, our team concluded that though media diffusion was clearly taking place in the gels, our intitial iteration of the diffusion testing protocol was likely at least partially responsible for the observed loss of mass. Based on previous research regarding gelatin's natural degradation properties, our team concluded that our initial iteration of the protocol, which did not account for minimizing the amount of times we transferred each gel from the mesh plate to the measuring scale, was likely the primary explanation for our observations. Therefore, in this second iteration of the protocol, we now outline how, rather than measuring the mass of each gel independently prior to diffusion and after each time interval, the mass of each mesh plate and hydrogel as a unit should be obtained.

Translucency Testing

SARA MOREHOUSE - Dec 14, 2022, 3:43 PM CST

Title: Translucency Testing Protocols

Date: 12/14/22

Content by: Althys Cao

Goals: To define a clear protocol for testing translucency of hydrogels.

Content:

1. Line the bottom of the gels (could either be separated in a petri dish or in plates) with a piece of paper.
2. Turn on a phone flashlight and put it under the paper.
3. Image gels as light was shone underneath.

Conclusions/action items:

Protocol for testing translucency is very simply as easy to understand and execute.

Updated translucency test - Quantitative test

Althys Cao - Dec 14, 2022, 5:53 PM CST

Title: Updated translucency test - Quantitative test

Date: 2022.12.14

Content by: Althys Cao

Present: Althys Cao

Goals: Update the current translucency test so that it can quantify how much light passes through / is absorbed by the gel.

Content:

1. Find a light source, can be any such as a phone flashlight or microscope light bulb; would recommend using a laser beam so that the light source is more concentrated / not as dispersed).
2. Turn off all other light sources (block them out if necessary) then turn on that light source and find the light intensity of that light source using a lux meter.
3. Put the gel on that light source and find the intensity of the light going through the gel using the lux meter.
4. Find the percentage (%) of how much light passes through the gel by: $\frac{\text{intensity of light going through gel}}{\text{intensity of light source}} \times 100$

Conclusions/action items:

If this project were to continue into the second semester, try this protocol out and make changes where necessary.

Reference:

P. Ciri, “How to measure light intensity: Understanding & using a lux meter,” *BIOS Lighting*, 30-Mar-2022. [Online]. Available: https://bioslighting.com/how-to-measure-light-intensity/architectural-lighting/. [Accessed: 14-Dec-2022].

Rheology Testing Protocol

SARA MOREHOUSE - Dec 14, 2022, 9:33 PM CST

Title: Rheology Testing Protocol

Date: 12-14-2022

Content by: Elijah Diederich

Present: NA

Goals: To keep a detailed document of the rheology protocol testing

Content:

Both of the tests will allow us to show that a PEG hydrogel can also be tested via both of these methods and values for the storage modulus (G'), loss modulus (G"), and the Young's Modulus (E) can be compared to native lung tissue samples. This proves that a hydrogel can accurately represent the mechanical properties of lung tissue and can also be tested.

Rheology Testing Protocol:

1. Once gelatin hydrogels have been formed and allowed to set for approximately 12-24 hours, rheometry testing may be performed.
2. Carefully remove 3 gels of each concentration (3.15% and 2.36%) from 48 well cell culture plates, keeping the gels of the same concentration 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 concentration (3.15% and 2.36%) were tested for a total of 6 separate frequency sweeps.
11. When testing is completed, results can be interpreted in MatLab

Conclusions/action items:

1. **Make sure to turn in Lab Archives Notebook**

Diffusion Testing Results

SARA MOREHOUSE - Dec 11, 2022, 8:20 PM CST

Title: Diffusion Testing Results

Date: 12/3/22

Content by: Sara Morehouse, Ana Martinez, Althys Cao

Present: Sara Morehouse, Ana Martinez, Althys Cao, Catriona Treacy, Elijah Diederich

Goals: To run through the protocols we developed to test diffusion of media through a hydrogel and make any necessary adjustments, as well as to determine the ability of media to diffuse through gels.

Content:

Results for 3.15 w/v% gelatin:

Gel #	Volume of Gelatin Solution	Initial Mass (g)	Mass after 1 hour	Mass after 23 hours
1	600 µL	0.583	0.552	0.544
2	600 µL	0.530	0.500	0.492

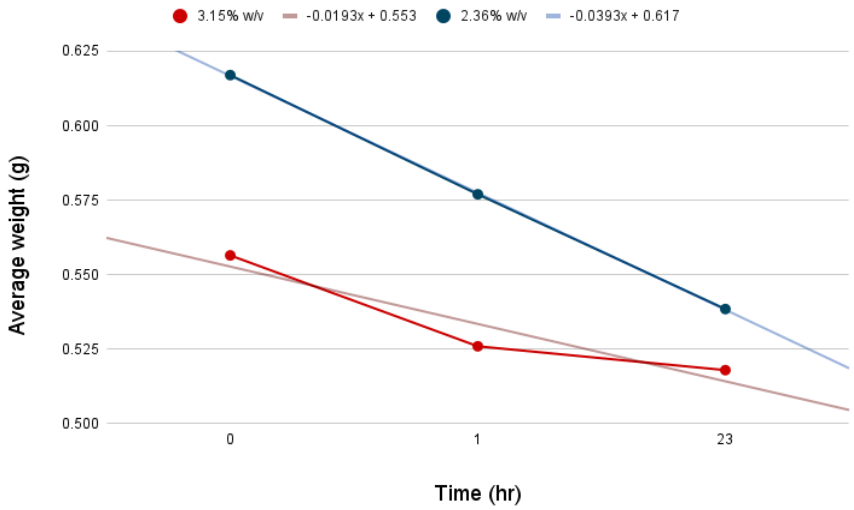
Results for 2.36 w/v% gelatin:

Gel #	Volume of Gelatin Solution	Initial Mass (g)	Mass after 1 hour	Mass after 23 hours
3	600 µL	0.598	0.552	0.514
4	600 µL	0.636	0.602	0.563

Image Results:

	Gel 1 (3.15 w/v%)	Gel 2 (3.15 w/v%)	Gel 3 (2.36 w/v%)	Gel 4 (2.36 w/v%)
Visual of gels prior to addition of media	 *same for all gels			
Visible diffusion after 1 hour				
Visible diffusion after 23 hours				

Graph Depicting Mass of Gels over Time:



Conclusions/action items:

From mass measurements, we found that mass decreased over time at air-liquid interface with media for all gels. However, media was clearly absorbed by the gels as demonstrated by the images taken at each time interval. The gradual darkening of the pink shade of the gel indicates that over time, more media was able to diffuse through the gel. These results indicate that when used in an actual experimental setting, media would be able to diffuse through the gels with fibroblasts embedded and epithelial cells could then be cultured at an air-liquid interface. While this experiment was only conducted with gelatin, similar results could be obtained when testing the PEG-NB/PEG-DT hydrogel using the protocol as outlined.

These results also prompted our team to consider possible ways we could alter our diffusion testing protocol so as to minimize mass decrease (and instead illustrate mass increase due to media diffusion) of the gels in future testing.

Translucency Testing Results

SARA MOREHOUSE - Dec 14, 2022, 4:04 PM CST

Title: Translucency Testing Results

Date: 12/14/22

Content by: Althys Cao

Goals: To describe results obtained of translucency testing.

Content:

After gelatin hydrogel formation, two gels of each concentration were tested. Both the 3.15 w/v% and the 2.36 w/v% gels appeared clear. When each gel was tested with the described protocols of shining the light on the underside of the gel, all displayed the desired property of allowing light to pass through the gel and out of its surface. By exhibiting translucency, gels are able to be imaged through for cell culture purposes and therefore pass the translucency test.

Figure 1: Gel before shining light (left), and gel during translucency testing viewed

Conclusions/action items:

All gels passed the test.

Rheology Testing Results

Caitriona Treacy - Dec 14, 2022, 8:08 PM CST

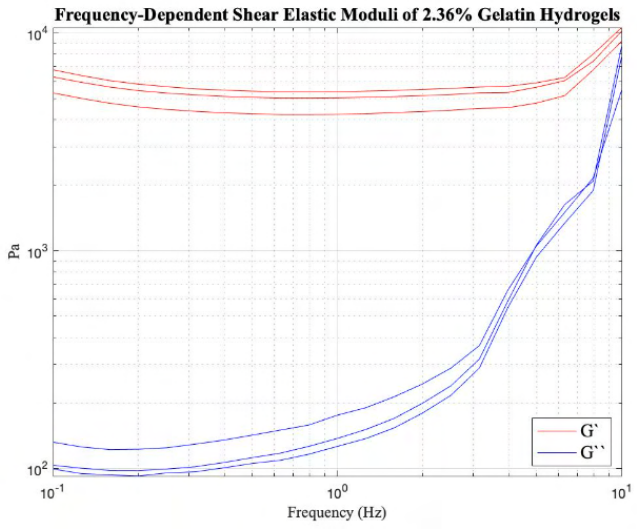
Content:

The results from the frequency test with the parameters described earlier in the testing section can be summarized in table 2. The Young's Modulus, Storage Modulus, and Loss Modulus of both concentrations of hydrogels are included as well as the values for native lung tissue. As the gelatin concentration increased in the hydrogels, the Young's Modulus, Storage Modulus, and Loss Modulus increased. This follows the pattern that increasing the degree of crosslinking leads to an increased hydrogel stiffness. Young's Modulus was derived from the equation E=2G(1+μ) where μ=Poisson's ratio. For gelatin, μ=0.5, classifying it as an incompressible material that is deformed elastically at small strain. At very low frequencies, G can be approximated as G' and the Young's Modulus can be derived using the storage modulus and Poisson's ratio at 0.1 Hz. In graphs #1 and #2, the storage modulus can be observed as constant until about the 5-10 Hz value, while the loss modulus appears to be constant until about the 1 Hz value. This follows the idea that at larger strains, these values will be much more prominent, especially the loss modulus. These constant regions seen in the graphs also correspond with that of a chemically linked hydrogel where time dependence plays no factor and the crosslinking of the gels does not change in a given concentration.

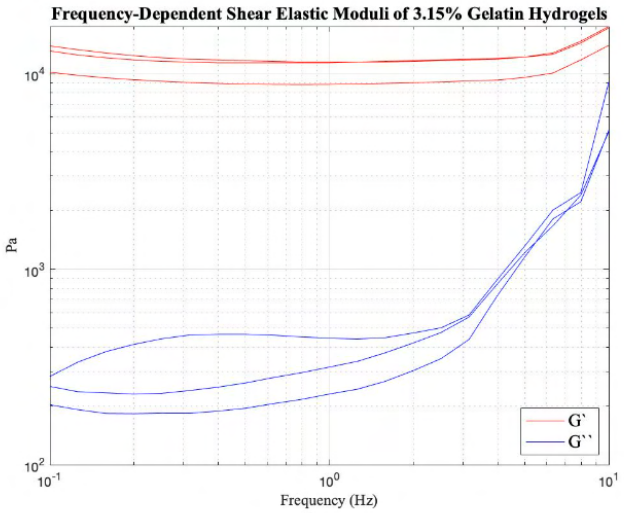
Table 2: Rheology Testing - (All results derived at 0.1 Hz)

Materials	Young's Modulus (E)	Storage Modulus (G')	Loss Modulus (G'')
Gelatin Hydrogel - 2.36%	18.375 ± 2.24 kPa	6.125 ± 0.747 kPa	0.112 ± 0.018 kPa
Gelatin Hydrogel - 3.15%	37.24 ± 5.82 kPa	12.41 ± 1.94 kPa	0.246 ± 0.04 kPa
Native Lung Tissue	0.44-7.5 kPa	2.12 ± 0.61 kPa	0.212 ± 0.061 kPa

Graph 1: LogLog plot of the Frequency-Dependent Shear Elastic Modulus of the 2.36% Gelatin Hydrogels



Graph 2: LogLog plot of the Frequency-Dependent Shear Elastic Modulus of the 3.15% Gelatin Hydrogels



Conclusions/action items:

Young's Modulus, Storage Modulus, and Loss Modulus were all successfully obtained via testing. For the most part, values fit within desired range.

Ethical Considerations

SARA MOREHOUSE - Oct 11, 2022, 7:03 PM CDT

Title: Ethical Considerations

Date: 10/11/22

Content by: Sara Morehouse

Goals: To gain an understanding of ethical concerns and considerations in our research.

Content:

Ethical Issues in Stem Cell Research - PMC (nih.gov)

- Human stem cells (hSC) raise ethical considerations as pluripontent stem cells can be derived from oocytes and embryos. This concern can be circumvented through use of induced pluripotent stem cells which are derived from somatic cells.

- Ethical dilemmas with any hSC research include consent for use of materials

Recognizing the ethical implications of stem cell research: A call for broadening the scope - PMC (nih.gov)

- In a positive effect, enables creation of new personalized therapies that have more value than non-personalized interventions. This reduces harm; for example, eliminating need for use of cell-derived hydrogel by using synthetic.

- This type of research has the potential to increase costs for healthcare as it can increase quality adjusted life years, which could cause financial harm. This could also impact the surrounding system of socialized healthcare as it places a greater financial burden on society.

- Cell culture and stem cell research can reduce need for animal studies as it is less morally problematic. It is also more proportional for discovering effective human treatments and therapies than animal studies, which would reduce need to inflict harm on animals for research purposes.

- By working to create a synthetic hydrogel base that is more accurate to the composition and properties of cell ECM and can be used for organoid and cell cultures, this increases the accuracy and efficacy of cell culture and organoid research, which aids the field evolving towards animal-free substitutes.

Conclusions/action items: Overall, our design will hopefully have an ethically positive result as it can lessen need for animal research.

2022/12/06 - "Rheology Analysis MATLAB"

Caitriona Treacy - Dec 06, 2022, 7:08 PM CST

Title: Rheology Analysis MATLAB Script

Date: 2022/12/06

Content by: Caitriona Treacy

Present: Caitriona Treacy & Elijah Diederich

Goals: Document the MATLAB script used to produce the graphs for the rheology testing as well as the standard deviations of the rheology results.

Content: Matlab script analyzing the rheology testing is attached as a pdf.

Caitriona Treacy - Dec 06, 2022, 7:08 PM CST

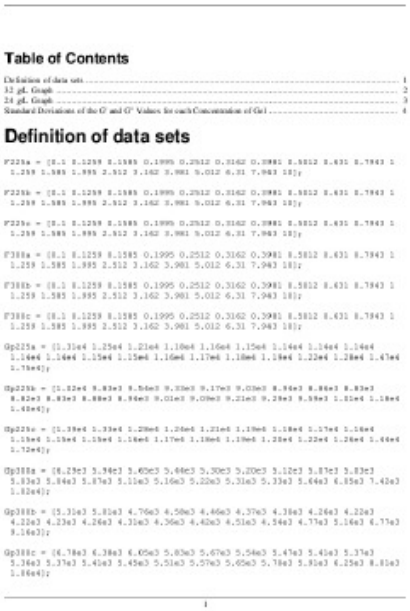


Table of Contents

Definition of data sets

Download

RheologyTesting.pdf (51.3 kB)

2022/14/09- Overview of EMTU

CARLEY SCHWARTZ - Sep 25, 2022, 8:31 PM CDT

Title: Overview of EMTU

Date: 09-14-22

Content by: Carley Schwartz

Present: Self

Goals: To gain a better understanding of the in vivo environment that is the basis of the project

Content:

Lung epithelial tissue mainly consists of epithelial cells, some goblet cells, basal cells and others

The ECM in simple terms is the basement membrane that the epithelial tissue adheres to and the interstitial space which includes the fibroblasts.

The ECM and its fibroblasts are extremely important for recovery after injury or disease

ECM has contents also called matrisome which have collagen and elastin as well

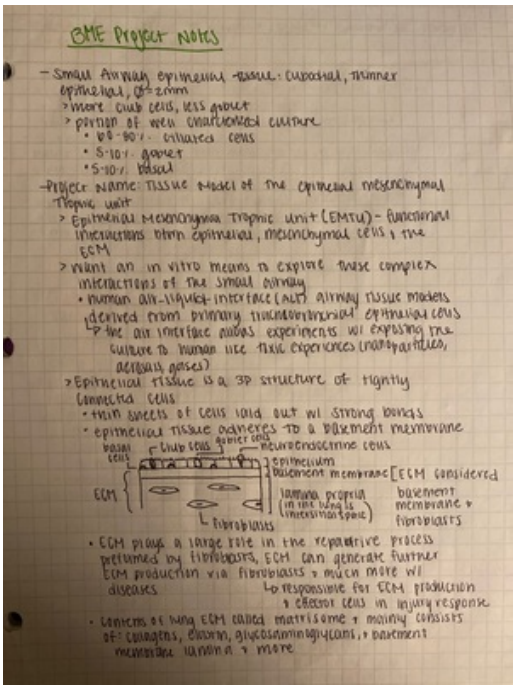
APA citation: White E. S. (2015). Lung extracellular matrix and fibroblast function. *Annals of the American Thoracic Society*, *12 Suppl 1*(Suppl 1), S30–S33. https://doi.org/10.1513/AnnalsATS.201406-240MG

Conclusions/action items:

Along with these and another image of notes I took on paper with the same source that helped me form a better understanding of lung epithelial tissue and how this will apply gto the project.

Picture of Notes with diagram

CARLEY SCHWARTZ - Oct 06, 2022, 8:04 PM CDT



Download

Notes_image_.jpg (814 kB)

09/15/22 Notes Pages with sources

CARLEY SCHWARTZ - Sep 27, 2022, 5:39 PM CDT

Sources Used in Order:

https://pubs.acs.org/doi/10.1021/acs.biomac.0c00045 [this source was used to cover the biochemical and structural factors that affect synthetic ECM]

https://www.frontiersin.org/articles/10.3389/fmats.2020.00076/full [this source was used for the section on 3D bio printing technologies]

https://pubs.rsc.org/en/content/articlelanding/2021/ra/d1ra03410c [this source was used to cover the 3D printed chitosan scaffold]

- 3D extracellular matrix has biochemical + structural factors that affect synthetic ECM
  - > cells + ECM has an "outside-in" + "inside-out" communication/signaling process
    - tensioned ECM will induce a stretching of cytoskeleton + nucleus of cells
    - Compressed ECM will result in altered local charge density + ion concentrations which affect ion channels?
  - > ECM changes in pathological states can result from
    - variations in composition bc of altered synthetic processes or degradation of an ECM component
    - post translational modifications
    - Spatial arrangements diff. bc of covalent or non-covalent modifications
  - > ~~typo~~ Major ECM components
    - Collagen - not just structure, also affect signaling + cell differentiation
    - non-collagenous glycoproteins - fibronectin (FN), react w/ integrins + other receptors
      - LD FN usually a coating in 3D models due to its plethora of roles
      - LD laminin also a component and play a role in cell adhesion/structural organization
    - Proteoglycans + glycosaminoglycans - keratin sulfate + plays a role w/ water retention, tumor suppression, + cell development
  - > Synthetic ECM mimicks allow us to tune certain biophysical parameters (mechanical properties or permeability of matrix) to investigate affect
    - Examples of synthetic materials that ~~[illegible]~~ make scaffold for cell-seeding: polyethylene glycol (PEG), polycaprolactone (PCL), poly(lactid acid) (PLA), + poly(glutamic acid) (PGA)
      - LD Disadvantages: inability to provide the biochemical signals need to communicate
    - bioactive motifs are needed to induce specific biological signals + cells (based on organ + role)

* rayometer
* bioprinting at UW → Dr. P will research + does stuff at Minnesota
  in vivo sciences → ISIA

- 3D bioprinting technologies
  - > cell-laden hydrogels can be used as ~~bio~~ bioink to 3D print layer by layer the scaffold
  - > ECM from animal tissues can be ~~pulverized~~ decellularized w/ detergents, pulverized + reconstituted as a hydrogel suitable for 3D cell culture
    - · stiffness of ECM-derived hydrogels is lower than that of native tissue but can be modified w/ chem. processes (photo-cross linking) but could be toxic

- 3D printed chitosan scaffold for lung tissue engineering
  - > chitosan (CS) is biorenewable, biodegradable, biocompatible, + bifunctional (has a positive charge)
    - · w/ 3D printing CS can be difficult bc of its mech. properties so can combine w/ collagen
    - · polycaprolactone (PCL) has strong mechanical properties + high potential in cell viability
  - > combining CS + PCL could be used for lung tissue engineering
    - · more CS + less PCL = soft tissue
    - · less CS + more PCL = hard tissue
  - > PCL low level = 1%, high level = 4%.
  - > CS low level = 0%., high level = 4%.
    - · key factors of pressure, temp, velocity of bio-ink adjusted
      - LP uses a 3D bioplotter, scaffolds: $10 \times 10 \times 5\ mm^3$, using 300 μm needle inner diameter (Magics 13 Envisiontec softwared + Bioplotter RP software used)

best printing results had these parameters
1 chit%., 1 PCL%., 4 chit%., 4 PCL%.
1 , 0 , 4 chit%., 2.5 PCL%.
LP chit% 4, PCL% 4: P(1.5 bar), T(25°), V(220 mm/min), 1340
★[chit% 4, PCL% 1: P(2), T(15), V(150), 1000
chit% 4, PCL% 2.5; P(1), T(25) V(230), 1270

Conclusions: These preliminary notes were prior to the client meeting and as a result of that many weren't relevant to the clients true requirements for the project. Instead of any sort of bio printing we will need to focus more on synthetic materials. Some of the useful take aways from these articles are how the tension of the ECM scaffold matter and how it can affect cellular behavior. It also detailed some of the major ECM components and a few potential synthetic materials that could provide as a scaffold that mimics ECM. These were mainly found in the first source used.

09/22/22 Notes on PGA synthetic material along with picture of preliminary notes

CARLEY SCHWARTZ - Oct 06, 2022, 8:07 PM CDT

Overview: To begin research I prefer taking handwritten notes so I will include those as well. Notes taken directly on this page will have sources next to any new ones. Any sources I believe to be a competing design rather than just background info will be in that section.

Source for Notes In Order:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8037451/ [This source was the first I found to mention PGA in any promising matter for the project-led to a deeper dive into its current uses]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8037451/ [This source provided an overview into some of PGA properties and how it fits in with the requirements of a scaffold]

Study of PGA vs Synthetic polymer mixture to see how degradation compares

- drawback to synthetic polymers is that polymer remnants can persist after cell culture
  - polymer fragments can degrade tissue mechanics → stimulate local inflammatory response (in vivo)
- → results showed that the polymers that degraded faster during tissue culture had improved engineer mechanics bc there would be less disruption of collagenous ECM

Polyglycolic acid

- synthetic braided polymer, less reactive → resists infection from bacteria
  - maintains 50% of its tensile strength for 25 days
- biodegradable polymer, sensitive to hydrolystic degradation
- Approx strength of 7 GPa
- Melting point ≈ 200°C
  - * other materials: PLGA, PLLA, → PU
  - * PGA w/ Pluronic-F-127
  - * PDLLA <> Elastic properties good
- → Artificial scaffolds need to be non-immunogenic, biocompatible non-toxic, chemically stable,
- research study found: adipose-derived stem cells embedded in PGA successfully regenerated vascular → Alveolar tissue
  - * Artificial scaffolds lack native integrin binding sites → bioactive cues for cellular attachment, proliferation → differation → cell seeding → graft coverage can help
    - → can provide cell attachment sites by increasing porosity or incorporating ECM components
  - * electrospinning to create porous scaffolds

10/02/22 PEG Reactive Ends Notes

CARLEY SCHWARTZ - Oct 07, 2022, 6:53 PM CDT

Title: PEG Reactive End Notes and Crosslinking

Date: 10-02-22

Content by: Carley

Present: Self

Goals: To learn more about the different reactive ends of PEG

Content:

Ortal Yom-Tov, Dror Seliktar, Havazelet Bianco-Peled, PEG-Thiol based hydrogels with controllable properties, European Polymer Journal, Volume 74, 2016, ISSN 0014-3057, https://doi.org/10.1016/j.eurpolymj.2015.11.002.

In PEGDA hydrogels, monomers are cross-linked *via* free-radical initiated polymerization of acrylate end groups; free radicals are created either by chemical activation or UV cleavage of a photo initiator. A major disadvantage of free-radical cross-linking is the cytotoxic effect caused from free radicals released during the cross-linking reaction, and the limited use of UV light in cloudy solutions. In contrast, thermo-gelation and Michael-type addition cross-linking avoids the use of cytotoxic free-radicals and UV light.

van de Wetering et al. used 8-arm PEG with different molecular weights; they observed diminished protein diffusion coefficients with decreasing PEG molecular weights from 10 kDa to 2 kDa. Yang and coworkers have reported that higher molecular weight between crosslinks is correlated with increased strain to failure and decreased modulus and strength of hydrogel networks synthesized

The swelling of these hydrogels were shown to increase with increasing PEG-acrylate and di-thiol molecular weights. Moreover, increasing the precursor's molecular weights leads to the combined effect of both higher initial swelling and faster degradation rate.

Implementing Michael-type addition chemistry to prepare hydrogels composed of PEG-Thiol and PEGDA requires preparation of single polymer precursor solutions as a first step, followed by joining those solutions together. This procedure is required since dissolving both polymers in the same vessel activates the polymerization process prior to their complete dissolution.

PEG-Thiol based hydrogels were prepared by mixing PEG-4SH (molecular weight 10 kDa) solutions with PEGDA (molecular weight 10 kDa) solutions.

- PEG-4SH powder was weighed inside a gloves chamber in order to prevent humidity from damaging samples followed by its addition to phosphate buffer saline pH 7.4
    - stirring time varied for testing of affect
- PEGDA powder was added to PBS and stirred until complete dissolution was achieved
- PEG-4SH and PEGDA solutions were mixed together, the mixture had a final concentration of 10% (w/v) PEG-4SH and 5% (w/v) PEGDA
- resulting precursor solution should be shaken for several seconds and 0.5 ml aliquots were transferred into round molds

2.2. Preparation of PEG-Acrylate hydrogels

PEG-4AC hydrogels were prepared by mixing PEG-4AC (molecular weight 10 kDa, Laysan Bio) solutions with PEGDA (molecular weight 10 kDa) solutions. Briefly, PEG-4AC powder and PEGDA powder were added to PBS to a final concentration of 10% (w/v) and 5% (w/v) respectively. The precursor solution was stirred until complete dissolution was achieved followed by the addition of 1% (v/v) photo initiator stock solution [10% (w/v) Irgacure® 2959 in 70% ethanol and 30% deionized water]. 0.5 ml aliquots of the precursor solution were transferred into a round Teflon mold with a 14 mm diameter, which was then irradiated with UV light (365 nm, 4–5 mW/m2) for 5 min in order to achieve chemical cross-linking of the resulted hydrogels.

Results: Mixing time for PEG-4SH resulted in less gelation time needed when there was longer mixing before hand(caps at 3 h), additionally higher young's modulus values as a result of longer gelation periods for each mixing.

	0.5 h mix	2 h mix	4 h mix
Gelation time (days)	Degradation time (days)	Degradation time (days)	Degradation time (days)
1	2	3	3
4	5	11	11
7	>30	22	>30

Significantly lower mesh size values were received at longer gelation periods for each mixing time. This supports the suggestion that the gelation process is very slow, and at longer times the number of crosslinks increase, resulting in a diminished mesh size.

In addition, significantly diminished mesh size values are obtained at increasing mixing times at each gelation period. The decreased mesh size is correlated with the increased Young's modulus values observed for the same samples.

Hence, hydrogels that were allowed to cure for longer times exhibited a greater number of crosslinks resulting in a much higher degradation time.

Conclusions/action items:

While originally the main PEG that I read about was PEGDA this gave another type used regularly that is formed with the crosslinking during a chemical reaction.

09-23-22 PEG hydrogel used in other studies

CARLEY SCHWARTZ - Oct 06, 2022, 7:33 PM CDT

Title: Examples of other researchers using PEG

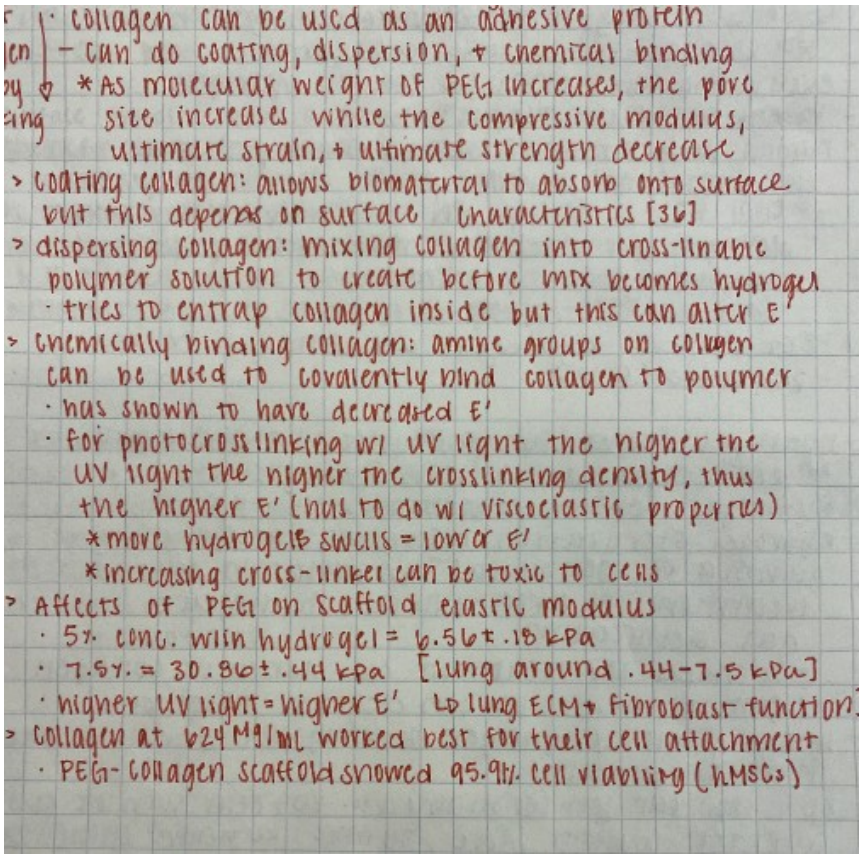
Date: 09-23-22

Content by: Carley

Present: Self

Goals: To find literature using PEG in similar applications

Content:



Source: https://etd.ohiolink.edu/apexprod/rws_etd/send_file/send?accession=akron1384720879&disposition=inline

Conclusions/action items:

This article was very informational because it shows that PEG is able to be fine tuned mechanically to mimic lung ECM with respect to young's modulus based on % concentration and the molecular weight

09-27-22 GelMA Competing Design

CARLEY SCHWARTZ - Oct 06, 2022, 7:55 PM CDT

Title: Brief Research on caitriona's design idea

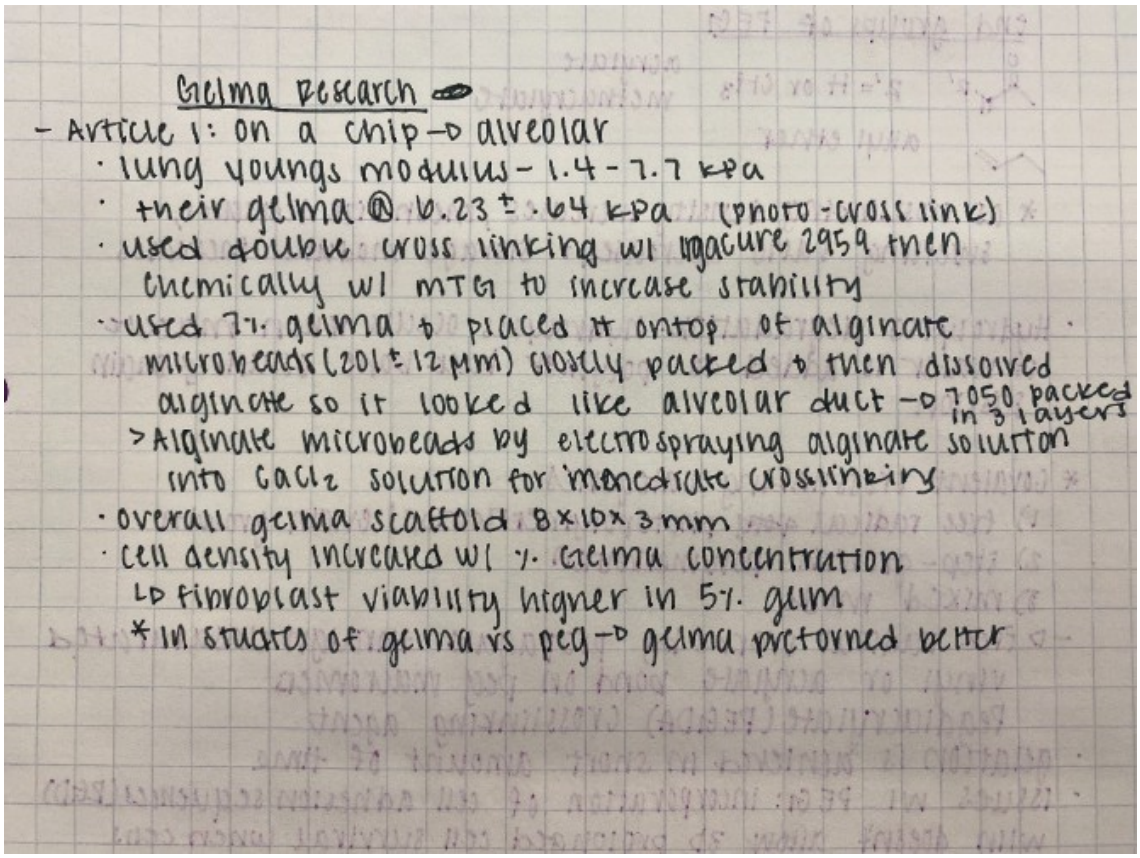
Date: 09-27-22

Content by: Carley

Present: self

Goals: to have background knowledge on all design ideas

Content:



Conclusions/action items:

This GelMA competing design was modeling the alveolar sac which is very similar to our design, thus providing new information. While GelMA worked well for their design there is also variations in its ability to provide stable results.

09-27-22 Matrigel Issues

CARLEY SCHWARTZ - Oct 07, 2022, 6:58 PM CDT

Title: Find out what is wrong with matrigel

Date: 09-27-22

Content by: Carley

Present: Carley

Goals: To understand what is wrong with matrigel

Content:

Matrigel, a mouse tumor extracellular matrix protein mixture, is an indispensable component of most organoid tissue culture. However, it has limited the utility of organoids for regenerative medicine due to its tumor-derived origin, batch-to-batch variation, high cost, and safety issues.

However, Matrigel has several undeniable drawbacks. As Matrigel is a raw material extracted from the Engelbreth–Holm–Swarm mouse sarcoma, it causes large batch-to-batch variation in cultured organoids. Given its origin, there is also a potential risk of transmission of animal pathogens that infect macrophages and affect the immune systems.

Matrigel is primarily composed of tumor ECM components such as laminin which is highly expressed in several types of tumors. In tumors the ECM is substantially different from that of normal tissues

Conclusions/action items:

We now understand why matrigel was dissatisfying for the client and what to avoid in our own design.

WRONG Design Idea 1: PGA synthetic scaffold

CARLEY SCHWARTZ - Sep 28, 2022, 3:05 PM CDT

Overview: For this design idea I looked further into synthetic materials and decided to focus on PGA which has been used for multiple scaffolds in other applications.

Article 1: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341840/

- PGA is highly crystalline due to its chain-structural regularity
- Biocompatible and biodegradable
- all synthetic scaffolds biodegrade but at different rates
- synthetic scaffolds lack the biochemical communication and adhesion that if found in native ECM(some people have used conducting polymers that have electrical properties)
- most extracellular proteins have a fibrous structure
    - ex: collagen has a fiber diameter of 50-500 nm
- to mimic the physical architecture of natural collagen it may needed to have a 3D nano-fibrous gelatin scaffold.

Article 2: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6408266/

- While this article does focus on the intestine this is much further researched than lung ECM but has similar properties
- PGA coated with poly-L-lactic acid (PLLA) which improved the mechanical properties
- PGA biofelt(2mm thick, 60mg/cm^3), flat scaffolds were cut and soaked in 5 wt% PLLA in chloroform, then ethanol for series of soaks, then coated with collagen type 1 for 30 min followed by washes(for cell culture there size was flat 5x5 mm)
- they placed each flat scaffold into the wells of 24 well plate and incubated for 1 hr without medium immersion to allow for cellular attachment to scaffold
    - each then received medium(1.5mL/well) for 12 hr of static culture followed by dynamic culture with gentle shaker
    - medium was changed every two days
    - 150 microliter of resazurin added to each well on days 0,2, and 4

Table 2

Properties of the Scaffolds

	PGA	PGA/PLLA	PCL
Porosity (%)	91.5 ± 4.1	81.9± 3.3	67.9 ± 2.9
Fiber size (μm)	14.4 ± 1.6	15.9 ± 1.5	8.6 ± 2.0
Pore size (μm)	157.9 ± 30.5	84.7 ± 23.2	45.0 ± 12.6
SRS (N)	30.686 ± 7.898	45.881 ± 10.923	3.250 ± 0.809
Compression Test (N)	0.092 ± 0.028	0.623 ± 0.115	0.363 ± 0.145
	90.3 ± 3.6	80.5 ± 2.5	68.7 ± 3.7

- for the in vitro cell culturing the best results were on the PLLA/PGA embedded with collagen

"PLLA coated tubular PGA scaffolds have appropriate pore size, mechanical properties, and degradation rate, and produce TEI with a similar architecture to that of native rat intestine."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3968884/ [biofelt PGA scaffold]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3215154/ [electrospun PGA scaffold]

09-23-22 PEG Design Idea

CARLEY SCHWARTZ - Oct 06, 2022, 7:50 PM CDT

Title: PEG design idea for matrix

Date: 09-23-22

Content by: Carley Schwartz

Present: Self

Goals: To collect mechanical and biological information on PEG hydrogel and then look into methods and materials once it is established the material is viable

Content:

Translucent: Yes

Young's Modulus: 3-300 kPa

Cross-linking: Can use UV light for free radical reaction

Biochemistry: No natural cell adhesive properties but can have collagen added with no toxic or immune response from cells

2D vs 3D: cells less constrained in the 2D but not as similar to native while 3D is

Able to sterilize before hand with ethanol on already formed scaffold or germicidal UV radiation

PEG crosslinking:

- mechanism depends on end chains
  - for this project will most likely have an acrylate(diacrylate) group on the end
- cross-linking is when the reactive ends are polymerized with a free radical initiator
  - this initiator can be UV light with wavelength at 365 nm which is same at BME labs
- can deposit cells inside before cross-linking
- swelling can be an issue because it makes the surface rounded and difficult to image(problem with ease of use for client-need to discuss)
- As cross linking density increases, the mesh size decreases, swelling decreases, and storage modulus increases
- gelation is achieved in short amount of time (seconds and minutes)

Issues with PEG: incorporation of cell adhesion sequences(RGD) w/in doesn't allow for 3D prolonged survival when cells encapsulated bc mesh size of PEG smaller than that of cell. This poses a physical barrier for incorporation. This can limit some models to 2D.

Source:

https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/materials-science-and-engineering/tissue-engineering/polyethylene-glycol-and-synthetic-peg-derivatives

Conclusions/action items:

This article helped gain a better understanding of how PEG works and what this will entail for our model. Further research into methods will be the next stop.

Looking into methods

CARLEY SCHWARTZ - Oct 14, 2022, 1:27 PM CDT

This entry is a summary of comparing different sources and their mechanical results

w/v% = (grams/mL)*100

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7766244/

- The 3.5% w/v PEGDA control condition gels had an average modulus of 5.1 ± 0.48 kPa
  - 3.5% w/v PEGDA hydrogels were made with 3.5 mM PEG-RGDS
  - Poly(ethylene glycol) diacrylate was synthesized by dissolving 6 kDa poly(ethylene glycol) (PEG, Sigma, St. Louis, MO, USA) in anhydrous dichloromethane (DCM, Fisher Scientific, Waltham, MA, USA)

https://advancedbiomatrix.com/pegda.html

PEGDA is prepared by dissolving the lyophilized solid in the DG Water. PEGDA hydrogels (1 mL) should be prepared in the following manner:

1. Allow the PEGDA to come to room temperature.
2. Under aseptic conditions add 1.0 mL of DG Water to 100 mg of PEGDA vial for a 10% (w/v) solution.
3. Invert several times to dissolve. The solution will be clear and slightly viscous.
4. To sterilize, filter through a 0.2 micron filter.
5. Add desired volume of photoinitiator to PEGDA. Place the vial on a rocker or shaker and allow solids to completely dissolve (approximately 10-20 minutes).
6. Keep solution protected from light until ready to crosslink. Pipette solution into desired format (i.e. 96 well plate) and photocrosslink.
7. Hydrogel properties may vary depending on time of exposure and type of light. Ensure the Photocrosslinking light is in close proximity to the hydrogel solution.
8. Freeze unused PEGDA solution at -20 °C and protected from light.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4829474/

The attraction for using PEGDA as a cell encapsulant and tissue scaffold arises from the combination of its tissue-like physical properties, which can be tailored to closely mimic extracellular matrices, cytocompatibility and synthetic versatility. Over a certain polymer composition range, highly water-swollen PEGDA hydrogel networks have been proven to be cytocompatible encapsulants for many cell types including fibroblasts, chondrocytes, vascular smooth muscle cells (SMCs), endothelial cells (ECs), osteoblasts, neural cells, and stem cells. Synthetic customization of PEGDA macromolecular architecture and chemistry provides a broad diversity of properties, making it an attractive alternative to natural hydrogels.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3773240/

Irgacure 2959 was added to a 70/30 mixture of ethanol and deionized (DI) water to create a 10% w/v photoinitiator solution. 10%, 15%, and 20% w/v solutions of PEG-DA (molecular weight 2000, 3400, and 6000 Da each) in PBS were prepared, and 10 µL of photoinitiator solution was added to each mL of PEG-DA solutions. The solutions were then placed in rectangular silicone rubber molds (~2 mm height), pressed between optical glass microscope slides, and exposed to 312 nm light (3.0 mW/m^2, XL-1000, Spectronics Corp.; Lincoln, NE) for five minutes on each side. Gel samples were cut using a 3 mm biopsy punch, and gels were transferred to PBS to equilibrate for at least 24 hours before use.

1. Mechanics and Porosity -> range of modulus

2. Porosity -> what size molecule needs to get [Dex strand label that is similar in properties to molecule- what needs to travel]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2629651/

2022/10/23-Research into PEG-thiol Chemistry

CARLEY SCHWARTZ - Oct 25, 2022, 7:17 PM CDT

Title: Peg Thiol Research

Date: 10-23-22

Content by: Self

Present: Self

Goals: To look into more research articles on other PEG types

Content:

Stable engineered vascular networks from human induced pluripotent stem cell-derived endothelial cells cultured in synthetic hydrogels

Poly(ethylene glycol) (PEG) hydrogels were formed using thiol- ene photopolymerization chemistry

Monomer solutions for cell encapsulation were prepared in 1X PBS with 40 mg/mL 8-arm PEG-NB in which 40–60% of the available norbornene arms were cross-linked with a matrix metalloproteinase

Cell Culture Viability: Cells were rinsed with 1X PBS and stained with cal- cein AM and ethidium homodimer-1 for 30 min using the manufacturer's recommended dilutions. Samples were then washed with 1X PBS and fixed in 10% buffered formalin (4% formaldehyde, Fischer) for 30 min.

Conclusions/action items:

10-13-22 PEG-DT and NB Crosslinking research

CARLEY SCHWARTZ - Dec 06, 2022, 8:25 PM CST

Title: PEg research

Date: 10-13-22

Content by: self

Present: self

Goals: to understand the photopolymerization of PEG and what concentrations to use

Content:

In combination between the literature below and the meeting with margot the protocol created(found in protocols section of lab archives) was created.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6262827/

Tetra-arm PEG-Nb and PEG-dithiol (PEG-DT, Mw = 3,400 Da, Laysan Bio.) were mixed off-stoichiometrically with cell-adhesive peptide ligand CGRGDS (1 m*M*) and LAP (2 m*M*). The final working concentration of PEG-Nb was 10 wt% and the [SH]:[ene] ratio for PEG-DT and PEG-Nb was 0.75:1 so that unreacted norbornene groups would be available for microgel assembly. Two PEG-Nb molecular weights (5 and 20 kDa)

Subsequently, varying volumes of 20 wt% PEG-DT (2, 4, or 8 μL) and 100 m*M* LAP (1, 2, or 4 μL) were added to achieve the desired concentrations and mixed by pipetting. The microgels were then assembled into scaffolds by UV irradiation (365 nm, 10 mW/cm2, 3 min)

Despite the lower scaffold porosity, cellularity was enhanced in the low concentration groups, and the cell volume was 2–3 fold higher compared to the high concentration groups

Conclusions/action items:

UV light: 365 3-5 min

varying concentrations lead to different kPa stiffness

PEG20 storage modulus .135

10-26-22 RGD Density Research

CARLEY SCHWARTZ - Dec 06, 2022, 6:18 PM CST

Title: RGD Density Research

Date: 10-26-22

Content by: self

Present: self

Goals: to learn more about RGD concentrations

Content:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC154359/

The RGD ligand concentration (tested at eight different concentrations ranging from 2.5 to 340 μM) mediated cell invasion in a biphasic manner

with the incorporation of 42.5 and 85 μM RGD significantly enhancing the level of HFF outgrowth ($P < 0.05$, compared with 10 and 140 μM, respectively). Matrices that were not functionalized with adhesion ligands or that contained low ligand concentrations (2.5 and 5 μM) did not support cell invasion at all, because cells, due to the weak cell–matrix interaction, were not able to exert sufficient traction for forward movement. A ligand density of 10 μM (i.e., the concentration of RGD peptides fixed three-dimensionally within the gel network) was found to be the minimal concentration necessary for HFF invasion.

Conclusions/action items:

From this article, the cells being used were not fibroblasts but the general ideas of RGD density having a minimum range for good cell adhesion was beneficial for understand and doing further research

11-13-22 RGD research

CARLEY SCHWARTZ - Dec 13, 2022, 5:28 PM CST

Title: further rgd research

Date: 11-13-22

Content by: self

Present: self

Goals: to get a set range of mM values for RGD peptides and understanding how they work

Content:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7842198/ - this article gave detailed information on RGD mM values that led to promising cell viability

Recent studies involving the use of adult human mesenchymal stem cells (hMSCs) in PEG hydrogels determined that incorporation of a pendant RGD at 2.8 mM maintained 75% viability after 1 week

In this regard, RGD presented to the cell as a loop, via attachments to the PEG backbone on either end of the sequence, may induce higher levels of hMSC survival. Also, creating a distance between the presented RGD and the material backbone can provide better access for the necessary integrin–ligand interactions. Therefore, creating a spacer arm between the RGD motif and the material surface provides mobility and removes steric hindrance for the extension of the adhesive ligand to reach the cell surface integrin

The RGD with a spacer arm sequence, when covalently tethered to the PEG network as a pendant functionality, provided the highest survival rate of hMSCs, reaching ∼84% after 2 weeks in culture, which was significantly higher than the survival seen with dually attached RGD

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5092185/#:~:text=RGD%20(arginylglycylaspartic%20acid)%20is%20a,polymeric%20network%20of%20the%20hydrogels. -This article gave some details into how RGD sequences work

RGD (arginylglycylaspartic acid) is a tripeptide that can mimic cell adhesion proteins and bind to cell-surface receptors, creating a hospitable microenvironment for cells within the 3D polymeric network of the hydrogels. RGD functionalization occurs through Huisgen 1,3-dipolar cycloaddition. Some PAA carboxyl groups are modified with an alkyne moiety, whereas RGD is functionalized with azido acid as the terminal residue of the peptide sequence

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3091033/ - This article provided the bulk for understanding how RGD peptides work and why they are the best option

The capacity of a material to support cell adhesion is not only critical for stimulating proper tissue development at implant/tissue interfaces, but also necessary for materials that serve as carriers for delivery of reparative cells to wound sites. Furthermore, cell attachment to a biomaterial scaffold is an important early step in the generation of in vitro-engineered tissue substitutes.
An exhaustive literature has established that RGD is highly effective at promoting the attachment of numerous cell types to a plethora of diverse materials. RGD is the principal integrin-binding domain present within ECM proteins such as fibronectin, vitronectin, fibrinogen, osteopontin, and bone sialoprotein.

The RGD sequence can bind to multiple integrin species, and synthetic RGD peptides offer several advantages for biomaterials applications. Because integrin receptors recognize RGD as a primary sequence (although conformation of the peptide can modulate affinity), the functionality of RGD is usually maintained throughout the processing and sterilization steps required for biomaterials synthesis, many of which cause protein denaturation. The use of RGD, as compared with native ECM proteins, also minimizes the risk of immune reactivity or pathogen transfer
Another benefit is that the synthesis of RGD peptides is relatively simple and inexpensive, which facilitates translation into the clinic. Finally, RGD peptides can be coupled to material surfaces in controlled densities and orientations. These advantages of straightforward synthesis, minimal cost, and tight control over ligand presentation cannot readily be achieved when using full-length native matrix proteins to functionalize material surfaces.

Conclusions/action items:

In combination with the first article and discussing potential concentrations with Margot the theoretical protocol was created.

Additionally, there is now a much better understand of how these peptides work and the motivation behind using them specifically.

12-01-22 Mesh Size

CARLEY SCHWARTZ - Dec 07, 2022, 5:24 PM CST

Title: A bit of background on mesh size

Date: 12-01-22

Content by: self

Present: self

Goals: to learn more about mesh size and mmp degradation

Content:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2962861/

The mesh size is significantly smaller than the size of a cell and therefore acts to retain the cells inside the gel. Typical mesh sizes for crosslinked PEG hydrogels used in cell encapsulation are in the range of ∼40 to 200 Å, which was estimated from equilibrium-swelling theory.35 The mesh size will not only control diffusion of nutrients and other biological signaling molecules (e.g., growth factors) from the surrounding medium to the entrapped cells, but will also control diffusion of tissue-specific molecules secreted by the cells.

Degradation

Specifically, the gel mesh size, which controls ECM diffusion increases with degradation and therefore must closely match secretion of newly synthesized ECM. If degradation occurs too quickly the cell-laden hydrogel will simply dissolve. If degradation is too slow, a buildup of ECM will occur in the pericellular regions. The latter event will not only affect tissue growth, but may also influence cell function

As linkages are cleaved, mass loss increases with time until there are no longer a sufficient number of cross-links to maintain a 3D network, at which time the remaining highly branched chains dissolve.

Conclusions/action items:

Overall, gained some insights into the usual mesh size of these hydrogels which fully allows for native protein movement and signals with MMP degradation

12-05-22 Gelatin Research

CARLEY SCHWARTZ - Dec 13, 2022, 5:07 PM CST

Title: Gelatin Research

Date: 12-05-22

Content by: self

Present: self

Goals: to learn more about gelatin idea

Content:

https://link.springer.com/content/pdf/10.1007/978-3-319-77830-3_53.pdf

It is a biodegradable natural polymer composed of different types of amino acids [18]. It is found in different parts of several mammals like cattle bones, pig skin, hides, and fish as well as in some plants and insects [19]. The structure of gelatin allows them to undergo interactions with other molecules and forms crystallites followed by further transformation into a three-dimensional network susceptible to immobilize the liquid [20]. Sometimes, chemical crosslinker enhances the gelation process through further networking

mammalian gelatin is the primary contributor of the total world gelatin production

Connective tissues and bones of vertebrate animals are the primary element from where the mammalian gelatins are generated. Available sources of mammalian gelatin are pig skin (46%), bovine hide (30%), and pork and cattle bones (23.1%) [61]. However, researches on two different types of mammalian gelatins – bovine and pig sources – disclosed that both sources comprised of different components with wide distribution of molecular weight ranging from 10 to 400 kDa as well as the outcome established strong co-relationship between the average molecular weight and gel strength of the gelatin, with high isoelectric and melting points [62]. These two are usually required where health is concerned.

Gelatin is very faint yellow to amber in color, and usually dry pure commercial gelatin is a tasteless, odorless, transparent, brittle, glass-like solid comprising a range of molecular weight about 40,000 to 90,000 Da. It can establish the equilibrium both with acids and bases which can explain the nature of polypeptides and determine the amino acid composition due to the availability of amino and carboxylic groups on its backbone protein chain molecules and also help to unravel the structural stabilization and reaction nature toward other substances with it by using its ionization constant and electronically charged group

Conclusions/action items:

Learned a lot of key information on gelatin and its properties

2022/09/13 - "Macrophages in Lung Injury, Repair, and Fibrosis"

Caitriona Treacy - Oct 11, 2022, 9:43 PM CDT

Title: "Macrophages in Lung Injury, Repair, and Fibrosis"

Date: 2022/09/13

Content by: Caitriona Treacy

Present: N/A

Goals: Gather background about the general physiology of the lung epithelium.

Search Term: PubMed: "Lung COPD Injury"

Citation:

P. Cheng, S. Li, and H. Chen, “Macrophages in Lung Injury, Repair, and Fibrosis,” *Cells*, vol. 10, no. 2, p. 436, Feb. 2021, doi: 10.3390/cells10020436.

Content:

- Macrophages play a significant role as heterologous phagocytes and express pattern recognition receptors to detect pathogens and pathogen-associated molecular patterns and damage-associated molecular patterns.
- The origin of lung macrophages is believed to be complex. They either formed by the differentiation of blood-derived monocytes or the proliferation of local macrophages.
- In the lungs, three different populations of macrophages exist: Airway, alveolar, and interstitial macrophages. They serve to sustain homeostasis.
- Alveolar macrophages account for 55% of lung immune cells and are situated on the inner surface of the lung. They participate in the onset of several lung diseases and are crucial for maintaining airway homeostasis.
- Interstitial macrophages preserve homeostasis and module tolerance toward non-threatening antigens.
- Although macrophages play a significant role in defending against invading organisms, their excessive numbers could cause tissue damage.
- Stimulated by different local environmental factors, macrophages can be divided into two distinct polarization states: classically activated (MI) which are closely linked to pro-inflammatory responses and the alternatively activated phenotype (M2) which play a key role in anti-inflammatory reactions.
- Inflammation is the host's necessary defense against immunogenic agents.
- Inflammation is originally protective, and when homeostasis is restored, the inflammation developed dissipates during repair. However, in case the inflammation is not suitably resolved, pathological fibrosis may develop, ultimately resulting in organ failure.
- Acute lung injury (ALI) usually leads to acute respiratory distress syndrome (ARDS), the primary cause of death in critical patients.
- ALI is characterized by leukocyte accumulation, epithelial injury, pulmonary edema, and increased alveolar permeability, as well as diffuse alveolar damage.
- The excessive lung inflammation in ALI is believed to be related to changes in alveolar macrophage activation and death. Although ALI was believed to be linked to apoptotic cell death, apoptosis elicits a non-inflammatory response, inconsistent with the severe inflammation in ALI.
- An increasing body of evidence has shown that there exists heterogeneous and complex resident epithelial progenitor or stem cells in the lung. They generally divide rarely but proliferate and differentiate rapidly to accelerate the restoration of the epithelium since the lung is injured.

- In response to epithelial injury, recruited and resident macrophages drive tissue repair. Macrophages could directly boost epithelial proliferation in the absence of other cell types within a co-culture system. *Check [82].*
- Disruptions in the macrophage function could lead to abnormal pairs, as the uncontrolled generation of growth factors and inflammatory mediators, deficient production of anti-inflammatory macrophages, or invalid communication among macrophages and epithelial cells, tissue stem or progenitor cells, and fibroblasts all contribute to persistent injury, leading to the development of pathological fibrosis.
- Th2 cells and group 2 innate lymphoid cells predominantly secrete IL-13 and IL-4, which enhance tissue repair and promote the initiation of matrix synthesis via activated macrophages.
- They cytokine modulates the M2-like biochemical functions and enhances their polarization, including collagen synthesis, refactoring the extracellular matrix, and anti-inflammation.
- Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive disease with no effective treatments.
- Increased myofibroblasts, deposition of collagen, and alveolar epithelial injury are characteristics of IPF resulting in impaired functional gas exchange, respiratory failure, and death.
- Macrophage apoptosis is related to pathological fibrosis. Elimination of macrophages prevents the development of lung fibrosis.
- There is no cure for pulmonary fibrosis. Abnormal lung repair after epithelial injury will also exacerbate pulmonary fibrosis, which is related to the infiltration of macrophages. The role of macrophages in lung injury repair and fibrosis is very complicated.
- There is relatively little information on cellular communication between macrophage and fibroblast cells, and endothelial cells in the lung at steady state and after lung injury.

Conclusion/Action Items: Macrophages interact closely with fibroblasts in tissue repair after lung injury. However these interactions are not well understood, and inflammation due to repair can sometimes lead to further injury and fibrosis. Further research should be done on source 82 of this article as it has information about co-culture systems within the lung.

Caitriona Treacy - Oct 11, 2022, 9:46 PM CDT

Note on 2022/11/10: After getting further in the design process, this research seems somewhat irrelevant to the context of what Dr. Brasier is studying in his lab and what he requires from the design. However, it is still a good start for understanding what structures are at play when the lung experiences injury. Furthermore, it was important research when gathering background before beginning the brainstorming phase and is included as it reflects time spent gathering information on the problem statement.

2022/09/18 - "The Physiology of Small Airways"

Caitriona Treacy - Oct 12, 2022, 12:04 AM CDT

Title: "The Physiology of Small Airways"

Date: 2022/09/18

Content by: Caitriona Treacy

Present: N/A

Goals: Understand the basic physiology surrounding the small airway of the lung.

Search Term: PubMed: "Lung Small Airway Properties"

Citation:

P. T. Macklem, “The Physiology of Small Airways,” *Am J Respir Crit Care Med*, vol. 157, no. 5, pp. S181–S183, May 1998, doi: 10.1164/ajrccm.157.5.rsaa-2.

Content:

- The small airways of less than 2 mm in diameter are pathways of low resistance and normally contribute about 10% of the total resistance to flow. If small-airway resistance is only 10% of the total, doubling it only increases total resistance by 10%. Thus, it is difficult to detect small airway obstruction by general lung function tests.
- Small-airway obstruction has very little effect on the mechanical properties of the lung, particularly if there is collateral ventilation, but it does affect the distribution of the inspired gas if air is displaced from one set of air spaces across collateral channels to alveoli beyond the obstructed airways.
- The total cross-sectional area of a given generation of small airways can be several orders of magnitude greater than the total cross-sectional area of the large airways. Because the linear velocity of gas is the flow divided by the cross-sectional area, gas velocity is much less in the small airways.
- Small airways have fully developed laminar flow. Fully developed laminar flow is the only flow regimen independent of gas density, so changes in gas density have little or no effect on small-airway resistance.
- The liquid lining small airways has the characteristics of a surfactant with low surface tension, particularly on expiration. This low surface tension protects the small airways from closing at low lung volumes.

Conclusion/Action Items: While helpful as a tool to define major differences between the small airway of the lung (where this project is focused), this article did not reveal many details about mechanical or chemical properties of the lung epithelium or extracellular matrix. Further research on these and their physiological functioning should be conducted.

2022/09/30 - "Bronchial Extracellular Matrix from COPD patients induces altered gene expression in repopulated primary human bronchial epithelial cells"

Caitriona Treacy - Oct 12, 2022, 12:37 AM CDT

Title: "Bronchial Extracellular Matrix from COPD Patients Induces Altered Gene Expression in Repopulated Primary Human Bronchial Epithelial Cells"

Date: 2022/09/30

Content by: Caitriona Treacy

Present: N/A

Goals: Explore how lung epithelium and ECM has been modeled in the past and results of such models.

Search Term: PubMed: "Small Airway OR Bronchial ECM Properties"

Citation:

U. Hedström *et al.*, "Bronchial extracellular matrix from COPD patients induces altered gene expression in repopulated primary human bronchial epithelial cells," *Sci Rep*, vol. 8, no. 1, Art. no. 1, Feb. 2018, doi: 10.1038/s41598-018-21727-w.

Content:

- Remodeling of the bronchial airways in chronic obstructive pulmonary disease (COPD) includes changes in both the bronchial epithelium and the subepitheliam extracellular matrix (ECM).
- Findings suggest that COPD-related changes in the bronchial ECM contribute to the defective regenerative ability in the airways of COPD patients.
- COPD is characterized by chronic airway inflammation, loss of small airways and emphysema. It is projected to be the fourth leading cause of death worldwide by 2030.
- The airway epithelium constitutes the first line of defense in the lungs and plays a crucial role in protection agains microbes, noxious gases and other inhaled environmental insults.
- The ECM is made up of a complex macromolecular network of proteins and proteoglycans providing both rigidity and flexibility to the tissue structure of all organs. The ECM also has important regulatory functions, influencing cell proliferation, differentiation, and migration. One way the ECM can modulate cell function is by acting as a reservoir for growth factors and inflammatory mediators. Several studies have shown that the ECM in the airways is remodeled in COPD.
- Gaining a better understanding of how a diseased ECM modulated epithelial cell phenotype inn COPD could provide more insight into mechanisms of pathological remodeling of the airway epithelium which may expand possibilities for pharmacological intervention.

Conclusion/Action Items: Gives global context and significance to the project.

Caitriona Treacy - Oct 12, 2022, 12:39 AM CDT

Note on 2022/10/10: The actual model used in this article was derived from the lungs of patients which were decellularized. The methods of this research have no application to our project, but the motivation behind their work reflects the work we are doing as well.

2022/11/12 - Degradation Timing Parameters

Caitriona Treacy - Nov 12, 2022, 2:54 PM CST

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5058441/

ECM Remodeling will require 5-9 days.

Title: "Preparation of extracellular matrices produced by cultured and primary fibroblasts"

Date: 2022/11/12

Content by: Caitriona Treacy

Present: N/A

Goals: Understand how long fibroblast ECM remodeling takes and how this affects rate of hydrogel degradation.

Search Term: PubMed: "Fibroblast ECM Reconstruction AND Degradation"

Citation:

J. Franco-Barraza, D. A. Beacham, M. D. Amatangelo, and E. Cukierman, “Preparation of extracellular matrices produced by cultured and primary fibroblasts,” *Curr Protoc Cell Biol*, vol. 71, p. 10.9.1-10.9.34, Jun. 2016, doi: 10.1002/cpcb.2.

Content:

- In general, fibroblasts function to secrete and organize extracellular matrix which later provides structural support for cell adhesion, cellular migration, and tissue organizations. In the context of our project, fibroblasts will be integrated into the model and later degrade the initial material to create a reconstructed extracellular matrix. In other words, our design must integrate fibroblasts into an environment which makes it possible for extracellular matrix reconstruction to occur. We must, therefore, also understand the length of time that will be required for this degradation and ECM reconstruction to take place before epithelial cells can be applied to the model.
- Matrices formed with fibroblast-derived 3-D ECM have been shown to closely resemble *in vivo* mesenchymal matrices and end up being primarily composed of fibronectin fibrillar lattices. Utilizing 3-D matrix models which mimic the *in vivo* environment allow for the acquisition of physiologically relevant information about cell-matrix interactions, structure, function, and signaling.
- **"Fibroblasts are plated and maintained in culture in a confluent state. After 5 to 9 days, unextracted 3-D matrix cultures can be sorted into normal or activated (myofibroblastic, fibrotic or desmoplastic) phenotypes."**
- After fabrication protocol is completed, these fibroblast-derived 3-D matrices can be stored for 2-6 weeks at 4˚C or up to 3 weeks at -80˚C.

Conclusion/Action Items: As outlined above, fibroblast ECM reconstruction takes around 5-9 days to occur. This information should be integrated into the information related to gel degradation rates in fabrication protocols for the project. Further, this article includes numerous other protocol outlines for general ECM preparation, ECM culture phenotypic evaluation, Fixing Extracted Matrices for Lack of Pliability Analysis, Cell Attachment Assay, Determination of Cell Shape, and Mechanical Compression of the Fibroblast-Derived 3-D Matrix which all have potential to be very useful in the context of the project once fibroblasts start being incorporated into the model.

2022/11/29 - "Peptide Concentration"

Caitriona Treacy - Nov 29, 2022, 4:28 PM CST

Title: "Harnessing Endogenous Circulating Angiogenic Cells"

Date: 2022/11/29

Content by: Caitriona Treacy

Present: N/A

Goals: Determine the desired concentration of MMP.

Search Term: Dissertation acquired through Dr. Murphy.

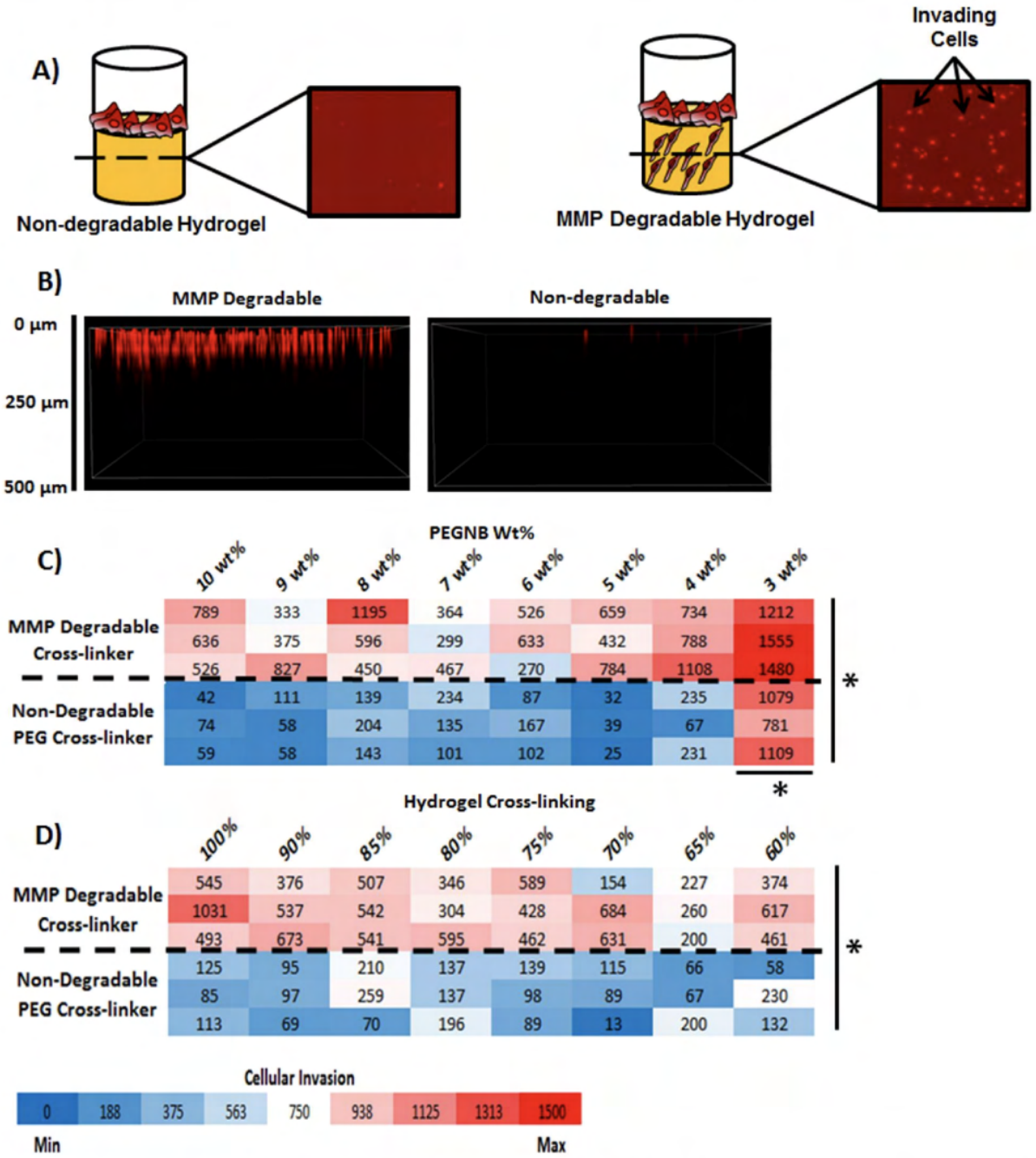
Citation:

M. Parlato, J. Molenda, and W. L. Murphy, “Specific recruitment of circulating angiogenic cells using biomaterials as filters,” *Acta Biomaterialia*, vol. 56, pp. 65–79, Jul. 2017, doi: 10.1016/j.actbio.2017.03.048.

Content:

- MMP degradable cross linker has been shown to permit more cell invasion than hydrogels cross-linked with a non-degradable PEG-dithiol cross-linker.
- Chapter 4, Figure 3 shows CAC invasion into PEGNB hydrogels with a decreasing number of MMP degradable cross-links, % cross links that are degradable.
- Use of an MMP degradable peptide as the cross-linker does not lead to change in stiffness of the hydrogels. However, formation of a 2 wt% PEGNB hydrogel and a 25% cross-linked hydrogel was poor and the resulting hydrogels were not robust enough fro mechanical testing.
- A 100% cross-linked hydrogel formed with an MMP degradable peptide had a lower stiffness than an equivalent hydrogel formed with a PEGDT cross-linker.
- Stiffness of the formed hydrogels were largely not dependent on cross-linker identity, except at the lowest and highest ends of the tested range.
- Hydrogel stiffness in general increases with increasing PEGNB wt% as well as increasing hydrogel cross-linking with either PEG-DT or the MMP degradeable cross-linker.
- Hydrogel shear modulus increased linearly with increasing PEGNB wt%.
- Regardless of PEGNB wt% or hydrogel cross-linking, hydrogels cross-linked with a peptide susceptible to MMP degradation allowed more cell invasion than hydrogels. cross-linked with non-degradable PEG-dithiol cross-linker.
- Data suggest that MMP-mediated degradation of the PEGNB hydrogels is critical for cell invasion in response to an SDF-1α gradient and that hydrogel susceptibility to MMP mediated degradation is a critical design parameter. (pg 134)

- Chapter 4, Figure 2 Directly from the article:



Chapter 4, Figure 2. PEGNB hydrogels cross-linked with an MMP degradable cross-linker permitted more CAC invasion than did hydrogels cross-linked with a non-degradable PEG-dithiol cross-linker. Heat-map legend presented at bottom of figure. **A)** Schematic representation of the assay results. PEG-dithiol cross-linked hydrogels did not permit any invasion into the hydrogel, while MMP degradable hydrogels permitted invasion of CACs. Representative maximum intensity projections are shown at right for both images. **B)** 3D reconstruction of CAC invasion into MMP degradable and non-degradable hydrogels. **C)** Heat-map of CAC invasion into MMP degradable and non-degradable hydrogels formulated from various PEGNB wt%. * indicates statistical significance relative to peers via two-factor ANOVA ($p < 0.05$). **D)** Heat-map of CAC invasion into MMP degradable and non-degradable hydrogels formulated with various amounts of hydrogel cross-linking. * indicates statistical significance relative to peers via two-factor ANOVA ($p < 0.05$).

- CACs produce high levels of MMP-9 in response to stimulation with soluble growth factors and are thus well equipped to proteolytically degrade the PEGNB hydrogels in response to soluble SDF-1α stimulations. That is to say, whether a cell type response with the production of MMP will affect the degradability of the hydrogel.

Conclusion/Action Items:

2022/11/29 - "Evaluation of MMP substrate concentration and specificity for neovascularization of hydrogel scaffolds"

Caitriona Treacy - Dec 14, 2022, 7:39 PM CST

Title: "Evaluation of MMP substrate and specificity for neovascularization of hydrogel scaffolds"

Date: 2022/11/29

Content by: Caitriona Treacy

Present: N/A

Goals: Determine the desired concentration of MMPs in the fabrication of our hydrogels.

Search Term: PubMed: "MMP concentration PEG hydrogel"

Citation:

S. Sokic, M. C. Christenson, J. C. Larson, A. A. Appel, E. M. Brey, and G. Papavasiliou, “Evaluation of MMP substrate concentration and specificity for neovascularization of hydrogel scaffolds,” *Biomater. Sci.*, vol. 2, no. 10, pp. 1343–1354, Aug. 2014, doi: 10.1039/C4BM00088A.

Content:

- "A critical biomaterial design criterion is the synchronization of the rates of scaffold degradation and vascularized tissue formation. Matrix metalloproteinases (MMPs) are key enzymes that regulate [...] extracellular matrix remodeling." While we are not so much concerned with vascularized tissue formation in the context of this project, the information regarding MMP enzymes and scaffold degradation are relevant to our model.
- "In this study, PEG hydrogels containing MMP-sensitive peptides with increased catalytic activity for MMPs expressed during neovascularization were investigated." These models were functionalized with MMP-2-, MMP-14-, or general collagenase-sensitive peptides with varying peptide concentration using crosslinkers containing one (SSite) or multiple (TSite) repeats of each protease-sensitive sequence.
- **Increasing peptide concentration enhanced the degradation kinetics of scaffolds functionalized with MMP-specific sequences.**
- Peptide concentration and specificity regulate scaffold degradation as well as matrix remodelling.
- Useful MMP background: "MMPs reportedly produced by endothelial cells include MMP-2, MMP-9, and MMP-14. Specifically, MMP-2 is responsible for the degradation of basement membrane proteins. MMP-14 has a well-documented role in guiding endothelial cell function including migration, formation of guidance channels and lumens, and vessel stabilization."
- Including peptide sequences with increased MMP catalytic activity and sensitivity in hydrogels significantly increases scaffold degradation when exposed to MMP-1 and MMP-2 enzymes. The presence of these peptide sequences also increases fibroblast cell proliferation and the spreading of fibroblasts within the matrices.
- Peptide sequences optimized for MMP-2 and MMP-14 cleavage were incorporated between network crosslinks in PEG scaffolds in order to vary degradation rates independent of mechanical properties to evaluate for sensitivity to degradation and tissue remodeling.
- The ratio of PEG to peptide molar ratio was 2:1. Hydrogel solutions were prepared using " 1× phosphate-buffered saline (PBS) (pH 7.4) with 37 mM *N*-vinylpyrrolidone (NVP), 0.05 mM of the photosensitive dye, eosin Y, 225 mM of the co-initiator, triethanolamine (TEA), with final **MMP-2-sensitive, MMP-14-sensitive, or collagenase-sensitive SSite or TSite PEGDA crosslinker concentrations of 3% (weight/volume (w/vol))** and 15 mg mL^{-1} acrylate-PEG-YRGDS (M_W = 3400 Da)."
- Scaffold were characterized using compression experiments which showed that concentration of cleavage sites and incorporation of MMP peptide substrates did not result in changes in crosslinking or, relatedly, the resulting mechanical properties of the hydrogels.

- Hydrogels synthesized with SSite MMP-substrates for cleavage by MMP-2 enzyme degraded within 6 days while MMP-14 substrates showed an increased degradation time of 7 days. TSite gels wiith substrates for cleavage by MMP-2 underwent complete hydrogel degradation in 4 days while the MMP-14 sensitive substrate completely degraded by 5 days.
- **MMP-2 and MMP-14-Sensitive hydrogels displayed relatively similar degradation profiles when incubated with MMP-14 enzyme regardless of incorporated peptide concentration.**

Conclusion/Action Items: Include the information about 3% weight/volume and the 2:1 PEG to MMP molar ratio in the project ideas for model fabrication.

2022/12/03 - "Gelatin Motivation"

Caitriona Treacy - Dec 03, 2022, 2:03 PM CST

Title:

Date: 2022/12/3

Content by: Caitriona Treacy

Present: N/A

Goals: Provide rationale as to why gelatin is a suitable alternative solution for the scaffold material.

Search Term:

Citation:

R. I. R. Ibañez, R. J. F. C. do Amaral, R. L. Reis, A. P. Marques, C. M. Murphy, and F. J. O'Brien, "3D-Printed Gelatin Methacrylate Scaffolds with Controlled Architecture and Stiffness Modulate the Fibroblast Phenotype towards Dermal Regeneration," *Polymers*, vol. 13, no. 15, Art. no. 15, Jan. 2021, doi: 10.3390/polym13152510.

Content:

- "Collagen is the main structural protein in skin, and its biocompatibility and low immunogenicity make it an ideal biomaterial for skin tissue applications. However, its use in 3D printing is challenging due to the processing required for printing and its low handling and mechanical properties. A common alternative to collagen is the use of gelatin, a derivative from the degradation of collagen, which has desirable gelling properties, biocompatibility and biodegradability. Although gelatin-based hydrogels are easily 3D-printed, their usage in vitro is limited by poor thermal stability."
- Both gelatin and gelatin-derived hydrogel materials can be used in 3D-printing applications which the client could seek out if a more advanced technique is desired to create "high-resolution scaffolds with controlled pore size and architecture."

Conclusion/Action Items:

2022/11/01 - "Specific Recruitment of Circulating Angiogenic Cells Using Biomaterials as Filters"

Caitriona Treacy - Nov 01, 2022, 6:53 PM CDT

Title: "Specific recruitment of circulating angiogenic cells using biomaterials as filters"

Date: 2022/11/01

Content by: Caitriona Treacy

Present: N/A

Goals: Understand the fabrication process used in other labs in the fabrication of hydrogels.

Search Term: Article obtained from and written by the team's faculty advisor (Dr. William Murphy)

Citation:

M. Parlato, J. Molenda, and W. L. Murphy, “Specific recruitment of circulating angiogenic cells using biomaterials as filters,” *Acta Biomaterialia*, vol. 56, pp. 65–79, Jul. 2017, doi: 10.1016/j.actbio.2017.03.048.

Content:

- Key Terms:
  - Angiogenesis: Formation of new blood vessels. Involves the migration, growth, and differentiation of endothelial cells. Controlled by chemical signals in the body.
  - Circulating angiogenic cells (CACs): Monocyte-like cells that appear to stimulate angiogenesis through secretion of growth factors.
  - Endogenous cell recruitment: A prospective therapy that might be more convenient and have a lower cost for clinical cartilage tissue engineer. Avoids the need for in vitro culture of seed cells and the risk of pathogen transmission.
- Study aims to understand how the design of biomaterials affects CAC recruitment and invasion using an enhanced-throughput cell invasion assay to systematically examine the effects of biomaterial design on CAC recruitment. As part of the study, the screens co-optimized hydrogel degradability and hydrogel stiffness for maximal CAC invasion. As part of the specificity of invasion, dermal fibroblast and mesenchymal stem cell invasion were examined individually and in co-culture with CACs to identify hydrogels specific to CAC invasion.
- Application goal of the study is that the design concept may be applied more broadly (beyond CAC recruitment) to specific subsets of biomaterials as "filters" to control which types of cell populations invade into and populate the biomaterial.
- Note: article states, "We found that these conditions also specifically recruited CACs and excluded the other tested cell types of dermal fibroblasts, mesenchymal stem cells, and lymphocytes. This suggests an intriguing new role for biomaterial as "filters" to control the types of cells that invade and populate that biomaterial." S**uggests that while the information included in the article may be relevant to our process, the specific information likely won't be usable for our fabrication because we specifically need fibroblasts to be able to invade the matrix.**
- Use of pro-angiogenic cell types to stimulate angiogenesis: Delivered cells can secrete a wide range of the appropriate soluble factors and can respond to changes within the diseased tissue (in this study, the cells that promoted angiogenesis were CACs which are involved in blood vessel formation, repair, and maintenance).
- Utilized a thiol-ene chemistry to rapidly polymerize hydrogels within a plate-based formate. Employed 8-arm, poly(ethylene glycol) (PEG)-based hydrogels because of the ability to easily modulate cross-linker molecule identity , stiffness, and soluble factor incorporation within the hydrogel system.

- **PEGNB Synthesis:**
    - Multi-arm, alcohol terminated, 8-arm PEG with a tripentaery-thritol core were functionalized with norborene groups via reaction with norborene anhydride. Briefly, norbornene acid at a 10x molar excess versus PEG alcohol groups and dicyclohyxylcarbodiimide (DCC) at 5x molar excess versus PEG alcohol were both dissolved in anhydrous dichloromethane (DCM). PEG, pyridine at a 5x molar excess versus PEG alcholol groups, and 4-(dimethylamino) pyridine at a 0.5x molar excess versus PEG alcohol groups were also dissolved within a separate DCM solution. Both DCM solutions were combined once the norbornene anhydride (white precipitate) had formed. Reaction was stirred overnight and then solution was filtered to remove precipitates. PEG was recovered via precipitation in ethyl ether and was purified via dialysis against deionized (DI) water.
    - Functionalization of the PEG polymer with norbornene groups (PEGNB) was confirmed via NMR.
    - PEGNB hydrogels were polymerized via a thiol-ene reaction with dithiol cross-linkers in all cases. P

Conclusion/Action Items:

2022/09/30 - "Design and Optimization of Biocompatible Polycaprolactone/Poly(l-lactic-co-glycolic acid) Scaffolds With and Without Microgrooves for Tissue Engineering"

Caitriona Treacy - Oct 11, 2022, 11:41 PM CDT

Title: "Design and Optimization of Biocompatible Polycaprolactone/poly (l-lactic-co-glycolic acid) Scaffold with and without Microgrooves for Tissue Engineering Applications"

Date: 2022/09/30

Content by: Caitriona Treacy

Present: N/A

Goals: Gather information about the use of PEG as a starting material for the tissue scaffold design.

Search Term: Scopus: "ECM Model PEG"

Citation: C. Alvim Valente *et al.*, "Design and optimization of biocompatible polycaprolactone/poly (l-lactic-co-glycolic acid) scaffolds with and without microgrooves for tissue engineering applications," *Journal of Biomedical Materials Research Part A*, vol. 106, no. 6, pp. 1522–1534, 2018, doi: 10.1002/jbm.a.36355.

Content:

- Fibroblasts adhered efficiently to smooth membranes of the PCL70/PLGA 30 blend and pure PLGA (compared to pure PCL and silicone).
- Micro-grooved membranes promoted similar cell adhesion for all groups.
- Microstructured membranes (15 and 20 µm wide grooves) promoted suitable orientation of fibroblasts in both PCL70/PLGA 30 and pure PLGA (compared to pure PCL).
- PLGA is biodegradable and approved by the FDA for clinical use.
- PLGA is one of the most widely used biodegradable polymers in scaffold preparation for tissue engineering – easy manufacturing, low inflammatory response, and adjustability of degradation rates.
- Degradation time can be modulated by tuning the ratio of its hydrophilic (polyglycolic acid) and hydrophobic (polylactic acid) constituents. High content of hydrophilic units shows faster degradation process.
    - PLGA (50:50) –> 2-4 weeks
    - PLGA (70:30) –> 6-8 weeks
    - PLGA (85:15) –> 23-26 weeks
- PLGA-based biomaterials with nano/microporous allow nutrient permeability and favor cell adhesion and proliferation.
- Surface roughness of scaffolds is an important characteristic involved in cell biocompatibility.
- Fabrication of smooth and micro-grooved membranes:
    - Smooth membranes: Pure PCL, pure PLGA, or mixtures of both polymers prepared at different ratios were solubilized in chloroform so that the final proportion of all solutions was 5% w/v. Solutions poured into glass petri-dishes at 0.5 mL/cm^2. Solutions were dissolved under an ultrasonic bath for 4 hours and poured into a glass petri-dish 5.5 cm in diameter. Solvent was evaporated at room temp for 48 hours. Then vacuum dried for 8 hours.
    - Micro-grooved membrane fabrication used the same methodology, but the solutions were poured on micro-structured silicon molds.
- Membrane Characterization
    - Atomic force microscopy (AFM) collected nano topography and surface roughness data of membranes.
    - Scanning electron microscopy examined the morphology of the membranes.

    - Mechanical properties (Young's Modulus) were measured using a Universal test machine (Lloyd Instruments).
    - Contact-angle measurements were performed using a goniometer.
- In vivo studies involved applications in bone, articular cartilage and the meniscus. In situ studies of muscle soft tissues sometimes suggested inflammatory responses.

Conclusion/Action Items: Report findings about PLGA to the team to be considered and incorporated into the first draft of the design matrix.

Caitriona Treacy - Oct 11, 2022, 11:44 PM CDT

Note after advisor meeting 2022/09/30: While widely used, PLGA will not be a feasible material to use for this product due to the fact that it is not translucent. Imaging cells with PLGA requires a lot of manual manipulation and intensive processing, so it would not be a usable material for the scaffold.

2022/10/04 - "GelMA Applications and Fabricaition"

Caitriona Treacy - Oct 12, 2022, 12:54 AM CDT

Title: "Design and Optimization of Biocompatible Polycaprolactone/poly (l-lactic-co-glycolic acid) Scaffold with and without Microgrooves for Tissue Engineering Applications"

Date: 2022/09/30

Content by: Caitriona Treacy

Present: N/A

Goals: Gather information about the use of GelMA as a starting material for the tissue scaffold design.

Search Term: Scopus: "GelMA Hydrogel"

Citation:

Y. Piao *et al.*, "Biomedical applications of gelatin methacryloyl hydrogels," *Engineered Regeneration*, vol. 2, pp. 47–56, Jan. 2021, doi: 10.1016/j.engreg.2021.03.002.

Content:

- Gelatin methacryloyl (GelMA) hydrogel has good biocompatibility and permeability and adjustable physical and chemical properties, especially a three dimensional network structure suitable for cell growth, which is condusive to cell adhesion and reconstruction.
- Tissue Engineering applications include bone, endochondral bone, heart tissue, cartilage, vascular networks, and corneas.
- Micromolding: involved formation of gel over pre-patterned templates. Suitable for biomaterials that can be converted from a liquid form into a rigified mass. Hydrogels (both natural and synthetic) are the most common type of material used with micromolding.
- Hydrogels have the particular advantage that they are inherently porous and can serve as mimics of the extracellular matrix.
- Micromolding-based methods are the most popular for generating microfluidic biomaterials. because they are not too technically demanding and are easy to learn. In principle, micromolding can be automated, but it is largely implemented as a technique for manual production of small numbers of samples.
- GelMA Synthesis/Tuning: Methacrylic Anhydride [MAA] is the most widely used chemical used for gelatin modification. GelMA has been used in various bio-applications including micropatterning, fluidic systems, 3D scaffolds, and bioprinting. It has been used with different cells (fibroblasts, stem cells, cartilage, hepatocytes), and composite materials (carbon nanotubes, graphene oxide, and natural and synthetic polymers).
- One method of fabrication is to react MAA monomers with lysine and hydroxyl lysine groups of gelatin by dissolving gelatin in PBS solution at 50 ˚C.
- GelMA synthesis routes remain suboptimal, especially in terms of controllability and efficacy.

Conclusion/Action Items: As it is one of the most commonly used synthetic materials in tissue models, there is a lot of information readily available on GelMA. It has been used many times in contexts which parallel the scaffold that is being created in this project, and it seems to meet all major design specifications. It appears that fabrication may be difficult in producing uniform results, but research should be brought back to the team and utilized to complete the second draft of the design matrix.

3-D Tissue Model Intro Video

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 11, 2022, 9:56 PM CDT

Title: Elijah Diederich

Date: 9-11-22

Content by: Elijah Diederich

Present: Myself

Goals: To gain introductory knowledge on 3-D tissue models via an informational video

Content:

YouTube Video link: https://www.youtube.com/watch?v=Ok6faE_NCmg

"In Vitro Human Airway Modeling Using Primary Airway Epithelial Cells: Webinar by Dr. Ronaghan"

Notes attached below

Conclusions/action items:

1. Continue to familiarize with the cell biology and biology of the lungs

2. Set up meeting with advisor and client

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 11, 2022, 10:01 PM CDT

Download

Carley_Intro_Video_Notes_2_.pdf (1.53 MB)

Fibroblasts and Myofibroblasts in Wound Healing

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 11, 2022, 10:22 PM CDT

Title: Fibroblasts and Myofibroblasts in Wound Healing

Date: 9-11-2022

Content by: Elijah Diederich

Present: Myself

Goals: To understand the role of fibroblasts in epithelial tissue injury

Content:

Citation:

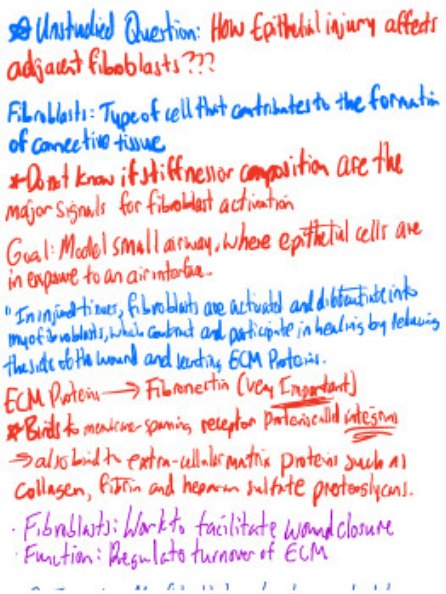
L. B. W. JH; “Fibroblasts and myofibroblasts in wound healing: Force generation and measurement,” *Journal of tissue viability*, 07-Dec-2009. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/19995679/. [Accessed: 06-Oct-2022].

Notes for article attached below

Conclusions/action items:

1. Continue to research the ECM proteins and how these cells communicate

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 11, 2022, 10:26 PM CDT



Download

Fibroblasts_Notes.pdf (1.16 MB)

Biology of the ECM

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 11, 2022, 10:44 PM CDT

Title: Biology of the ECM

Date: 10-11-2022

Content by: Elijah Diederich

Present: Myself

Goals: To better understand the ECM and how it functions

Content:

Citation:

> B. Yue, “Biology of the extracellular matrix: An overview,” *Journal of glaucoma*, 2014. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4185430/. [Accessed: 06-Oct-2022].

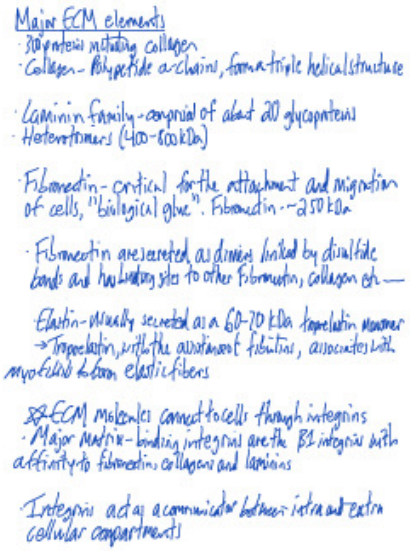
Notes attached below

Conclusions/action items:

1. Meet with group about possible design ideas

2. Finish presentation

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 12, 2022, 11:21 AM CDT



Download

Biology_of_the_ECM_Matrix_.pdf (1.2 MB)

Fibroblasts in Lung Homeostasis and disease

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 12, 2022, 11:25 AM CDT

Title: Fibroblasts in lung homeostasis and disease

Date: 10-11-2022

Content by: Elijah Diederich

Present: Myself

Goals: To further my knowledge on fibroblasts and how they change in times of disease

Content:

Citation:

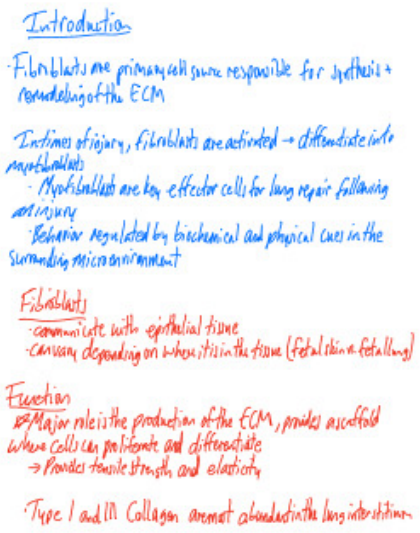
> U. F. O. Themes, “Fibroblasts in lung homeostasis and disease,” *Thoracic Key*, 11-Jan-2017. [Online]. Available: https://thoracickey.com/fibroblasts-in-lung-homeostasis-and-disease. [Accessed: 06-Oct-2022].

Notes attached below in PDF

Conclusions/action items:

1. Update Lab archives for submission next week

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 12, 2022, 11:51 AM CDT



Download

Fibroblasts_in_lung_homeostasis_and_disease.pdf (1.16 MB)

Harnessing Endogenous Circulating Angiogenic Cells

ELIJAH DIEDERICH (ediederich@wisc.edu) - Nov 01, 2022, 7:24 PM CDT

Title: Harnessing Endogenous Circulating Angiogenic Cells

Date: 11-1-2022

Content by: Elijah Diederich

Present: Myself

Goals: To continue my understanding of this project and further my knowledge of cells biology and processes

Content:

Angiogenesis - the physiological process through which new blood vessels form from pre-existing blood vessels through the process of sprouting and splitting

Harnessing cells through 2 approaches:

1. In-vivo recruitment and 2. Ex-vivo expansion

In-vivo recruitment has specific parameters of biomaterial degradability and soluble factor content. This biomaterial was able to act as a "cellular filter", allowing for control of cells types into the biomaterial

Ex-vivo expansion also had specific parameters of biomaterial stiffness and the introduction of integrin-binding peptides (probably for increased cell adhesion). Integrins work in cellular signaling. Dissertation goes further into the set of fundamental biomaterial design rules for biomaterials.

For this dissertation, I will be focusing specifically on Chapter 3: Polyethylene Glycol Hydrogels as Adaptable Substrates for screening cellular interactions with the Extracellular Matrix

Chapter 3 Notes:

Abstract

Multi-arm Polyethylene Glycol-based hydrogels allow for biomaterial stiffness, peptide content and identity, and soluble factor diffusion to be decoupled and effectively studied independently. The cellular interactions between injured epithelial tissue and fibroblasts are client is hoping to study may be best tested using HTS or High-throughput screening.

As stated in previous notes, the composition of the ECM may control specific cellular interactions and this is something that can be studied and tested through this screening. In this particular study of angiogenesis, the endothelial growth factor signaling (VEGF) is tightly regulated by ECM stiffness, biomolecule composition and soluble protein sequestering. Incorporating various concentrations of the VEGF-binding peptide (VBP) was introduced to examine the effects of VEGF sequestering on endothelial cell populations.

Protein sequestration - Where an active protein is bound to an inactive complex by an inhibitor, usually leads to ultra-sensitivity. Hydrogel stiffness, peptide content and identity, and soluble transport will be constant through this experiment.

3.3 Introduction

HTS can be very useful when examining cellular interactions with the ECM. Using HTS, the number of conditions that can be examined has greatly increased allowing for better research results. HTS can be very useful when examining the these interactions because the ECM influences cellular populations through biomolecule composition, stiffness, and sequestering of soluble proteins. *Binding of endothelial cells integrins to components of the ECM and ECM stiffness simultaneously influence VEGF signaling within the endothelial cells. The use of PEG hydrogels allow for a "blank slate" in which specific biological cues can be presented. These biological cues can be adjusted independently depending on the research question.

These hydrogels were created using thiol-ene chemistry involving the reaction or norbornene-functionalized, multi-arm PEG molecules with dithiol cross-linkers and thiol containing peptides. Once the hydrogels were formed to the correct properties of stiffness, peptide concentration and identity, and soluble transport, VBP was incorporated into these hydrogels to probe the effects of ECM-mediated sequestering of VEGF on in vitro endothelial cell behavior.

3.4 Methods

All materials purchased from Sigma-Aldrich besides the 8-arm PEG (JenKem Technology USA). The PEGNB synthesis is included in this article and may be of use when performing this in the BME team lab. Instead of using PBS as seen with the grad student at WIMR last week, this process involves the use of DCM. This process was also exposed to 10 minutes of UV light compared to 5 minutes of UV light which was observed with the

grad student last week as well. The UV light used was at a wavelength of 365 nm. If using a peptide in our experiment which seems like the best course of action, peptides will need to be purchased and a matrix degradable peptide used for hydrogel cross-linking may also be a good idea. Details are mentioned in the paper.

3.5 Results

After the hydrogels were created, they were left to sit in PBS overnight at 37 degrees Celsius. Hydrogel equilibrium swelling was determined after these gels were taken out of solution. Increasing PEGNB wt% correlated with a decrease in equilibrium swelling. Hydrogel crosslinking also demonstrated this trend. Incorporating a CRGDS peptide or a model protein did not affect hydrogel equilibrium swelling. This will need to be investigated further in our project and a size comparison between these proteins and fibroblasts will need to be looked at. Solute transport was also unaffected when these peptides and protein were incorporated.

Chapter 3 has some great figures and charts that are worth looking at for equilibrium swelling. Include changing the PEGNBwt%, hydrogel crosslinking, CRGDS Peptide addition, and the concentration of the BSA protein.

Mathematical modeling was used to predict hydrogel stiffness. The model predicted that the hydrogel stiffness would increase with increasing PEGNB wt & and hydrogel cross-linking. Addition of CRGDS peptide content was predicted to have limited effects on hydrogel stiffness. The changing of hydrogel mesh size was originally thought to have an effect on cellular transport but after modeling this, the mesh size was seen to be much larger than soluble proteins so this had minimal effects on the protein transport.

The incorporation of VEGF-binding peptides caused a total increase in hydrogel stiffness of approximately 100 Pa, which was shown to be a very small chance when comparing it to differences caused by increased PEGNB wt% and hydrogel crosslinking.

Conclusions/action items:

1. Meet with client and send them a sheet of questions to review before meeting

2. Make sure to share notes taken with the grad student to all team members and start to try to lockdown chemistry

3. Start to get ready for show and tell this Friday

Fibroblasts in 3D Keratin Hydrogel

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 12, 2022, 7:26 PM CST

Title: Culturing Fibroblasts in 3D human hair keratin hydrogels

Date: 11-30-2022

Content by: Elijah Diederich

Present: NA

Goals: To find an adequate fibroblast concentration used in hydrogels that show excellent cell viability

Content:

Citation:

> A. (2015, February 17). *Culturing fibroblasts in 3D human hair keratin hydrogels*. ACS Applied Material and Interfaces. Retrieved November 30, 2022, from https://pubs.acs.org/doi/10.1021/acsami.5b00854

Introduction:

- Limited research on using keratin-based 3D scaffolds for cell culture in vitro, studying viability and proliferation of L929 murine Fibroblasts
- Results show that the keratin based hydrogel performed comparably to collagen hydrogels in supporting viability and proliferation
- Using protein based natural materials (Collagen, gelatin, silk, elastin), is advantageous because they are made of amino acids which can be degraded enzymatically (important to client)

Materials and Methods

- Human hair was collected from local hair salons and washed and decontaminated with ethanol (70%)
- Oscillatory Rheology was performed using a parallel plate rheometer
    - Keratin hydrogel concentrations tested (15, 25, 40, and 50 mg/ml)
    - Height of each sample was measured by lowering the parallel plate until it touched the sample surface without compressing it.
    - Preliminary strain sweep was ran to determine the viscoelastic response range of the hydrogels to be below 5% before the frequency sweep was performed
- Cells were encapsulated at 50000 per ml, cell culture media was changed every 2 days
- Control sample was 1 mg/ml rat tail collagen 1 hydrogels

Results

- From 15 g/ml to 50 g/ml keratin hydrogels, the storage modulus increased from 3 Pa to 800 Pa
- For in-vivo studies, subcutaneous keratin hydrogels were implanted into rodents
    - Results showed significant cell infiltration
    - At day 90 post implantation, only about 30% of the hydrogels remain detectable based on volume showing that these hydrogels can be used as a viable in vivo
- Cell viability in these hydrogels showed to be successful across a 16 day culture period
- Human hair keratin hydrogels are also shown to have tunable physical and chemical properties, showing that they are a viable 3D cell culturing system

Conclusions/action items:

1. Continue research on fibroblast concentrations in hydrogels

RGD Peptide effects on Ovarian Cancer stem-like cells

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 12, 2022, 9:28 PM CST

Title: Effect of RGD content in poly(ethylene glycol)-crosslinked poly(methyl vinyl ether-alt-maleic acid) hydrogels on the expansion of ovarian cancer stem-like cells

Date: 11-10-2022

Content by: Elijah Diederich

Present: NA

Goals: To continue research on RGD peptides and their effect in hydrogels

Content:

Citation:

> Zhou N;Ma X;Hu W;Ren P;Zhao Y;Zhang T; (2021, January). *Effect of RGD content in poly(ethylene glycol)-crosslinked poly(methyl vinyl ether-alt-maleic acid) hydrogels on the expansion of ovarian cancer stem-like cells*. Materials science & engineering. C, Materials for biological applications. Retrieved November 10, 2022, from https://pubmed.ncbi.nlm.nih.gov/33255056/

Introduction

- RGD stands for arginine-glycine-aspartic acid sequence is present in many ECM proteins such as fibronectin, collagen 1, and vitronectin
- Study consisted of fabricating PEG hydrogels with varying concentrations of RGD to investigate the effects of RGD content on cancer stem cell enrichment (CSC)
- Results were evaluated with the following criteria: Morphology, Proliferation, viability, the expression of CSC markers, malignant signaling pathway-related genes, and drug resistivity
- Integrins play a critical role in cell adhesion to the RGD sequence with the ECM proteins (very important factor in regulating the proliferation, self-renewal and metastatic potential of tumorigenic cells, ovarian cancer cells in this study)
    - Specific RGD binding integrins can activate a variety of cellular signaling pathways
- Synthetic peptides that contain the RGD sequence seen in fibronectin can essentially mimic cell attachment activity

Materials and Methods

- Polyethylene glycols used in study had Mw=20K, The human ovarian cancer cell line (SK-OV-03) was used
- 2 concentrations of PEG crosslinked hydrogels were created (140:1. and 280:1, which stands for molar ratio of carboxyl groups in P(MVE-alt-MA) to hydroxyl groups in PEG)
- Varying concentrations of peptides (25, 50 and 200 uM (Micromolar)) were added to the hydrogels as w
- ell

Results

- Difference in the mean pore sizes of the 2 kinds of hydrogels is related to the degree of crosslinking that occurs. The results showed that the difference in RGD content had no effect on the porosity of the gels created
- PEMM-2 hydrogels (140:1 ratio) average Young's Modulus in the 240 kPa range, while the PEMM-1 (280:1 ratio) gels had an average Young's Modulus in the 57 kPa range. Both of these Young's Moduli were consistent across all RGD concentrations showing that RGD content had no effect on Young's Moduli
- Results also showed there was no effect on the swelling behavior of the hydrogels with a variation in RGD content
- Results showed that an increase in RGD concentration results in stronger cell-matrix interaction, meaning the formation of cell spheroids is much more difficult
    - Results also showed that the adherent cells predominated the hydrogels with a higher RGD content
- A majority of cells were in a viable state in all RGD concentrations showing that the hydrogel despite having different stiffness's and RGD concentrations can still successfully support cells
- Many cell aggregates formed on the PEG hydrogels with the lower RGD content, resulting in an enhanced drug resistance.
- Drug sensitivity on cells on the higher RGD content surface increased due to less CSC properties shown by the ovarian cancer cells

Conclusions/action items:

1. Continue to research RGD peptide concentrations and consult with group on whether or not similar findings were found

Selectively Crosslinked PEG Hydrogels

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 12, 2022, 10:43 PM CST

Title: Selectively Cross-linked Tetra-PEG Hydrogels Provide Control over Mechanical Strength with minimal impact of Diffusivity

Date: 10-24-2022

Content by: Elijah Diederich

Present: NA

Goals: To explore diffusion in PEG hydrogels and the impact of selective crosslinking

Content:

Citation: Lust ST;Hoogland D;Norman MDA;Kerins C;Omar J;Jowett GM;Yu TTL;Yan Z;Xu JZ;Marciano D;da Silva RMP;Dreiss CA;Lamata P;Shipley RJ;Gentleman E; (2021, June 20). *Selectively cross-linked tetra-peg hydrogels provide control over mechanical strength with minimal impact on diffusivity*. ACS biomaterials science & engineering. Retrieved October 24, 2022, from https://pubmed.ncbi.nlm.nih.gov/34151570/]

Introduction

- Cells responds much differently in 3D structures compared to when on 2D surfaces (ex: cell elongation and morphology concerns from client)
- By changing polymer concentrations, macromer arm size, and the number of arms, scientists are able to vary the degree of cross-linking in hydrogels
- Crosslinking the cell with matrix metalloproteinases(MMP)-sensitive peptides allows for encapsulated cells to actively remodel and migrate through gel
- In this study A4+B4/Tetra PEG hydrogels are being synthesized. In simple terms the A4+B4 stands for mixing two polymer macromers with difference reactive terminal ends. The 4 in each of these cases stands for 4-arm.
- This tetra-PEG design allows for tunable physical and biological properties while maintaining network connectivity

Materials and Methods

- A 4-arm 10 kDa PEG-NPC (20 or 15 uL) was crosslinked with a 10 kDa PEG tetramer solution (20 or 15 uL) with a vinyl-sulphone end group through a base catalyzed Michael-type addition
- This strategy was performed to create gels of these concentrations (2.5%, 5%, and 10% w/v hydrogels)
- Rheology Measurements were performed using small-amplitude oscillatory time-sweep measurements
    - Strain was kept at 5% as was the angular frequency of 1 rad/s at 37 degrees C
    - An amplitude and frequency sweep were also performed indicating this gel was in the viscoelastic region
    - G' and G'' values were recorded for all tests

Results

- Model predicted that the increased in polymer concentration (from 2.5% to 5% to 10%) in tetra-PEG hydrogels results in smaller mesh sizes
- G' values proved to be significantly different among all 3 samples although G'' showed the exact opposite conclusion among the 3 samples
- Results from this study also showed that mesh size does not have a large influence on the diffusion of small solutes but can have a much larger effect on larger solutes
    - In this specific test, Dextran size varied from 10-70 kDa
- Results also showed that the diffusion of larger solutes is far more impacted by changing polymer concentrations compared to that of smaller solutes

Conclusions/action items:

1. Continue research on PEG hydrogels and methods that team can perform in BME lab

2. Meet with grad student recommended by Dr. Murphy to see PEG gels created in person

Bicyclic RGD peptides effect on Nerve Growth in PEG Anisogels

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 13, 2022, 9:19 PM CST

Title: Bicyclic RGD peptides enhance nerve growth in synthetic PEG-based Anisogels

Date: 11-24-22

Content by: Elijah Diederich

Present: NA

Goals: To continue to look for RGD peptide concentrations in PEG hydrogels

Content:

Citation:

> Vedaraman S;Bernhagen D;Haraszti T;Licht C;Castro Nava A;Omidinia Anarkoli A;Timmerman P;De Laporte L; "Bicyclic RGD peptides enhance nerve growth in synthetic PEG-based Anisogels," *Biomaterials science*, 16-Mar-2021. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/33724266/. [Accessed: 24-Nov-2022].

Introduction

- Linear RGD peptides suffer from low stability towards degradation and lack integrin selectivity
- Cyclization of RGD improves the affinity towards integrin subtypes but lacks selectivity
- New class of short bicyclic peptides with RGD in a single loop and random screened tri-amino acid peptide sequences in the second loop
    - Shown to be selective towards certain integrin subunits
    - Enzymatic conjugation to the PEG backbone enables the formation of a hydrogel that supports nerve growth
- Mouse fibroblasts and primary nerve cells shows superior growth in bicyclic RGD peptide conjugated gels

Results and Discussion

- Influence of RGD peptides on fibroblast growth
    - Results showed that monocyclic RGD peptide leads to better cell invasion (due to higher affinity for integrin $\alpha v \beta 3$) when compared to the linear RGD peptide
    - Results also showed higher cell spreading for bicyclic RGD peptides when compared to monocyclic and bicyclic

Conclusions/action items:

1. Create protocols for adding RGD peptides into PEG hydrogels

Stability of Proteins Encapsulated in Michael-Type Addition Polyethylene Glycol Hydrogels

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 14, 2022, 10:11 AM CST

Title: Stability of Proteins Encapsulated in Michael-Type Addition Polyethylene Glycol Hydrogels

Date: 11-3-2022

Content by: Elijah Diederich

Present: NA

Goals: To explore the stability of proteins in hydrogels

Content:

Citation:

G. Z. R. S. L. J. B. Z. SP; “Stability of proteins encapsulated in Michael-type addition polyethylene glycol hydrogels,” *Biotechnology and bioengineering*, 11-Oct-2021. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/34606089/. [Accessed: 03-Nov-2022].

Introduction

- Degradeable PEG hydrogels are often used as drug delivery systems
- Proteins encapsulated in this study were lysozyme and alcohol dehydrogenase (ADH), encapsulated through Michael-type addition
- PEG hydrogels can be made degradable either hydrolytically or enzymatically
- Molecular weight of PEG was varied to achieve gel mesh sizes of 7.5x and 11.6x the radius of gyration of lysozyme, and 2.7-3.9x the radius of gyration of ADH
- Unfolding free energy was measured via the max. emission wavelength which is greater for proteins that are denatured

Results

- Stability of proteins is often measured by the degree of denaturation
- Difference in accessible surface area between the unfolded and folded state of lysozyme was smaller compared with lower confinement, free solution, and crowded (PEG-OH) environments ----> hence lower unfolding
- Results also showed that in the formation of the PEG hydrogels, a conjugation reaction is observed between PEG-DA and lysozyme
    - After conjugation, lysozyme activity was unchanged and maintained at an activity level comparable to free lysozyme
- Enzymatic activity was also examined for a rheology perspective, under mild stress conditions(50 degrees C), and shaking at 300 RPM, results showed that all samples retained >80% enzymatic activity under these two conditions

Conclusions/action items:

1. Continue research on proteins for presentation

Alginate Hydrogels

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 11, 2022, 7:53 PM CDT

Title: Alginate Hydrogels notes

Date: 10-03-2022

Content by: Elijah Diederich

Present: Myself

Goals: To familiarize myself with alginate and how we would use it in our 3-D tissue model

Content:

"Notes attached below in PDF*

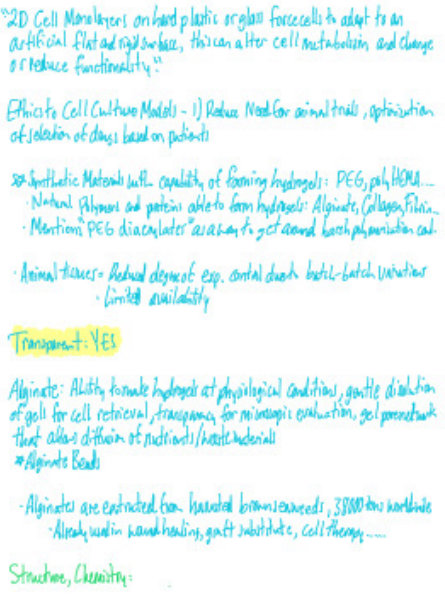
Citation:

T. Andersen, P. Auk-Emblem, and M. Dornish, “3D cell culture in alginate hydrogels,” *Microarrays (Basel, Switzerland)*, 24-Mar-2015. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4996398/#B89-microarrays-04-00133. [Accessed: 11-Oct-2022].

Conclusions/action items:

1. Complete slides for presentation and practice what you are going to say

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 11, 2022, 9:43 PM CDT



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Alginate_Hydrogels_.pdf (3.72 MB)

PEG Hydrogels

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 11, 2022, 2:00 PM CST

Title: Capillary Morphogenesis in PEG-collagen hydrogels

Date: 10-1-2022

Content by: Elijah Diederich

Present: NA

Goals: To determine the effects on capillary morphogenesis when including collagen in a PEG hydrogel

Content:

Introduction

- PEG hydrogel conjugated with type 1 collage, looking to research how the ECM regulates capillary morphogenesis (process that causes capillary tissue to develop its shape)
- Fibroblasts and endothelial tissue were encapsulated inside the hydrogel and MMP activity (allows for breakdown of collagen) was inhibited to cause the prevention of capillary formation

Materials and Methods

- Cell culture used was human umbilical vein endothelial cells (EC), fibroblasts cultured in DMEM (can be used for diffusion testing later on)
    - Both cell types above have been previously shown to support robust capillary formation in vitro.
- PEG-diacrylamide was the hydrogel created using chemistry beyond what we could do, might just need to do PEG hydrogels. (Margo's Method should be sufficient)
- PEGDAm and Collagen reaction was then run to form a PEG-collagen conjugated hydrogel (photoinitiator Irgacure 2959 was used)
- Rheological measurements to assess the kinetics of hydrogel formation were performed using a frequency oscillation (1% strain, 1 rad/s, 5 min exposure to UV light)
- Bulk transport was assessed using Dextran, used to simulate diffusion of macromolecular species
    - Used 24 well plates with PBS, incubated at 37 degrees C

Results

- Formation of PEG diacrylamides was shown to have been performed at a 95% efficiency rate
    - Final mass of PEGDA was shown to be 2.52 +/- 0.02 mg per mg of collagen for a stoichiometric ratio of 57:1
    - Gels showed an increase in G'(storage modulus) and G"(loss modulus) after the gels were exposed to UV light (crosslinking)
    - Loss modulus is usually an order lower in magnitude compared to the storage modulus which indicates extensive crosslinking of the material
    - Storage modulus also did not significantly change over 14 days in buffer solution
- Data collected suggests that PEG-collagen hydrogels are hydrolytically stable in an inert aqueous environment
- Dextran diffusion results showed [PEG-Collagen] (PC), PC +0%PEG gels showed the significantly greatest release of Dextran compared to other formulations of gels
- Cell viability showed to be more than 60% in all constructs after 24 hours

Citation:

AJ;, S. R. K. S. D. P. (n.d.). *Capillary morphogenesis in peg-collagen hydrogels*. Biomaterials. Retrieved October 1, 2022, from https://pubmed.ncbi.nlm.nih.gov/24021759/

Conclusions/action items:

- **Continue to research MMP and rheology of hydrogels to increase understanding of these concepts**
- **Meet with group to determine best hydrogel option**

Rheology Testing

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 12, 2022, 11:14 AM CST

Title: Baseline stiffness modulates the non-linear response to stretch of the ECM matrix in Pulmonary Fibrosis

Date: 12-3-2022

Content by: Elijah Diederich

Present: NA

Goals: To get a better understanding of rheology and how to apply it to the gelatin hydrogels that we are making

Content:

Citation:

Júnior C;Narciso M;Marhuenda E;Almendros I;Farré R;Navajas D;Otero J;Gavara N; (n.d.). *Baseline stiffness modulates the non-linear response to stretch of the extracellular matrix in pulmonary fibrosis*. International journal of molecular sciences. Retrieved December 12, 2022, from https://pubmed.ncbi.nlm.nih.gov/34884731/

Introduction

- The Young's Modulus (EM) is equivalent to the slope on a stress strain curve, although a useful measurement, the EM does not provide an accurate representation of the mechanical environment sensed locally be cells in the lungs (In this case, very important. Viscoelasticity is an intrinsic feature of many biological tissues)
- Viscoelastic properties such as G' (storage modulus), and G''(loss modulus) are much more accurate representative of the actual mechanical properties sensed by fibroblasts.
- Using low amplitude oscillatory forces at different frequencies, it is possible to estimate G' and G''.
- This study was conducted using healthy rat lung tissue and rat lungs with pulmonary fibrosis

EM, G' and G'' Values

- The median EM baseline value for healthy rat lung tissue was 2.7 kPa, and in fibrotic lung tissue was 10.1 kPa
- G' values at 0.1 Hz in healthy lung tissue were 2.12 +/- 0.61 kPa
- G'' values at 0.1 Hz in healthy lung tissue were 0.212 +/- 0.061 kPa
- At frequencies higher than 1 Hz, G'' is much more prominent and graph becomes non-linear, this is why values are taken at 0.1 Hz.

Procedures

- Frequency varied between 0.1-10 Hz, G' and G'' values were taken.

Conclusions/action items:

- **Work to complete rheology testing on gelatin hydrogels and write procedure**
- **Get in contact with Dr. Puccinelli to get grad student help**

Hydrogel Characterization Lecture

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 12, 2022, 11:23 AM CST

Title: Hydrogel Characterization Lecture

Date: 12-4-2022

Content by: Elijah Diederich

Present: NA

Goals: To get a better understanding about the mechanical properties of Hydrogels in order to adequately perform rheology testing

Content:

Youtube Video Link: https://www.youtube.com/watch?v=YJB6Z14NuKk&t=819s

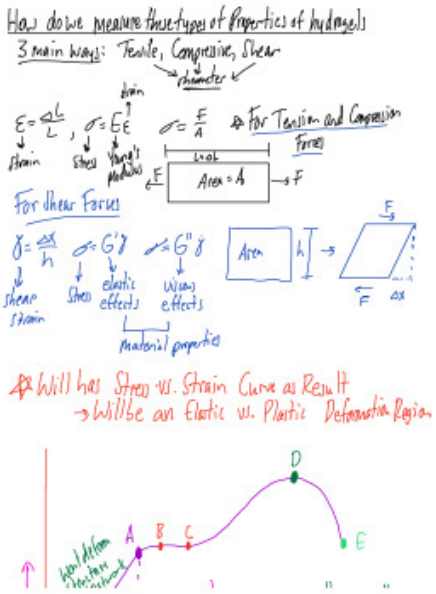
**********Notes taken on Ipad and Included in PDF form below**********

Conclusions/action items:

1. Perform Rheology Testing

2. Find G' and G'' values of lung tissue

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 12, 2022, 11:28 AM CST



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Hydrogel_Characterization_Lecture_YouTube_Video_Notes.pdf (1.53 MB)

Rheology Testing Protocol

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 14, 2022, 8:12 PM CST

Title: Rheology Testing Protocol

Date: 12-14-2022

Content by: Elijah Diederich

Present: NA

Goals: To keep a detailed document of the rheology protocol testing

Content:

Both of the tests will allow us to show that a PEG hydrogel can also be tested via both of these methods and values for the storage modulus (G'), loss modulus (G"), and the Young's Modulus (E) can be compared to native lung tissue samples. This proves that a hydrogel can accurately represent the mechanical properties of lung tissue and can also be tested.

Rheology Testing Protocol:

1. Once gelatin hydrogels have been formed and allowed to set for approximately 12-24 hours, rheometry testing may be performed.
2. Carefully remove 3 gels of each concentration (3.15% and 2.36%) from 48 well cell culture plates, keeping the gels of the same concentration 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 concentration (3.15% and 2.36%) were tested for a total of 6 separate frequency sweeps.
11. When testing is completed, results can be interpreted in MatLab

Conclusions/action items:

1. **Make sure to turn in Lab Archives Notebook**

2022.09.13 - Initial research on the EMTU

Althys Cao - Oct 11, 2022, 7:25 PM CDT

Title: 2022.09.13 - Initial research on the EMTU

Date: 2022.09.13

Content by: Althys

Present: self

Goals: have an idea on what the epithelial-mesenchymal trophic unit (EMTU) is about.

Content:

- Airway structural cells (i.e. epithelial cells, mesenchymal cells, ECM, etc.) are important in controlling tissue homeostasis.
- Functions of the lung related include: differentiation during lung growth, repair of damaged tissue, and regulation of the inflammatory response.
- These functions require local responses to stimuli.
- Fibroblasts are likely to be regulators of these responses.
- Asthmatic lung: fibroblasts play a key role as a resident mesenchymal cell beneath the epithelium.
- EMTU: anatomic and functional relationship between the attenuated fibroblast sheath, epithelial and neural tissue, and also the extracellular matrix (ECM).

Source:

> M. J. Evans, L. S. Van Winkle, M. V. Fanucchi, and C. G. Plopper, “The attenuated fibroblast sheath of the respiratory tract epithelial–mesenchymal trophic unit,” *American Journal of Respiratory Cell and Molecular Biology*, vol. 21, no. 6, pp. 655–657, Dec. 1999.

Conclusions/action items: To understand more about the EMTU, I will need to focus on fibroblasts, epithelial cells and the ECM. I will decide on what to focus on the most following the 1st meeting with the client.

2022.09.19 - More information on the ECM

Althys Cao - Oct 11, 2022, 10:48 PM CDT

Title: 2022.09.19 - More information on the ECM

Date: 2022.09.19

Content by: Althys

Present: self

Goals: after the first meeting with the client and knowing the goal of this project is to build a scaffold, I decide to do more research on the ECM as it is what the scaffold will be mimicking

Content:

The ECM is a network of proteins and polysaccharide molecules, known as glycosaminoglycans (GAGs), and GAGs linked to protein forming proteoglycans.

Around 300 different molecules in the ECM.

The ECM structure includes two distinct entities:

- Basement membranes (BM): 50-100 nm layer, consisting predominantly of collagen type IV, laminins, nidogens, and perlecan. Collagens and laminins are important in establishing BM stability, while nidogens and perlecan link with collagen type IV and laminins in the preservation of BM structural integrity.
- Interstitial Matrix (IM): located under the BM, acts as one of the major structural layers of the lamina propria and submucosa. Main components: Collagens I and III, fibronectin, elastin-tropoelastin, decorin, hyaluronan.

ECM does not represent a static structure, but a dynamic tissue component that constantly undergoes continuous remodeling.

Source:

S. Pompili, G. Latella, E. Gaudio, R. Sferra, and A. Vetuschi, “The Charming World of the extracellular matrix: A Dynamic and protective network of the intestinal wall,” *Frontiers in Medicine*, vol. 8, Apr. 2021.

Conclusions/action items: The ECM is a very complex structure. I feel like 3D-printing may be the best option if we want to make a model that most accurately represents in vivo ECM. I will do some research on this idea, but I also want to note that this may not be a viable option for this project based on the resources we have.

2022.10.05 - Degradable PEG ideas

Althys Cao - Dec 14, 2022, 5:52 PM CST

Title: Degradable PEG ideas

Date: 2022.10.05

Content by: Althys Cao

Present: Althys Cao

Goals: Figure out ways to make PEG hydrogels degradable

Content:

- PEG hydrogels can be degraded using oxidation; however, this process also affects any proteins and cells incorporated inside the gels, which is unideal because that is also one requirement from the client.

- Can utilize enzymatic degradation of peptides. The peptide mentioned is matrix metalloproteinase (MMP) sensitive linkages. However, rate of release is not able to be controlled.

Conclusions/action items:

Moving forward with MMP. Next is to figure out ways to incorporate MMP into PEG hydrogels

Reference:

A. Kasko, “Degradable Poly(ethylene glycol) Hydrogels for 2D and 3D Cell Culture,” *Degradable poly(ethylene glycol) hydrogels for 2D and 3D cell culture*. [Online]. Available: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/materials-science-and-engineering/tissue-engineering/degradable-polyethylene-glycol-hydrogels. [Accessed: 14-Dec-2022].

2022.09.13 - Some models of the EMTU

Althys Cao - Oct 11, 2022, 10:31 PM CDT

Title: 2022.09.13 - Initial research on the EMTU

Date: 2022.09.13

Content by: Althys

Present: self

Goals: find out attempts and current ways to model the EMTU

Content:

- No studies have been performed in which an intact epithelial cell layer and fibroblasts are in intimate contact surrounded by their own matrix.
- The most "natural" system to date: placing epithelial cells onto the surface of collagen gels containing embedded fibroblasts. The models have a relatively short life.
- The article's model involves using Matrigel (the full protocol is found in the methods section - The 3D "EMTU" model). It currently lacks a circulation or the dynamic effect of interstitial flow.

Source:

F. Bucchieri, A. Pitruzzella, A. Fucarino, A. M. Gammazza, C. C. Bavisotto, V. Marcianò, M. Cajozzo, G. Lo Iacono, R. Marchese, G. Zummo, S. T. Holgate, and D. E. Davies, “Functional characterization of a novel 3D model of the epithelial-mesenchymal trophic unit,” *Experimental Lung Research*, vol. 43, no. 2, pp. 82–92, Feb. 2017.

Conclusions/action items: This model in the article can be a great starting point. However, I will still need to meet with the client first before deciding on any next steps.

2022.09.20 - Bioink used in 3D printing to mimic the ECM

Althys Cao - Oct 11, 2022, 11:40 PM CDT

Title: 2022.09.20 - Bioink used in 3D printing to mimic the ECM

Date: 2022.09.20

Content by: Althys

Present: self

Goals: research some bioink materials that have been used to mimic the ECM, preferably lung ECM

Content:

There are quite a few materials. I have chosen 2 to highlight:

- Collagen: pure collagen has low viscosity which makes bioprinting extremely difficult. Collagen blends have emerged as a result to improve the viscosity issue.
- Silk fibroin (SF): high viscosity and decent shear-thinnig properties. SF+PEG with lower concentrations of PEG support cellular viability as well as preserving cellular activity.

Conclusions/action items: Collagen and PEG seem like good materials to start researching for design ideas. Will need to meet with advisor to confirm.

2022.11.01 - PEG hydrogels to Matrigel for vascular toxicity screening and stem cell expansion

Althys Cao - Nov 01, 2022, 4:16 PM CDT

Title: Versatile synthetic alternatives to Matrigel for vascular toxicity screening and stem cell expansion

Date: 2022.11.01

Content by: Althys

Present: self

Goals: take notes on how the gels were made (i.e. the chemistry)

Content:

Hydrogel characteristics:

- 120-uM-thick synthetic hydrogel arrays
- To mediate cell adhesion: use pendant linear H-Cys-Arg-Gly-Asp-Ser-NH2 (linear RGD) / head-to-tail cyclized Arg-Gly-Asp-[D-Phe]-Cys (cyclic RGD)
- Mechanical properties:
  - soft: 0.45 $\pm$ 0.04 kPa
  - medium: 1.16 $\pm$ 0.08 kPa
  - stiff: 4.72 $\pm$ 0.17 kPa
- change stiffness by tuning concentrations of 20kDa, eight-arm PEG norborne (PEGNB) and dithiol-terminated crosslinking molecules
- all hydrogel solutions were polymerized using a crosslinking peptide H-Lys-Cys-Gly-Gly-Pro-Gln-Gly-Ile-Trp-Gly-Gln-Gly-Cys-Lys-NH2, degradable by metalloproteinases (MMPs)
- peptides were covalently attached to PEG via thiol-ene 'click' chemistry: couples thiols to norbornene groups on the PEG molecules
- successful culture environments promoted endothelial-network assembly within 24h of seeding and maintained stability 48h after seeding
- optimal amount of photoinitiator and volume of hydrogel: 0.2% w/v Irgacure 2959 (I2959) and 9uL solution per well

(not very important so I'll give n overview, but there were many tests comparing PEG hydrogels' sensitivity to toxic chemicals and generally they were more responsive compared to Matrigels).

Conclusions/action items:

E. H. Nguyen, W. T. Daly, N. N. Le, M. Farnoodian, D. G. Belair, M. P. Schwartz, C. S. Lebakken, G. E. Ananiev, M. A. Saghiri, T. B. Knudsen, N. Sheibani, and W. L. Murphy, “Versatile synthetic alternatives to Matrigel for vascular toxicity screening and stem cell expansion,” *Nature Biomedical Engineering*, vol. 1, no. 7, Jul. 2017.

2022.03.10 - PEG+Collagen hydrogel

Althys Cao - Oct 12, 2022, 12:00 AM CDT

Title: 2022.03.10 - PEG+Collagen hydrogel

Date: 2022.03.10

Content by: Althys

Present: self

Goals: from the meeting with advisor, I have decided that PEG would be a suitable material to build a scaffold. However, just PEG will not be enough because the scaffold will lack adhesive properties (and they are needed so that cells can grow on top). I have decided to go with PEG+Collagen instead because collagen provides adhesiveness (and the client also request collagen being inserted into collagen anyway).

Content:

Method will follow the article closely.

The article made 2 models: 1 model with collagen type I and 4-arm PEG succinimidyl glutarate, and 1 with collagen type I and 8-arm PEG succinimidyl glutarate.

The models both have long shelf life (there are some variations depending on PEG types and concentrations, but overall shelf life for all models is over 1 month).

4-arm PEG-star hydrogels can withstand more compressive forces and can swell a more considerable amount compared to the 8-arm PEG-star hydrogels. Thus, I will go with 4-arm PEG-star hydrogels.

The models can facilitate cell attachment and proliferation.

The models are also injectable, which makes it easy to inject into 12-well or 24-well plates later on.

However, the drawback is that the hydrogels in the article is used for wound healing in a medical scheme, so unsure if it will still be applicable to the client's needs.

Source:

G. M. Fernandes-Cunha, K. M. Chen, F. Chen, P. Le, J. H. Han, L. A. Mahajan, H. J. Lee, K. S. Na, and D. Myung, “In situ-forming collagen hydrogel crosslinked via multi-functional peg as a matrix therapy for corneal defects,” *Scientific Reports*, vol. 10, no. 1, Oct. 2020.

Conclusions/action items: My design idea is a hydrogel made from collagen type I and 4-arm PEG succinimidyl glutarate

2022.11.27 - brainstorm ideas for diffusion testing

Althys Cao - Dec 14, 2022, 3:20 PM CST

Title: Brainstorm Ideas for Diffusion Testing

Date: 2022.11.27

Content by: Althys

Present: Althys

Goals: find some ideas on how to do diffusion testing

Content:

The paper determined diffusion via changes in mass over time --> will use changes of mass for diffusion testing

For time stamps, choose 1 hour and 24 hours (or simply overnight), instead of the full range stated in the paper (1h, 2h, 4h, 6h, 8h, 12h, 18h, 24h), because this diffusion test is meant to be more of a proof-of-concept test.

Conclusions/action items: During actual testing, the two time stamps were actually 1h and 23h simply (still overnight) because they were more convenient. The next action item is to do the actual testing and find ways to mimic the tranwell inserts because the gels need to be placed at an ALI

Reference:

M. Parlato, J. Molenda, and W. L. Murphy, “Specific recruitment of circulating angiogenic cells using biomaterials as filters,” *Acta Biomaterialia*, vol. 56, pp. 65–79, Jul. 2017.

Biology of the Extracellular Matrix

ANA MARTINEZ CAVAZOS - Oct 08, 2022, 12:25 AM CDT

Title: Biology of the Extracellular Matrix

Date: 9/19/22

Content by: Ana Martinez

Present: Ana Martinez

Goals: To begin learning about the biology and physiology behind our project, specifically about the extracellular matrix and how it functions.

Content:

[1] B. Yue, “Biology of the extracellular matrix,” *Journal of Glaucoma*, vol. 23, 2014.

Defining the ECM

- The ECM is an intricate network composed of an array of multidomain macromolecules
- Organized in a cell and tissue-specific manner.
- Components link together to form a stable composite structure
- The ECM structure contributes to the mechanical properties of tissues
- “Core matrisome” of the ECM is made up of ~ 300 proteins --> collagens, proteoglycans, elastin, and cell-binding glycoproteins, etc.

More on Collagen

- Protein that is made up of 3 polypeptide α chains forming a triple helical structure
- Vertebrates = 46 different collagen chains come together to form 28 collagen types
    - Fibril-forming collagens (e.g. types I-III)
        - Contain continuous triple-helix domains "bound" by amino acid and carboxyl-terminal, non-collagen domains
            - Non-collagen domains are then removed via proteolysis --> triple helices formed are connected from the sides into fibrils
    - Network-forming collagens (e.g. basement membrane collagen - type IV)
        - Include non-fibril supramolecular structures --> formed by nonfibrillar collagens
    - Fibril-associated collagens with disruptions in triple helixes (FACITs) (e.g. types IX and XII)
        - These are associated with collagen fibrils
        - Do not assemble into fibrils by themselves
    - Miscellaneous (e.g. type VI)
- Certain proline residues in collagens are hydroxylated by prolyl 4-hydroxylase and prolyl 3-hydroxylase.
- Certain lysine residues are also hydroxylated by lysyl hydroxylase
- After processing occurs, fibrillar procollagens are secreted into the EC space --> propeptides are removed
    - Resulting collagens assemble into fibrils via covalent cross-links
        - Covalent cross-links = formed between lysine residues of two collagen chains by a process catalyzed by extracellular enzyme lysyl oxidases (LOX)
- Takeaway: The collagen backbone dictates the architecture, shape, and organization of tissues

More on Fibronectin

- Protein that is critical for the attachment and migration of cells --> “biological glue”
- Monomer (~250 kDa) is made of subunits of collagen comprising of repeat types I, II, and III
- Fibronectin is secreted as dimers linked by disulfide bonds
    - Dimers can form multimers
- Has binding sites to other fibronectin dimers, collagen, heparin, and cell surface receptors
    - FNIII10 repeat = important Arg-Gly-Asp cell-binding site
- As dimers, other proteins, and cell surface receptors continue "depositing", fibronectin fibrils are lengthened and thickened
    - Fibrils --> deoxycholate-insoluble matrix

Cellular Receptors

- ECM molecules connect to cells through integrins, syndecans, etc
- Integrins:
  - Heterodimeric receptors made up of α and β subunits
    - Subunits = transmembrane proteins with large modular EC domains, single transmembrane helices, and short cytoplasmic regions that aid in cytoskeletal interactions
  - Major matrix-binding (β1) integrins = associated with fibronectin, collagens, and laminins
  - Known as "links" between the ECM and the cytoskeleton of the cell

ECM Remodeling/Modulation

- ECM is constantly undergoing remodeling --> components are deposited, degraded, or modified
- Intermolecular cross-linking by LOX = key posttranslational modification for collagens and elastin.
  - Excess LOX can lead to "expanded" cross-linking activity --> increased tissue tensile strength and matrix stiffness
    - This can affect cellular behaviors
- Collagens and other ECM elements are substrates for:
  - matrix metalloproteinases (MMPs)
    - produced in precursor forms
    - remain "dormant" until activated.
    - Most are secreted, but membrane type MMPs are not
    - MMP activities are counteracted by tissue inhibitors of MMPs (TIMPs) and other inhibitors
  - a disintegrin and metalloproteases (ADAMs)
  - ADAM with thrombospondin motifs (ADAMTS)
  - proteases --> cathepsin G, elastase, etc.
  - ** Many of these can also cleave precursor proteins, release ECM-bound growth factors, and help release bioactive fragments with new bioactivities (ex: endostatin)
- ECM is also modulated by exogenous stimuli:
  - cytokines
  - glucocorticoids
  - oxidative stress
  - pressure
  - mechanical stretch

Chemical & Physical "Cues" of ECM

- The ECM's biochemical properties allow cells interact with their EC environment using various signal transduction pathways
  - Chemical "cues" = given by ECM components:
    - fibronectin
    - integrin
    - non-integrin receptors
    - growth factors
    - associated signaling molecules
- Different receptors sets --> different matrices -- different interactions --> distinct cellular responses
- Physical properties of the ECM provide physical cues to the cells
  - Rigidity - "stiff" = integrin clustering, strong focal adhesions, Rho/MAP kinase activation --> increased proliferation and contractility
  - Density
  - Porosity
  - Insolubility
  - Spatial arrangement/orientation

- **Conclusions/action items:**

This article gave me a very thorough background on the different components, functions, and interactions within the ECM. Some action items for the following days include continuing to learn more about the ECM, as well as begin learning more about the epithelial mesenchymal trophic unit -- the system we will focus on for our project.

Extracellular Matrix in the Lung

ANA MARTINEZ CAVAZOS - Oct 08, 2022, 9:21 PM CDT

Title: The ECM in the Lung

Date: 9/21/22

Content by: Ana Martinez

Present: Ana Martinez

Goals: To learn more about the ECM found in the lung, specifically its composition and alterations caused by chronic lung disease.

Content:

[2] 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.

Pulmonary ECM - General

- Organized into two main structural types:
  - 1) basement membranes
    - These are thin sheets of glycoproteins covering the basal side of epithelia and endothelia
    - Surround muscle, fat and peripheral nerve cells
  - 2) interstitial matrices
    - These form a loose, fibril-like meshwork that interconnects structural cell types within tissues
    - Meshwork maintains the lung's 3D cohesiveness and biomechanical characteristics
  - Both 1) and 2) form tissue-specific “niches” that influence:
    - Stemness and differentiation of progenitor/stem cell populations
    - Proper function of tissue/compartment-specific differentiated cell types

Pulmonary ECM Composition

- Fibrillar collagens (types I, II, III, V and XI)
  - Have great tensile strength and low elasticity
  - Contribute to the overarching architecture of lung
- Large elastic fibers
  - Have low tensile strength and high elasticity
  - Provide lung with its necessary compliance and elastic recoil
  - Composed of two distinct components:
    - 1) ECM protein elastin = found in crosslinked form in the inner core of elastic fibers
    - 2) Outer periphery of elastic fibers = contains 10–15 nanometer-sized microfibrils
- The interstitial ECM of the Alveoli
  - Composed of a relaxed meshwork --> core proteins: type I and III collagens, elastin, etc.
  - 3D arrangement allows for nonlinear stress–strain behaviors characteristic of soft connective tissues (i.e. hysteresis, viscoelasticity)
  - During respiration, the energy dissipation that causes hysteresis and viscoelasticity is driven by "fiber-fiber" contacts in collagen-elastin ECM
    - Contractile cell types and surfactant in the ALI likely contribute to these effects as well

The ECM as a "Reservoir"

- ECM serves as a reservoir for a number of growth factors and cytokines --> crucial for cell differentiation and proliferation
- ECM-bound factors (i.e. fibroblast growth factor (FGF), hepatocyte growth factor, latent and active TGF-β1):
  - 1) can be latent, masked or have a different activity than with their soluble form
  - 2) can form immobilized gradients crucial for cell migration
  - 3) as imprints of former cellular activity, they might have a memory function for directing cellular behavior
  - 4) facilitate cell adhesion growth
  - 5) when "stored", they may be activated rapidly by proteolytic release
    - This can generate local signals that are independent of processes like gene expression

More on Myofibroblasts

- Fibroblastic foci contain activated fibroblasts and/or highly contractile myofibroblasts
  - Myofibroblasts are incorporated into contractile stress fibers
  - Produce high amounts of ECM molecules in response to pro-fibrotic stimuli
  - Origin of myofibroblasts in fibrotic foci is under debate
    - In some studies, shown to stem from bone marrow-derived progenitor cells, peripheral blood circulating fibrocytes, alveolar epithelial cells undergoing epithelial–mesenchymal transition, resident fibroblasts, and pericytes
    - In other studies, a yet unidentified population of resident stromal cells within fibrotic foci were shown to be the source of myofibroblasts --> ruled out pericytes and epithelial cells as sources

Conclusions/action items:

To continue researching the ECM, specifically the different models that are currently being used to model the ECM of the lung. Researching current work/competing designs will help our team begin making decisions about which biomaterials are best for modeling the EMTU of the lung.

GelMA - Alveolar Lung-on-a-chip Model

ANA MARTINEZ CAVAZOS - Oct 08, 2022, 9:25 PM CDT

Title: GelMA - Alveolar Lung-on-a-Chip Model

Date: 10/3/2022

Content by: Ana Martinez

Present: Ana Martinez

Goals: To learn more about an innovative design using GelMA focused on a very similar anatomical area than our prospective model.

Content:

[3] 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.

General Information

- Huang et al. developed a physiologically relevant model of the human pulmonary alveoli
    - "lung-on-a-chip" - made from 3D hydrogel comprised of GelMA with inverse opal structure and a compartmentalized dimethicone chip
        - GelMA inverse opal structure is made of well-defined, interconnected pores resembling highly alveolar sacs
- Functional epithelial monolayers were formed readily by populating sacs with 10 human epithelial cells
- Cyclic strain was integrated into model - to allow for biomimetic breathing
    - This later helped Huang et al. to begin studying pathological effects (i.e. cigarette smoking, COPD, pneumonia, lung cancer, severe acute respiratory syndrome COVID2)
- Purpose: to create an accurate cell culture-based model of the lung's tissue and extracellular matrix (ECM) physiology

Specifics of "Lung-on-a-Chip"

- Model successfully reconstructed the following of the alveoli:
    - microstructure
    - ECM properties
    - air-cell interface
    - breathing "events" (mimics inhalations and exhalations)
- PDMS Chip
    - Was bonded to GelMA
    - Provides the medium supply, air-liquid interface (ALI), and the cyclic mechanical movements
- GelMA Inverse Opal Structure
    - Very closely resembles native alveolar sacs
    - Possesses sac-like pores
    - Interconnecting windows between sacs
    - Stiffness is similar to native lung

Creating Model

- ~7050 "alveoli" constructed within a 8x10x3 mm^3 model (so as to allow for good physiological emulation of lung & alveoli)
- Primary human alveolar epithelial cells populated on sac surfaces -- formed a monolayer epithelial cell
- Model was tested/subjected to cyclic inhalation and exhalation moments
    - This property was later used to begin investigating impacts of pathological effects on lung

Results and Discussion

- Fabrication Steps (specifics)
    - 1) Alginate microbeads (201 +- 12 micrometers) were assembled into a lattice
    - 2) 7% GelMA solution was infiltrated into void spaces within lattice and was photo-crosslinked
        - 7% solution = balance of good stability of sac-like structures and stiffness close to native lung tissue
        - GelMA = biocompatible, photo-crosslinkable, easy to access, inexpensive
            - GelMA also possesses essential ECM properties:
                - arginine-glycine-aspartic acid sequences = increased cell attachment

        - matrix metalloproteinase-responsive peptide motifs = allow cells to proliferate
    - 3) Remove alginate alginate microbeads via 0.01 M EDTA solution
    - 4) Alveoli-like 3D hydrogel with uniform pores and connecting windows should form.
- Results
    - General Young's Modulus Values of Lung (depending on anatomical compartments of lung and on mechanical testing used): ~1.4 to 7.2 kPa
        - Model's Young's Modulus Values: 6.23 +- 0.64 kPa --> within range for native tissue
    - Swelling ratios of GelMA hydrogels (~8 and 10% for 2D and 3d) were significantly smaller than when using Irgacure 2959 (radical photoinitiator) alone
        - "Successful" process - photocross-link GelMA with Irgacure 2959, then repeat with mTG --> this allows for greater stability

Conclusions/action items:

This GelMA alveolar "lung-on-a-chip" model turned out to be very similar in nature to our prospective design. This study provided our team with further information regarding GelMA's biochemical properties, its benefits, and the solution amounts that seem to work for models of the lung. However, our team also learned through this study (among other literature) that GelMA can be significantly unreliable; it often leads to a variation of successful vs. failed model "batches". The route that Huang et al. took to compensate for this was to combine the use of GelMA with other materials like the alginate lattice beads. However, given our limited timeframe for our own project, it might not be feasible for us to follow suit other than to combine GelMA with collagen/fibronectin. Therefore, we might need to find a more stable biomaterial to use for our scaffold.

Poly(e-caprolactone) - General Characteristics

ANA MARTINEZ CAVAZOS - Oct 11, 2022, 7:50 PM CDT

Title: Poly(e-caprolactone) (PCL) - General Characteristics

Date: 9/27/22

Content by: Ana Martinez

Present: Ana Martinez

Goals: To begin learning about poly(e-caprolactone)'s biochemical properties, and how it has succeeded/failed/been used in previous work to determine whether it is a viable biomaterial for us to incorporate into our model.

Content:

> [4] S. M. Espinoza, H. I. Patil, E. San Martin Martinez, R. Casañas Pimentel, and P. P. Ige, "Poly-ε-caprolactone (PCL), a promising polymer for pharmaceutical and biomedical applications: Focus on nanomedicine in Cancer," *International Journal of Polymeric Materials and Polymeric Biomaterials*, vol. 69, no. 2, pp. 85–126, Feb. 2019.

- PCL is a biodegradable (synthetic) polymer member of the aliphatic polyester family
- Uses: development of wound dressings, contraceptive devices, fixation devices and drug delivery systems
- Has also extended to include proteins, peptides, vaccines, other reactive molecules
- PCL microspheres (50 **µm** and 2 mm) have been used to deliver drugs, antigens, antibodies, ribozymes, nerve growth factor, heparin, steroids, hormones and vitamins
- Once freeze-dried and stored for further uses, PCL can be used in the obtention of nanoparticles, microparticles and scaffolds
    - Useful because of its advantageous viscoelastic and rheological properties
    - For these reasons, PCL has been most extensively applied in bone, cartilage, cardiovascular, blood vessel, skin, nerve, tendon, and ligament engineering (tissue repairment/replacement)

Conclusions/Action Items:

- PCL's biochemical properties, mainly its viscoelastic, biodegradable, and rheological properties, make the polymer ideal for bone, cardiac, cartilage, and similarly based scaffolds.
- Conversely, PCL will likely not be as effective on a model of the small lung airway/EMT unit.
- Action Items: to incorporate my findings on PCL and apply them in the decision-making process for the best design to use for our EMTU model; to research more on the selected design so I can better contribute to the model's fabrication, modification, and testing process.

Poly(e-caprolactone) (PCL) - General Characteristics (Part 2)

ANA MARTINEZ CAVAZOS - Oct 11, 2022, 7:49 PM CDT

Title: Poly(∈-caprolactone) - General Characteristics (Part 2)

Date: 9/28/22

Content by: Ana Martinez

Present: Ana Martinez

Goals: To learn more about poly(e-caprolactone)'s biochemical properties, and how it has succeeded/been used in previous work to determine whether it is a viable biomaterial for us to incorporate into our model.

Content:

[5] B. Azimi, P. Nourpanah, M. Rabiee, and S. Arbab, "Poly (∈-caprolactone) fiber: An overview," *Journal of Engineered Fibers and Fabrics*, vol. 9, no. 3, p. 155892501400900, Sep. 2014.

- PCL fiber samples generally vary from 3000-80,000 g/mol in weight and can be graded according to the molecular weight
- Temperature:
  - Semi-crystalline with a melting point of 59-64 degrees Celsius
  - Has a glass-transition temperature of 60 degrees Celsius
- Solubility:
  - Soluble in chloroform, dichloromethane, carbon tetrachloride, benzene, toluene, cyclohexanone, and 2-nitropropane at room temperature
  - Low solubility in acetone, 2-butanone, ethyl acetate, dimethylformamide, and acetonitrile
  - Insoluble in alcohol, petroleum ether, and diethyl ether
- Why is PCL so versatile?
  - PCL allows modification of its physical, chemical, and mechanical properties by co-polymerization/blending with many other polymers efficiently
  - Co-polymerization altering the chemical properties indirectly affects other properties (crystallinity, solubility, and degradation pattern)
  - Result: This blending that leads to property alteration/biodegradation is why PCL is preferred for making tissue engineering scaffolds, fibers, and films
- Previous Work with PCL: Attachment displays the methods and outcomes of several studies investigating the fabrication of PCL-based scaffolds in tissue engineering applications

Conclusions/action items:

- PCL's ability to modify its physical, chemical, and mechanical properties via co-polymerization makes PCL versatile
  - This makes PCL a great biomaterial to use in cardiac and bone tissue scaffolds
  - However, PCL has not proven to be quite as versatile in the lung, so it might not be the best biomaterial choice for our model of the EMT unit.
- Action Items: To incorporate my findings on PCL (from this source and the previous) and apply them in the decision-making process for the best design to use for our EMT unit model. After coming to a conclusion, I need to help my team research more on the selected design so I can better contribute to the model's fabrication, modification, and testing process.

TABLE I. Comprehensive data on PCL fiber degradation.

Workers	Year	...	Brief method and outcome	Refs
N. BÖLGEN et al.	2005	In vitro& in vivo	In vitro and in vivo degradation studies of non-woven materials made of PCL nanofibers showed that electrospun PCL materials were degraded much faster in vivo as compared with in vitro due to the enzymatic degradation of PCL in addition to the hydrolytic degradation.	[30]
Lam. C.X.F. et al.	2007	In vivo	Over 6 months, composite PCL/ b-Tri-calcium phosphate (TCP) scaffolds degrade faster than PCL homopolymer scaffolds in vivo.	[31]
Pektok. E. et al.	2008	In vivo	In vivo healing and degradation characteristics of small-diameter vascular grafts made of PCL nanofibers compared with expanded polytetrafluoroethylene (ePTFE) grafts were evaluated.	[32]
Lam et al.	2008	In vitro	PCL and PCL/ TCP scaffolds degraded via a surface degradation pathway in the alkaline accelerated setting; however, this appeared to switch to a bulk degradation pathway under the long term simulated condition.	[33]
Wan et al.	2008	In vitro	Degradation of the PCL component with chitosan could be accelerated at various rates depending on the compositions of the scaffolds and the media, and the chitosan component could effectively buffer the acidic degradation products of the PCL component.	[34]
Mobaraksh et al.	2008	In vitro	By increasing gelatin content the biodegradability of PCL/ gelatin nanofibrous scaffolds increased in PBS over 2-week period.	[35]
Tillman. B.W. et al.	2009	In vivo	PCL/collagen electrospun scaffolds maintain a high degree of patency and structural integrity in vivo without eliciting abnormal inflammatory response over the course of 1 month.	[36]
Johnson et al.	2009	In vitro	The net effects of biological and non-biological environments on PCL electrospun structures following 7 and 28 days of in vitro exposure are established. Material degradation, as well as biological deposition, was responsible for the changes in mechanical properties.	[37]
Vieira et al.	2011	In vitro	Hyper elastic constitutive models were used to predict the mechanical behavior and biodegradation of a blend composed of PLA and PCL fiber.	[38]

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Matrigel - 3D Model

ANA MARTINEZ CAVAZOS - Oct 11, 2022, 7:50 PM CDT

Title: 3D Model of the EMT Unit Using Matrigel

Date: 10/9/22

Content by: Ana Martinez

Present: Ana Martinez

Goals: To learn more about a 3D model of the EMT unit that utilized matrigel as the primary biomaterial.

Content:

> [6] F. Bucchieri, A. Pitruzzella, A. Fucarino, A. M. Gammazza, C. C. Bavisotto, V. Marcianò, M. Cajozzo, G. Lo Iacono, R. Marchese, G. Zummo, S. T. Holgate, and D. E. Davies, “Functional characterization of a novel 3D model of the epithelial-mesenchymal trophic unit,” *Experimental Lung Research*, vol. 43, no. 2, pp. 82–92, Apr. 2017.

Aim of Product

- Communication across the epithelial-mesenchymal trophic (EMT) unit contributes greatly to tissue homeostasis
- EMT unit communication also is a key factor in abnormal signaling, which contributes to chronic lung diseases like COPD.
- Most *in vitro* models are limited in complexity and do not accurately represent the EMT unit.
    - This research team hypothesized that cellular outgrowth from the bronchial tissue would allow them to develop a mucosal structure
    - This mucosal structure would, in theory, would represent the "architecture" of in vivo tissue as well as the EMT unit.

Methodology

- Team embedded bronchial tissue in Matrigel to create 3D scaffold
- Outgrowth cultures were monitored using several procedures:
    - Time-lapse microscopy
    - Electrical resistance
    - Light and electron microscopy
- Testing: cultures were repetitively challenged with cigarette smoke extract (CSE) to mimic smoking activity (which is linked with development of chronic lung diseases like COPD)

Results

- Once the outgrowths formed as a multicellular sheet with mobile cilia, the Matrigel was remodel to provide an air-liquid interface (ALI)
- Team utilized immunofluorescence and electron microscopy (EM) and found the following:
    - An upper layer of mucociliary epithelium
    - A lower layer of highly organized extracellular matrix (ECM)
        - Layer was interspersed with fibroblasts
        - Layer was also separated by a basement membrane
- Team utilized EM to analyze the mucosal structure after exposure CSE, and observed several characteristics that were similar to what occurs in vivo:
    - Epithelial damage
    - Significant loss of cilia
    - Remodeling process of the ECM

Conclusions

- Bucchieri et al. were able to develop a 3D model of the bronchial mucosa, which encompasses the EMT unit.
- The structural changes that were found via EM analysis after exposing the mucosal structure to CSE suggest that this model may be useful for drug discovery and preclinical testing procedures
    - This applies especially for drugs/pre-clinical testing that focus on airway remodeling

Conclusions/action items:

This research article was helpful in understanding how matrigel could be a helpful biomaterial in some instances when trying to accurately develop a model of the EMT. Although our client has expressed issues with using matrigel to develop our specific model of the small airway EMT unit, learning about the model demonstrated by Bucchieri et al. may help to consider matrigel for developing a future model (from another specific area of the lung, etc) that is known to tolerate matrigel well as a biomaterial.

Next steps include researching more on more viable biomaterials and some competing designs using those biomaterials (particularly GELMA and PEG). Furthermore, I need to research more on specific PEG iterations (PEGDA, PEG-4SH) and which ones would be most suitable and effective for our model. Additionally, I need to research more on both chemical and photo-crosslinking methods for PEG, and whether chemical crosslinking should even be considered given our limited space/resources at the BME lab.

PEGDA - Cell Adhesion Characteristics

ANA MARTINEZ CAVAZOS - Oct 21, 2022, 10:55 PM CDT

Title of Article: Synthetic Biodegradable Hydrogels with Excellent Mechanical Properties and Good Cell Adhesion Characteristics Obtained by the Combinatorial Synthesis of Photo-Cross-Linked Networks

Date: 10/20/22

Content by: Ana Martinez

Present: Ana Martinez

Goals: To learn more about how PEG hydrogels are being generated so as to maximize cell adhesion characteristics.

Content:

E. Zant and D. W. Grijpma, “Synthetic biodegradable hydrogels with excellent mechanical properties and good cell adhesion characteristics obtained by the combinatorial synthesis of photo-cross-linked networks,” *Biomacromolecules*, vol. 17, no. 5, pp. 1582–1592, 2016.

Synthesis of PEG Oligomers

- Hydroxyl-group terminated linear oligomers with number-average molecular weight, *M*n, of 4 and 10 kg/mol were prepared by ring-opening polymerization (ROP) of TMC, DLLA, and CL monomers using hexanediol as initiator and Sn(Oct)2 as catalyst.
- The PEG synthesized oligomers were PEG 4k and PEG 10k
    - These were dried at 120 degrees C under a vacuum for 2 hours, then cooled to room temp. under argon
    - These were functionalized in DCM (3 mL/g oligomer) by reaction with MAAh (4 mol/mol oligomer) in the presence of TEA (4 mol/mol oligomer) -- functionalization reaction proceeded at room temp. for 5 days.
    - After the 5 days, the PEG-dMA-functionalized oligomers were purified via precipitation in cold diethyl ether (-25 deg. C).
    - PEG-dMA was then dried under reduced pressure for 40-50 deg. C for 3 days
- How were molecular weight and degrees of functionalization determined?
    - Used a Varian Inova 400 MHz NMR spectrometer and deneuterated chloroform (solvent)

Synthesizing (via Photo-Crosslinking) a Combination of 255 Single- and Mixed-Macromer Networks

- Macromers utilized: PTMC-dMA 4k, PDLLA-dMA 4k, PCL-dMA 4k, PEG-dMA 4k, PTMC-dMA 10k, PDLLA-dMA 10k, PCL-dMA 10k, and PEG-dMA 10k
    - Macromers were dissolved at 50 °C at a conc. of 33 wt % in PC
    - Contained hydroquinone inhibitor (0.1 wt % with respect to the macromer)
    - Contained Irgacure 2959 photoinitiator (1 wt % with respect to the macromer)
    - Mixed-macromer solutions were combined in 96-well plates --> yielded 255 different macromer mixtures
    - Volume of mixed-macromer solutions = 250μL; macromer concentration in solutions was 33 wt %
- Photo-crosslinking: 20-microliter amounts of each of the 255 solutions were transferred into separate 96-well plates and photo-crosslinked via UV irradiation
- Final Steps
    - After photo-crosslinking, the networks were extracted using:
        - 1) a 50/50 vol/vol mixture of PC and ethanol for 3 days
        - 2) a 25/75 vol/vol mixture of PC and ethanol for 1 day
        - 3) ethanol only for 1 day
    - Networks were dried for 1 day atmospherically, then dried for 3 days under reduced pressure at 45 deg. C

Networks and the Varying Levels of Water Uptake and Cell Adhesion

*Note: The makeup of each network is illustrated in the (first) figure attached.

- Network 30-2
    - Showed no adhesion nor cell proliferation
    - Note: this was expected, as these consisted only of PEG hydrogel --> without adhesive peptides or other mechanisms that would aid the hydrogel with cell adhesion
- Networks 76-2, 143-2, and 159-2
    - Showed high uptakes of water (257 wt %, 197 wt %, and 235 wt %, respectively)
    - However, these networks showed very limited initial cell adhesion and proliferation of human mesenchymal stem cells (hMSCs)
- Network 27-2
    - Showed no initial cell adhesion

    - Note: this was unexpected --> it is possible that this was a "false negative" result due to screening of large numbers of specimens in high throughput
- Networks 166-2 and 175-2
    - Additionally, showed good initial cell adhesion and high rates of proliferation of the hMSCs similar to that found in gelatin-based networks (ex: GelMA)
    - Showed high uptakes of water similar to the 76-2, 143-2, and 159-2 networks

Conclusions/action items:

PEG-dMA hydrogels' cell adhesion abilities can be enhanced by the mixed-macromer network method outlined by Zant and Grijpma. Specifically, photo-crosslinking PEG-dMA (4k and 10k iterations) with PTMC-dMA 4k, PDLA-dMA 4k, and either PCL-dMA 4k or PTLC-dMA 10k seem to result in the most successful balance between water uptake and cellular adhesion. As we continue to explore different methods to increase PEG's cell adhesion abilities, researching further into mixed-macromer network methods like this one might be useful. Although it may be possible that increasing PEG's cell adhesion abilities with adhesion peptides like RGD and GFOGR might be more feasible given our limited resources and timeframe, Zant and Grijpma's method is nevertheless insightful and could aid our group if we decide to continue this project into upcoming semesters.

ANA MARTINEZ CAVAZOS - Oct 21, 2022, 10:55 PM CDT

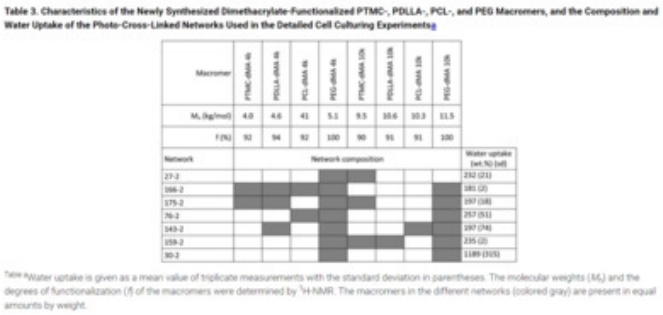

Table 3. Characteristics of the Newly Synthesized Dimethacrylate-Functionalized PTMC-, PDLLA-, PCL-, and PEG Macromers, and the Composition and Water Uptake of the Photo-Cross-Linked Networks Used in the Detailed Cell Culturing Experiments[a]

Macromer	PTMC-dMA 4k	PDLLA-dMA 4k	PCL-dMA 4k	PEG-dMA 4k	PTMC-dMA 10k	PDLLA-dMA 10k	PCL-dMA 10k	PEG-dMA 10k
M_n (kg/mol)	4.0	4.6	41	5.1	9.5	10.6	10.3	11.5
F (%)	92	94	92	100	90	91	91	100

Network	Network composition								Water uptake (wt %) (sd)
27-2				■	■				232 (21)
166-2	■	■	■	■				■	181 (2)
175-2	■	■		■	■			■	197 (18)
76-2			■	■				■	257 (51)
143-2		■		■			■	■	197 (74)
159-2				■	■	■		■	235 (2)
30-2				■				■	1189 (315)

[a] Water uptake is given as a mean value of triplicate measurements with the standard deviation in parentheses. The molecular weights (M_n) and the degrees of functionalization (F) of the macromers were determined by ^{1}H-NMR. The macromers in the different networks (colored gray) are present in equal amounts by weight.

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ANA MARTINEZ CAVAZOS - Oct 21, 2022, 10:55 PM CDT

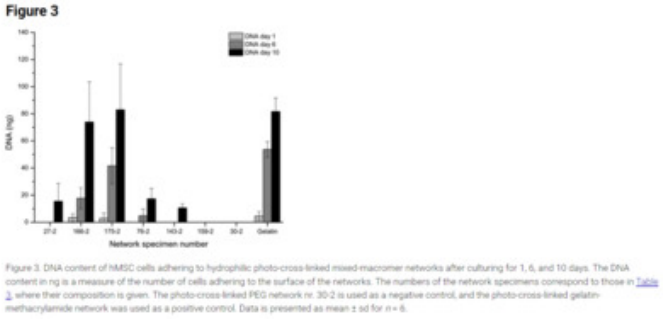

Figure 3. DNA content of hMSC cells adhering to hydrophilic photo-cross-linked mixed-macromer networks after culturing for 1, 6, and 10 days. The DNA content in ng is a measure of the number of cells adhering to the surface of the networks. The numbers of the network specimens correspond to those in Table 3, where their composition is given. The photo-cross-linked PEG network nr. 30-2 is used as a negative control, and the photo-cross-linked gelatin-methacrylamide network was used as a positive control. Data is presented as mean ± sd for $n = 6$.

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Successful PEG hydrogel models using thiol-ene chemistry

ANA MARTINEZ CAVAZOS - Nov 01, 2022, 7:27 PM CDT

Title: Notes on: ***A Quantitative Comparison of Human HT-1080 Fibrosarcoma Cells and Primary Human Dermal Fibroblasts Identifies a 3D Migration Mechanism with Properties Unique to the Transformed Phenotype***

Date: 10/29/22

Content by: Ana Martinez

Present: Ana Martinez

Goals: To learn more about the methodology in this PEG hydrogel model formed via thiol-ene chemistry and that allows for successful function of embedded hydrogels.

Content:

M. P. Schwartz, R. E. Rogers, S. P. Singh, J. Y. Lee, S. G. Loveland, J. T. Koepsel, E. S. Witze, S. I. Montanez-Sauri, K. E. Sung, E. Y. Tokuda, Y. Sharma, L. M. Everhart, E. H. Nguyen, M. H. Zaman, D. J. Beebe, N. G. Ahn, W. L. Murphy, and K. S. Anseth, “A quantitative comparison of human HT-1080 fibrosarcoma cells and primary human dermal fibroblasts identifies a 3D migration mechanism with properties unique to the transformed phenotype,” *PLoS ONE*, vol. 8, no. 12, Dec. 2013.

Synthetic ECM Preparation

- Synthetic ECM formed via a thiol-ene reaction
  - involves photoinitiated coupling of thiol groups and alkenes
  - Starting products = thiol group and alkene; added reactants = radical, catalyst; product = thiol-ene compound
- Thiol-ene reaction was used to incorporate cysteine-containing peptides into PEG matrix
- Crosslinks were formed with a matrix metalloproteinase (MMP)-degradable peptide --> modified from a collagen amino acid sequence
- Cell adhesion was promoted by adding "pendant" peptides that presented RGD amino acid sequence derived from fibronectin
  - This RGD amino acid sequence was chosen because it binds several integrins important for motility (cell migration through ECM)
- *PEG hydrogel synthesis*
  - Synthetic ECM monomer solutions were prepared in PBS as a 2.5 wt% or 3 wt. % solution of 4-arm PEG-norbornene (MW 20,000) + MMP-degradable peptide cross-linker
    - 2.5 wt% = 140 Pa shear modulus and 3.0 wt% = 220 Pa shear modulus
  - CRGDS amino acid sequence was incorporated for cell adhesion
    - Scrambled, non-bioactive C*RDGS* amino acid sequence added to maintain a constant 1.5mM total pendant peptide concentration
  - 0.05 wt% (final concentration) Irgacure 2959 (Ciba) was then incorporated as a photoinitiator
  - Cell Encapsulation
    - Cell pellet was suspended in monomer solution at 200,000 cells/mL
    - 30 µL aliquots were added to the cut end of a 1 mL syringe tip and polymerized under ~10 mW cm^-2, ~352 nm centered UV light (XX series UVP lamp) for 3 minutes
    - Once polymerized, PEG hydrogels were suspended in appropriate media and allowed to swell overnight before beginning experiments

Collagen Preparation

- Before beginning migration experiments, collagen was prepared at a final density of 1.7 - 3.5 mg/mL using a 1:1 mixture of i) high concentration type I rat tail collagen and 100 mM HEPES buffer in 2X PBS with ii) 1X PBS or serum free AMEM added to dilute to the final density
- Cell pellet was re-suspended at a final concentration of 200,000 cells/mL
- 200 µL of the cell/collagen mixture was added to the bottom of a 48-well plate.
- Collagen Gel Formation
  - Incubated cell/collagen mixture at 37° C, 5% CO2 for 60 minutes
  - After 60 minutes, serum-containing media was added
  - Cell/collagen constructs were incubated overnight before beginning time-lapse imaging

Conclusion

- Study compared migration and morphologies for HT-1080 fibrosarcoma cells and primary human dermal fibroblasts (hDFs) using engineered culture platforms to provide defined biochemical and biophysical ECM properties

- In synthetic ECM, hDFs tended to display features consistent with those previously reported for fibroblasts in 3D culture
    - Multipolar morphologies
    - Organized actomyosin filaments
    - Vinculin enrichment on the tips of protrusions.
- On the other hand, HT-1080s displayed cytoskeletal and adhesion properties that were distinct from hDFs in 2D and 3D culture
    - More rounded (and sometimes elongated) morphologies
        - These cells migrated through contractility-dependent, proteolytic mechanisms in synthetic ECM gels
        - The following were also observed:
            - Cortical F-actin expression
            - Cortex rupture and blebbing
            - Cortical contraction waves/rings
            - Rear end uropod-like structure
    - Decreased cell adhesiveness
- In short, Schwartz et al, demonstrated that:
    - HT-1080s adopt a distinct phenotype than hDFs under a wide range of 2D and 3D culture conditions
    - Due to the HT-1080s transformed phenotypes, these aggressive tumorigenic cell types migrate distinctly through the ECM compared to normal primary cells

Conclusions/action items:

Knowing that the cells and fibroblasts embedded onto Schwartz et al.'s PEG hydrogels behaved similarly to how they would in collagen gels (which mimic the actual ECM more closely), our team will likely incorporate the methodology for synthesizing the PEG hydrogels (materials used, concentrations used, and crosslinking methods used) into our own project. We will finish up last stages of ordering initial materials and begin test-formulation of PEG hydrogels in the near future.

Fibroblast Concentrations Used by Parlato et al. (2017)

ANA MARTINEZ CAVAZOS - Dec 10, 2022, 4:50 PM CST

Title: Fibroblast Concentrations Used by Parlato et al. (2017)

Date: 12/10/22

Content by: Ana Martinez

Present: Ana Martinez, Sara Morehouse

Goals: To learn more about the different concentrations of fibroblasts used by Parlato et al. (2017) and further define the range we will suggest our client should use as part of our project.

Content:

Citation: M. Parlato, J. Molenda, and W. L. Murphy, “Specific recruitment of circulating angiogenic cells using biomaterials as filters,” *Acta Biomaterialia*, vol. 56, pp. 65–79, 2017.

General Culture Information

- Human dermal fibroblasts (DFs) were obtained from Lonza
- Cultured directly on TCPS in Minimal Essential Media-a (CellGro) with 1% Penicillin/Streptomycin (Pen/Strep) and 10% v/v fetal bovine serum (FBS).
- DFs were used between passage 6 and passage 9 for experiments
- Detachment for passage involved treatment with trypsin for 5 minutes, followed by washing the cell culture surface with PBS
    - DFs were recovered via centrifugation at 0.2 kRCF for 5 min
    - DFs counted on a hemocytometer
    - Finally, they were seeded directly into experiment or into a new TCPS culture flask (as needed)

Concentrations

- For all assays, these were seeded directly into the wells of the transwell plate at a density of 1000 cells/well (12,500 cells/cm^2)

Conclusions/action items:

We wish to put together a range of fibroblast concentrations appropriate for our project parameters that are in units of cells/mL. This paper did not provide such units, so we might need to convert the given value to such units and/or keep looking for other literature that provides fibroblast concentrations in our desired units.

Fibroblast/Cell Concentrations Used by Arcaute et al. (2006)

ANA MARTINEZ CAVAZOS - Dec 10, 2022, 5:13 PM CST

Title: Fibroblast Concentrations Used by Arcaute et. al (2006)

Date: 12/10/22

Content by: Ana Martinez

Present: Ana

Goals: To learn more about the different concentrations of fibroblasts used by Arcaute et al. (2006) and further define the range we will suggest our client should use as part of our project.

Content:

Citation: K. Arcaute, B. K. Mann, and R. B. Wicker, “Stereolithography of three-dimensional bioactive poly(ethylene glycol) constructs with encapsulated cells,” *Annals of Biomedical Engineering*, vol. 34, no. 9, pp. 1429–1441, 2006.

General Culture Information

- Human dermal fibroblasts (HDFs) were maintained on Dulbecco’s Modified Eagle Medium (DMEM)
- HDFs were upplemented with 10% Fetal Bovine Serum, 200 mM L-glutamine, 10,000 units/ml of penicillin, and 10 mg/ml of streptomycin

- Experiments conducted using cultures at passage 10 or less

Fabrication

- PEG-dma was dissolved in 10mM HEPES buffered saline (pH 7.4), followed by the addition of I-2959 and Acrylolyl-PEG-RGDS into solution
- Solution was then filter sterilized using a 0.22 um filter
- Cell suspension in complete media was mixed in an equivolume amount with PEG solution to form a cell-polymer solution
    - 10^6 cells/mL
    - 30% w/v PEG-dma
    - 0.5% w/v I-2959
    - 5 mg/mL acrylolyl-PEG-RGDS
- Once the cell-containing scaffold was fabricated, it was placed with media in a 37 degree C, 5% CO2 incubator

Conclusions/action items:

The concentration provided by this research team is not really in line with the general range of 100,000 to 200,000 cells/mL fibroblast concentrations we have seen in other papers. Regardless, the additional concentrations of PEG, photoinitiator, and the RGD sequence are helpful in further development of our protocol for PEG gel fabrication.

Fibroblast/Cell Concentrations Used by Singh et al. (2015)

ANA MARTINEZ CAVAZOS - Dec 10, 2022, 5:47 PM CST

Title: Fibroblast Concentrations Used by Singh et al. (2015)

Date: 12/10/22

Content by: Ana Martinez

Present: Ana Martinez

Goals: To learn more about the different concentrations of fibroblasts used by Singh et al. (2015) and further define the range we will suggest our client should use as part of our project.

Content:

Citation: S. P. Singh, M. P. Schwartz, E. Y. Tokuda, Y. Luo, R. E. Rogers, M. Fujita, N. G. Ahn, and K. S. Anseth, “A synthetic modular approach for modeling the role of the 3D microenvironment in tumor progression,” *Scientific Reports*, vol. 5, no. 1, 2015.

General Culture Information:

- Primary neonatal human foreskin dermal fibroblasts (hDFs) were obtained from the University of California-Davis, Department of Dermatology
- hDFs were cultured in Dubellco’s Modified Eagle’s Medium (DMEM) supplemented with 10% Fetal Bovine Serum and 1% penicillin/streptomycin.

"Cluster growth by WM239A cells seeded on top of PEG hydrogels" --> More on Concentrations:

- 30 µL of monomer solution (8 mM/8 mM, 9 mM/9 mM, or 10 mM/10 mM -ene/thiol) was pipetted into a 1 mL syringe
- Photocrosslinking: solution exposed to 365 nm light (XX-40 light source, UVP) at 10 mW/cm^2 for 90 seconds using LAP as the photoinitiator
- After hydrogel was placed in a 24-well plate with PBS and allowed to equilibrate for 1 hour, fibroblast/cell concentrations were incorporated...
- After preparation, 2,000 WM239A cells were seeded on top of each hydrogel (cultures maintained at 37 degrees C at 5% Coo2 for 14 days)
- hDFs were then added --> cells were pelleted and resuspended in a 3mg/mL PureCol® collagen solution at a cell concentration of 600,000/mL
    - Transwell Insert Incorporation:
        - 0.5 mL of the resulting collagen solution was placed in the bottom of the 24-well plate containing insert
        - Insert was then placed into the collagen solution
    - Cells were again maintained at 37 degrees C at 5% CO2

"Cell migration and cluster growth for WM239A cells cultured within PEG hydrogels" --> More on Concentrations:

- After trypsinizing and counting the cells, WM239A cells were pelleted and re-suspended in monomer solution at the following concentrations:
    - 300,000 cells/mL for single cell migration studies
    - 400,000 cells/mL for cluster growth studies
- After hydrogel was formed, these cells were grown from a single cell suspension for 7 days
- hDFs were then added --> pelleted and resuspended in a 3 mg/mL PureCol® collagen solution at a cell density of 500,000 cells/mL
- The rest of the steps were similar to those for seeding cells on top of PEG gels (outlined above)

Conclusions/action items: Although a higher concentration than the general range our team has found of 100,000-200,000 cells/mL for fibroblasts, it is reasonable for our team to include this value as part of our suggested range while including the differences in concentrations depending on use.

Fibroblast Concentrations/General Fabrication Methodology Using TrueGel3D Hydrogels

ANA MARTINEZ CAVAZOS - Dec 10, 2022, 7:49 PM CST

Title: Fibroblast Concentrations Using TrueGel3D Hydrogels

Date: 12/10/22

Content by: Ana Martinez

Present: Ana Martinez

Goals: To learn more about the different concentrations of fibroblasts using TrueGel3D Hydrogels proposed by Millipore Sigma and finalize the range we will suggest our client should use as part of our project.

Content:

Citation: “Utilization of TrueGel3D™ Hydrogels to Study Fibroblast Spreading,” *Utilization of TrueGel3D™ hydrogels to study fibroblast spreading*. [Online]. Available: www.sigmaaldrich.com/US/en/technical-documents/technical-article/cell-culture-and-cell-culture-analysis/3d-cell-culture/truegel3d-fibroblast-spreading. [Accessed: 01-Dec-2022].

Hydrogel Preparation:

- 6 uL of 3T3 fibroblasts cell suspension was prepared in culture medium, D-PBS (sterile without Ca^{++}/Mg^{++} or any other physiological solution)
    - Final concentration of fibroblasts: 1*10^5 cells/mL
- 15 uL of water, 2.4 uL of 10X buffer (pH 5.5) and 2.0 uL FAST-PVA (at a final concentration of 2.0 mmol/L) were mixed in a reaction tube
- 1.6 uL of TrueGel3D™ RGD integrin adhesion peptide (at a final concentration of 1 mmol/L) was added to the reaction tube from step 2
    - Contents were mixed immediately to ensure homogenous distribution
    - Contents were then incubated for 5 minutes to allow attachment of the RGD peptide to the FAST-PVA polymer's maleimide groups
- 3.0 µL of PEG non cell-degradable crosslinker or CD cell-degradable crosslinker (at a final concentration of 2 mmol/L) was pipetted and spotted on the surface of a sterile culture plate (8-chambers slide) compatible with inverse microscopy
- Cell suspension was transferred to the reaction tube containing the polymer
- 27 µL of cell suspension mixture was added to the culture dish containing 3.0 µL of crosslinker and mixed quickly
    - Mixture was then incubated for 3 minutes to allow gel formation
- Once gel formed, 350-400 µL of cell culture medium was added
- Culture plate was placed in the incubator and the medium was replaced after 1 hour and in future time intervals for proper cell growth

Conclusions/action items:

The final concentration of fibroblasts used was 100,000 cells/mL, which fits within the range of concentrations we have found in prior research and can therefore be cited in our report as part of that range. Although this particular hydrogel fabrication procedure does not completely match the one outlined by our project, the materials used are quite similar, and this methodology could potentially be worthwhile for the client to consider as well.

Gelatin Hydrogels as an Alternative Model: Properties, Fabrication, and General Info

ANA MARTINEZ CAVAZOS - Dec 14, 2022, 4:24 PM CST

Title: Gelatin Hydrogels as an Alternative Model (Properties, Fabrication, General Info)

Date: 12/10/22

Content by: Ana Martinez

Present: Ana Martinez

Goals: To learn more about the properties and general fabrication procedures for gelatin hydrogels, which our team will use as an alternative model for testing purposes due to difficulties with getting PEG gels to set.

Content:

Citation: J. Skopinska-Wisniewska, M. Tuszynska, and E. Olewnik-Kruszkowska, “Comparative study of gelatin hydrogels modified by various cross-linking agents,” *Materials*, vol. 14, no. 2, p. 396, 2021.

Properties:

- Pros
  - Contains binding moieties that are important for cell attachment/adhesion
  - Non-toxicity and biocompatibility to cells and fibroblasts
  - Biodegradable
- Cons
  - Gelatin-based materials are generally characterized to have poor mechanical properties, short degradation time, and thermal instability
- The "cons" of gelatin materials can be overcome via photocrosslinking
  - Physical Methods
    - Plasma
    - UV radiation
    - Dehydrothermal treatment (DTH)
    - Combined freeze-drying/leaching methods
  - Chemical Methods
    - Use of enzymes (not as effective)
    - Chemical crosslinking (seem to be most effective)
      - Non-Zero-Length Crosslinkers: react with amino/carboxyl groups of amino acids --> incorporated into gelatin network structure
      - Zero-Length Crosslinkers: activate carboxyl groups to direct reaction with amino groups present on adjacent gelatin chain --> reagent is not built into gelatin matrix

Hydrogel Preparation:

- 40% aqueous solution of gelatin was prepared by mixing gelatin in distilled water at 50 degrees C for 30 minutes
- Crosslinker solutions were prepared by dissolving a molar ratio of 1:5 EDC-NHS and SQ in distilled water in separate beakers
- Dialdehyde starch was stirred in water at 60 degrees C until clear solution was obtained
  - Refilled to 30 cm^3 (mL) of total volume with distilled water --> obtained a 20% gelatin solution
- 15 mL of 40% aqueous gelatin solution was mixed with desired crosslinker amount
- 1% of DAS and EDC-NHS, as well as 1% and 3% of SQ were added to gelatin solution
- 25 cm^3 (mL) of mixed solutions were poured to the bottom of levelled dishes (so liquid layer was 1 mm thick) and left for solutions to gel

Conclusions/action items:

We conclude that using gelatin hydrogels as an alternative model for testing purposes will work reasonably well due to the similarities between gelatin and PEG in biocompatibility, biodegradability, and cell adhesion properties (gelatin has these naturally). However, due to gelatin's natural and significant degradation properties, we will likely advise the client to consider fabricating gelatin hydrogels with chemical crosslinking methods in order to minimize this and other "bad" properties of the material. For our purposes of proving whether or not our testing protocol is reasonable and works well, gelatin hydrogels will serve their purpose.

Culture Systems of the Small Airway Epithelium

SARA MOREHOUSE - Sep 21, 2022, 12:56 AM CDT

Title: Notes on Cell Culture Systems of the Small Airway Epithelium

Date: 9/21/2022

Content by: Sara Morehouse

Goals: To gain an understanding of the functions of the small airway epithelium as well as how 2D and 3D cell culture systems are used to model it.

Content:

The small airway epithelium is where gas exchange mainly occurs, and so it is an area of high immunological activity.

Protein complexes like tight junctions and adherens junctions form a barrier between circulation and the external environment.

In 3D culture systems, small airway epithelial cells are cultured in scaffolds. There are synthetic and natural hydrogels that currently exist that are used as ECM substitutes. Puramatrix (a synthetic peptide hydrogel) and microporous moldable silicon membranes have been developed.

When cells are cultured with fibroblasts and collagen matrices at air-liquid interfaces, they show fibronectin deposition and more in-vivo like inflammatory responses. Additionally, 3D cultures yield a better representation of epithelial physiology including tight junctions and inflammatory responses.

Citation:

Bhowmick, R., & Gappa-Fahlenkamp, H. (2016). Cells and Culture Systems Used to Model the Small Airway Epithelium. Lung, 194(3), 419–428. https://doi.org/10.1007/s00408-016-9875-2

Conclusions/action items:

This article gave a brief overview of the benefits of 3D cell culture systems and some products and methods that are currently used in this field.

Cells and Culture Systems Used to Model the Small Airway Epithelium

Rudra Bhowmick[1] · Heather Gappa-Fahlenkamp[1]

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Cells_and_Culture_Systems_Used_to_Model_the_Small_Airway_Epithelium.pdf (823 kB)

Crosslinking Notes

Title: Cross-linking in Hydrogels - A Review

Date: 10/11/22

Content by: Sara Morehouse

Goals: To learn more about the process of cross-linking for formation of hydrogels.

Content:
http://article.sapub.org/10.5923.j.ajps.20140402.01.html#:~:text=Hydrogels%20represent%20a%20class%20of%20high%20water%20content,exte

- Crosslinking = stabilization process of polymers that leads to network structure forming from the polymeric chain
- Cross link bond can be ionic and covalent
- Changes liquid into solid or gel
- Increases molecular mass of polymer
- More mechanically strong, resistant to heat, wear, attack by solvents
- Hydrogels are crosslinked hydrophilic structures that can take in large amounts of water or fluids.
- Elasticity: decreases as number of cross-links increases
- Decreases viscosity
- Insoluble - chains are tied together with strong covalent bonds and can't dissolve in solvents but can absorb them.
- Increases strength and toughness
- Lowers melting point
- Methods:
    - Irradiation
    - Sulfur vulcanization
    - Chemical reactions (adding different chemicals in combination with heat and sometimes pressure)
- Physical crosslinks:
    - Entangled chains, hydrogen bonding, hydrophobic interaction, crystallite formation
    - Yields reversible hydrogels
    - Defects may occur
- Chemical crosslinking:
    - Covalent crosslinking of polymers
    - Polymerize end-functionalized macromers
    - Can be crosslinked with glutaraldehyde, formaldehyde, epoxy compounds, dialdehyde.

PEGDA cell adhesion using nucleic acid aptamers

Title: Cell Adhesion on an Artificial Extracellular Matrix Using Aptamer-Functionalized PEG hydrogels

Date: 10/16/22

Content by: Sara Morehouse

Goals: To learn more about a potential method for promoting cell adhesion in a PEG hydrogel.

Content:
https://pubmed.ncbi.nlm.nih.gov/22079002/#:~:text=Importantly%2C%20the%20results%20also%20showed%20that%20the%20aptamers,the%20s

- Nucleic acid aptamers applied to PEG hydrogel to mimic adhesion sites of ECM
- Hydrogels functionalized with nucleic acid aptamers to control release of growth factors
- Nucleic acid aptamers = single-stranded oligonucleotides
    - High affinity, high specificity, low immunogenicity
- Natural adhesion proteins can be used for cell adhesion however:
    - they can be limited by fast degradation
    - May lose capability to recognize receptors
    - (ex: collagen should be further functionalized by adhesion peptides to improve cell recognition function)
- Small adhesion peptides
    - Easy synthesis, easy conjugation, little immunogenicity
    - Require circularization, multimerization, presentation in context of parent proteins in order to be sufficiently stable
- Nucleic acid aptamers incorporated into PEG hydrogel using free radical polymerization
- This study used PEGDA (700 Da) and TMSPM, phosphate buffered saline (PBS), ammonium persulfate (APS), TEMED, NaOH, and a variety of oligonucleotides
- Hydrogels were synthesized on glass slides, acrylate groups generated on surface through silanization (mix 0.5 mL TMSPM in 50 mL ethanol with 1.5 mL of 10%
- PEGDA diluted w PBS to **20% w/v**
- Rxn solution: 5 µL PEGDA/aptamer mixture, 15 µL APS (10% w/v), 0.15 µL TEMED (50% w/v)
- Solution deposited on large glass slide then covered with silanized glass slide. Cured for 2 hours, silanized glass slide was then lifted.
- Examination of Cell Adhesion:
    - Used Dulbecco's phosphate buffered saline (DPBS) supplemented with glucose, magnesium chloride, and BSA.
    - Hydrogel samples placed in 24-well plate, incubated with cell samples at 37 degrees C, taken out for washing after a certain amount of time in order to rem attached.
    - Certain aptamers could bind to specific cells

PEGDA cell adhesion using RGD peptides

SARA MOREHOUSE - Oct 18, 2022, 4:16 PM CDT

Title: Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing

Date: 10/18/22

Content by: Sara Morehouse

Goals: Gain understanding of methods for using RGD peptides to promote cell adhesion in PEGDA hydrogel and analyze results.

Content:

Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing

- PEGDA and RGD peptides
- Functionalized amine terminus of peptide with acrylate moiety, enabled adhesion peptide to copolymerize with the PEGDA upon photoinitiation
- Studied ability to promote spreading of human foreskin fibroblasts
- Hydrogels without peptides permitted spreading of only 5% of seeded cells
- Hydrogels with RGD/RDG supported spreading of 50% of cells at 1 pmol/cm^2 and 15% of cells at 0.1 pmol/cm^2 (lower concentrations did not promote spreading)
- Adding a MW 3400 PEG spacer arm between hydrogel and peptide linkage, there was 70% spreading with incorporation of 1pmol/cm^2 RGD (no spreading with same concentration of RDG)
- When cells seeded in serum-free medium, only RGD peptides incorporated with spacer arm were able to promote spreading
- Short peptide sequences are stable and have been proven sufficient for cell adhesion and spreading when chemically incorporated into biomaterial surfaces in large enough numbers
    - Small size = much higher concentrations possible
- Used peptide radiolabeling - this peptide did not exceed 1 molar percent of total peptide except for gels w lowest peptide concentration of 0.001pmol/cm^2 (molar percent = 10)
- Peptide activation
    - Peptide dissolved to [1 mg/mL] in 50mM of sodium bicarbonate buffer (pH 8.2)
    - Succinimidyl ester of acrylic acid dissolved in DMF, added dropwise to aq. peptide solution to final volume fraction of 33%
        - Synthesized acrylated peptide with aminolysis of N-hydroxy succinimide ester of acrylic acid by the α-amine terminus of peptide sequence
        - Produces amide linkage btwn peptide and acrylic group
    - Vials agitated continuously at room temp for certain amount of time, frozen in liquid nitrogen and lyophilized
        - Two parameters: molar ratio of peptide to succinimide ester (1, 5, 10, 25), reaction time (2, 6, 24 hrs)
        - Acrylated and unacrylated peptides were separated from by-products by dialysis against DI water for 48hrs
            - Have to remove residual acrylic acid which could alter adhesivity of gel
        - Final product lyophilized overnight
    - Synthesis of acrylated peptide containing MW 3400 PEG chain done by reaction peptide with acryloyl-PEG-NHS ester
    - MW 3400 acryloyl-PEG-NHS dissolved in 50 mM of bicarbonate buffer so final molar ratio to peptide was 2
    - PEG solution (200 microliters) added dropwise to 1 mL peptide solution, reacted room temp 2 hrs, lyophilized

- They made their own PEGDA through PEG acrylation
- Polymerization of PEGDA:
    - PEGDA dissolved in PBS to get 10% or 23% w/v solution
    - Photoinitiator solution: 2,2-dimethoxy-2-phenyl-acetophenone in 1-vinyl-2-pyrrolidinone (600 mg/mL)
    - Mixed photoinitiator with 1.5 µL/mL PEGDA solution
    - Thin film of crosslinked PEGDA formed by pipetting 100 µL of this solution onto Teflon-coated glass plate, covered with coverslip, exposed to 100 W medium pressure mercury UV light source (365 nm) at flux of 10mW/cm^2 for 90s. Films lifted from plate with razor blade
- Co-photopolymerization of peptide acrylate and PEGDA
    - ….
- Results:
    - On PEGDA-only hydrogels, cell spreading was less than 5% after 24hrs which was actually less than spreading observed only on tissue culture wells without a gel
    - PEGDA hydrogels containing peptide incorporated via linkage without a PEG spacer arm: cells seeded on it adhered nonspecifically - for both 10% and 23% PEGDA hydrogels
        - Active peptide YRGDS promoted cell adhesion and spreading in dose-dependent manner (greatest with 1.0 pmol/cm^2 peptide surface concentration)
        - Extent of spreading did not depend on concentration of PEGDA
        - Similar results with inactive peptide YRDGS
        - Repeated with GRGDS and GRDGS, again similar results
        - Results showed that **serum proteins were required for cell spreading** and bioactivity of the YRGDS peptide
    - Hydrogels with peptide linkage via MW 3400 PEG spacer:
        - Found cell adhesion and spreading on YRGDS peptide but NOT the YRDGS peptide
        - Extent of spreading again dependent on peptide surface concentration
            - Over 80% of cells fully spread on hydrogel at 1 pmol/cm^2 after 70hrs
        - Inactive YRDGS peptide did not support cell spreading
        - Cells seeded in serum-free medium had only slightly less cell spreading than in serum-containing medium

PEG-DMA cell adhesion with RGD peptides

SARA MOREHOUSE - Oct 19, 2022, 8:26 PM CDT

Title: Transient cellular adhesion on poly(ethylene-glycol)-dimethacrylate hydrogels facilitates a novel stem cell bandage approach

Date: 10/19/22

Content by: Sara Morehouse

Goals: To learn more about success of using RGD peptides to promote cell adhesion in PEG-DMA hydrogels

Content:

Transient cellular adhesion on poly(ethylene-glycol)-dimethacrylate hydrogels facilitates a novel stem cell bandage approach - PMC (nih.gov)

- RGD is an adhesive peptide found in the cell attachment region of fibronectin
- Found that the number of cells on hydrogels containing RGD was much higher as time passed compared to cells on hydrogels without RGD
- Cell spreading occurred more on RGD-containing hydrogels
- Used PEG-DMA hydrogel
- Mesenchymal stromal cells were able to attach to PEG-DMA without RGD surprisingly, however they did not have as long of a life (declined after about 9 days)
- PEG-DMA synthesized using method from:https://pubmed.ncbi.nlm.nih.gov/15244441/
    - PEG MW 3350 Da mixed in glass vials with excess methacrylic anhydride (10:1 ratio)
    - Vials placed in microwave for 30s, 1 min (4x), 30s, allowed to cool between intervals
    - Cooled to room temp, added 10mL of anhydrous ethyl ether, vigorously scraped bottom of vial
    - Washed product in 10mL of anhydrous ethyl ether (2x) to remove unreacted MA, isolated with Buchner filtration
    - Removed remaining solvent with rotovaporation, stored product in -20°C freezer
- Used H-Gly-Arg-Gly-Asp-Ser-OH peptide (RGD from Calbiochem), reacted with acrylated-PEG-succinimidyl valerate spacer (A-PEG-SVA, MW 3400) in 1:2 molar ratio
    - Combined in sodium bicarbonate buffer, pH 8.1-8.3, while stirring at room temp for 2.5 hrs
    - Dialyzed in DI water overnight x2 using dialysis membrane (MW cutoff 1000 Da)
    - Dialyzed polymer solution rotovapped for 24hrs and stored at -80°C
- PEG-DA RGD hydrogel
    - 1 µmol acrylated RGD/g swollen hydrogel crosslinked between glass slides using thermal radical initiators (APD, 0.3M; TEMED, 0.3M) for 10 mins at 37°C
    - Swollen in DI water overnight, cut into discs with cork borer
- PEG-DMA RGD hydrogel
    - MW 3.4 kDa
    - 1 µmol acrylated RGD/g swollen hydrogel
    - Crosslinked with same method as PEG-DA

The Influence of Biomaterials on Cytokine Production in 3D Cultures notes

SARA MOREHOUSE - Dec 11, 2022, 8:25 PM CST

Title: notes on The Influence of Biomaterials on Cytokine Production in 3D Cultures

Date: 10/25/22, 12/4/22

Content by: Sara Morehouse

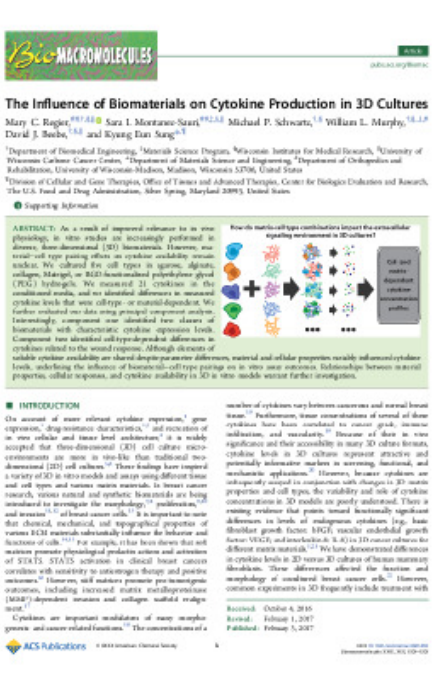
Goals: Learn more about epithelial cell attachment to PEG-NB hydrogels

Content:

- 3D cultures of mammary fibroblasts yielded better cytokine levels than 2D cultures, which affected function/morphology of cocultured breast cancer cells
- MCF10A normal breast epithelial cells
    - Selected to represent normal mammary epithelium
    - DMEM/F12 supplemented w horse serum (5%), epidermal growth factor (EGF, 20 ng/mL), hydrocortisone (0.5 mg/mL), cholera toxin (100 ng/mL), insulin (10 µg/mL), penicillin/streptomycin (1%)
    - Cultures all showed relatively high levels of epidermal growth factor
- Human normal dermal fibroblasts
    - Selected to represent normal stroma
    - high-glucose DMEM (4.5 mg/mL), fetal bovine serum (FBS, 10%), and penicillin/streptomycin (1%)
- Cytokines:
    - Studying various cytokines that are important in breast cancer
    - Found that PEG hydrogels resulted in highest levels of soluble proteins
    - Cytokine availability dependent on multiple factors including cell-matrix interactions, matrix mechanics, matrix-cytokine interactions
- 3D culture in PEG hydrogel
    - Thiol-ene photopolymerization used
    - Crosslinked 8-arm PEGNB (20000 MW) with KCGGPQGIWGQGCK (MMP-degradable peptide), functionalized with CRGDS peptide for cell adhesion
    - Hydrogel formation:
        - Cells detached using 0.05% trypsin, resuspended in PBS to 400000 cell/mL
        - Mixed with Irgacure 2959 photoinitiator (0.2% in 1X PBS) in 1:1 ratio
        - 100µL of mixture mixed with 100µL of 2X hydrogel monomer (in 1X PBS) → **final concentration of 100000 cells/mL in 40 mg/mL PEG-NB, 60% peptide cross-links and 2mM CRGDS**
        - 40µL of final mixture added to wells of 96-well plate, polymerized under UV lamp at 5-10 mW cm-2 for 2 mins (placed on top shelf of exposure stand for UVP XX-15L lamp)
        - 200 µL of MEM added on top of gels
        - Incubated at 37°C and 5% CO2
    - Elastic modulus of 0.1-6 kPa

Conclusions: From this paper shared with us by Dr. Murphy, I was able to learn more about the specific types of PEG and crosslinkers that we could possibly use for our design. Also, there was helpful information about different peptides that we could possibly incorporate in order to promote cell adhesion and degradation. Lastly, I was able to find a value for fibroblast concentrations within the matrix which was a necessarily spec for our design.

SARA MOREHOUSE - Nov 01, 2022, 12:37 PM CDT



The Influence of Biomaterials on Cytokine Production in 3D Cultures

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Regieretal_Biomacromolecules_2017_1032_.pdf (2.44 MB)

Fibroblasts in 3D Matrices: cell migration and matrix remodeling

SARA MOREHOUSE - Dec 04, 2022, 6:04 PM CST

Title: Fibroblasts in three dimensional matrices: cell migration and matrix remodeling

Date: 12/4/22

Content by: Sara Morehouse

Goals: To learn more about fibroblasts in matrices in order to figure out the concentration of fibroblasts we need to use in protocols.

Content: Fibroblasts in three dimensional matrices: cell migration and matrix remodeling | Experimental & Molecular Medicine (wisc.edu)

This article gives a fairly general overview of how matrix remodeling works, specifically in a collagen matrix. They focused on fibroblast-mediated remodeling and how it depends on growth factors and mechanical environments. The article discusses how matrix remodeling can increase stiffness. However, it did not discuss how concentration of fibroblasts relates to matrix remodeling which is what I am specifically looking for.

Conclusions/action items: I still need to find information on how concentration of fibroblasts will affect the matrix.

Normal Human Dermal Fibroblasts (3D Cell Culture)

SARA MOREHOUSE - Dec 08, 2022, 11:14 AM CST

Title: Normal Human Dermal Fibroblasts...

Date: 12/1/22

Content by: Sara Morehouse

Goals: To figure out concentration of fibroblasts needed for our model.

Content:

Normal Human Dermal Fibroblasts Skin Model | TheWell Bioscience

- Using Vitrogel RGD, used a fibroblast cell suspension concentration of 1-1.5 x 10_6 cells/mL

In this source, I was primarily searching for a fibroblast concentration that could help define a range for our model. This article described a 3D model of fibroblast culture using a commercially available gel called Vitrogel. While not the same composition as our design, it had similar components such as RGD peptides which was helpful in learning more about fibroblast culture in 3D hydrogels.

Conclusions/action items: This source yielded the value of fibroblast concentration to be 1-1.5e6 cells/mL.

A Quantitative Comparison of Human HT-1080 Fibrosarcoma Cells and Primary Human Dermal Fibroblasts Identifies a 3D Migration Mechanism with Properties Unique to the Transformed Phenotype

SARA MOREHOUSE - Dec 11, 2022, 8:34 PM CST

Title: A Quantitative Comparison of Human HT-1080 Fibrosarcoma Cells and Primary Human Dermal Fibroblasts Identifies a 3D Migration Mechanism with Properties Unique to the Transformed Phenotype

Date: 12/5/22

Content by: Sara Morehouse

Goals: To find a value of fibroblast concentration to be used in our protocols.

Content:

This article described an approach for measuring fibroblast migration, morphology, and adhesion in PEG hydrogel. I primarily was interested in finding a value for fibroblast concentration in order to set a protocol for our final design. The value I found was:

200000 cell/mL + 30 µL aliquot

Conclusions/action items: The fibroblast concentration described in this paper will be useful for setting the protocol for fibroblast concentration in our final design.

Notes from "Harnessing Endogenous Circulating Angiogenic Cells"

SARA MOREHOUSE - Dec 11, 2022, 8:52 PM CST

Title: Notes from "Harnessing Endogenous Circulating Angiogenic Cells"

Date: 11/8/22

Content by: Sara Morehouse

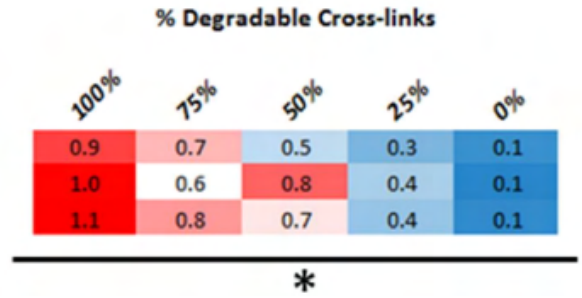
Goals: To find values for MMP peptide concentrations for our design to be fully degradable.

Content:

When reading this paper, I was primarily searching for information on how to control degradation of PEG hydrogels via MMP peptides. I was able to find a table listing different combinations of crosslinkers and the purposes for combining them at certain ratios. There was also a section of the paper that discussed degradability, PEG concentrations, and use of
MMP peptides vs PEGDT.

- “Regardless of PEGNB wt% or hydrogel cross-linking, hydrogels cross-linked
- with a peptide susceptible to MMP degradation (hereafter referred to as the MMP
- degradable peptide) allowed more CAC invasion than did hydrogels cross-linked
- with a non-degradable PEG-dithiol cross-linker”
- Decreasing PEG wt% promotes higher levels of cell invasion (3% vs 4-10%)
- Replacing MMP degradable peptide crosslinkers with PEG-dithiol crosslinkers reduces cell invasion (dosage-dependent)
- Best combination for highest level of cell invasion is 3 wt% PEGNB with 100%
- MMP degradable peptide cross-linker

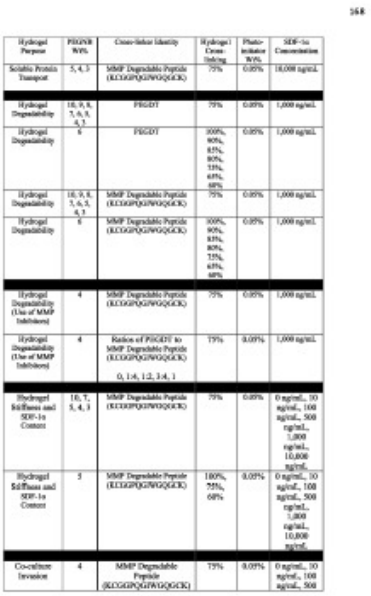
Table: Red = most cell invasion and blue = least cell invasion (heat map)



Conclusions/action items:

This paper gave a helpful background for figuring out how to incorporate MMP peptides in order to promote degradation of the hydrogel.

SARA MOREHOUSE - Dec 11, 2022, 8:52 PM CST



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Hydrogel Purpose	PEGNB Wt%	Cross-linker Identity	Hydrogel Cross-linking	Photo-initiator Wt%	SDF-1α Concentration
Soluble Protein Transport	5, 4, 3	MMP Degradable Peptide (KCGGPQGIWGQGCK)	75%	0.05%	10,000 ng/mL
Hydrogel Degradability	10, 9, 8, 7, 6, 5, 4, 3	PEGDT	75%	0.05%	1,000 ng/mL
Hydrogel Degradability	6	PEGDT	100%, 90%, 85%, 80%, 75%, 65%, 60%	0.05%	1,000 ng/mL
Hydrogel Degradability	10, 9, 8, 7, 6, 5, 4, 3	MMP Degradable Peptide (KCGGPQGIWGQGCK)	75%	0.05%	1,000 ng/mL
Hydrogel Degradability	6	MMP Degradable Peptide (KCGGPQGIWGQGCK)	100%, 90%, 85%, 80%, 75%, 65%, 60%	0.05%	1,000 ng/mL
Hydrogel Degradability (Use of MMP Inhibitors)	4	MMP Degradable Peptide (KCGGPQGIWGQGCK)	75%	0.05%	1,000 ng/mL
Hydrogel Degradability (Use of MMP Inhibitors)	4	Ratios of PEGDT to MMP Degradable Peptide (KCGGPQGIWGQGCK) 0, 1:4, 1:2, 3:4, 1	75%	0.05%	1,000 ng/mL
Hydrogel Stiffness and SDF-1α Content	10, 7, 5, 4, 3	MMP Degradable Peptide (KCGGPQGIWGQGCK)	75%	0.05%	0 ng/mL, 10 ng/mL, 100 ng/mL, 500 ng/mL, 1,000 ng/mL, 10,000 ng/mL
Hydrogel Stiffness and SDF-1α Content	5	MMP Degradable Peptide (KCGGPQGIWGQGCK)	100%, 75%, 60%	0.05%	0 ng/mL, 10 ng/mL, 100 ng/mL, 500 ng/mL, 1,000 ng/mL, 10,000 ng/mL
Co-culture Invasion	4	MMP Degradable Peptide (KCGGPQGIWGQGCK)	75%	0.05%	0 ng/mL, 10 ng/mL, 100 ng/mL, 500

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crosslinkers.pdf (378 kB)

SARA MOREHOUSE - Dec 11, 2022, 8:53 PM CST

Harnessing Endogenous Circulating Angiogenic Cells

By

Matthew B Parlato

A dissertation submitted in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy
(Biomedical Engineering)

At the
University of Wisconsin-Madison
2015

Date of final oral examination:

The dissertation is approved by the following members of the Final Oral Committee:
William L. Murphy, Professor, Biomedical Engineering (*Thesis Advisor*)
David J. Beebe, Professor, Biomedical Engineering
Wan-ju Li, Assistant Professor, Biomedical Engineering
Bo Lin, Associate Professor, Surgery
Lucian Lozonschi, Associate Professor, Surgery

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MattParlato_Dissertation_2016_1033_.pdf (12.3 MB)

PEGDA/Collagen Hydrogel

SARA MOREHOUSE - Oct 11, 2022, 7:22 PM CDT

Title: Characterization of Sequential Collagen-Poly(ethylene glycol) Diacrylat Interpenetrating Networks and Initial Assessment of their Potential for Vascular Tissue Engineering

Date: 10/11/22

Content by: Sara Morehouse

Goals: To learn about methods used to create a PEGDA-collagen hydrogel.

Content:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5469296/

- Polyethylene Glycol Diacrylate
- Covalently-crosslinked PEGDA network combined with physically-crosslinked collagen network
    - PEGDA has biocompatibility, low thrombogenicity, resistance to cell-mediated compaction
    - Degradation rates and mechanical properties can be tailored
        - Degradation by introducing hydrolytically or enzymatically-degradable segments within the PEG network
        - Mechanical properties by varying molecular weight and concentration in solution of PEGDA
- First formed collagen hydrogel then infusing this with PEGDA
    - Purpose to prevent encapsulated cells from taking on a rounded cell phenotype
- Exposed to longwave UV light to form PEGDA network interlaced with collagen network
    - Vary time between infusion to control degree of cell spreading
- PEGDA formation: 0.1 mmol/ml dry PEG, 0.4 mmol/ml acryloyl chloride, and 0.2 mmol/ml triethylamine in anhydrous dichloromethane, stirred under argon overnight. Washed with 2 M K_2CO_3 and separated into aqueous and dichloromethane phases to remove HCl. Dried with anhydrous $MgSO_4$. PEGDA was precipitated in diethyl ether, filtered, and dried under vacuum.
- Fabrication process:
    - Physical crosslinking of pure collagen network
        - Collagen diluted and neutralized to yield desired concentration (1.5 mg/mL, 3 mg/ml, or 5 mg/mL)
        - 300mL pipetted into culture inserts
        - Polymerization by 30 mins incubation at 37 °C and 5% CO_2
        - Immersed for 30 mins in DMEM supplemented with 1% sodium pyruvate and 1% Glutamax (overall called SFM solution)
    - Infiltration of network with 3.4 kDa, 6.0 kDa or 10.0 kDa PEGDA solution
        - SFM solution replaced with 1.7 ml of sterile-filtered SFM containing 11.7% w/v PEGDA and 0.26% w/v photoinitiator (Irgacure 2959)
        - Will eventually yield 10% w/v PEGDA within the hydrogel
        - Infiltrate at 37 °C for 15-60 mins
        - Excess solution removed, hydrogel was then polymerized by 6 mins exposure to longwave UV light
        - Immersed in culture medium (SFM with 10% FBS)
        - Found 45 mins to be sufficient infiltration time, did 60 mins anyway to ensure
- Results:
    - decrease in PEGDA molecular weight led to a significant increase in tensile modulus
    - Shear storage modulus increased with increasing collagen concentration
    - Network elasticity increased with increasing collagen concentration

- Pure PEGDA hydrogels do not allow for sufficient cell elongation, even when cell adhesion ligands are incorporated, but delaying PEGDA infiltration allowed for spindle-shaped cells to form
- PEGDA/collagen network lasted 14 days without significant changes in lateral dimensions whereas collagen-only shrunk by about 60% by day 14

Notes on The Diffusion Properties of the Hydrogels

SARA MOREHOUSE - Dec 13, 2022, 12:24 PM CST

Title: "The Diffusion Properties of the Hydrogels"

Date: 11/27/22

Content by: Sara Morehouse

Goals: To learn more about how diffusion through hydrogels is measured.

Content:

In this approach, a spectroscopy method was used to measure diffusion by measuring light intensity passing through gels with diffusing substance to measure concentrations. They tested a silicic acid gel and an agarose gel. For diffusing substances, potassium permanganate solution and fuchsin solution were used. They then measured the diffusion coefficient for each.

Conclusions/action items:

While a good method for measuring diffusion of media through gels, it uses equipment that our team does not have access to and so an alternative method will have to be created.

unusuable Design Idea: Matrigel

SARA MOREHOUSE - Sep 28, 2022, 8:50 PM CDT

Title: Design Idea: Matrigel

Date: 9/28/22

Content by: Sara Morehouse

Goals: To outline a design idea for the scaffold using Matrigel as the hydrogel base.

Content:

Fabrication of thin-layer matrigel-based constructs for three-dimensional cell culture - PubMed (nih.gov)

- Handled as a liquid below 10degrees C
    - Above this temp, assembles into clear hydrogel that supports cell growth and survival
- Technique used by Kim et al. to dilute Matrigel past manufacturer's recommended gelling point to generate thin protein coating around cells to form 3D cell cultures
    - Reduced evaporation effects, interfacial tension
    - Downside - large numbers of cells needed, cells at the bottom attached directly to plate (too stiff)
- This team used method of dispensing Matrigel into cold medium-filled wells and allowing it to settle, which condenses Matrigel at the bottom of the plate immediately before gelation, minimizes cell adherence to plate
    - Medium used was Dulbecco's modified Eagle's medium (DMEM), a basal medium for supporting cell growth supplemented with 10% fetal bovine serum and 1% antibiotic antimycotic solution (70 microliters per well)
    - Used growth factor-reduced Matrigel at concentration of 5 mg/mL
- Liquid-liquid interface

Development and Functional Characterization of Fetal Lung Organoids - PubMed (nih.gov)

- Using Matrigel as a scaffold to culture a cell suspension of epithelial, endothelial, mesenchymal and hematopoietic cells at the ALI on a floating membrane filter to generate 3D lung cell cultures called organoids
- Used in this case to study fetal lung development
- Model consisted of transparent permeable transwell inserts (ThinCert) that were coated in growth factor reduced Matrigel (3 mg/mL). This Matrigel GFR was dissolved in a medium called LO-Med (DMEM/F12, 10% FCS, 1% insulin-transferrin-selenium-ethanolamine, 1mM HEPES, 100 units penicillin /mL, streptomycin - 100µg/mL, amphotericin B - 0.25µg/mL).
- Cell mix combined with Matrigel GFR (0.4 mg/mL in LO-Med), transferred to coated inserts. Lower compartment of insert filled with LO-Med, upper compartment containing cells in Matrigel was not submerged in medium - this allows for ALI.
- Cells cultured at 37 degrees C, medium exchanged every 2 days. After 15 days, organoids further cultured in mesenchymal stem cell-conditioned medium
- Results found that cells cultured in the ALI had more complex branched and cystic morphology
- Noted that there is an undefined culture condition - solution varies from batch to batch, can be complex and undefined.

Conclusions/action items:

Recap only the most significant findings and/or action items resulting from the entry.

10/4/22 Design Idea: Alginate

SARA MOREHOUSE - Oct 09, 2022, 5:44 PM CDT

Title: Design Idea: Alginate

Date: 10/4/22

Content by: Sara Morehouse

Goals: To learn more about alginate as a hydrogel and to see if it is a viable option for our design.

Content:

Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3223967/

- Naturally occurring polymer obtained from seaweed
- Alginate hydrogels can be prepared using cross-linking
- Form gels by reaction with divalent cations such as Ca^{2+}, Sr^{2+}, or Ba^{2+} (typically calcium)
- Chemical structure (M/G residue ratios, sequence, molecular weight, etc) has significant impact on functional properties of hydrogel (porosity, swelling, stability, biodegradability, strength, biocompatibility) → can vary depending on source of alginate
- Mechanical properties enhanced by increasing length of G-block and molecular weight, however high weight solutions become greatly viscous
    - Proteins or cells mixed with high-viscosity solution can be damaged from high shear forces
    - By using a combination of high and low weight polymers, can increase elastic modulus while keeping viscosity of solution lower.
- Biocompatibility -
    - No significant inflammatory response associated with gels formed from commercially available, highly purified alginate
- Cell adhesivity -
    - Inherently lacks mammalian cell adhesivity
    - However alginate derivatives that contain cell-adhesive peptides have been used recently
    - RGD-containing peptides used as adhesion ligands due to presence of integrin receptors
    - Minimum concentration of peptides needed depends on cell type
    - Cyclic RGD peptides are more potent
- Methods of gelling:

    Ionic crosslinking:
    - Combine aq alginate solution with divalent cations (Ca^{2+})
        - Calcium chloride will lead to rapid and poorly controlled gelation → Use phosphate buffer to slow gelation
        - Instead could do calcium carbonate, add Glucono-δ-lactone to dissociate Ca^{2+} → will then initiate gelation much more gradually
    - Gelation rate critical for determining uniformity and strength of gel
        - Slower = more uniform
        - Slower at lower temperatures
    - Stiffness:
        - Alginates with higher content of G residues will be stiffer
    - Limited long term stability
        - Water migration leads to plastic deformation

    Covalent crosslinking (probably not an option with our resources)
    - Reagents can be toxic - have to remove all unreacted chemicals
    - Mechanical properties and swelling of hydrogels can be tightly regulated by using different cross-linking molecules and controlling cross-linking densities

- Introduce hydrophilic cross-linking molecules as second macromolecule (PEG) to compensate for loss of hydrophilic character during cross-linking
- Photo-crosslinking uses laser

Conclusions/action items: This article provided a useful background on how to make hydrogels out of alginate and how its properties can vary depending on the composition of the alginate.

Comparative Study of Gelatin Hydrogels modified by Various Cross-linking Agents

SARA MOREHOUSE - Dec 14, 2022, 4:56 PM CST

Title: Comparative Study of Gelatin Hydrogels modified by Various Cross-linking Agents

Date: 12/14/22

Content by: Sara Morehouse

Goals: To find out if gelatin can be used for cell culture at 37C despite its thermal degradation at 25-40C.

Content:

Comparative Study of Gelatin Hydrogels Modified by Various Cross-Linking Agents - PubMed (nih.gov)

In this article, they crosslinked gelatin with 3 different crosslinkers: EDC-NHS, squaric acid (SQ), and dialdehyde starch (DAS).

After physically crosslinking the gelatin with each crosslinker, they found that both SQ and DAS were successful in reducing weight loss of gels at increased temperatures. While DAS was most effective at reducing weight loss, this was due to a higher density of crosslinks that increased its mechanical stiffness and Young's Modulus. SQ was able to reduce weight loss of gelatin at high temperatures while also keeping a low Young's Modulus that would fit within our client's desired range for lung ECM.

Table 4

Mechanical parameters of the measured unmodified and cross-linked gelatin hydrogels.

Sample	Tensile Strength [kPa]	Elongation [%]	Young's Modulus [kPa]
Gelatin 20%	31.68 ± 9.66	39.23 ± 2.98	37.60 ± 5.00
EDC-NHS	67.91 ± 7.13	74.77 ± 11.71	99.43 ± 11.83
SQ 1%	22.41 ± 6.30	40.54 ± 8.27	53.96 ± 4.34
SQ 3%	10.21 ± 1.60	37.71 ± 5.07	25.00 ± 3.39
DAS	111.91 ± 12.04	25.40 ± 2.84	168.00 ± 40.00

Table 1

The parameters of the thermal decomposition of unmodified and cross-linked gelatin hydrogels.

Sample	I Stage 240 °C		II Stage From 240 °C to 500 °C	
	T [°C]	Δm [%]	T [°C]	Δm [%]
Gelatin 20%	204	12.47	305	57.38
EDC-NHS	212	11.87	316	58.34
SQ 1%	214	11.65	316	56.03
SQ 3%	217	11.04	309	55.12
DAS	214	11.66	326	57.52

Conclusions/action items: This source was very helpful as it allowed us to overcome the challenge of gelatin thermal degradation and to provide a solution for using gelatin as a possible final model.

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: