BME Design-Fall 2023 - CARLEY SCHWARTZ
Complete Notebook

PDF Version generated by

ANURAAG SHREEKANTH BELAVADI

on

Dec 13, 2023 @07:17 PM CST

Table of Contents

Project Information 2
- Team contact Information 2
- Project description 3

Team activities 4
- Client Meetings 4
  - 10/05/2023 Client Meeting 1 4
  - 10/19/2023 Client Meeting 2 5
  - 11/02/2023 Client Meeting 3 6
  - 11/16/2023 Client Meeting 4 7
  - 11/20/2023 Client Meeting 5 8
- Advisor Meetings 9
  - 09/15/2023 Advisor Meeting 1 9
  - 09/20/2023 Advisor Meeting 2 10
  - 09/29/2023 Advisor Meeting 3 11
  - 10/13/2023 Advisor Meeting 4 12
  - 10/27/2023 Advisor Meeting 5 13
  - 11/17/2023 Advisor Meeting 6 14
  - 12/01/2023 Advisor Meeting 15
- Design Process 16
  - 09/22/2023 Product Design Specifications 16
  - 11/01/2023 Meeting 17
  - 11/03/2023 Show and Tell 18
  - 2023/11/06-Team Meeting 19
  - 11/13/2023 Whole Team Meeting 20
  - 11/20/2023 Whole Team Meeting 2 21
  - 11/27/2023 Whole Team Meeting 3 22
- Materials and Expenses 23
  - 10/11/2023 Initial Materials List 23
  - 11/02/2023 Updated Materials List 24
  - 12/07/2023 Final Materials List 25
- Fabrication 26
  - 09/14/2023 GelMA Hydrogel via Pipette/Molds Co-Culture Protocol 26
  - 09/17/2023 Team Bioprinter Orientation 27
  - 09/24/2023 GelMA Print 1 Team Meeting 30
  - 10/01/2023 Team Bioprinter Troubleshooting 31
  - 10/08/2023 GelMA Bioprinting Thermal Insulator Attempt 32
  - 10/15/2023 GelMA Bioprinting (Droplet Printing) 33
  - 10/22/2023 Team Meeting 34
  - 2023/11/05- GelMA Bioprinter Group Meeting 35
  - 2023/11/12 - Bioprinter Sub-Team Meeting 36
  - 12/12/2023 Bioprinter Optimization Summary 39
- Testing and Results 40
  - Protocols 40
    - 12/03/2023 ImageJ Cell Counting Protocol 40
    - Rheology Protocol Updated - 12-12-23 41
    - 12/13/23 Live / Dead Staining Protocol 43

Experimentation 45
11-29-2023 - Fibroblast encapsulation Round 2 45
12-1-23 - Rheology/Cell Group meeting 46
12/12/2023 Rheology Testing Semester Summary 47
12/12/2023 Gel Imaging Semester Summary 48
Project Files 51
10/06/2023 - Preliminary Presentation 51
10/11/2023 - Preliminary Report 52
12/08/2023 - Poster Presentation 53
12/13/2023 - Final Report 54
Carley Schwartz 55
Research Notes 55
Biology and Physiology 55
Bioprinting 55
GelMA Bioink LAP .25% 55
2023/10/05-GelMA Protocol and My Notes 56
2023/10/4 Bioprinting Research 57
2023/09/15-Biofabricating Airway Model Notes 58
2023/11/30-Bioprinter Notes 61
Biological Assays 62
2023/10/11- Live/dead Cell Staining for embedded cells 62
2023/10/11 - Cell Culture Info 63
2023/10/09- Cell Marker for Fibrotic Expression in Fibroblasts and Epithelial Cells 64
Summer GelMA Notes/Trials 65
GelMA Bioprinter Notes 1 65
2023/12/13-End of Semester Reflection 67
Design Ideas 69
2023/10/25 GelMA Droplet Bioprinting 69
2023/11/30 GelMA Fabrication Protocol 70
Elijah Diederich 74
Research Notes 74
Bioprinted GelMA Hydrogels - Intro Research 74
Direct-Write Bioprinted GelMA Hydrogels 75
3D Bioprinting Strategies - Lung/ECM 77
PrestoBlue - Cell Viability Assay 78
Fibroblast Encapsulation - GelMA and Collagen Hydrogels 79
Fibroblast Encapsulation - Acrylated Alginate Hydrogel 80
Fluid Transport Analysis - Collagen/Matrigel Hydrogel 81
Collegenase Protocol - GelMA 82
Collagenase Protocol - GelMA/PEGDA Hydrogel 83
Design Ideas 84
Cellink GelMA Variations 84
Caitriona Treacy 87
Research Notes 87
Biology and Physiology 87
9/28/2023 - 3D Printed GelMA Scaffolds 87
10/4/2023 - Baseline Stiffness ECM Modulates 90
10/26/2023 - Fibroblast encapsulation in gelatin methacryloyl 91
11/2/2023 - Guidelines for Cell Viability Assays 92
11/12/2023 - Calcein Staining in GelMA 96
12/4/2023 - Collagenase degradation of GelMA 99
11/10/2023 - Tong Lecture Series 102
William Onuscheck 103
Research Notes 103
Bioprinting 103
09/13/2023 Notes on CellLink Application Note: Biofabricating Airway Epithelial Models for COVID-19 Research 103
09/15/2023 Notes on Bioprinting - General 106
Anuraag Shreekanth Belavadi 109
Research Notes 109
Biology and Physiology 109
Cell Viability 109

11/27/2023 Update- 10/20/2023 Cell Viability Assays Introduction 110
11/27/2023 Update- 10/28/2023 Comparison of Trypan Blue Dye Exclusion and Fluorometric Assays for Mammalian Cell Viability Determination 111
11/27/2023 Update- 10/28/2023 Use of GelMA for 3D printing of cardiac myocytes and fibroblasts 114
Bioprinting 115
09/20/2024_Bioprinting Standards 115
09/24/2023_3D Bioprinted GelMA Based Models for the Study of Trophoblast Cell Invasion 116
09/16/2023_GelMA Bioprinting Applications in TE 117
10/08/2023_Protocols of 3D Bioprinting of Gelatin Methacryloyl Hydrogel Based Bioinks 118
10/11/2023_Gelatin-Methacryloyl (GelMA) Hydrogels for 3D Cell Culture and Extrusion Bioprinting 119
11/27/2023 Updated GelMA synthesis Protocol 120
Training Documentation 121
09/24/2023 Training Documentation 121
Nick Herbst 122
Research Notes 122
09/09/2023 Lung Extracellular Matrix Background Research 122
09/09/2023 Bioprinting Lung Tissue 124
09/16/2023 CELLINK Bioprinter 128
09/21/2023 Bioprinting GelMA 131
09/29/2023 GelMA Bioink SDS and Specifications 133
10/17/2023 Cell Viability Assays in 3D Hydrogels 135
10/30/2023 Bioprinting With Fibroblasts 137
11/11/2023 Mixing Bioink and Cells 139
12/02/2023 Hydrogel Scaffold Porosity for Lunge Model 141
Design Ideas 143
09/20/2023 GelMA Options 143
10/23/2023 Effect of Cells in Bioink 144
11/01/2023 Current State of Affairs 145
11/05/2023 Scaffold Design 1 149
Training Documentation 150
09/09/2023 Prior Completed Trainings 150
2014/11/03-Entry guidelines 153
2014/11/03-Template 154

Team contact Information

Nick Herbst - Sep 08, 2023, 2:24 PM CDT

Last Name	First Name	Role	E-mail	Phone	Office Room/Building
Puccinelli	Tracy Jane	Advisor	tjpuccinelli@wisc.edu	608-265-8267	2158 ECB
Brasier	Allan	Client	abrasier@wisc.edu	N/A	4246 Health Sciences Learning Center
Schwartz	Carley	Co-Leader	cischwartz@wisc.edu	630-621-7554	N/A
Diederich	Elijah	Co-Leader	ediederich@wisc.edu	920-517-9419	N/A
Treacy	Caitriona	Communicator	ctreacy2@wisc.edu	262-599-4130	N/A
Onuscheck	Will	BSAC	onuscheck@wisc.edu	847-868-5393	N/A
Shreekanth Belavadi	Anuraag	BWIG	shreekanthbe@wisc.edu	224-300-9722	N/A
Herbst	Nick	BPAG	nherbst2@wisc.edu	920-493-0020	N/A

Project description

Nick Herbst - Sep 13, 2023, 8:26 PM CDT

Course Number: BME 400

Project Name: Tissue Model of the Epithelial Mesenchymal Trophic Unit

Short Name: Tissue Model

Project description/problem statement: A multitude of chronic lung diseases such as pulmonary fibrosis, asthma, and chronic obstructive pulmonary disease (COPD) can cause damage to epithelial tissues of the lungs. This presents a problem because when this tissue is damaged, a fibrotic response is triggered in sub-epithelial fibroblasts that results in further disease and fibrosis. There are currently no tissue models that accurately recreate the lung extracellular matrix and its changes due to cell injury. Such a model would need to have tunable mechanical stiffness and porosity, as well as be cell adhesive and degradable. Dr. Brasier of the UW School of Medicine and Public Health requires a scaffold that meets these criteria to be fabricated with a bioprinter. The scaffold must have a uniform and replicable composition that allows for epithelial cell culture in an air-liquid-interface (ALI) so that his lab can study the effects of fibrosis on small-airway lung epithelial cells.

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

10/05/2023 Client Meeting 1

Nick Herbst - Oct 05, 2023, 11:20 AM CDT

Title: Client Meeting 1

Date: 10/05/2023

Content by: Nick

Present: Nick, Will, Dr. Brasier, Dianhua

Goals: Update Dr. Brasier on the current status of the project

Content:

- Told Dr. Brasier about how Carley trained us on bioprinter
- Dr. Brasier and Dianhua thinks that incorporating fibroblasts will tune stiffness and help with cell adhesion
- Asked Dianhua if he can provide 3T3 fibroblasts for us to see cell viability/cytotoxicity
    - He said needs heads up (1 week in advance)
    - Dr. Brasier only wants us to add the fibroblasts once we can get healthy and fibrotic stiffness scaffolds
        - Negates advisor's suggestion that the team divide and conquer by having some people take GelMA sample and test cell viability while printing optimization is still ongoing
- Dr. Brasier said to add the fibroblasts as soon as we can reliably print
- They say we need to keep it sterile in the printer
    - Can print directly into 12 or 24 well plate

Action Items:

- present preliminary presentation
- work on preliminary report
- try to print GelMA again, making sure to use the insulator nozzle and make sure that the yellow and/or red lights are *on* when using the temperature-controlled printhead

10/19/2023 Client Meeting 2

Nick Herbst - Oct 19, 2023, 11:23 AM CDT

Title: Client Meeting 2

Date: 10/19/2023

Content by: Nick

Present: Nick, Will, Dr. Brasier, Dianhua

Goals: Update Dr. Brasier on the current status of the project and discuss cell viability testing

Content:

- Told Dr. Brasier that we can print GelMA but not consistently and we are looking into splitting the team into bioprinting and cell viability
- Discussing calcein / propidium iodine staining OR degrade gel and typan blue
- Dianhua
- Brasier lab does flow cytometry for live/dead
    - contact Melissa from Brasier lab because Dianhua says we can't do it
- Brasier suggests dissolving gels and counting cells
    - collagenase then trypan blue for cell counting
- Asked Dianhua to prepare cells for us
    - 3T3 grow fast, epithelial cells are slower
    - he will tell us the day before they are ready
- Got Dr. Master's old UV light for making GelMA gels by hand for cell viability

Action Items:

- send Dr. Brasier information on purchasing collagenase
- obtain cells from Dianhua
- half team is cell viability, half is bioprinter

11/02/2023 Client Meeting 3

Nick Herbst - Nov 02, 2023, 11:16 AM CDT

Title: Client Meeting 3

Date: 11/02/2023

Content by: Nick

Present: Nick, Anuraag, ~~Dianhua~~

Goals: Update Dianhua on the current status of the project

Content:

- Dr. Brasier had a conflict so the meeting was supposed to be with Dianhua
- Planned on telling Dianhua that the bioink had a shelf life of 2 months so the bioink we are currently using (which is about a year old) is not good anymore
    - **Dianhua did not show up, so after 15 minutes of waiting without hearing from him the team decided to end the Zoom meeting**

Action Items:

- Have BPAG organize materials to order and have Communicator request materials from client
- Present at Show and Tell

11/16/2023 Client Meeting 4

Nick Herbst - Nov 16, 2023, 3:58 PM CST

Title: Client Meeting 4

Date: 11/16/2023

Content by: Nick

Present: Will, ~~Dianhua, Dr. Brasier~~

Goals: Update Dr. Brasier on the current status of the project

Content:

- Planned on asking Dr. Brasier about what to do regarding the bioprinter and ask Dianhua for a status update about the materials shipment
    - **Neither of them showed up**

Action Items:

- Email Dr. Brasier to ask the questions we wanted to ask during the client meeting

11/20/2023 Client Meeting 5

ANURAAG SHREEKANTH BELAVADI - Nov 20, 2023, 11:36 AM CST

Title: Whole Group Client Meeting

Date: 11/20/2023

Content by: Anuraag

Present: Carley, Caitriona, Will, Nick

Goals: Discuss ongoing challenges with regard to construction happening in the client's lab, scope of project moving forward, and discussion regarding communication moving forward.

Content:

- The client isn't willing to move the 3D printer during construction

- Wants to focus addressing the sterility issues with the GelMA

- Try to have one person (preferably Will or Carley due to extensive cell-culture experience) work on making the matricies to avoid mistakes that could cause sterility issues

- The team is allowed to work with the bioprinter around the construction schedule

- The team will perform cell culture using the 3T3 cells in ECB with bench-made GelMA to narrow down the sterility

- Discussed having Dr. Brasier join in on bioprinting trials (hopefully reliable printing) after construction in order for the lab to have a better understanding of how the team has worked to use the bioprinter appropriately.

Conclusions/action items:

Update team members who were not present about the discussion, work on drafting an email or setting-up a meeting with the client to convey better communication needs, and plan on developing a schedule to work around the construction schedule.

09/15/2023 Advisor Meeting 1

ELIJAH DIEDERICH (ediederich@wisc.edu) - Sep 15, 2023, 1:38 PM CDT

Title: Advisor Meeting 1

Date: 09/15/2023

Content by: Nick

Present: Carley, Elijah, Caitriona, Will, Anuraag, Nick

Goals: Update our new advisor on the current state of our project and the planned future directions

Content:

- Need to keep it a design process
  - Cellink has different kinds of GelMA ink, can do design matrix for bioink
  - Can explore structure/morphology of print
  - Optimize nozzle issues (not heated, gelation occurs in tip)
- Look at BME 402 report requirements to get head start and know what we need to do for the spring
  - no design matrix, yes PDS
- Presentation is what we have done, what we need to work on, what we are doing now, and have a clear plan
- Grading
  - notebook can be looked over quickly before grading
  - need consistent and thorough research
  - need team meetings
  - everyone needs to talk during meetings
- Next week advisor meeting 2158 ECB 12:30 on Wednesday

Action Items:

- Go to Dr. Brasier's lab to learn how to operate the bioprinter
- Begin work on the PDS (due end of next week)

09/20/2023 Advisor Meeting 2

Nick Herbst - Sep 20, 2023, 1:40 PM CDT

Title: Advisor Meeting

Date: 9-20-2023

Content by: Elijah Diederich

Present: Nick, Elijah, Will, Caitriona

Goals: Discuss how bioprinter training went with out advisor

Content:

- Come up with clear plan so that when we present in two weeks, it is very clear. Go with 402 requirements for preliminary presentation

- Have draft of plan and presentation done for next weeks meeting

- Tracy will be available Wednesday of presentation week for meeting

- Look at evaluation form for 402

- Make sure to include brief summary of previous work in preliminary presentation

- Get PDF of BioX printer and send to Tracy Puccinelli

- See if tech support can come to the lab to help us with clogging issue and UV light placement; if not call tech support to get started

    - Also use user's manual

- Month at a time for plan on presentation; just this semester plan (3 slides)

Conclusions/action items:

1. Continue to work on PDS this week

2. Make plan and presentation for next week meeting

09/29/2023 Advisor Meeting 3

WILLIAM ONUSCHECK - Sep 29, 2023, 12:33 PM CDT

Title: Advisor Meeting 3

Date: 9-29-2023

Content by: William Onuscheck

Present: Nick, Elijah, Will, Caitriona, Anaruug

Goals: Establish expectations for prelim presentations, update on printing

Content:

- Managing timeline expectations from a perspective of optimism vs what would be realistic to accomplish

  -Ere on the side of optimism

- Update on printing issues

  - Print pressure errors

  - Crosslinking issues associated with the temperature that the GelMA prints at

  - Have to find a low enough print temperature that won't kill cells, allow for smooth printing of GelMA

  - Try water bath

- Establish contact with tech line

- Add stats to broader impact, image there

- Image of previous semesters design

-Concurrent cell viability - cytotoxicity for LAP, UV, GelMA

Conclusions/action items:

Finish final draft of the presentation. Plan out cytotoxicity tests / cell viability tests (split group into cell focused vs printer focused?).

10/13/2023 Advisor Meeting 4

ANURAAG SHREEKANTH BELAVADI - Oct 13, 2023, 12:45 PM CDT

Title: Advisor Meeting 4

Date: 10/13/2023

Content by: Anuraag Belavadi

Present: Will, Nick, Elijah, Carly, Caitriona

Goals: Update advisor on bioprinting progress, discuss cell viability, and go over presentation.

Content:

- Went over Presentation comments

- Reduce the amount of information; focus on the information that we need feedback on from peers

- Part of the presentation needs figures updated on the slides (Figures on the Background and Broader Impact Slides)

Went over progress in bioprinting

- 37C to liquefy, 27C to print

- Longer wait time to reach lower printing temperature (More than 5 mins).

- Planned for droplet-based printing and establishing parameters for the next team meeting

- The client wants to focus on GelMA printing and fibroblast encapsulation before cell viability assays.

- Tell the client that cell viability needs to be addressed before/regardless of bioprinting progress

Cell Viability

- Culture in GelMA solution

- Try different concentrations of cells embedded.

- Adjust the time between mixing cells in GelMA solution and UV light exposure

- Update advisor on details of upcoming client meeting.

Conclusions/action items:

Figure out the logistics of viability testing before the next client meeting and continue adjusting the parameters of bioprinting to address encapsulation of fibroblasts.

10/27/2023 Advisor Meeting 5

ANURAAG SHREEKANTH BELAVADI - Nov 01, 2023, 1:59 PM CDT

Title: Advisor meeting

Date: 10/27/2023

Content by: Anuraag Shreekanth Belavadi

Present: Nick, Will, Catriona

Goals: Discuss progress made with making gels, bioprinting progress, and details regarding Show and Tell

Content:

- Move forward with Calcine staining for cell viability

- Progress for Crosslinking can be improved with less time in the fridge, shorter crosslinking, time

- Discussion regarding CELLINK GelMA concentration

  - Concentration is fixed and as a result, crosslinking efforts need to be addressed using other faactors

- Need to make more progress in terms of fibroblast encapsulation (Client Dependent)

- Discussed conversations and next steps following recent client meetings

Show and Tell

- Focus on tailoring show and tell to receive feedback on what needs improvement
- Discuss and refine what the scope of the project is within the team before preparing for Show and Tell
- No cartridges or gels required

Conclusions/action items:

Refine the crosslinking process and focus on enhancing fibroblast encapsulation to improve the progress of the project and prepare for show and tell.

11/17/2023 Advisor Meeting 6

Nick Herbst - Nov 17, 2023, 2:12 PM CST

Title: Advisor Meeting 6

Date: 11/17/2023

Content by: Nick

Present: Carley, Caitriona, Will, Anuraag, Nick

Goals: Update our new advisor on the current state of our project and ask for advice with current challenges

Content:

- We gave Tracy a status report
  - Dianhua wants to indefinitely pause the project
  - Dr. Brasier gave us a heads up about the construction only 2 days before hand
  - Client has not shown up for the past 2 scheduled client meetings
  - Out of GelMA bioink and have not heard any status update on that shipment
  - Dr. Masters' GelMA is likely contaminated
- Tracy's feedback
  - possibly make own gelatin methacryloyl
  - Try to have an in-person meeting
    - If can't, then have Tracy intervene
  - If everything goes south
    - abandon ship and get new project
    - continue design project (but modified) with department as client
  - For deliverables, go deeper theoretically/hypothetically with printed scaffold
    - porosity, architecture

Action Items:

- Schedule in-person meeting with Brasier
  - Reestablish expectations/directions
  - Be very assertive and persistent
- Make own GelMA
- Look into structure of scaffold

12/01/2023 Advisor Meeting

ANURAAG SHREEKANTH BELAVADI - Dec 01, 2023, 2:09 PM CST

Title: Advisor Meeting 7

Date: 12/01/2022

Content by: Anuraag

Present: Elijah, Will, Caitriona, Nick

Goals: Discuss and get advice on the poster presentation and receive feedback on the preliminary presentation

Content:

- Try live/dead assay with gels intact (Quantifiable)

- Try a lower seeding density

- Do percent live/dead based on ImageJ analysis

- Probably look into other literature in terms of seeding density protocol

- If we do cell counts again, try to get qualitative data/ pictures

- Make at least 6 gels along with practice with the three cell-laden gels to try to get data

- Get an update on whether the calcein stain is available in the teaching lab.

- Iron out the protocol

Conclusions/action items:

09/22/2023 Product Design Specifications

Nick Herbst - Nov 01, 2023, 1:41 PM CDT

Title: Product Design Specifications

Date: 09/22/2023

Content by: Everyone

Present: Everyone

Goals: Complete the PDS

Content:

- We need to update the PDS from last semester

- Since the project has taken a new direction, we also need to modify the PDS to be relevant for bioprinting

- See the attached file for the full PDS

Action items:

- Complete preliminary presentation

- Work on preliminary deliverables

Nick Herbst - Oct 03, 2023, 4:44 PM CDT

Tissue Model of The Epithelial Mesenchymal Trophic Unit

BME Design

Date: September 22, 2023
BME 400

Product Design Specification

Client: Dr. Allan Brasier
Advisor: Dr. Tracy Jane Puccinelli

Team Members:
Carley Schwartz cischwartz@wisc.edu (Co-Leader)
Elijah Diederich ediederich@wisc.edu (Co-Leader)
Caitriona Treacy ctreacy2@wisc.edu (Communicator)
William Ornscheck ornscheck@wisc.edu (BSAC)
Amirag Shreekanth Belavadi shreekanthbel@wisc.edu (BWIG)
Nick Herbst nherbst2@wisc.edu (BPAG)

Download

BME_400_PDS.pdf (222 kB)

11/01/2023 Meeting

WILLIAM ONUSCHECK - Nov 01, 2023, 3:23 PM CDT

Title: November 11th Meeting

Date: November 11th

Content by: William Onuscheck

Present: Caitriona, Elijah, Anuraag, Will

Goals: Refocus project

Content:

Split team into

Cell Viability

Conclusions/action items:

11/03/2023 Show and Tell

CARLEY SCHWARTZ - Nov 06, 2023, 5:50 PM CST

Title: Show and Tell

Date: 11/03/2023

Content by: Everyone

Present: Everyone

Goals: Present at Show and Tell session and ask peers for advice/feedback

Content:

- We presented to various design teams during Show and Tell and asked for advice

- Peer comments:

    - we got comments that calcein staining works well from a couple people that work with hydrogels (they specifically said this with respect to PEG gels)

    - we didn't get a ton of advice on the bioprinter and how to handle troubles but thats expected as it is very niche

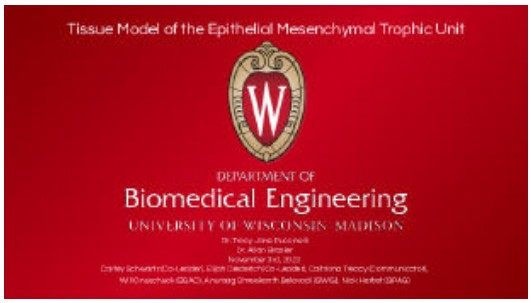
- See the attached file for slides used during Show and Tell

Action items:

- Continue optimizing bioprinting

- Another group will be working on cell viability assays

Nick Herbst - Nov 06, 2023, 1:57 PM CST



Download

Show_and_Tell.pdf (573 kB)

2023/11/06-Team Meeting

CARLEY SCHWARTZ - Nov 06, 2023, 5:57 PM CST

Title: Team Meetings

Date: 11-6-23

Content by: Carley

Present: Whole Team

Goals: To discuss activities between subgroups

Content:

- Nick, Carley and Caitriona discussed their hedge way on the bioprinter and how they tried doing cylindrical printing rather than droplet and it has more potential but similar flow rate issues
- Anuraag, Elijah, and Will discussed how they plan to do fibroblast embedding on wednesday and trypan blue staining on friday

Conclusions/action items:

To continue subgroup work, specifically move forward with fibroblast embedding plans and for bioprinting attempt to use CAD file or molds to help control for overflow

11/13/2023 Whole Team Meeting

Nick Herbst - Nov 13, 2023, 5:50 PM CST

Title: Whole Team Meeting

Date: 11/13/2023

Content by: Nick

Present: Everyone

Goals: To discuss activities between subgroups

Content:

- Printer group (Nick, Carley, Caitriona)
    - Tried GelMA printing again, mixed results
    - First attempt had big overflow and was stiff (looked like UFO) so decided to have 20 min equilibration time rather than 15 min
        - also decreased pressure by 2 kPa and decreased photocrosslinking time by 2 sec
        - While setting up for next trial, turned temp up briefly before waiting to equilibrate to prevent too high viscosity
    - Second attempt was going very very good but then all of a sudden stopped because the cartridge ran dry
        - Currently can not print more until get new GelMA shipment
    - Saw construction schedule: construction done 12/6
- Cell group (Will, Anuraag, Elijah)
    - Made gels with molds and Dr. Masters' GelMA and added fibroblasts in it and swelled it in media
        - photocrosslinking was *very* weird, took much longer than it should have
    - Good cell viability 24 hr after
    - After weekend, gels contaminated but 3T3 flask was fine
        - Dr. Masters' GelMA is most likely contaminated
- Discussed making own GelMA from Masters' old equipment
    - We could make it and then somehow get it into
- Sterility of GelMA
    - Autoclave: Carley found a study that it does make it sterile but the hydrogels that come from it are lower elastic modulus
        - Gamma radiation: sterilizes but gives higher elastic modulus
    - Re-lyophilize?

Conclusions/action items:

- Things to address in client meeting (Nick and Will)
    - bioink shipment update
    - bioprinter access (ask after we know shipping date)
    - Dianhua wants to give tutorial on BSC
- Come up with list of equipment to make our own GelMA

11/20/2023 Whole Team Meeting 2

Nick Herbst - Nov 20, 2023, 5:51 PM CST

Title: Whole Team Meeting 2

Date: 11/20/2023

Content by: Nick

Present: Nick, Caitriona, Will, Elijah

Goals: Update the rest of the team on the in person client meeting

Content:

- Told Elijah and Will about how the meeting with Dr. Brasier went
  - Dr. Brasier does not want us to move the bioprinter
  - He really emphasized the sterility issue
- Possibly do gels with 511's 3T3 cells with Dr. Masters' GelMA in ECB
- GelMA bioink shippment has arrived
- Forgot to ask Dr. Brasier about why he hasn't come to the last 2 client meetings
  - Last client meeting of the semester will be 11/30

Conclusions/action items:

- Figure out plan for making own GelMA
- Make gels in ECB

11/27/2023 Whole Team Meeting 3

Nick Herbst - Nov 27, 2023, 6:02 PM CST

Title: Whole Team Meeting 3

Date: 11/27/2023

Content by: Nick

Present: Nick, Caitriona, Will, Elijah, Anuraag, Carley

Goals: Discuss goals leading up to the poster presentations next week

Content:

- Discussed if we should try to methacrylate our own GelMA before the poster presentation
  - It is a ~2 week process but we don't have the time
  - Decided to wait
  - **ALSO,** the materials were stored in improper conditions in Dr. Brasier's lab so they might not be of good quality
- Will suggested that our results for the final deliverables should focus on cell viability
  - daily time points of of 3T3s on gels using Masters' gels
  - look at different stiffness affect on viability
- 3T3 culture in ECB need some TLC since media wasn't changed over Thanksgiving break
  - Will is taking care of the cell culture
- Discussed poster
  - Design section split to talk about bioprinted gels and mold-made gels and reasons for using each
  - Have a longer future work section

Conclusions/action items:

- Wednesday go into ECB and make gels in the afternoon
  - Will, Elijah, Anuraag
- Ask Dr. P for replacement bulbs of UV light or a different UV light
  - If not, then buy a 365-405 nm UV nail light
- Start working on the poster
  - get some done for advisor meeting
- Client meeting this Thursday
  - send reminder this Wednesday
  - discuss communication issues with client meeting absences
  - mention moving onto poster work, plan methacrylating own GelMA for next semester, doing gel and cell work at ECB

10/11/2023 Initial Materials List

Nick Herbst - Nov 02, 2023, 11:18 AM CDT

Title: Initial Materials List

Date: 10/11/2023

Content by: Nick

Present: Everyone

Goals: Make an expenses table for the preliminary report

Content:

Item	Description	Cost/Quantity
Fall 2022/Spring 2023 Items	Unusable PEG materials and GelMA materials	$1,091
Bioprinter	CELLINK Bio X 3D Bioprinter	Provided by client
GelMA Bioink	CELLINK GelMA Bioink LAP 0.25% 3mL Cartridge	Provided by client
Temperature-Controlled Printhead	CELLINK Temperature-Controlled Printhead	Provided by client
Nozzle Cover	CELLINK Thermal Insulator Nozzle Cover	Provided by client

We have $3,909 left from our initial $5,000 budget

Action items:

- work on preliminary deliverables

11/02/2023 Updated Materials List

Nick Herbst - Nov 02, 2023, 11:29 AM CDT

Title: Updated Materials List

Date: 11/02/2023

Content by: Nick

Present: Everyone

Goals: Update the expenses table

Content:

Item	Description	Cost
Fall 2022/Spring 2023 Items	Unusable PEG materials and GelMA materials	$1,091
Bioprinter	CELLINK Bio X 3D Bioprinter	Provided by client
GelMA Bioink	CELLINK GelMA Bioink LAP 0.25% 3mL Cartridge	Provided by client
Temperature-Controlled Printhead	CELLINK Temperature-Controlled Printhead	Provided by client
Nozzle Cover	CELLINK Thermal Insulator Nozzle Cover	Provided by client
GelMA Bioink	CELLINK GelMA Bioink LAP 0.25% 3mL Cartridge	$325
Collagenase	Collagenase to degrade GelMA gels	$70

We have $3,514 left from our initial $5,000 budget

Action items:

- show and tell session

12/07/2023 Final Materials List

Nick Herbst - Dec 07, 2023, 11:31 AM CST

Title: Final Materials List

Date: 12/07/2023

Content by: Nick

Present: Everyone

Goals: Finalize the Materials List

Content:

Item	Description	Cost/Quantity
Fall 2022/Spring 2023 Items	Unusable PEG materials and GelMA materials	$1,041
Molds	Silicone molds to make GelMA hydrogels by hand	$50
GelMA	Lyophilized GelMA gifted by Dr. Masters	Provided by former advisor
Bioprinter	CELLINK Bio X 3D Bioprinter	Provided by client
GelMA Bioink	CELLINK GelMA Bioink LAP 0.25% 3mL cartridge x3	Provided by client
Temperature-Controlled Printhead	CELLINK Temperature-Controlled Printhead	Provided by client
Nozzle Cover	CELLINK Thermal Insulator Nozzle Cover	Provided by client
GelMA Bioink	CELLINK GelMA Bioink LAP 0.25% 3mL cartridge x3	$325
Collagenase	25 mg collagenase to degrade GelMA gels	$70
LIVE/DEAD Viability Kit	Calcein/Ethidium stains for cell viability	Provided by BME Teaching Lab
Total		$1,486

We have spent $1,486 so far and we have $3,514 left from our original $5,000 budget

Action items:

Final deliverables

09/14/2023 GelMA Hydrogel via Pipette/Molds Co-Culture Protocol

Nick Herbst - Sep 20, 2023, 1:51 PM CDT

Title: GelMA Hydrogel via Pipette/Molds Co-Culture Protocol

Date: 9/14/23 (Summer work)

Content by: Carley Schwartz

Present: Carley

Goals: To establish co-culture protocol for hydrogels made by hand

Content:

1. Set water bath to 50 ℃
2. Measure out 50 mg of GelMA and place in 5ml sterile tube
3. Add 850µL of PBS and place in water bath
4. While GelMA is dissolving, prepare a 20 million cells/ml stock solution
    1. This will mean that you had 2 million cells being placed into GelMA and if homogenous there will be 200,000 cells embedded into each hydrogel
5. Sanitize molds with ethanol and UV light [Maximum 5 minutes, can deform the molds]
6. Press silicone molds to a petri dish, making sure that the seal is tight to prevent polymer solution from leaking
7. Get dissolved GelMA solution from water bath
8. Moving quickly to prevent temperature dependent gelation of the GelMA solution, add 50 µL of LAP and 100 µL cell solution and mix well
9. Place 100 µL of solution into each 9mm silicone mold
10. Place gels under UV light
    1. Used bioprinter UV light with settings of 405 nm wavelength and 1 minute of setting with 5 cm distance
11. Place gels in well with media and incubate overnight to allow for swelling

- Protocol above will make 10 100 µL gels

Conclusions:

These gels became contaminated during the incubation time and were unfit for cell culture.

09/17/2023 Team Bioprinter Orientation

WILLIAM ONUSCHECK - Sep 17, 2023, 6:45 PM CDT

Title: Team Bioprinter Orientation

Date: September 17th, 2023

Content by: William

Present: Carley, William, Anuraag, Nick, Caitriona

Goals: Carley teaches the team how to use the bioprinter, its features, parameters, difficulties, etc.

Content:

-Print integrity and success is determined mainly by honing print speed and extrusion pressure

-Bioprinter is capable of being loading with three materials simultaneously,

-UV light for crosslinking must be centered manually

-UV crosslinking may be done layer by layer, or as a whole body at the end

-Theoretical printer resolution is not accurate due to sagging / gel behavior of printed material.

-Bioprinter has pre loaded geometries and infills

Figure 1: Brainstorming

Figure 2: Teaching

Conclusions/action items:

The team should begin research and design on optimal geometries to model airway epithelium. The team should also spend time determining optimal printing parameters for GelMA. Experimental determination of a reasonable actual resolution would be beneficial for future design as well. The team has requested key card access to the lab as well.

09/24/2023 GelMA Print 1 Team Meeting

Caitriona Treacy - Sep 24, 2023, 11:10 AM CDT

Title: "WIMR Team Meeting"

Date: 2022/09/24

Content by: Caitriona Treacy

Present: Nick Herbst, Elijah Diederich, Carley Schwartz, Caitriona Treacy

Goals: GelMA Printing Tutorial

Content:

- Heat Up the GelMA. Amber cartridge is photo blocking (won't cross-link). Apply the 22 gauge tip (blue). Take off cap on top, apply printing top piece with tube which attaches to the printer. Insert into temperature controlled ink head.
- Tighten the base adjustment on the ink head. Temperature set 35˚. Wait for cartridge to heat up. Will take at least 25 mins. You can view cartridge temperature in the print toolbar.
- Droplet printing = simple structure similar to when we were doing it by hand. Droplet print asks for how many wells. Select 6 well if using petri dish.
- Bioprint –> 3D Models –> 10x10x1 mm –> Surface –> Petri dish –> Printer –> Clean chamber Fan ON –> Tool 1 –> Temperature Controlled tool type –> Print profile –> GelMA 1 (gives presets for this material)
- Crosslinking –> for 1 mm thickness 405nm for 10 seconds at a distance of 5 cm
- Layers –> Grid Lattice - Rectilinear
- Print –> Menu bar in corner, check through tools before the run to make sure it's all set correctly.
- Do the homing: Sidebar –> Move
- 25-27˚ temperature range once you start to print.
- Test flow: 25 kPa. Temperature decreased to the recommended 27˚C. Calibrate with printer door open. Put calibration pin in vertical position. xy-auto calibrate. Adjust the plate in the z axis manually. Start print.
- Error found in pressure. Had to change tip and therefore recalibrate printer. Pressure tested print head. Tested flow. No movement of GelMA.

What has been unsuccessful:

- 25˚
- Droplet bioprinting n 6 well. UV 405 10 seconds distance of 5 cm. Test flow shot out, got stuck in tube. Nozzle switched after. 30 ish kPa and let it run. Got cloggled within the 10 seconds of UV light.
- Repeated with Dianhua to demonstrate the issues. Contacted the field scientist and the authors of the paper provided by Dr. Brasier.
- Co-culture model on GelMA got contaminated.

Conclusion/Action Items: Established that for any future prints using GelMA, at least 3 team members must be present. The hope here is that this will allow for more thoughtfully-completed prints and fewer repeats in protocols that may be unsuccessful. Nick located a nozzle insulator to keep the ink in the printer tip heated. Next trial should utilize the nozzle insulator to keep the tip from clogging.

10/01/2023 Team Bioprinter Troubleshooting

Nick Herbst - Oct 08, 2023, 10:31 AM CDT

Title: Team Bioprinter Troubleshooting

Date: 10/01/2023

Content by: Anuraag and Nick

Present: Nick, Caitriona, Anuraag

Goals: attempt to print with the insulator nozzle

Content:

- Attached thermal insulation nozzle cover following user manual

- tried to find the water bath to warm the GelMA

- The temperature controlled printhead did not heat up, so the bioink didn't go to solution

    - blue light means on, yellow is warming, red it hot

Conclusions/action items:

- attempt printing again while being sure that the temperature controlled printhead is properly working

- find the water bath

10/08/2023 GelMA Bioprinting Thermal Insulator Attempt

Nick Herbst - Oct 08, 2023, 11:18 AM CDT

Title: GelMA Bioprinting Thermal Insulator Attempt

Date: 10/08/2023

Content by: Nick

Present: Nick, Caitriona, Will, Elijah, Carley

Goals: Attempt to print with the insulator nozzle and temperature-controlled printhead

Content:

- Attempted to use the water bath, but there were no stands so we were worried about the water would contaminate the bioink
    - decided to continue to heat to 37 C with the temp-controlled printhead
- With the nozzle and the heating, the bioink melted and was very very fluid (low viscosity) at 37 C
- preemptively de-clogging with needle nozzle in between test flows
- Settings: 27 C and 25 kPa, 20 sec 405 nm photocrosslinking, single layer 1cm^2 lattice
    - didn't wait long from switch form 37 to 27, so bioink still liquid-y
        - printed, but very very low viscosity so the lines spread-out/dispersed/transversely propagated into a blob
    - printed blob had high stiffness (determined by touch)

Conclusions/action items:

- print again but give bioink more time to reach 27 after heating to 27
- concretely figure out temp, pressure, and phototime to get a droplet
    - do stiffness vs time curve w rheometry testing
- cooling the printbed might help with the spreading of the printed lines
- future future work, stain cultured epithelial cells for biomarker

10/15/2023 GelMA Bioprinting (Droplet Printing)

ANURAAG SHREEKANTH BELAVADI - Nov 01, 2023, 1:51 PM CDT

Title: Droplet Printing

Date: 10/15/2023

Content by: Anuraag

Present: Anuraag, Caitriona, Will, Elijah, Carley

Goals: Establish a replicable printing protocol using droplet printing tests to be carried forward and applied to bioprinting of the GelMA scaffold.

Content:

- Discussed outreach activity and presentation requirements

- Attempted to print GelMA

Conclusions/action items:

10/22/2023 Team Meeting

CARLEY SCHWARTZ - Oct 22, 2023, 10:57 AM CDT

Title: Team meeting covering

Date: 10-22-23

Content by: Carley Will Nick

Present: Carley Will Nick

Goals: Cell viability assay discussion

Content:

- Discussed plans for how to take GelMA from cell link to create molds and also create them with old GelMA
  - Needed to consider how to remove GelMA properly from cell link container
- Plans to characterize both with rheometer before doing cell viability assays
  - Want to compare cell link and our Gelma following the same pipette method protocol and UV time (using Master's UV light) n
- Planning to meet again this week to work on bioprinter
- Will's halloween costume plans

Conclusions/action items:

Overall made progress on GelMA cell link characterization plans and cell viability discussion after client meeting

2023/11/05- GelMA Bioprinter Group Meeting

CARLEY SCHWARTZ - Nov 05, 2023, 11:19 AM CST

Title: Bioprinter Subgroup Meeting

Date: 11-05-23

Content by: Carley

Present: Carley, Caitriona, Nick

Goals: To discuss gcode file for bioprinting set structure and attempt using bioprinter setting

Content:

Gcode file: Cylindrical structure, infill density over 50% will get the inside of the structure to be completely filled (concerns with GelMA not being solid enough and spreading)

Trouble-shooting could involve creating a gcode file that is the exact dimensions of the mold we have previously used, print into this, and photocrosslink which would prevent any spreading.

Used concentric cylinder(10-1), 40% infill, 22 kPa pressure, with a pre-flow time of 50 ms and it formed a very large glob

used concentric cylinder(5-03), 25% infill, 19 kPa pressure, with a pre-flow of 70 ms and it formed nothing

Conclusions/action items:

cylinder could work but will need to figure out optimimized infill density and sizing with speed and pressure

2023/11/12 - Bioprinter Sub-Team Meeting

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 01, 2023, 1:47 PM CST

Title: Bioprinter Team Meetings

Date: 11-12-23

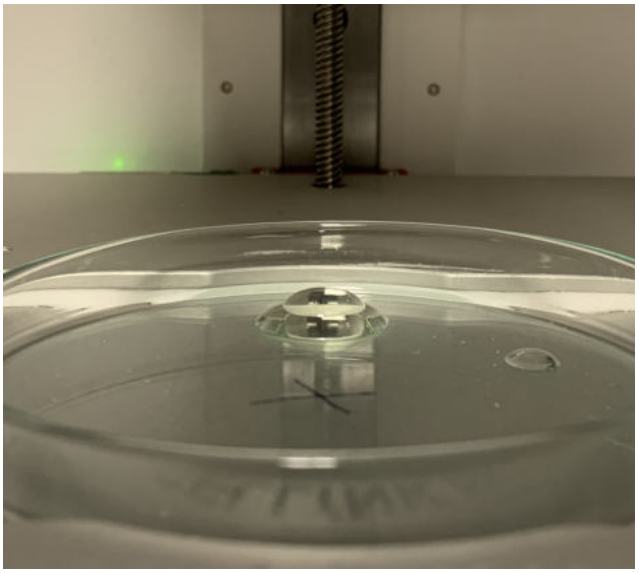
Content by: Caitriona

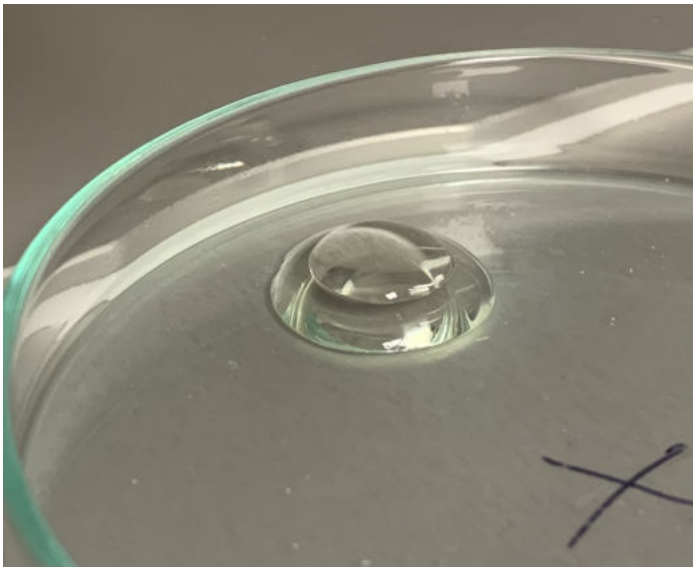
Present: Nick & Caitriona

Goals: Conduct another trial of GelMA bioprinting, specifically using varying infill densities.

Content:

GelMA was first heated to 37˚C, checked for liquid state visually, and temperature was then lowered to 27˚C. A time interval of 15 minutes passed before printing began to allow for uniform cooling of the GelMA to occur. The first trial used 25% infill density, 33 kPa. Stiffness was not quantitatively tested, but upon removal from the petri dish, it was observed to have a very high stiffness, well above the desired value.

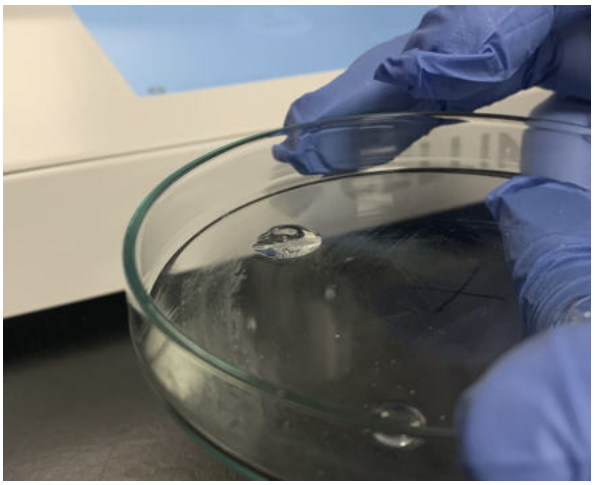


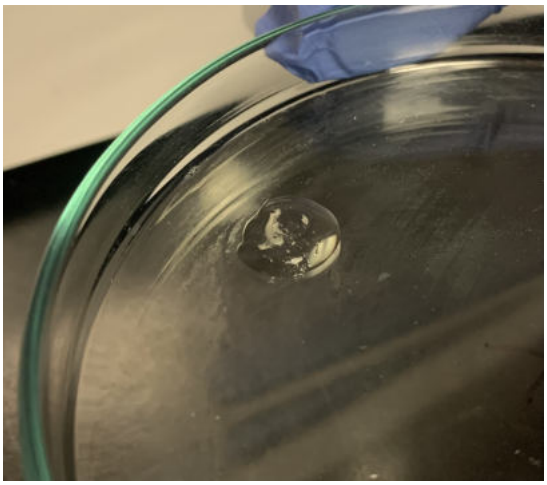


Pictured above are photos of the first trial, from different angles. As can be seen in the images, two layers were printed (10mmx1mm cylinder), but the bottom layer experienced a high level of spreading whereas the second layer did not.

Team discussion led to the belief that 15 minutes is not a sufficient cooling time – the viscosity of the GelMA leaving the cartridge was much too low, contributing to the spreading that occurred in the bottom layer and an excess of GelMA being dispensed.

Crosslinking time was shortened, and an additional five minutes passed to allow for GelMA cooling. The result was inconsistent GelMA dispensing patterns (as can be seen in the trial 2 photos with the imperfectly shaped circular cross-section below).





Conclusions/action items: This was the final print that could be done before the new order is delivered. Now, our subgroup should focus on design elements that do not directly use the printer until this delivery. The CAD file that was developed and loaded onto the printer specifically for this design was oriented incorrectly with what is desired. This can be easily modified while the team waits for supply delivery.

12/12/2023 Bioprinter Optimization Summary

Nick Herbst - Dec 12, 2023, 4:37 PM CST

Title: Bioprinter Optimization Summary

Date: 12/12/2023

Content by: Nick

Present: Nick, Carley, Caitriona

Goals: Summarize bioprinting trials that was done throughout the semester in an effort to optimize bioprinting

Content:

Print Trial	Structure	Infill Density	Print Temperature	Equilibrium Time	Extrusion Pressure	Success
1	Cubic	15%	27℃	2-3 min	35 kPa	-/-
2	Cubic	15%	27℃	2-3 min	35 kPa	-/-
3	Cubic	15%	27℃	2-3 min	27 kPa	-/-
4	Cubic	15%	27℃	10 min	30 kPa	-/-
5	Cylindrical	15%	28℃	10 min	33 kPa	-/-
6	Cylindrical	15%	27℃	10 min	27 kPa	-/-
7	Cylindrical	60%	28℃	15 min	23 kPa	-/-
8	Cylindrical	50%	28℃	20 min	25 kPa	-/-
Final	Cylindrical	35%	28℃	20 min	22 kPa	+/-

Conclusions:

- This entry serves as an overarching summary of all of the fabrication entries throughout the semester
- While not noted in the table, UV time was around 4-5 seconds for each trial except the final trial, which was 2 seconds
    - Will need to do longer in future
- Several runs were done for each trial
- Success was determined by the gel having the designed printed structure and by the gel having the desired stiffness. Success in both of these metrics is denoted by +/+ while failure in both is denoted by -/-.
- The final trial was the only one to have good structure and was actually testable, which is likely due to the new GelMA and longer equilibrium time

12/03/2023 ImageJ Cell Counting Protocol

Nick Herbst - Dec 12, 2023, 3:31 PM CST

Title: ImageJ Cell Counting Protocol

Date: 12/03/2023

Content by: Nick

Present: Nick, Caitriona, Carley

Goals: Use this protocol with ImageJ to count calcien-stained cells

Content:

1. Check your options: Edit → Options → Conversions → Scale when converting
2. Change the image to greyscale: Image → Type → 16-bit
3. Threshold the image: Image → Adjust → Threshold
    1. Check Dark Background and Red
    2. Move lower and upper bounds such that the threshold box is in the upper tails of the value curve and the bright stained nuclei are highlighted
    3. Click Apply
4. Count the cells: Analyze → Analyze Particles
    1. Set the size range to about 25-100 sq pixels (may need to set it to 20-100)
    2. Set the circularity to about 0.5-1.0 (
    3. Select Outlines in the Show tab
    4. Check Display Results, Clear Results, and Summarize
    5. Click OK
5. Save the Summary table as a .csv file (the Count column gives the number of calcien-stained nuclei)

Conclusions:

- See link for protocol source: https://cpb-us-w2.wpmucdn.com/voices.uchicago.edu/dist/c/2275/files/2020/01/cell_counting_automated_and_manual.pdf
- This protocol assumes that the bright stained dots are the nuclei of live cells, and also only uses the live cell images (not the dead cell images) so the data can only be compared between conditions and not used as a true measure of viability
- **UPDATE:** This protocol may not be completely relevant because what I counted were not nuclei; the staining went wrong

Rheology Protocol Updated - 12-12-23

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 12, 2023, 7:57 PM CST

Title: Rheology Protocol

Date: 12-12-23

Content by: Elijah Diederich

Present: Myself

Goals: To inform fellow notebook readers about the rheology protocol used

Content:

Frequency Sweep Rheometry Protocol

1. Once GelMA hydrogels have been formed and allowed to set and swell for approximately 24 hours, rheometry testing may be performed.
2. Carefully remove 3-4 hydrogels of each type; healthy lung ECM and fibrotic lung ECM, keeping the gels of the same type in their respective petri 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, 3-4 hydrogels of each type were tested for a total of 6-8 separate frequency sweeps.
11. When testing is completed, results can be interpreted in Excel.

Conclusions/action items:

1. Proofread final report sections

2. Finish client evaluation

12/13/23 Live / Dead Staining Protocol

Nick Herbst - Dec 13, 2023, 5:17 PM CST

Title: Live dead staining protocol

Date: 12/13/2023

Content by: Will

Goals: Use this protocol to perform live / dead imaging

Content:

Sample preparation

1. Aspirate media from scaffold wells
2. Wash multiple times with PBS

Staining

The following should be done under dark conditions, or shielded from light exposure as best as possible.

1. Add 1µM, 5µM calcein-AM, ethidium homodimer
2. Allow to incubate of 15 minutes at 37°C

Imaging

The following should be done under dark conditions, or shielded from light exposure as best as possible.

1. Image scaffold under using multichannel imaging of FITC and TRITC filters
2. Adjust exposure and filter cutoffs as necessary to reduce background noise,

Reference:

Cohen, Noam et al. “PEG-fibrinogen hydrogel microspheres as a scaffold for therapeutic delivery of immune cells.” Frontiers in bioengineering and biotechnology vol. 10 905557. 9 Aug. 2022, doi:10.3389/fbioe.2022.905557

11-29-2023 - Fibroblast encapsulation Round 2

ELIJAH DIEDERICH (ediederich@wisc.edu) - Nov 29, 2023, 1:09 PM CST

Title: Fibroblast encapsulation ECB

Date: 11-29-2023

Content by: Elijah Diederich and Will Onuscheck

Present: Elijah, Will

Goals: To attempt fibroblast encapsulation in Master's (Old Advisor) GelMA

Content:

1. Goal of today is to create 3 batches of GelMA hydrogels with varying stiffness to determine and quantify cell viability of varying stiffness's.

- Cells are at a concentration at 4.5 x 10 ^9/ml. This equates to 450,000 cells per fabricated hydrogel. Cells were counted using a microscope and counting squares.

- GelMA hydrogel fabrication protocol found in appendix C (preliminary/final report) was used to fabricate gels

- To vary gel stiffness, fridge times were varied from 3,5, and 10 minutes. UV time was consistent at 5 minutes.

- Gels look fantastic after UV light and placed into culture media. Gels were then placed into incubator shortly after.

Conclusions/action items:

1. Rheology of Gels (1 gel/batch)

2. Image degraded gels to determine viability after 24 hours

12-1-23 - Rheology/Cell Group meeting

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 01, 2023, 1:16 PM CST

Title: Cell group Meeting

Date: 12-1-23

Content by: Elijah Diederich

Present: Will, Elijah, Caitriona

Goals: To check on seeded hydrogels and perform DAPI staining to draw conclusions on if gels are supporting cells

Content:

11-30-23:

Will came into lab and did degradation of one of each groups (cross-linking conditions) of gels, noticed what appeared to be dark blotches (spots) in each gels

Some cells inside appeared to have proper morphology but others appeared to be floating

12-1-23:

Elijah and Will both present in lab

The same observations were noticed compared to 11-30-23. Some cells still appeared to be 'floating' but others had spindly-like fibroblast morphology

Elijah also confirmed the presence of dark blotches in the gels (pictures taken on microscope)

A decision was made to do DAPI staining on one gel per group to determine the viability of cells --> Got good pictures, to identify what is alive and what is crap, we then degraded the gels with collagenase. Pictures were also taken of these results. Caitriona also made an appearance during this portion.

These protocols can be found in the appendix of the final report (also in lab archives)

Gels were degraded in each group of crosslinking conditions and images taken. Team is unsure how to interpret these and will bring them to advisor meeting.

Conclusions/action items:

1. Advisor meeting

2. Keep last gel to determine next steps

3. Get ready for final poster presentation next week

12/12/2023 Rheology Testing Semester Summary

Nick Herbst - Dec 12, 2023, 4:00 PM CST

Title: Rheology Testing Semester Summary

Date: 12/12/2023

Content by: Nick

Present: Elijah and Will

Goals: Summarize the mechanical testing of the hydrogels that was done throughout the semester

Content:

Pipette-Based Hydrogels		
Gel Batch	Condition (4 °C, UV)	Young's Modulus (kPa)
Normal Batch 1	3 min, 5 min	4.2 ± 0.92
Normal Batch 2	5 min, 5 min	6.63 ± 2.6
Normal Batch 3	10 min, 5 min	13.24 ± 2.8
Fibrotic Batch 1	5 min, 7 min	49.65 ± 22
Fibrotic Batch 2	5 min, 10 min	277 ± 155

Bioprinted Hydrogels		
Gel Batch	**Condition (UV)**	**Young's Modulus (Pa)**
Normal Batch 1	2 seconds	995 ± 915

Conclusions:

- The Young's Modulus was determined though relating the storage modulus of the gels (obtained from rheology) to the assumed Poisson's ratio of 0.5 (used for all hydrogels)
- Rheology testing was done for multiple batches of pipette-based hydrogels but only for one batch of bioprinted hydrogels
- Notebook entries were not maintained in real time for the results, but data/results were maintained in the team's shared files. This entry serves as an over-arching summary
- Much longer UV time is needed for the bioprinted gels to reach the desired stiffnesses, which will be done this upcoming semester

12/12/2023 Gel Imaging Semester Summary

Nick Herbst - Dec 12, 2023, 4:29 PM CST

Title: Gel Imaging Semester Summary

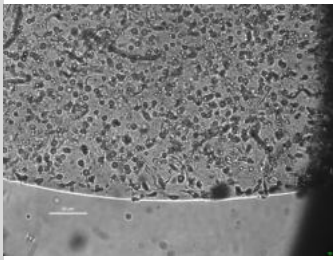
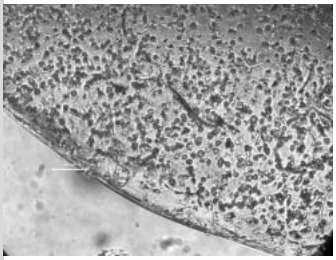
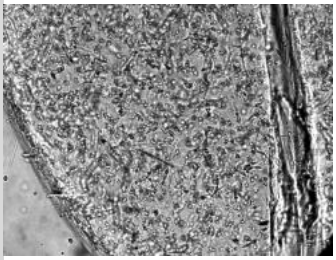
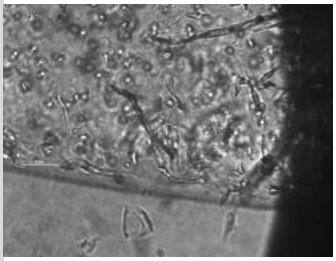
Date: 12/12/2023

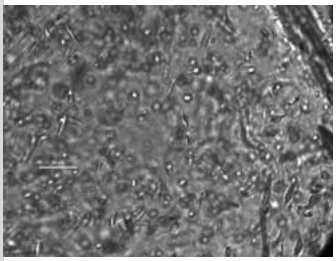
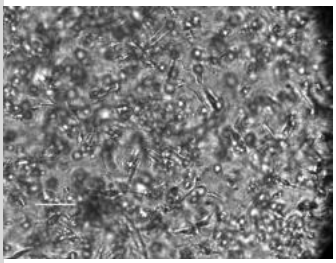
Content by: Nick

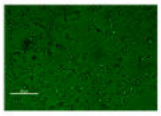
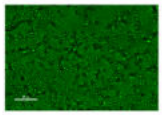
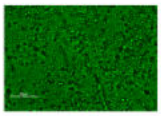
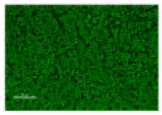
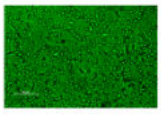
Present: Will, Elijah, Anuraag

Goals: Summarize the imaging and attempted cell viability testing of the hydrogels that was done throughout the semester

Content:

Zoom	Timepoint	Image
10x	24 hours	
	48 hours	
	72 hours	
20x	24 hours	

ZoomTimepoint	Image
48 hours	
72 hours	

Stiffness (kPa)	24hr Cell Count	24hr Images	48hr Cell Count	48hr Images
4.2 ± 0.92	275		430	
6.63 ± 2.6	398		696	
13.24 ± 2.8	644		965	

Conclusions:

- The team's advisor said that the collagenase + trypan blue method for cell viability was suspicious so the team moved to LIVE/DEAD staining with calcein and ethidium, but those images came out unusable
- ImageJ cell counting protocol was used to count the "alive" "cells" in from the LIVE/DEAD stain, but it turns out the green dots weren't live cells since calcein-positive cells should be completely green, not just a dot within the cell. Also, no ethidium staining showed up at all ever
    - The team needs to identify a new cell viability method that will work properly with the cell-in-gel system
- Brightfield images of the cells in gels were taken to qualitatively observe morphology of the cells, only top layer looked fibroblastic/spindly
- Notebook entries were not maintained in real time for the results, but data/results were maintained in the team's shared files. This entry serves as an over-arching summary

10/06/2023 - Preliminary Presentation

Nick Herbst - Nov 01, 2023, 1:40 PM CDT

Title: Preliminary Presentation

Date: 10/06/2023

Content by: Everyone

Present: Everyone

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

Content:

- See attachment for full presentation slides

Action items:

- Work on preliminary report

Nick Herbst - Nov 01, 2023, 1:40 PM CDT

Download

BME_400_Preliminary_Presentation.pdf (1.4 MB)

10/11/2023 - Preliminary Report

Nick Herbst - Dec 12, 2023, 3:28 PM CST

Title: Preliminary Report

Date: 10/11/2023

Content by: Everyone

Present: Everyone

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

Content:

- See attachment for full report

Action items:

- Work on other preliminary deliverables

Nick Herbst - Nov 01, 2023, 1:41 PM CDT

Tissue Model of the Epithelial Mesenchymal Trophic Unit

BME Design

Date: October 11, 2023
BME 400

Preliminary Report

Client: Dr. Allan Brasier
Advisor: Dr. Tracy Jane Puccinelli

Team Members:
Carley Schwartz cschwartz@wisc.edu (Co-Leader)
Elijah Diederich ediederich@wisc.edu (Co-Leader)
Caitriona Treacy ctreacy2@wisc.edu (Communicator)
Will Orascheck orascheck@wisc.edu (BSAC)
Amrrag Shreekanth Belavadi shreekanthbel@wisc.edu (BWIG)
Nick Herbst nherbst2@wisc.edu (BPAG)

Download

BME_400_Preliminary_Report.pdf (2.24 MB)

12/08/2023 - Poster Presentation

Nick Herbst - Dec 08, 2023, 2:47 PM CST

Title: Poster Presentation

Date: 12/08/2023

Content by: Everyone

Present: Everyone

Goals: Present our design project to our peers at a poster session

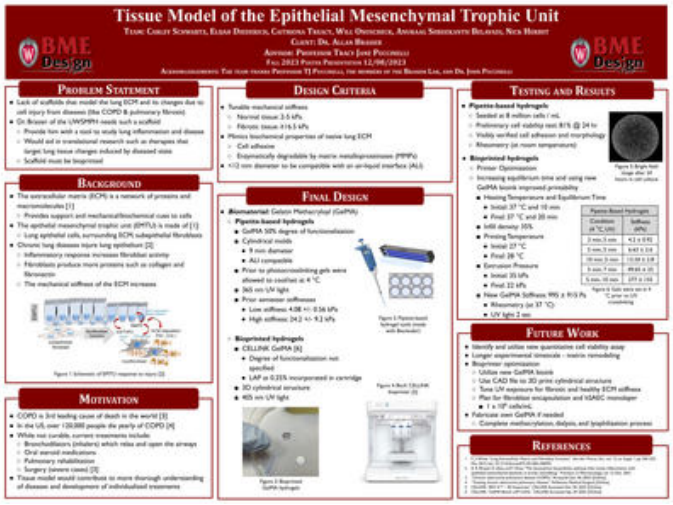
Content:

- See attachment for full poster

Action items:

- Work on final report

Nick Herbst - Dec 08, 2023, 2:47 PM CST



Download

BME_400_Poster.png (1.32 MB)

12/13/2023 - Final Report

Nick Herbst - Dec 12, 2023, 3:28 PM CST

Title: Final Report

Date: 12/13/2023

Content by: Everyone

Present: Everyone

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

Content:

- See attachment for full report

Action items:

- Work on other final deliverables

Nick Herbst - Dec 13, 2023, 5:31 PM CST

Tissue Model of the Epithelial Mesenchymal Trophic Unit

BME Design

Date: December 13, 2023
BME 400

Preliminary Report

Client: Dr. Allan Brasier
Advisor: Dr. Tracy Jane Puccinelli

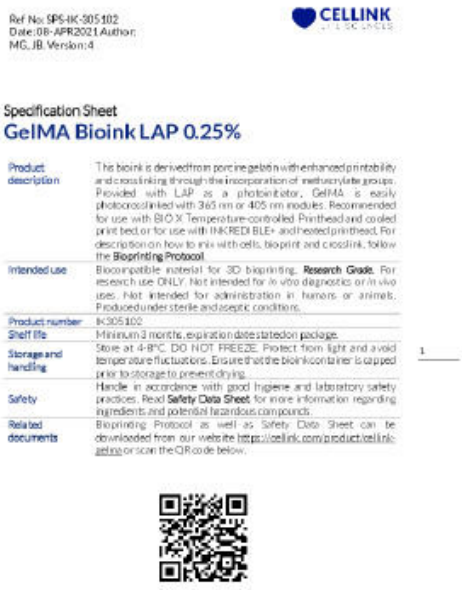
Team Members:
Carley Schwartz cjschwartz@wisc.edu (Co-Leader)
Elijah Diederich ediederich@wisc.edu (Co-Leader)
Caitriona Treacy ctreacy2@wisc.edu (Communicator)
Will Orrascheck orrascheck@wisc.edu (BSAC)
Amrrag Shreekanth Belavadi shreekanthbel@wisc.edu (BWIG)
Nick Herbst nherbst2@wisc.edu (BPAG)

Download

BME_400_Final_Report.pdf (3.01 MB)

GelMA Bioink LAP .25%

CARLEY SCHWARTZ - Oct 11, 2023, 11:32 AM CDT



Ref No: SPS-IK-305102
Date: 08-APR2021 Author: MG, JB Version: 4

CELLINK

Specification Sheet

GelMA Bioink LAP 0.25%

Product description	This bioink is derived from porcine gelatin with enhanced printability and crosslinking through the incorporation of methacrylate groups. Provided with LAP as a photoinitiator, GelMA is easily photocrosslinked with 365 nm or 405 nm modules. Recommended for use with BIO X Temperature-controlled Printhead and cooled print bed or for use with INKREDIBLE+ and heated printhead. For description on how to mix with cells, bioprint and crosslink, follow the **Bioprinting Protocol**.
Intended use	Biocompatible material for 3D bioprinting. **Research Grade.** For research use ONLY. Not intended for *in vitro* diagnostics or *in vivo* uses. Not intended for administration in humans or animals. Produced under sterile and aseptic conditions.
Product number	IK305102
Shelf life	Minimum 3 months, expiration date stated on package.
Storage and handling	Store at 4-8°C. DO NOT FREEZE. Protect from light and avoid temperature fluctuations. Ensure that the bioink container is capped prior to storage to prevent drying.
Safety	Handle in accordance with good hygiene and laboratory safety practices. Read **Safety Data Sheet** for more information regarding ingredients and potential hazardous compounds.
Related documents	Bioprinting Protocol as well as Safety Data Sheet can be downloaded from our website https://cellink.com/product/cellink-gelma or scan the QR code below.

1

Download

Specification-Sheet-GelMA-Bioink-LAP-0.25_08-Apr-2021-1-4_1_.pdf (107 kB)

2023/10/05-GelMA Protocol and My Notes

CARLEY SCHWARTZ - Oct 11, 2023, 11:38 AM CDT

Title: CELLINK PROTOCOL GELMA

Date: 10-5-23

Content by: Carley

Present: Self

Goals: To make notes on protocol based on experiences

Content:

Based on previous experiences with the bioprinter I think the most difficult aspect of the protocol is the timing and temperature. The extrusion pressure is relatively accurate at the given as well as their other provided parameters such as nozzle recommendations. I think that the temperature warming of GelMA needs to be at least 30 minutes and then once switching to 26-27 for printing we need to wait 15-20 min again because while the print head may be at that temperature the GelMA solution will take longer to adjust. I also believe that our preliminary structures should focus on

Conclusions/action items:

Plan to adjust protocol to these temperature based timing changes in next GelMA trial.

CARLEY SCHWARTZ - Oct 11, 2023, 11:38 AM CDT


CELLINK

BIOPRINTING PROTOCOL

GelMA Bioink

This is a suggested procedure, please adjust according to your experimental needs.
To maintain the sterility of the product work under sterile conditions.

Protocol aim

Materials needed

Download

Bioprinting-Protocol-GelMA-Bioink_7-Feb-2023.pdf (104 kB)

2023/10/4 Bioprinting Research

CARLEY SCHWARTZ - Oct 04, 2023, 5:56 PM CDT

Title: Bioprinter Ideas

Date: 10-04-23

Content by: Carley Schwartz

Present: Carley Schwartz

Goals: To learn more about bioprinting

da Rosa, N. N., Appel, J. M., Irioda, A. C., Mogharbel, B. F., de Oliveira, N. B., Perussolo, M. C., Stricker, P. E. F., Rosa-Fernandes, L., Marinho, C. R. F., & de Carvalho, K. A. T. (2023). Three-Dimensional Bioprinting of an In Vitro Lung Model. *International journal of molecular sciences*, *24*(6), 5852. https://doi.org/10.3390/ijms24065852

Content:

- They used A549 cells, adenocarcinomic alveolar epithelial cells from humans, to establish a 3D bioprinted structure, which they infected with influenza A virus. Also used 549 cells, with MRC5 (human lung fibroblasts) and EA.hy926 (human endothelial cells), for the development of a triple-layered alveolar lung model that simulates the blood–air barrier system.
- After differentiation, an immunocytochemistry assay was performed on the lung spheroids and the cells that remained adhered to the plate during differentiation
- Furthermore, a live/dead assay was performed after 24, 48, and 72 h of bioprinting, and the structures presented a cell viability of 92.15%, 92.02%, and 91.55%, respectively, proving the biocompatibility of the bioink

Conclusions/action items:

Provides some ideas for potential assays to conduct for assessment of the success of the hydrogel. This could be useful later on when deciding what we want our live/dead assay protocol to be for assessing cell viability and compatibility of GelMA.

2023/09/15-Biofabricating Airway Model Notes

Title: Biofabricating airway epithelial models for covid-19 research

Date: 09-15-23

Content by: Carley

Goals: To learn more about a successful model made on the same 3D printer

Content:

Note: this article is only available if subscribed to the bioprinter cellink website, Dr. Brasier printed this out for me so I currently do not have a link or a way to site this article

Article Information

Title: Biofabricating Airway Epithelial Model for COVID-19 Research

Author(s): Himjyot Jaiswal

Publication: Cellink

Date Accessed: 09-15-23

Introduction

- Wanted to create a 3D model for understanding the disease mechanisms of COVID-19
- They focused on bioprinting primary human bronchial epithelial (Calu-3 cells) to generate an ALI
    - Bioprinted models were cultured in transwell insert for 14 days (similar to what we want)

Method

3D structure	Designed as a disk with a solid bottom layer and 5 layered brim [created this way to facilitate entrapment of post-seeded cells]
Bioink	Cellink GelMA-Laminin 521
Cells/Protocol	Fibroblasts (2*10^6 m resuspended in 100 uL of media and placed into 1 mL of GelMA bioink), this mixture was pre-warmed to 37C and transferred to amber cartridge to prevent LAP from reacting. Bioprinting temp was 26-27 C for their GelMA Photocured for 10 s at 405nm with distance of 5cm Disks left to incubate for 3 days in fibroblast growth media at 37C For adding lung epithelial layer: mixed fibroblast to epithelial media at 1:9 ratio and cultured the disks and cells submerged in this mixture for 4 days and then transferred to ALI (transwell inserts).

Results

1. Successful had polarization of cells in ALI by analyzing expression of ACE2 at the apical membrane of the culture

Conclusions/action items:

This is a good start for us to base some ideas off of and may be very useful when designing a 3D structure for the cells to be seeded in.

2023/11/30-Bioprinter Notes

CARLEY SCHWARTZ - Nov 30, 2023, 1:43 PM CST

Title: Bioprinter Trial Notes

Date: 11-30-23

Content by: Carley

Present: Self

Goals: To one see if we can work around construction in room and two to locate the new materials and make sure everything is ready for our use.

Content:

Bioprinter room is abit of a mess but still useable and new GelMA cartridges have arrived and are ready for use. I ran through the calibration cycle on the bioprinter and did a practice run through with the sample ink. Practiced with some new setting for cylindrical shapes and plan on bringing in one of our new extra molds to see if this will help with retaining structure.

Conclusions/action items:

Want to practice more cylindrical structures so may consider using a mold to help hold the form. Might also need to make the speed on the printer to be slightly faster to reduce spreading before UV time.

2023/10/11- Live/dead Cell Staining for embedded cells

CARLEY SCHWARTZ - Oct 11, 2023, 12:47 PM CDT

Title: Live/dead assay for embedded cells

Date: 10-11

Content by: Carley

Present: Self

Goals: To determine appropriate live/dead assay for embedded cells

Content:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6696429/

In this study they were using a chitosan and collagen hydrogel with fibroblasts embedded in the matrix. When attempting to do typical live/dead staining on hydrogels the gels themselves would absorb the staining dye as well as the cells making it difficult to tell the difference during quantification processes. Hydrogels perform well using the Click-iT or Base-Click proliferation stains. You have to pre-incubate the gels in a nucleotide solution prior to staining. You can stain for the presence of Caspase-3 with an antibody guaranteed for IF or ICC. They then did imaging of these scaffolds.

Proliferation and myofibroblast differentiation are important modifiers of collagen matrix contraction . To test for proliferation, aFPCM were incubated for 3 h in the presence of 10 μM ethynyl deoxyuridine (EdU; a modified nucleotide; BaseClick; Millipore Sigma, ST. Louis, MO, USA) and half-volume of fresh media plus treatments prior to fixation. To properly evaluate the nature of myofibroblasts and their proliferative activity on a three-dimensional level, control and treatment aFPCM were fixed with 4% paraformaldehyde and fluorescently stained with anti-α-smooth muscle actin (α-SMA, clone 1A4), Baseclick-EdU 594, and DAPI (4′,6-diamidino-2-phenylindole; Millipore Sigma). Previously prepared aFPCM were separated into halves by use of a scalpel. Halves were carefully transferred to microcentrifuge tubes containing phosphate-buffered saline. Preceding the fluorescent staining, tissue was treated with -20 °C methanol for 10 min, which precipitated the proteins and opened the cellular membranes. Shortly thereafter, the tissues were submerged with 100 μL goat serum/PBS (1:10) followed by the primary antibody; alpha smooth muscle actin/PBS azide (1:500) overnight at 4 °C. After PBS washes, goat anti-mouse IgG Alexa 488/PBS azide (1:200), working as the secondary antibody, was introduced to the lattices for 45 min. Detection of proliferative activity within cells was achieved through Baseclick-Edu 594 stain for 30 min. The DAPI/PBS azide (2:1000) stain was then administered to the lattices, wherein all nucleated cells absorbed the highly specific blue-fluorescent dye excited by 405 nm laser light.

Following the fixation and staining procedure, the matrices were prepared for analysis under fluorescent microscopy. Prior to mounting the matrices on microscope slides, 80% glycerol in PBS at 4 °C was applied to each microcentrifuge tube containing the matrices for 10 min. Tissues were teased out of the microcentrifuge tube with forceps and deposited on the microscope slides containing 40 μL of previously placed glycerol/PBS. Then, 22 mm2 coverslips placed over the flattened tissues were sealed with coverslip sealant and allowed to dry before microscopy or storage at −20 °C.

Conclusions/action items:

This is useful for our project as we would likely run into similar issues with the GelMA based hydrogel with live/dead staining of embedded cells. This provides a way to overcome this potential issue later one.

2023/10/11 - Cell Culture Info

CARLEY SCHWARTZ - Oct 11, 2023, 12:46 PM CDT

Title: Cell Culture Info on 3T3s and hSAECs.

Date: 10-11-23

Content by: Carley

Present: Self

Goals: To have info on cell lines being used in these experiments.

Content:

3T3: NIH/3T3 is a fibroblast cell line that was isolated from a mouse NIH/Swiss embryo. This cell line is a multiclonal population. Adherent growth properties, DMEM is typical media with serum (this is also what Brasier lab uses) [https://www.atcc.org/products/crl-1658]

hSAECs: Primary Small Airway Epithelial Cells; Normal, Human (HSAEC) is a cell line that provides an ideal cell system to propagate small airway epithelial cells in serum-free conditions when grown in Airway Epithelial Cell Basal Media. Adherent growth properties. [https://www.atcc.org/products/pcs-301-010]

The results showed that the SFM enabled fibroblast and epithelial cell proliferation while maintaining a morphology, cell size and metabolism similar to those of FBSCM. SFM has repeatedly been found to be better suited for epithelial cell proliferation and clonogenicity. [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9455993/#:~:text=The%20results%20showed%20that%20the,epithelial%20cell%20proliferation%20and%20clonogenicity.]

Conclusions/action items:

When doing a co-culture of both of these cell lines we need to consider what media we want to use. Will it be combination of both or due to hSAECs not being cultured with serum will this impact things.

2023/10/09- Cell Marker for Fibrotic Expression in Fibroblasts and Epithelial Cells

CARLEY SCHWARTZ - Oct 11, 2023, 12:55 PM CDT

Title: Cell based assays for fibrotic tissue state

Date: 10-9-23 (I edited on 10-11 too)

Content by: Carley

Present: Self

Goals: To learn more about markers of cell lines under fibrotic conditions

Content:

We describe high-content screening based phenotypic assays with primary normal human lung fibroblasts and primary human airway epithelial cells. For both cell types, TGFβ-1 stimulation is used to induce fibrotic phenotypes *in vitro*, with alpha smooth muscle actin and collagen-I as readouts for FMT and E-cadherin as a readout for EMT.

Mechanistically, epithelial to mesenchymal transition (EMT) is one of the major drivers of fibrosis, and approximately 30% of ECM-producing (myo-)fibroblasts derive from epithelial cells through EMT. During EMT, tightly organized epithelial cells lose expression of the tight junction marker E-cadherin and transform to mesenchymal cells with expression of the mesenchymal cell markers N-cadherin, vimentin and fibronectin.

Fibroblast to myofibroblast: Antibodies targeting collagen-I and aSMA, each diluted 1:1000 were added to the cells for 1 h at 37°C after an additional wash step with PBS. The anti-fibronectin antibody was used in a final concentration of 0.05 µg/ml. Primary antibodies were removed by 3 wash steps with PBS and the secondary antibodies labeled with AF568 for the anti-collagen-I- and anti-fibronectin-IgG1 and AF647 for the anti-aSMA-IgG2a diluted 1:1000 were incubated for 30 min at 37°C together with the nuclear dye Hoechst. Antibodies were removed by 3 washes with PBS and cell cytoplasm was stained by addition of 1:50000 diluted HCS CellMaskGreen dye for 30 min at ambient temperature. After an additional PBS washing step, plates were sealed and images were acquired with an IN Cell Analyzer 2200. Nuclear, cytoplasm, aSMA and collagen-I or aSMA and fibronectin images were acquired in the DAPI, FITC, Cy7 and Cy5 channels, respectively.

Epithelial to mesenchymal: Following 3 washes with PBS, non-specific antibody binding was blocked by incubation with 2%BSA in PBS for 30 min at ambient temperature. The blocking solution was removed by 3 wash steps with PBS, and the cells were incubated with the primary mouse-anti-E-cadherin antibody (final concentration 0.4 µg/ml) for 1 h at 37°C. Three washes with PBS were followed by an incubation with the secondary AF568-labed goat anti-mouse-IgG1 (1:1000 diluted) for 1 h at 37°C. Following 3 PBS wash steps, cells were permeabilized with 0.1% Triton-X-100 for 20 min at ambient temperature, washed again and incubated for 30 min at ambient temperature with HCS CellMask Green (1:50000 diluted). Images were acquired with a PerkinElmer Opera Phenix High Content Screening System, after a final wash step and plate sealing. Nuclear, cytoplasm and Ecadherin images were acquired in the Hoechst, AF488 and AF568 channels, respectively.

Conclusions/action items:

This article gives possible protocols or at the very least markers we could use to determine if they hydrogel stiffness is triggering fibrotic phenotype in the cells and the healthy tissue is providing healthy cell phenotype. These will need to be adapted to take into consideration the hydrogel.

GelMA Bioprinter Notes 1

CARLEY SCHWARTZ - Sep 15, 2023, 8:20 AM CDT

Download

IMG_5228.HEIC (2.66 MB)

CARLEY SCHWARTZ - Sep 15, 2023, 8:20 AM CDT

Download

IMG_5229.HEIC (2.89 MB)

CARLEY SCHWARTZ - Sep 15, 2023, 8:20 AM CDT

Download

IMG_5230.HEIC (2.8 MB)

CARLEY SCHWARTZ - Sep 15, 2023, 8:20 AM CDT

Download

IMG_5231.HEIC (2.23 MB)

CARLEY SCHWARTZ - Sep 15, 2023, 8:25 AM CDT

Title: Summer notes while working on the bioprinter

Date: 09-15-21

Author: Carley Schwartz

Content:

Each image is note from a trial run of creating GelMA hydrogels on the bioprinter.

The main content of these is unsuccessful trials runs but could be used to know what not to do when attempting again.

Conclusion:

Overall, the GelMA gets clogged very easily in the bioprinter nozzle tip. This is because the tip isn't temp controlled like the rest of the cartridge is in the printer head. This could mean that we need to switch out the nozzle between each dispensing run. Additionally, need to determine set pressure, speed, and other parameters depending on the shape we want to create.

2023/12/13-End of Semester Reflection

CARLEY SCHWARTZ - Dec 13, 2023, 7:03 PM CST

Title: End of Semester Reflection

Date: 12/13/23

Content by: Carley

Content:

Bioprinting:

I believe that are final print trial with the parameters attached as a note are promising to build off of to achieve both high and low stiffness. Concerns still present for me is the clogging issues in the print head which is currently being mitigated by changing the head between each print run or declogging with sterilized needle. This functions well now but clogging could increase when cells are encapsulated in the solution.

The true goal is to create protocols that the client can follow when we graduate, I hope that after meeting post break with Dr. Brasier to have him sit through a bioprinting trial he will understand that difficulties encountered and overcame. I also believe he may feel that the his own lab will have difficulties in the future as well and may wish to have the project continued for further optimization with a new engineering design team.

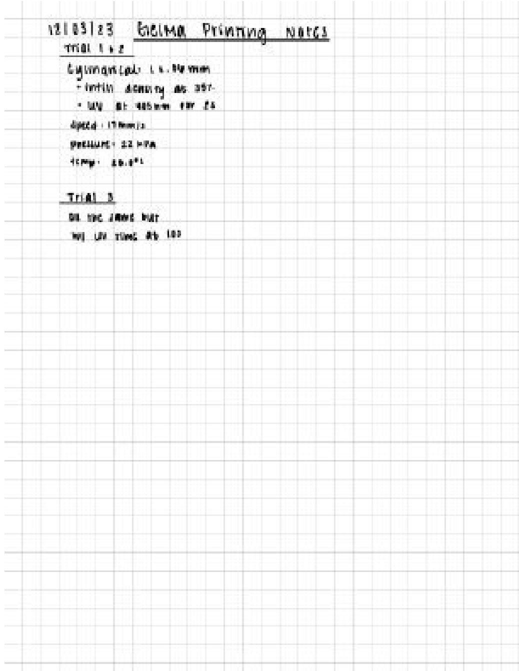
I also feel that at the beginning of next semester the team needs to rejoin as a big group rather than subgroups. We are all busy and as a team communication is not our strong suit which has led to confusion and a lack of awareness towards all the moving pieces of this project. I think it will be in our best interest, with the recent success on the bioprinter, to have the entire team come together and retrain with the machine. As the leader, one of my own goals has been through this experience to provide as much learning as possible for all members and knowing how to use the bioprinter is an amazing skill to have.

With respect to cell viability and pipette based models:

From conversations with Dr. Brasier he feels very concerned with the contamination issues occuring in the lab with the pipette based models - thus we should not use these in his lab. I believe the work that has previously been done this semester with cell viability assays was worth while and aided our understanding of the work but until we have set protocols from literature I think we should hold off on going forward. I believe that as a team we should have a meeting focused on everyone being on the same page with cell viability but also really nailing down what purpose does the quantitative data serve to meet our end goals. Are the other assays the better characterize the gels such as cell markers for fibrotic or healthy expression?

Overall, I am happy with the strides we have made with the bioprinting and that we have gotten our toes dipped on the biological assay side of things. I'm excited to see where the coming semester takes us!

CARLEY SCHWARTZ - Dec 13, 2023, 7:04 PM CST



Download

BME_Project_Notes.pdf (489 kB)

2023/10/25 GelMA Droplet Bioprinting

CARLEY SCHWARTZ - Oct 25, 2023, 9:44 AM CDT

Title: GelMA Bioprinting

Date: 10-25-23

Content by: Carley

Present: Carley

Goals: To spend time with the bioprinter attempting to print droplets

Content:

Bioprinter settings

- Droplet Bioprinting
- Extrusion time: 1.5 s
- Pressure: 20 kPa
- Temperature: 28 degrees
- UV 405 nm
    - 6 cm distance
    - 10 seconds
- Swelling for 24 in DMEM

Conclusions/action items:

First time having success with bioprinting GelMA! There was still clogging that I needed to remove but that is expected. There was significant width/height differences between the gels so lots of room for optimization. Likely, will need to figure out better extrusion time and pressure relationship so that a more cylindrical shape is made rather than a blob due to it spreading outward slightly when it is still liquid.

2023/11/30 GelMA Fabrication Protocol

Title: GelMA Fabrication

Date: 11-30-23

Content by: Carley

Present: self

Content:

1. **Dissolve gelatin type A from porcine skin at 10% w/v in warm PBS at 50° C, stir vigorously**

a. Cover a 400 ml beaker with foil.

b. Add 200 ml 1X PBS (and a stirring bar) to the beaker and set up the temperature so that the PBS reaches (and stays at) 50*C

i. If you input 50*C in the hotplate the solution would not heat at that temperature. I have found that inputting 115 *C in our hotplates makes the rxn solution heat at 50*C but every hot plate is different. Make sure you do a test to standardize the temperature to the hotplate you are using. Always use a thermometer to corroborate.

ii. In order for the rxn to occur accordingly, we need to make sure the rxn mix is at 50*C, not below that or more than 55*C.

c. Add 20 g of Gelatin type A from porcine skin (Different gelatin types and gelatin sources will have different properties)

d. After adding gelatin, give it 15-20 minutes to solubilize

This would make a large batch of about 16 tubes

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

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

a. Addition of methacrylic anhydride (MA) will depend on the methacrylation level you want to achieve. For example, if you want to create a 3% M (Methacrylation level) I would perform the following calculation: 3% = 0.03; 0.03 * 200 mL PBS = 6mL MA to be added dropwise.

b. For this step, cover your beaker top with parafilm (parafilm needs to be removed and replaced with saran wrap at the end, otherwise the parafilm would melt into your solution ruining it)

c. Load an 18 gauge needle syringe with the calculated MA.

d. Puncture the parafilm in towards the center and add the MA dropwise.

i. Make sure the gelatin solution is stirring vigorously to keep the slowly added MA well distributed in the solution; this is incredibly important to maintain the reaction as homogeneous as you can.

e. The final concentration can range from between 0.1-10% v/v, with large [MA] eventually yielding stiffer gels

3. **Let the reaction solution incubate overnight while stirring at 50° C**

a. Generally, a good idea to protect it from light by fully wrapping it in foil (should already be covered from step 1)

b. You'll also want to cover the top of the reaction vessel (beaker) with saran wrap and foil or you could use screw-top glass bottles.

On this day I also like to prepare the PBS wash to be used the next day and prewarm overnight. This will accelerate the process of waiting for the 3L to preheat at the right temperature. For pre-warming the 3L of PBS I usually set up the plate at 70*C which makes the solution heat at 50*C. Again, always corroborate with a thermometer and make sure you standardized according to the hotplate you'll be using.

Next day...

4. **Prewarm the centrifuge to 37*C at least 30 mins before collecting the rxn volume;** this will keep the gelatin liquid during the separation of the phases. In addition, you should **pre-warm the PBS that will be used to dilute the rxn at 37*-40*C at least 1hr before.**

5. **Collect reaction volume in 50 ml tubes (4 tubes total), spin down at 3500xg for 5 minutes, discard opaque pellet and collect supernatant.**

a. The opaque pellet is unsolubilized MA and precipitated protein

i. Too much methacrylation destabilizes the gelatin and it precipitates out of solution. Some precipitation is unavoidable, however, if you notice a lot and are getting a low yield, you might want to decrease the amount of MA

ii. If the solution looks clear and there is low precipitation this is indicative of an efficient reaction. (We have prepared batches ranging from .1M to 10M, but more than that can be tricky)

6. **Dilute gelatin-methacrylate (gelMA) solution 1:4 in prewarmed (to 37C) PBS and transfer to 12-14 kDa MWCO dialysis tubing.**

For the example stated above, if we have 200mL of rxn solution we will need to pre warm 600mL of 1X PBS.

a. Add the gelMA solution to the prewarmed PBS at a 1:4 dilution.

i. All throughout this protocol, you'll want to handle gelatin solutions at 37C - 40C to keep it from solidifying. For this reason, you prewarm the PBS to 37C - 40C before using it to dilute the concentrated gelMA.

ii. We dilute the gelMA to make it easier to handle and increase the efficiency of dialysis. Lower concentrations of gelatin don't gel as quickly and it makes diffusion of unreacted (and cytotoxic MA) through the dialysis membrane much more efficient (lower density of large, hydrophobic gelatin molecules means it's easier for the MA to move faster out of the solution).

b. With 50 ml serological pipettes transfer about 200 mL. You should cut the dialysis tubes long enough to allow for swelling (we cut about 3.5 feet approximately). Make sure you secure the ends with the clips by folding inside twice. Once you have filled up the tube grab both ends, take out the clip that was previously secured, and then place both ends of the membrane together. Fold it inside twice and then place the clip again.

i. We put the diluted gelMA into dialysis to remove unreacted MA because, as mentioned above, it is cytotoxic. It is much smaller than the gelMA, so it can diffuse out of the pores in the dialysis tubing while the gelMA stays trapped within the tube.

7. **Place into dialysis (at least 10X total sample volume)**

a. Replace buffer daily until desired purification level achieved

b. Currently, I dialyze into PBS for 3 days then switch to ddH20 for 3 days.

i. When you lyophilize, you are removing all water from the system. So, if you lyophilize your gelMA while its in PBS, your final product will be a mix of salt (from the PBS) and gelMA – this product is hard to work with. We switch to plain water for the final 3 days so that when we lyophilize, the end product is only gelMA (and a negligible amount of salt).

ii. Why not just do water for all 6 days? MA is more soluble in a salt solution, so we start out in PBS to get better diffusion of MA out of the gelMA solution. If we started with water, we wouldn't be able to get all the MA out as easily (and the cells would die when they came into contact with it).

c. Up until dialysis, its normal for gelMA solutions that were made with a high MA content (5-10%) to remain cloudy (even after centrifuging). However, after dialysis, the solution should be mostly clear (maybe a little bit of opaqueness).

8. **Filter through 0.2 µm filter, treat as sterile from here on out.**

a. After this step, all conditions should be clear with a slight tan tint

b. If you start this with a cloudy solution, it will quickly clog the filter and you will end up going through a lot of filters.

c. How you filter will depend on the size of your reaction. For small volumes >10 mL, you can use a syringe and filter attachment. For ~50mL, the steriflips work well. For >150mL, the screwtop bottle filters are the best option.

9. **Freeze and lyophilize, store at -20° C/-80 until use**

a. Flash freeze in 50mL conical tubes in liquid nitrogen. I've noticed that if the gelMA freezes over a long period of time, there is heterogeneity in the final product (i.e. some parts of the stock are more methacrylated than other parts, giving you a lot of variability in gel-to-gel mechanics and, thus, data). In addition, you want to store you product in an ice making freezer (-20*C) or a -80*C since placing the gelma in a thaw-freeze cycle freezer would affect the mechanical properties of your material.

b. Lyophilization takes 3-5 days, depending on total volume and instrument condition. If the tube feels like its room temp, its probably done. Always better safe than sorry, though, so leave it on until you think its done and then leave it on for a little longer.

c. I usually just put a kimwipe over the top of the conical tube and rubber band it in place. This isn't extremely sterile, but I've never had problems with it (and bacteria doesn't survive lyophilization very well.)

Here is an in depth paper on gelMA synthesis, I'd definitely recommend reading before getting started:

Loessner et al**. Functionalization, preparation and use of cell-laden gelatin methacryloyl-based hydrogels as modular tissue culture platforms.** Nat Protoc. 2016 Apr;11(4):727-46.

Conclusions/action items:

GelMA synthesis protocol created in collaboration with Dr. Master incase we want to make our own GelMA due to contamination. We will also want to make our own LAP.

Bioprinted GelMA Hydrogels - Intro Research

ELIJAH DIEDERICH (ediederich@wisc.edu) - Sep 12, 2023, 7:26 PM CDT

Title: Synthesis, properties, and Biomedical Applications of gelatin methacryloyl (GelMA) hydrogels

Date: 9-12-2023

Content by: Elijah Diederich

Present: Myself

Goals: To better understand bioprinting of GelMA hydrogels

Content:

Citation: [1] K. Yue et al., “Synthesis, properties, and biomedical applications of gelatin methacryloyl (gelma) hydrogels,” Biomaterials, https://www.sciencedirect.com/science/article/pii/S014296121500719X (accessed Sep. 12, 2023).

2.5 Bioprinted GelMA Hydrogels

- Sequential deposition of layers of a "bioink", constructs provide tissue-like environment for cells to proliferate and mature

- Chondrocyte (Cells responsible for cartilage formation) when mixed with GelMA solutions showed viabilities of 83 +/- 13% (Day1) and 73 +/- 2% (Day 3) within the hydrogels

- Bioprinting along with 3D molding can be used to design cell-laden cylindrical microchannels that resemble vasculature

- Agarose (polysaccharide) used as a sacrificial template to form these cylindrical hydrogels, agarose sections could then easily be removed and create a hollow vasculature network

- Bioprinting allows for precise processing of biomaterials and also allows for 3D spatial control

Conclusions/action items:

1. Continue to research bioprinting to get more comfortable with terminology/machine

2. Schedule lab day with Dr. Brasier

3. Start working on PDS

Direct-Write Bioprinted GelMA Hydrogels

ELIJAH DIEDERICH (ediederich@wisc.edu) - Sep 13, 2023, 4:06 PM CDT

Title: Direct-write Bioprinting of Cell-laden Methacrylated Gelatin Hydrogels

Date: 9-13-2023

Content by: Elijah Diederich

Present: Myself

Goals: To better understand the process of bioprinting GelMA hydrogels

Content:

Introduction:

- Cell-laden GelMA hydrogels at conc. 7-15% were printed with varying cell densities; correlation found between printability and hydrogel mechanical properties

- Encapsulated HepG2 (Liver Cancer Cell Line) proved viable for at least 8 days after encapsulation

- Direct-Write bioprinting (Extrusion) - **same as one we have access too** - promotes the extrusion of a viscous polymer precursor to build up a tissue layer

********This article contains a modified NovoGen MMX Bioprinter. Purpose of modification was to bio-print cells encapsulated in the GelMA hydrogel in one step*******

Methods:

- **Printability** determined with GelMA hydrogel precursor solution (5-15% w/v) and cross-linking time between 10-60 seconds

  - Printability also tested with varying cell densities (1x10^6, 1.5x10^6, 3x10^6, 6x10^6 cells/ml) with a constant 10% w/v hydrogel

- **Mechanical Properties** tested included the elastic modulus (E)

- **Cell Viability** determined using a Live/Dead assay

Conclusions/action items:

ELIJAH DIEDERICH (ediederich@wisc.edu) - Sep 20, 2023, 11:30 AM CDT

Continued Notes - 9-20-2023

3.1 - Results and Discussion

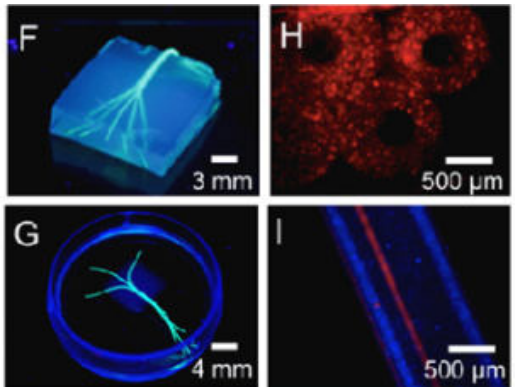
- GelMA hydrogels can be successfully printed at a range of concentrations (7-15% w/v) for all UV exposure times

- Observed that at lower concentrations, hydrogels were not easily printed to generate uniform and well-structured fibers

- Lower UV light times also consistently reduced printability (does not apply due to our bioprinter applying UV light after gels are printed)

- Increase of GelMA concentration and UV light exposure drastically increased mechanical properties (Young's Modulus) of hydrogels

- Using Sacrificial Polysaccharide (Agarose), can be used to introduce vascularization into model (Picture Shown Below)



- Constructs in paper including an array, 5-layer stack, and 3D lattice designs all showed ~75% cell viability

- Bioprinting allows for the ability to control macroscale structure

- Results also showed that cell viability can be preserved at levels higher than 80% for periods of at least 8 days in bioprinted constructs

Conclusion/Action Items:

1. Finish PDS sections and revise

2. Bio-print Gels for Brasier Lab

3D Bioprinting Strategies - Lung/ECM

ELIJAH DIEDERICH (ediederich@wisc.edu) - Sep 27, 2023, 6:59 PM CDT

Title: 3D Bioprinting Strategies, Challenges, and Opportunities to model the Lung Tissue Microenvironment and Its Function

Date: 9-27-2023

Content by: Elijah Diederich

Present: Myself

Goals: To continue to explore bioprinting and its application to our final design

Content:

Abstract:

- Lungs are very challenging to model in-vitro due to its intricate hierarchical structure and complex composition

- 3D bioprinting can be used to create 3D structures that are key to bridging the gap between current cell culture methods and living tissues

Introduction:

- The lung presents over 60 different kinds of cells that perform a wide variety of functions; these cells are supported by and distributed within the Extracellular Matrix (ECM)

- The Lung ECM constitutes almost 50% of the nonalveolar tissue in the lungs

Conclusions/action items:

PrestoBlue - Cell Viability Assay

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 18, 2023, 8:46 PM CDT

Title: PrestoBlue Cell Viability Assay

Date: 10-18-2023

Content by: Elijah Diederich

Present: Myself

Goals: To discover potential cell viability assays that could be used for our GelMA hydrogel

Content:

Link: https://www.thermofisher.com/order/catalog/product/A13261

- Uses reducing power of living cells to measure proliferation
- Blue reagent will be turned Red by viable cells, can be detected using absorbance or fluorescence measurements
- Results in as little as 10 min
- Does NOT require cell lysis
- Know use in hydrogels (Notes for paper from 10-18-2023)

25 ml bottle for $320

Conclusions/action items:

1. Finish Progress report

2. Continue to look at fibroblasts encapsulation cell densities

3. Cell viability assays

Fibroblast Encapsulation - GelMA and Collagen Hydrogels

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 18, 2023, 9:29 PM CDT

Title: Fibroblast encapsulation in GelMA vs. Collagen Hydrogels as substrates for oral mucosa tissue engineering

Date: 10-18-2023

Content by: Elijah Diederich

Present: Myself

Goals: To try and determine fibroblasts encapsulation densities

Content:

Citation: [1] F. Tabatabaei, K. Moharamzadeh, and L. Tayebi, “Fibroblast encapsulation in gelatin methacryloyl (gelma) versus collagen hydrogel as substrates for oral mucosa tissue engineering,” Journal of oral biology and craniofacial research, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7479286/ (accessed Oct. 18, 2023).

2.3 Methods - GelMA Preparation

- Cellink GelMA (REP-VL-350000) --> LAP photoinitiator (UV light 395 nm for 30 seconds)

- Solution aliquoted into 6 well tissue culture plastic inserts

- **10% w/v hydrogel** and **fibroblasts at 1 x 10^6 cells/ml**

2.4 - Assessment of viability of encapsulated fibroblasts

- Viability determined using PrestoBlue

- Hydrogels washed with PBS and solution with 10% v/v PrestoBlue reagent in phenol red-free DMEM was applied to each sample

- Following this was a 3 hour incubation at 37 degrees C and 5% CO2 --> Fluorescence intensity of triplicate aliquots of each sample was measured with a microplate reader (Synergy HTX, BioTEK) at excitation/emission wavelengths of 540 nm and 590 nm

- Viability measured at 24 hours and 72 hours

- Surface seeding of isolated oral keratinocytes (50 microliters(u) of cell suspension containing 1 x 10^6 cell/ml)

- Keratinocytes added after 4 days

Conclusions/action items:

1. Progress report

2. Inform group members about values above

3. Continue to research cell viability assays/fibroblasts seeding densities

Fibroblast Encapsulation - Acrylated Alginate Hydrogel

ELIJAH DIEDERICH (ediederich@wisc.edu) - Oct 25, 2023, 12:19 PM CDT

Title: Study on a 3D Hydrogel-Based Culture Model for Characterizing Growth of Fibroblasts under Viral Infection and Drug Treatment

Date: 10-25-2023

Content by: Elijah Diederich

Present: Myself

Goals: To continue to figure out fibroblast encapsulation densities

Content:

Citation: [1] X. Zhu and X. Ding, “Study on a 3D hydrogel-based culture model for characterizing growth of fibroblasts under viral infection and drug treatment,” SLAS discovery : advancing life sciences R & D, https://pubmed.ncbi.nlm.nih.gov/28340537/ (accessed Oct. 25, 2023).

Introduction:

- Study of Fibroblast topological changes and proliferation variation when exposed to HSV-1 in the presence of acyclovir (ACV; treatment drug)
- Use of 3T3 fibroblasts in study with experimental groups of HSV-1 treated alone, ACv treated alone, HSV-1 and ACV co-treated, and control samples
- Hyaluronic Acid hydrogel functionalized with acrylate groups used as 3D cell culture substrate (also functionalized with 100uM RGD for cell adhesion)

Methods:

- NIH 3T3 fibroblasts used and cultured in DMEM, FBS and Pen-strep (prevents bacterial contamination)
- To harvest cells from AHA hydrogel, cell incubated with trypsin-EDTA for 5 min in incubator and then removed
- Cells counted via hemocytometer calculator
- Cells were plated at a density of **2 x 10^5 cells/plate** (meaning per 96 well plate which contained AHA hydrogels)
- Rheology measurements were also taken via rheometry (G' and G" values considered)

Conclusions/action items:

1. Continue to share research with team

2. Discuss show and tell with team for next week

3. Sunday WIMR meeting

Fluid Transport Analysis - Collagen/Matrigel Hydrogel

ELIJAH DIEDERICH (ediederich@wisc.edu) - Nov 01, 2023, 2:03 PM CDT

Title: Computational and Experimental Analysis of Fluid Transport Through Three-Dimensional Collagen–Matrigel Hydrogels

Date: 11-1-2023

Content by: Elijah Diederich

Present: Myself

Goals: To better understand ways to assess fluid transport through hydrogels

Content:

Abstract:

- Assessment of quantitative nutrient delivery (mass transport) to develop an accurate in-vivo model

- Object of the study is to develop an in-vitro/computational model to measure mass transport from a perfusion system into a 3D ECM

- Perfusion-flow bioreactor system was used to control and quantify the mass transport of a within an ECM hydrogel

- CFD (computational fluid dynamics) simulation ran with material properties, fluid mechanics, construct structure as system inputs

- Advection and diffusion played a complementary role in mass transport (will elaborate on for results section)

-

Conclusions/action items:

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 04, 2023, 7:55 PM CST

Idea ended up being abandoned due to team focus on bioprinting and cell viability assays

Collegenase Protocol - GelMA

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 04, 2023, 8:35 PM CST

Title: Impact of the biophysical features of a 3D gelatin microenvironment on glioblastoma malignancy

Date: 12-4-2023

Content by: Elijah Diederich

Present: Myself

Goals: Discover a collagenase concentration to add for our degradation protocol

Content:

Citation: S. Pedron, “Impact of the biophysical features of a 3D gelatin microenvironment on glioblastoma malignancy,” Journal of biomedical materials research. Part A, https://pubmed.ncbi.nlm.nih.gov/23559545/ (accessed Dec. 4, 2023)

Materials:

- Collagenase purchased from Worthington Biochemical

- GelMA degradation decreases significantly with decreasing degree of funcitonalization

- With 85% DOF, GelMA hydrogels in 2.5 U/ml collagenase did not fully degrade, but did with 25 U/ml after 30 days of incubation

- With using a hydrogel at 50% DOF in our lab, 2.5 U/ml collagenase should be a sufficient amount but further articles will be looked at to determine this concentration

Conclusions/action items:

1. Continue to look at collagenase concentrations

2. Finish poster and rehearse assigned section

Collagenase Protocol - GelMA/PEGDA Hydrogel

ELIJAH DIEDERICH (ediederich@wisc.edu) - Dec 04, 2023, 8:52 PM CST

Title: Development of a Photo-Crosslinking, Biodegradable GelMA/PEGDA Hydrogel for Guided Bone Regeneration Materials

Date: 12-4-2023

Content by: Elijah Diederich

Present: Myself

Goals: To find a collagenase concentration for GelMA hydrogel degradation

Content:

Citation: Y. Wang, M. Ma, J. Wang, and B. Zhang, “Development of a photo-crosslinking, Biodegradable Gelma/Pegda hydrogel for Guided Bone Regeneration Materials,” Materials (Basel, Switzerland), https://pubmed.ncbi.nlm.nih.gov/30081450/ (accessed Dec. 4, 2023).

Materials:

- GelMA is known to have a fast degradation rate, in this study PEG polymer was added to decrease this degradation time (make it last longer)

- Collagenase concentration was used at 2 Units/ml for 4 weeks in an incubator (full degradation achieved for a 72% DOF with G10 (w/v%) - 21days and G15(w/v%)-24 days hydrogels)

- DOF percentage was found using an HNMR spectrum analysis

- Collagenase Type 1 purchased from Solarbio (China)

Conclusions/action items:

1. Talk to group about collagenase concentrations

2. Practice poster and make sure it is up to standard before printing

Cellink GelMA Variations

ELIJAH DIEDERICH (ediederich@wisc.edu) - Sep 27, 2023, 3:49 PM CDT

Title: GelMA Cell Link Variations

Date: 9-27-2023

Content by: Elijah Diederich

Present: Myself

Goals: To understand potential design options for GelMA and effectiveness in our project application

Content:

Source: Cellink.com/bioinks/

- Celllink website offers 5 different premixed GelMA Solutions --> Client prefers to buy GelMA vs. Fabrication by design group

Option 1: GelMA Bio-ink

- Plain GelMA bio-ink w/ 0.25% LAP (Photo initiator) --> Can be cured at either 365nm or 405nm UV Light

- 45-55% Degree of Methacrylation

- Gelation temp: 24.5-27 degree Celsius

**** This is currently the GelMA we are using in the Bioprinter; having troubles with printability and extrusion of material onto petri dish****

Option 2: GelMA A

- GelMA A variation is a blend of GelMa and Alginate (seaweed derived natural biomaterial) --> Offers a larger printability window due to softening by alginate (Could reduce shear stresses onto cells?!?)

- Shear thinning bio-ink that prints at low pressure and forms filaments once deposited

- Can be either photocured or ionically crosslinked

- 45-55% degree of methacrylation

- Viscosity 30-150 Pa x s

Option 3 : GelMA C

- GelMA C is a blend of GelMA and nanofibrillated cellulose (polysaccharide from plants)

- Nanosized fibrils help to keep the material translucent which is key for cell imaging and analysis

- Offer smooth printability at ambient conditions and low extrusion pressures

Option 4 : GelMA XG

- GelMA XG is a blend of GelMA and Xantham Gum

- The addition of Xantham gum increases the printability window and also the stability of the GelMA

- 45-55% Degree of Methacrylation

- Gelation Temperature: 23-27 degrees Celsius (larger window here compared to normal GelMA - option 1)

- Viscosity: 50-300 Pa x s

Option 5 : GelMA XA

- GelMA XA is a blend of GelMA, Xanthum Gum and Alginate

- Both of these additives help to increase printability, stability and ease of use

- Cons: Semi-translucent (will affect analysis and imaging of gel)

- 45-55% Degree of Methacrylation

- Gelation temp: 23-27 Degrees Celsius

- Viscosity: 90-350 Pa x s

- Can be crosslinked ionically or via UV light

Conclusions/action items:

1. Continue to Troubleshoot GelMA printing errors

2. Finish Preliminary Presentation Sections

3. Finish Semester Plan for Friday Meeting

9/28/2023 - 3D Printed GelMA Scaffolds

Title: 3D-Printed Gelatin Methacryloyl-Based Scaffolds with Potential Application in Tissue Engineering

Date: 9/28/2023

Content by: Caitríona Treacy

Present: N/A

Goals: To understand existing models of GelMA and how they may differ from the bioink that we are currently using, in an effort to identify areas where our fabrication is lacking and challenges are arising.

Search Term: 3D Printed GelMA Scaffold Application

Citation:

Leu Alexa, R., Iovu, H., Ghitman, J., Serafim, A., Stavarache, C., Marin, M. M., & Ianchis, R. (2021). 3D-Printed Gelatin Methacryloyl-Based Scaffolds with Potential Application in Tissue Engineering. Polymers, 13(5), 727. https://doi.org/10.3390/polym13050727

Content:

- The goal of the research was to obtain suitable 3D-printed scaffolds based on methylated gelatin (GelMA), the same material we are working with in our design. In this work, three degrees of GelMA methacrylation, three different concentrations of GelMA (10%, 20%, and 30%) were tested and also combined with two concentrations of the photoinitiator I-2959. Characterization of the final hydrogels was based on structure, mechanic properties, and morphology. In order to test for the presence of methacryloyl groups bounded to the surface of GelMA, FTIR an H-NMR analysis was run.
- 3D printing is considered the newest method used to design three-dimensional networks with versatile properties. It is a noninvasive method with the advantage that is allows for the use of the material without affecting its vital properties. As printing materials, hydrogels must present a shear thinning behavior, a property that allows continuous flow and printing under a high shear rate.
- Photopolymerization using UV light provides many advantages over thermal and chemical crosslinking processes. Namely, the crosslinking process takes place at room temperature, and provides thermal stability and mechanical stability. GelMA-based hydrogels exhibit high morphological and mechanical stability with tunable mechanical properties, inherent bioactivity, and physicochemical tailorability and allow cells to attach and spread in the scaffold to proliferate and regenerate tissue.
- In terms of the ink, achieving almost perfect homogeny of the mixture is important. This could be a variable we are not adequately considering in our trials. We must be sure that the ink is adequately mixed before printing, especially after being stored over a period of time when settling is very likely to have occurred.
- H-NMR spectrometry can be used to determine the methacryalation degree of GelMA. This will be important testing for us to consider once we have fabricated successful models.
- Fourier-Transform Infrarred Spectrometry was used in the structural characterization of the raw material, as well as the GelMA with different methacrylation degrees. This is also a useful evaluation technique to be aware of once fabrication has taken place.
- Testing to find the isoelectric points of our models may also be useful, depending on PDS requirements for analysis.
- A syringe of 3 mLwas attached to a cylindrical nozzle of 25 G or 23 G. Printing speeds ranging from 5 to 10 mm/s and pressures in the range of 150-300 kPa were tested. All printing took place at room temperature. BIOCAD software was used for the scaffold architecture. This software allowed for 2D objects to be drawn and viewed in 3D after generating the G code. As a standard, scaffolds were printed to have 20 layers.
- Swelling ratios will also be an important characteristic to quantify in the fabricated models.

- Mechanical properties: The nanoindentation technique was used to determine the Young's modulus and hardness of the samples. The storage and loss moduli were measured using rheometry, something that we have done in past semesters. Frequency sweep measurements were preformed at a stress of 5 Pa.

Conclusion/Action Items: Overall, this piece presented a systematic study to synthesize appropriate GelMA hydrogel ink formulations suitable to be functional in the 3D printing process. Even though we are currently planning to continue using the GelMA from the printing company, we have run into issues with it. The hope is that the understanding found in this article can be applied to troubleshooting and might help in understanding whether there is another variant of GelMA sold by the company that is more appropriate for our application. I would like to specifically look into the BIOCAD software that was used for developing the scaffold architecture in this case to see how it might have potential to be applied to our model.

10/4/2023 - Baseline Stiffness ECM Modulates

Caitriona Treacy - Dec 13, 2023, 4:07 PM CST

Title: Baseline Stiffness Modulates the Non-Linear Response to Stretch of the Extracellular Matrix in Pulmonary Fibrosis

Date: 10/4/2023

Content by: Caitríona Treacy

Present: N/A

Goals: To gather more in-depth information about the mechanical characteristics of the tissue that we are modeling. We need more reliable ranges for healthy and fibrotic tissue elastic moduli both to include in our preliminary presentation and to have specific numerical values to test for.

Search Term: "Young's modulus of healthy vs fibrotic small airway"

Citation:

C. Júnior *et al.*, "Baseline Stiffness Modulates the Non-Linear Response to Stretch of the Extracellular Matrix in Pulmonary Fibrosis," *International Journal of Molecular Sciences*, vol. 22, no. 23, Dec. 2021, doi: 10.3390/ijms222312928.

Content:

- Pulmonary fibrosis is a progressive disease which disrupts the mechanical homeostasis of the lung extracellular matrix. The effects of this are particularly relevant in the context of the lung, given the cyclic stretch that the ECM is subjected to during breathing.
- There is a wide evidence that mechanical alterations of the ECM are correlated with severe respiratory diseases. Fibroblasts are highly sensitive to the mechanics of their surroundings, and the presence of aberrant tissue mechanics drives the fibrotic response.
    - The reparative response of the cells becomes dysregulated, resulting in uncontrolled fibroblast proliferation and deposition of collagenous ECM. ECM repair processes are hindered and abnormal remodeling activity and scaring occur.
    - Fibroblasts on softer ECM (< 3 kPa) engage an apoptotic program, whereas fibroblasts on stiffer ECM become highly proliferative.
- A common approach to assess the micromechanical characteristics of the ECM at the cellular scale is based on atomic force microscopy. The Young's Modulus f the ECM sample at the microscale as well as the viscoelastic properties such as shear modulus of elasticity (G*) can be measured in this way.
- Findings in this article suggest that breathing-induced ECM stretch leads to a dynamic, cyclical decrease in the stiffness of fibrotic ECM.
- Fibrosis broadly stiffens pulmonary tissue at the macroscale, but does not alter the characteristic nonlinear behavior displayed by the stress-strain curves.
- Up to one order of magnitude difference in stiffness exists between healthy and fibrotic samples through tensile tests, even though no increase in collagen content is found.

Conclusion/Action Items: This study reported values within the range of 2-5 kPa for healthy lung tissue and a range of 10-30 kPa for fibrotic lung tissue. Compare this to other values in literature to gain a better idea of what values we are aiming to obtain with our models.

10/26/2023 - Fibroblast encapsulation in gelatin methacryloyl

Caitriona Treacy - Dec 13, 2023, 4:07 PM CST

Title: Fibroblast encapsulation in gelatin methcryloyl (GelMA) versus collagen hydrogel as substrates for oral mucosa tissue engineering

Date: 10/26/2023

Content by: Caitríona Treacy

Present: N/A

Goals: Start to gather information about existing protocol for fibroblast suspension in GelMA tissue models that can be applied once GelMA printing is consistent.

Search Term: "Fibroblast suspension in GelMA"

Citation:

> "Fibroblast encapsulation in gelatin methacryloyl (GelMA) versus collagen hydrogel as substrates for oral mucosa tissue engineering," *Journal of Oral Biology and Craniofacial Research*, vol. 10, no. 4, pp. 573–577, Oct. 2020, doi: 10.1016/j.jobcr.2020.08.015.

Content:

- This study was not specific to the small airway of the lung, nor was cell seeding done with bronchial epithelial cells. Rather, scaffolds were made to model connective tissue and seed oral epithelial keratinocyte cells. Concentration of GelMA used in the study was 10%.
- A mixture of fibroblasts with GelMA were aliquoted using six-well tissue culture plate inserts and cross-linked using visible light. Viability of fibroblasts was investigated after one and three days of cultivation using PrestoBlue assay.
- Preparation: Once pH was verified to be in the range of 7-7.5, fibroblast cell suspension that had been prepared in FBS was dispersed in the GelMA solution along with antibiotic/antimycotic (percentages of solution content can be found in section 2.3 of the article). The solution was kept warm at 37˚ and aliquoted into six-well tissue culture plate inserts. Crosslinking using LED light occurred. Polymerized hydrogels were washed with PBS.
- Fibroblast Viability Testing: At 24 and 72 h, fibroblast cell viability was investigated using a PrestoBlue assay – *investigate the application of this method further.* Generally, samples were washed with PBS and a solution of PrestoBlue reagent in phenol red-free DMEM. After 3 hours of incubation, the fluorescence intensity of triplicate aliquots of each sample was measured through excitation/emission wavelengths of 540/590 nm. Cell viability was calculated using the values of the percentage of the fluorescence intensity in all groups.
- After four days, cells were seedded onto the surface of the scaaffold. After two hours of incubation (allows for adhesion) all samples were raised to the air-liquid interface for 10 days.
- Results: The percentage of cell viability in GelMA 24 hours after cell encapsulation was 34.78%. After 3 days of culture, this increased to 112.5%.
- In the discussion, it is mentioned that encapsulation of fibroblasts in GelMA can be optimized by changing parameters such has methacrylation degree, cross-linking density, sample thickness.

Conclusion/Action Items: Need to work out how fibroblasts can be encapsulated and then ink replaced in the cartridge. Investigate whether the PrestoBlue assay might be applicable. The article mentions a specific protocol developed by CELLINK, the company of ink we are using, that outlines cell encapsulation. This protocol (Cellink, Ref No: REp-VL-350000) should be consulted.

11/2/2023 - Guidelines for Cell Viability Assays

Title: Guidelines for Cell Viability Assays

Date: 11/2/23

Content by: Caitríona Treacy

Present: N/A

Goals: Understand general details of cell viability assays as the team moves into testing so that these ideas can be effectively applied to the project.

Search Term: "Cell viability testing in hydrogels"

Citation:

S. Kamiloglu, G. Sari, T. Ozdal, and E. Capanoglu, "Guidelines for cell viability assays," *Food Frontiers*, vol. 1, no. 3, pp. 332–349, 2020, doi: 10.1002/fft2.44.

Content:

- Cell viability refers to the number of healthy cells in a sample. This measurement is of utmost importance when talking about any form of cell culture. Sometimes, cell viability might be the main purpose of an experiment (ex: toxicity assays), but it can also be used generally to correlate cell behavior to the number of cells. Cell viability assays are essential in screening the response of the cells against a drug or chemical agent.
- Different types of assays can be used to determine how many viable cells are in a model. They can be base on enzyme activity, cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity.
- Cell viability assays generally fall under the following classifications: dye exclusion assays, colorimetric assays, fluorometric assays, luminometric assays, and flow cytometric assays.
    - Dye exclusion essays include trypan blue, eosin, congo red, and erythrosine B stain assays.
    - Resazurin and 5-carboxyfluorescein diacetate acetoxymethyl ester (5-CFDA-AM) assays are based on fluorometric measurements.
    - Luminometric assays comprise adenosine triphosphate and real-time viability assays.
    - Major flow cytometric assays include membrane asymmetry, membrane permeability, and mitochondria assays.
- Ideally, cell viability assays should be safe, rapid, reliable, efficient, time- and cost-effective, and should not interfere with the test compound. Often, more than one cell viability assay should be applied in order to obtain reliable results. One assay can be used to emphasize or validate the results of another.
- Most critical factors for accurate and reproducible measurements:
    - Use of controlled and consistent source of cells to set up experiments
    - Performing suitable characterization of reagent concentration and incubation time for eaach experimental model system.
- **Trypan blue stain assay:** Used as a confirmatory test for measuring changes in viable cell number caused by adrug ortoxin. Trypanblule stain is a largelly negative chargedmolecule and is oon eof the simplets assays that aare used to determmine the nubmerof viable cells in a cell suspension. **Living cells have intact cell membranes that exclude the trypan blue stain, whereas dead cells do not. A viable cell will have a clear cytoplasm, whereas a nonviable cell will have a blue cytoplasm.**
    - 0.4% trypan blue stain and phosphate-buffered saline (PBS) or serum-free medium are obtained (trypan blue stain would bind to serum proteins if present and skew results)
    - Cell suspension is centrifuged at 100 x g for 5 minutes. The supernatant is discarded, and the pellet should be resuspended in 1-ml PBS solution or serum-free medium. A portion of this cell suspension is mixed

with one portion of trypan blue stain. The mixture stays at room temperature for 3 minutes. Longer incubation periods lead to cell death, so cells should be counted within 3–5 minutes of trypan blue mixing. Then, a drop of mixture is applied to hemocytometer which can then be counted (unstained and stained showing viable and nonviable, respectively).
    - Total number of viable cells per mL of aliquot is found by multiplying the total number of viable cells by two (dilution factor for trypan blue). Total number of cells per mL is found by adding nonviable and viable cell counts and multiplying by two. The percent viability can then be calculated using these values.
- **Eosin, congo red, and erythrosine B stain assays:** Eosin is a fluorescent red dye that can be used to stain cytoplasm, collagen, and muscle fiber. Congo red is a sulfonated azo dye use to stain cytoplasm. Erythrosine B (FD&C Red No. 3) is a tetraiodofluorescein dye widely used as a biological stain and also as a color additive and food in drugs. **Again, relies on the integrity of a cell membrane.** Nontoxic, does not bind to serum proteins, and does not require incubation period before counting (advantages over trypan blue staining).
    - Eosin, congo red, or erythrosine B stain(0.1%) are first obtained from the manufacturer
    - Cell suspension in PBS and stain are mixed at a 1:1 ratio and loaded into a hemocytometer. Nonviable (stained red) and viable (unstained) are counted.
    - Counting calculations are the same as for trypan blue.
- **MTT Assay:** Simple colorimetric test of cell proliferation and survival. Based on the conversion of MTT into formazan crystals by living cells (signals mitochondrial function). Tetrazolium salt is reduced to insoluble formazan dye by a dehydrogenase enzyme present in the viable cells at 37˚. Insoluble formazan salt is dissolved by the addition of solubilizing agents, and the colored product is quantitatively measured at 570 nm using a spectroscopic multiplate reader. A variety of methods have been used in each step, but **dead cells lose the ability to reduce tetrazolium salts into colored formazan products. Viable cells with active metabolism convert MTT into a purple-colored formazan product with an absorbance maximum near 570 nm. The intensity of the colored product is directly proportional to the number of viable cells present in the culture.**
    - MTT is dissolved in Dulbecco's phosphate-buffered saline (DPBS) at pH 7.4 (5 mg/mL). Solution is filtered and sterilized using a 0.2 µm filter into a sterile and light-protected container. MTS stored at -20˚C until analysis or at 4˚C for immediate use and should be protected from light.
    - Cell suspensions seeded into 96-well plates with our without the test compounds and incubated at 37˚C in a humidified incubator with 5% CO2 for the required exposure time. MTS solution of 20 µL is added to each well to reach a final concentration of 0.33 mg/ml and incubated at 37˚C for 1–4 hours. Absorbance is then measured at 490 nm with a multiplate reader.
    - The percentage of cell viability is found by dividing the mean OD of the sample by that of the blank.
- **XTT Assay:** Bioreduction of XTT yields a highly colored formazan product with only viable cells. A difference here between other tetrazolium salts is that formazan dye is soluble in aqueous solutions and directly quantified using a scanning multiplate spectrophotometer (ELISA) which enables a high degree of accuracy, allows online data processing by computers and thus allows a high number of samples to be handled quickly and conveniently. Cells are incubated in a yellow XTT solution in a 96-weell tissue culture plate. During incubation time, orange formazan solution is produced and is quantified spectrophotometrically using an ELISA plate test. **An increase in the number of living cells results in an increase of the sample's total activity of mitochondrial dehydrogenazes.** This rise is closely associated with the quantity of formed orange formazan as is measured by the absorbance.
    - XTT solution preparation includes dissolving 1 mg/ml XTT in sterile Hanks' balances salt solution, followed by addition of PMS at 5 µL/ml (5mM stock solution) *immediately before use.*
    - Cell suspensions seeded to 96-well plates. Incubated with or without test compound (200 µl/well) at 37˚C in a humidified incubator with 5% CO2 for the required exposure time. Following this incubation period, 100 µl of XTT solution mix is added to each well for a final concentration of 0.3 mg/ml and plates are incubated at 37˚C for 4 hours. Absorbance is measured at 450 nm against a reference wavelength of 650 nm using a microplate reader.
    - Percent of cell viability is calculated using: $$\%\ \text{Viability} = \frac{(A_{450} - A_{650})\ \text{of test cells}}{(A_{450} - A_{650})\ \text{of control cells}} \times 100.$$

Conclusion/Action Items: This article serves as a guideline that outlines the mechanisms and practice of assessment of the most common cell viability assays applied in research labs are discussed in detail.

11/12/2023 - Calcein Staining in GelMA

Title: Cell-laden microengineered gelatin methacrylate hydrogels

Date: 11/12/2023

Content by: Caitríona Treacy

Present: N/A

Goals: Gather information about calcein staining previously done on 3T3 cells embedded in GelMA hydrogels, mainly to validate it as a method in our design evaluation.

Search Term: "Calcein Staining 3T3 in GelMA"

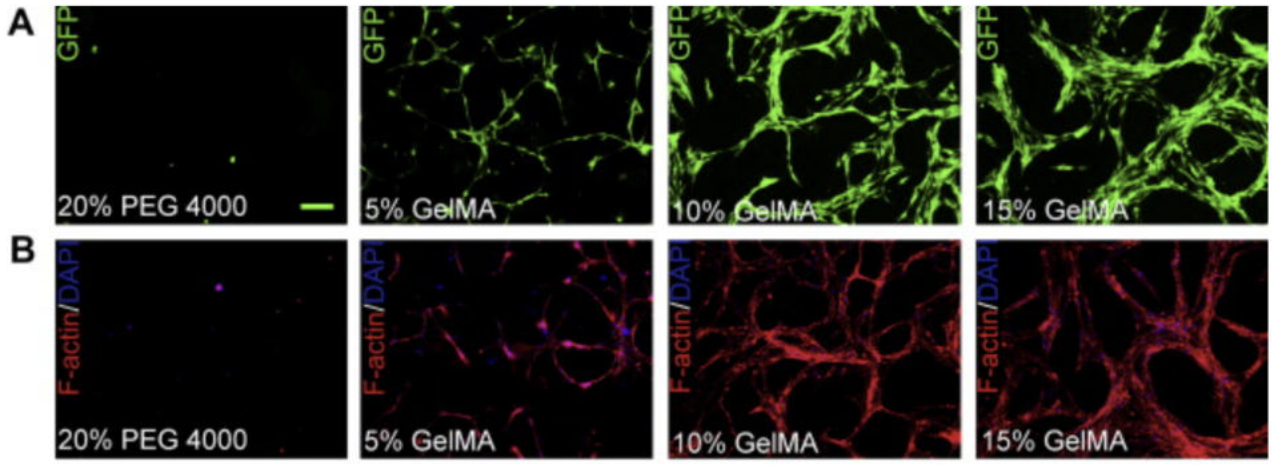
Citation:

J. W. Nichol, S. Koshy, H. Bae, C. M. Hwang, S. Yamanlar, and A. Khademhosseini, “Cell-laden microengineered gelatin methacrylate hydrogels,” *Biomaterials*, vol. 31, no. 21, pp. 5536–5544, Jul. 2010, doi: 10.1016/j.biomaterials.2010.03.064.

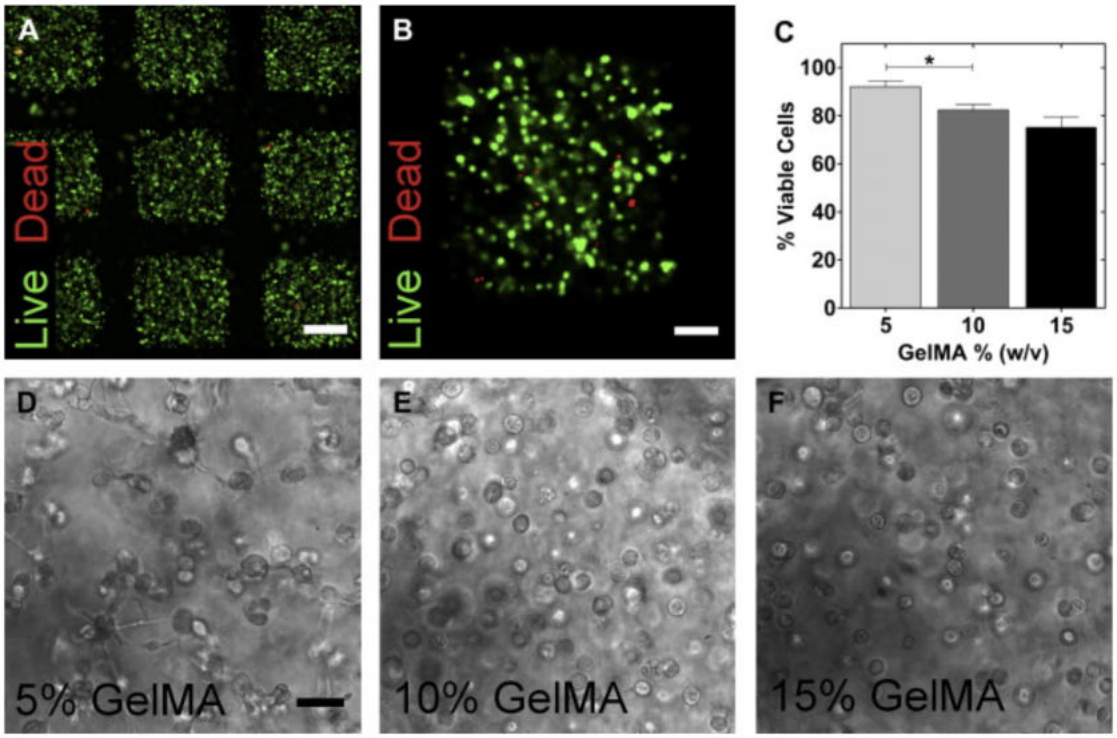
Content:

- The publication overall demonstrates that GelMA is a cell-responsive hydrogel platform for creating cell-laden microtissues. It was shown that cells readily bound to, proliferated, elongated, and migrated both when seeded on micropatterned GelMA and when encapsulated in microfabricated GelMA hydrogels. For the current application to the project, we are most interested in the protocol that was followed to demonstrate this cell behavior and viability, as we are looking to demonstrate the same in our fabricated gels.
- Cell Culture: NIH 3T3 fibroblasts were maintained in DMEM supplemented with 10% FBS and passaged 2 times per week.
- Cell adhesion: Comprehensive protocol described in article. GFP fluorescence was visualized using an inverted fluorescence microscope equipped with a GFP filter cube. GFP images were then used to quantify total cell area using ImageJ. After five days, cells were fixed and **stained with rhodamine-phalloidin (Invitrogen) and DAPI to visualize F-actin filaments and cell nuclei, respectfully. Total cell number was quantified using ImageJ by counting DAPI stained nuclei.**
- Cell encapsulation: NIH 3T3 fibroblasts were trypsinized and resuspended in GelMA macromer containing 0.5% (w/v) photoinitiator at a concentration of 5e6 cells/mL. The glass slides containing microgels were washed with DPBS and incubated for 8 hours in the 3T3 medium under standard culture conditions. A calciein-AM/ethidium homo dimer Live/Dead assay (Invitrogen) was used to quantify cell viability within the microgels "according to the manufacturer's instructions."
- Cell adhesion, proliferation and migration on GelMA surfaces shows HUVEC cells readily adhered to GelMA of all macromer concentrations. Images are provided below to provide an example of what imaging can be achieved with our

materials.



- 3T3 fibroblasts embedded in GelMA micropatterns of various macromer concentration were stained with calcein-AM (green)/ethidium homodimer (red) LIVE/DEAD assay 8 hoours after encapsulation shown at low (scale bar = 250 μm) and high (scale bar = 100μm) magnification. Images are shown below, again in an effort to display what we should be aiming for when we attempt cell imaging on our gels. Quantification of the cell viability in the images provided demonstrated excellent cell survival at all testing conditions.



Conclusion/Action Items: Talk to team and subsequently possibly advisor about whether the application of the Invitrogen and DAPI imaging described under 'Cell Adhesion' could be applied to obtain cell counts for our gels. This study overall shows that Live/Dead assays with calcein stain can be successful with 3T3s in GelMA. Therefore, it is possible for us to complete our cell viability assays in this way, but our protocol must first be refined to produce useful images.

12/4/2023 - Collagenase degradation of GelMA

Title: **Photocrosslinkable Gelatin Hydrogel for Epidermal Tissue Engineering**

Date: 12/4/2023

Content by: Caitríona Treacy

Present: N/A

Goals: One of the design specifications is that the tissue model be degradable. The goal of this entry is to understand a protocol describing some of the collagenase degradation we have been using, and to more comprehensively describe what has been accomplished or erroneous in these attempts.

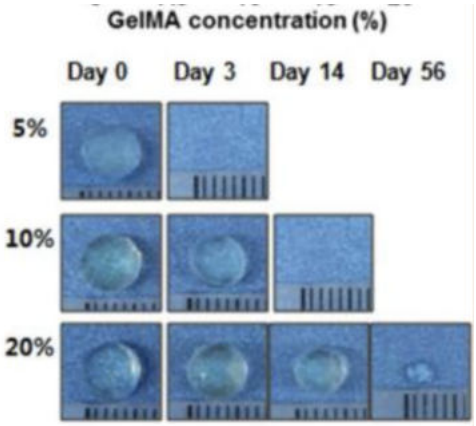
Search Term: "Collagenase degradation of GelMA hydrogel"

Citation:

X. Zhao *et al.*, "Photocrosslinkable Gelatin Hydrogel for Epidermal Tissue Engineering," *Adv Healthc Mater*, vol. 5, no. 1, pp. 108–118, Jan. 2016, doi: 10.1002/adhm.201500005.

Content:

- In this study, GelMA hydrogels with varying concentrations were synthesized for epidermal reconstruction. The physical properties of the hydrogels were fine-tuned by systematically varying GelMA concentrations to control keratinocyte adhesion, proliferation, and differentiation.
- To evaluate hydrogel degradation, GelMA hydrogels were incubated in collagenase solution. The figure below (1F-G in the article) shows the degradation rate decreasing with increasing GelMA concentrations, with complete degradation by less than 3 days to upwards of 8 weeks.



- Characterizing a hydrogel's swelling ratio (indicative of water sorption capacity) can be used to predict the rate of hydrogel degradation. If was found in this article that increasing GelMA concentrations from 5% to 20% resulted in reduced swelling ratios from 1500% to 500%. This is likely due to increased crosslinking densities at 20% GelMA, limiting the rate and amount of water penetration, but also thought to slow down degradation.
- It is worth mentioning the techniques used to evaluate cell viability and adhesion. Representative live/dead fluorescence images of HaCaT cells on CelMA surrfaces are included in the full paper. Phalloidin/DAPI fluorescence images of HaCaT cells are also shown after seven days of culture. Quantification of the staining was done using ImageJ software.
- Samples were placed in 1.5 ml Eppendorf tubes with 500 µL of DPBS with 2 U mL^{-1} of collagenase type II at 37°C continuously for 3 weeks, then replaced with 500 µL of DPBS with 0.2 U mL^{-1} of collagenase for 5 weeks, which corresponds to the collagenase concentration during wound healing.The collagenase solution was refreshed every 2–3 days to maintain constant enzyme activity. At predetermined time points, the collagenase solution was removed, and the samples were washed with sterile deionized water two times, freeze-dried and weighed. Morphology of the samples

at different time points was also recorded. The percentage degradation (D%) of the gels was determined using the equation: $D\% = \frac{W_0 - W_t}{W_0} \times 100\%$ where W_0 is the initial sample dry weight and W_t is the dry weight after time t.

Conclusion/Action Items: While this article was helpful in describing how tunable gels were fabricated and tests used to characterize their mechanical properties, its protocol was more general. It should also be noted that the degradation tests being done so far by the group have been degradation and imaging by trypan blue to observe cell viability. Additional protocols for this process should be gathered, as current methods have not led to fruitful images.

11/10/2023 - Tong Lecture Series

Caitriona Treacy - Dec 13, 2023, 6:19 PM CST

Title: Tong Lecture Series – One Engineer's Story: When preparation meets opportunity

Date: 11/10/23

Content by: Caitríona Treacy

Present: N/A

Goals: One engineer's story: When preparation meets opportunity.

Content:

- Travelle (Franklin-Ford) Ellis, MD, PhD.
- Had to define her own path at University of Pittsburgh.
- Using technology to assist people have better outcomes and better lives. Worked with micospheres in Dr. Murphy's lab during graduate school at UW-Madison. Is it clear, is it concise, and what are you trying to present? Spent her time mentoring other students (high schoolers primarily) who were interested in science about drug delivery.
- Through graduate school, her life was relatively linear. After that point, it started getting complicated, but this is where she learned her best lessons.
- Big 3 pieces of advice:
    - Find your people. They do not have to be engineers (maybe they shouldn't be). It is so important to lean on your network after graduation. "The people that come behind me are also part of me."
    - Do things that scare you. Too comfortable = too easy, probably means you're not tapping into your true passions. There are people there with you. Use your networks, lean into your passions, and take risks.
    - Laugh until you cry, cry until you laugh. Resilience something that you learn. Did not match for residency. She felt that she had failed at her goal. She had to learn how to lean into that. To embrace her strengths. She redefined herself for herself.
    - Extra: Someone is counting on you.
- Now she is living her dream as the Health Equity Director at Exact Sciences.
- "Your passions today will not leave you tomorrow." Practice is important, lean into the practice you have here at the UW-Madison. Do things that you are really curious about.
- From Dr. Murphy: "Pivots are not the exception, pivots are the norm."

Conclusion/Action Items: Go home, write down what *actually* makes you happy, what you want to do, what you don't want to do, where you want to end up, etc. Check in with yourself regularly and trust that it will all come together in the end.

09/13/2023 Notes on CellLink Application Note: Biofabricating Airway Epithelial Models for COVID-19 Research

Title: Notes on CellLink Application Note: Biofabricating Airway Epithelial Models for COVID-19 Research

Date: September 13th, 2023

Content by: William Onuscheck

Present: William Onuscheck

Search Term: Google: Cell link bioprinters

Reference: CellLink Life Sciences, *Biofabricating Airway Epithelial Models for COVID-19 Research*. CellLink Life Sciences, Gothenburg, Sweden, 2020

Goals: Our project will involve the use of a CellLink 3D bioprinter. The manufacturer has released recommendations for use of their printers in different contexts. One of these contexts is in biofabrication of airway epithelial models for COVID-19 research. Given the similarity to our project, the goal of this entry is to understand application of the technology in a relevant context.

Content:

-Combination of 3D bioprinting and Air liquid interface as a means to capture respiratory tract features in vivo in healthy and diseased states

-Brochure cites relevance for lung related disease (COPD, COVID 19, idiopathic pulmonary fibrosis)

-Context of angiotensin-converting enzyme 2, a protein required for the internalization of coronavirus

-Context of bioprinting primary human bronchial epithelial tissue (Calu -3) to generate an ALI.

-Bioprinted lung disease model culture in transwell insert for 14 days, stained for ACE2 and MUC5AC (mucin)

-Human pulmonary fibroblasts used as support cell

-Culture media described in depth (different media for epithelial cells than for fibroblasts=

-Lung model was bioprinted as a disk, topped with a 5 layer brim

-Disk was 3D printed using GELMA (+laminin), with fibroblasts mixed in. Cells were gently centrifuged into the bioink in their culture media

-Epithelial cells were added dropwise atop the disks post printing, and were left to settle for 10 min, before transfer into an incubator for 20 min, before transfer to a submerged culture in a mixture of respective mediums.

-Submerged culture for 4 dyas, followed by transfer into an ALI

-Tight monolayer of epithelial cells on day 7

Conclusions/action items:

The method presented in this application note (or brochure, which is what I cited it as, but it reads like an abstract) describes a method to create a model bronchial epithelial tissue. It however, does not mention mechanical characterization of produced models, nor does it mention how to control the stiffness of culture substrates. When mentioning the addition of fibroblasts to the bio ink there are many nuanced modifications that are preformed to keep

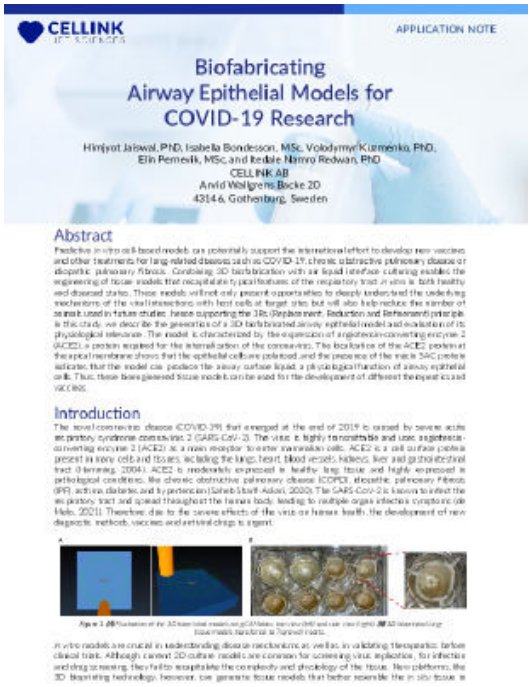
the fibroblasts homogenously distributed, further research should be done on how these techniques may be affected by bioinks intended for stiffer prints.

Read and take notes on referenced:

Biofabrication: An interesting tool to create in vitro model for COVID-19 drug targets

3D culture models to study SARS-CoV-2 infectivity and antiviral candidates: From spheroids to bioprinting

WILLIAM ONUSCHECK - Sep 13, 2023, 12:44 PM CDT



CELLINK

APPLICATION NOTE

Biofabricating Airway Epithelial Models for COVID-19 Research

Himjyot Jaiswal, PhD, Isabella Bondesson, MSc, Volodymyr Kuzmenko, PhD, Elin Pernevik, MSc and Imelda Nimmo Redwan, PhD
CELLINK AB
Arvid Wallgrens Backe 20
413 46, Gothenburg, Sweden

Abstract

Introduction

Download

App_Note_Biofabricated_Airways_for_COVID___Lumen_X_.pdf (748 kB)

09/15/2023 Notes on Bioprinting - General

Title: Notes on Bioprinting - General

Date: September 15th, 2023

Content by: William Onuscheck

Present: William Onuscheck

Search Term: NA

Reference:

Yu Zhang *et al.*, “3D Bioprinting for Tissue and Organ Fabrication,” *Annals of Biomedical Engineering*, vol. 45, Jan. 2017. doi:10.1007/s10439-016-1612-8

Goals: Our project will involve the use of a CellLink 3D bioprinter. The purpose of this entry is to understand the general principles of bioprinting, as well as to guide the development of criteria for our product design specifications.

Content:

Advantages of bioprinting include codelivery (of both cells and materials) as well as the compositional, spatial, and architectural control.

- Hydrogels often used as bioink due to their liquid like behavior during extrusion, with swift structural recovery

- Cells must be able to survive the high shear stresses during liquid printing

- Consider the vascularization needed to support physiological cell function

  - Introduction of vessel like structures necessary for the construction of in vivo tissue models.

  - Consider that vessels are fairly complex structures to begin with with 3 layers of endothelial and smooth muscle cells

  - Engineered / Printed vessel mimics should possess at least hollow lumens, ideally covered by endothelium, pericytes

  - Angiogenic growth factors being explored

  - Use of vessel like, scaffold free tubules has been reported as a substitute for actual vascularization (I think this means adding channels within the CAD model of a print)

  - Consider use of sacrificial technique to create vessel like

  - Unfortunately no numbers given for effective and reasonable diameters for vessel like structures

Conclusions/action items:

This article gives more of an overview into the printing of specific organs / the progress that has been made toward them, rather than general concept of 3D bioprinting. Vascularization may be a part of our project, so notes were taken on that. Given that if we were to add vessel like structures, its likely that we would not endothelialize them, it will be necessary to find recommendations on an effective diameter to facilitate transport without losing integrity.

WILLIAM ONUSCHECK - Sep 17, 2023, 8:38 PM CDT

3D Bioprinting for Tissue and Organ Fabrication

Download

s10439-016-1612-8.pdf (6.59 MB)

11/27/2023 Update- 10/20/2023 Cell Viability Assays Introduction

ANURAAG SHREEKANTH BELAVADI - Nov 27, 2023, 11:50 AM CST

Title: Cell Viability Assays: Introduction

Date: 10/20/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Understand the different available methods of determining cell Viability

Content:

- Cell viability methods can be categorized into those which analyze whole populations and those that involve analysis of individual cells.
- The trypan blue dye exclusion assay is one of the earliest methods for assessing cell viability.
- Metabolic dye assays are becoming increasingly popular as they can be performed on adherent cells and therefore lend themselves to high-throughput analysis.
- Live/dead viability staining describes a number of potential dyes where one specifically stains live cells (usually resulting in green fluorescence) while the other dye stains dead cells (usually with red fluorescence).
- Multiplexing assays is one mechanism by which many sets of data can be obtained from a small number of samples.
- A number of kits are now commercially available for both single assays and multiplexing, and often the underlying technology the different kits used are comparable.
- Apoptosis is a programmed sequence of events that is responsible for removing unwanted cells during normal development.
- Necrosis is an alternative mechanism of cell death which is generally considered more traumatic.
- Caspase activity can be investigated in a number of ways and as such has a chapter dedicated only to this method of investigating apoptosis.
- Alternatively, using a combination of DNA binding compounds such as SYTO probes (10) and propidium iodide it is possible to determine the type of cell death by investigating the cell staining pattern.
- Increasingly, investigators would like to determine cell viability within 3D tissues.

Source:

Stoddart, M.J. (2011). Cell Viability Assays: Introduction. In: Stoddart, M. (eds) Mammalian Cell Viability. Methods in Molecular Biology, vol 740. Humana Press. https://doi.org/10.1007/978-1-61779-108-6_1

Conclusions/action items:

Do further research on Trypan Blue dye exclusion assay and multiplexing assays

11/27/2023 Update- 10/28/2023 Comparison of Trypan Blue Dye Exclusion and Fluorometric Assays for Mammalian Cell Viability Determination

Title: Comparison of trypan blue dye exclusion and fluorometric assays for mammalian cell viability determinations

Date: 10/28/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Understand TB dye exclusion and fluorometric assays as they apply to the scope of the current project.

Content:

- Trypan Blue Exclusion Assay
    - A simple and inexpensive method for measuring cell viability.
    - Based on the principle that live cells have intact cell membranes that exclude trypan blue, a blue dye.
    - Dead cells, with compromised membranes, allow trypan blue to enter and stain the cells blue.
    - The number of viable cells is then counted manually using a hemocytometer.
- Fluorescence-Based Viability Dyes
    - Offer several advantages over the trypan blue exclusion assay.
    - Fluorescent dyes emit light when excited by a light source.
    - This allows for automated counting of viable and dead cells using a flow cytometer or an image-based cytometer.
- Examples of fluorescence-based viability dyes include:
    - Acridine orange (AO)
    - Propidium iodide (PI)
    - SYTOX orange and red
- Studies have compared the trypan blue exclusion assay and fluorescence-based viability dyes.
- These studies have found that the two methods often give different results.
- In general, the trypan blue exclusion assay tends to overestimate cell viability compared to fluorescence-based methods.
- Several reasons exist for the differences between the trypan blue exclusion assay and fluorescence-based methods.
- Trypan blue is a toxic dye, and prolonged exposure to the dye can kill cells. This can lead to an underestimation of cell viability.
- The trypan blue exclusion assay is based on the principle of membrane integrity. However, not all dead cells have compromised membranes. For example, cells that undergo apoptosis, a programmed form of cell death, may have intact membranes until they are in the late stages of apoptosis. Fluorescence-based dyes, such as propidium iodide, can stain these cells, even though they would not be counted as dead by the trypan blue exclusion assay.
- The trypan blue exclusion assay is a simple and inexpensive method for measuring cell viability.
- However, it is important to be aware of the limitations of this method and to consider using a fluorescence-based viability dye for more accurate results.
- The Trypan Blue Exclusion Assay is prone to non-specific binding to cellular artifacts, particularly in clinical and primary cell samples.
- There is no standardization of TB concentration to analyze cell viability.
- Image-based cytometry allowed researchers to examine the morphological changes that occur in TB-stained cells.
- These morphological changes may explain the differences in viability counts reached by the two methods.

Source:

Altman SA, Randers L, Rao G. Comparison of trypan blue dye exclusion and fluorometric assays for mammalian cell viability determinations. Biotechnol Prog. 1993 Nov-Dec;9(6):671-4. doi: 10.1021/bp00024a017. PMID: 7764357.

Conclusions/action items:

Proceed with trypan blue for cell viability but keep in mind that the dye is toxic so limit exposure before performing cell count.

11/27/2023 Update- 10/28/2023 Use of GelMA for 3D printing of cardiac myocytes and fibroblasts

ANURAAG SHREEKANTH BELAVADI - Nov 27, 2023, 12:21 PM CST

Title: Use of GelMA for 3D printing of cardiac myocytes and fibroblasts

Date: 10/28/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Figure out factors of 3D bioprinting that may affect the proliferation of cultured fibroblasts

Content:

- Cardiac myocytes (CMC) and cardiac fibroblasts (CFB) are fundamental to cardiac muscle, with CFBs providing structural support and extracellular matrix protein production.
- Engineered heart tissue (EHT) requires a combination of CMCs and CFBs for successful formation, as attempts with pure CMC populations failed.
- 3D bioprinting, particularly extrusion-based printing with Gelatin Methacryloyl (GelMA), is used to create complex multicellular EHTs.
- GelMA, with gelatin as the main component, enhances tissue regeneration and printability. The photopolymerizable methacrylamide group allows for covalent cross-linking by UV light after printing.
- Bioprinting parameters such as bioink viscosity, needle gauge, extruder temperature, and extrusion pressure must be optimized to reduce shear stress on cells and ensure high-precision 3D constructs.
- Viability assessment methods include LDH assay, LIVE/DEAD assay, and bioluminescence imaging (BLI), the latter providing a way to monitor long-term effects.
- GelMA's hydrogel properties and mechanical stability make it suitable for cell printing, offering a protective layer during the printing process.
- UV exposure for GelMA cross-linking negatively impacts cell viability, with extended exposure times leading to halted proliferation and cell death.
- GelMA's gelatin composition hinders traditional staining methods, requiring alternative approaches like low molecular weight stains.
- CMC-laden GelMA constructs are more sensitive to extruder pressure and GelMA concentration than CFB-laden constructs.
- Future applications aim to print spatially defined 3D constructs for studying physiological scenarios, with strategies suggested to improve cardiomyocyte survival, including adhesion molecules and optimized bioink compositions.

Source:

Koti P, Muselimyan N, Mirdamadi E, Asfour H, Sarvazyan NA. Use of GelMA for 3D printing of cardiac myocytes and fibroblasts. J 3D Print Med. 2019 Mar;3(1):11-22. doi: 10.2217/3dp-2018-0017.

Conclusions/action items:

The paper highlights that adding cells doesn't hinder printability but stresses the need for timely printing to preserve fibroblast viability—an important point for discussions with teammates and the client.

09/20/2024_Bioprinting Standards

ANURAAG SHREEKANTH BELAVADI - Sep 25, 2023, 11:36 AM CDT

Title: Standards and Specifications for Bioinks and Bioprinting

Date: 09/20/2024

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Understand the current standards required to appropriately use bioprinting and the appropriate bio-inks for applications regarding the project goals

Content:

- ASTM WK72274: This standard provides a way to measure how well a bioink can be printed, including how easily it flows through a printer nozzle and how well it maintains its shape after printing. It is helpful for manufacturers and researchers to compare different bioinks and to ensure that they are using a bioink that is compatible with their printer and their application. The standard does not cover bioinks containing cells, as these bioinks have additional requirements not addressed in this standard.

-ASTM WK74668: This document is about bioinks and biomaterial inks used in biofabrication to make living constructs for tissue regeneration or drug testing. It lists ranges of material compositions and properties that promote the survival of living cells in bioinks. It also describes compositions and properties of materials that can be printed without cells to provide mechanical support or as sacrificial materials to create boundaries or porosity in the bioprinted constructs. This standard may apply to 3D bioprinting methods such as laser-assisted bioprinting, inkjet bioprinting/droplet bioprinting, and extrusion-based bioprinting.

As an emerging field with many different manufacturers and academic researchers independently developing products, the bioprinting field currently lacks measures to ensure product consistency. Bioinks lack standards for properties such as printability and for printing parameters such as the distance between the nozzle and printing space. The standards and specifications regarding bioprinting and bioinks are still mostly under development.

Source: Standards Coordinating Body. The Regenerative Medicine Standards Portal. [Online]. Available: https://portal.standardscoordinatingbody.org/

Conclusions/action items:

Follow the ASTM WK72274 and ASTM WK74668 standards whilst bioprinting the scaffold necessary for the client. Although these standards are still under revision, they will still be used to guide the team's future efforts.

09/24/2023_3D Bioprinted GelMA Based Models for the Study of Trophoblast Cell Invasion

ANURAAG SHREEKANTH BELAVADI - Sep 25, 2023, 11:49 AM CDT

Title: 3D Bioprinted GelMA-Based Models for the Study of Trophoblast Cell Invasion

Date: 09/24/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Understand how GelMA is optimized for bioprinting in migration studies

Content:

- Bioprinting is an emerging technique used for creating 3D structures containing live cells, with applications in biomedicine.
- The study focuses on employing 3D bioprinted models made from GelMA (gelatin methacryloyl) to investigate trophoblast cell invasion in the context of placental functions during pregnancy.
- The study begins by optimizing various parameters for 3D bioprinting, including GelMA concentration, UV crosslinking time, and printing configurations.
- A systematic study is conducted to determine optimal printing parameters for GelMA, including concentration and UV crosslinking time.
- Researchers aim to balance factors like GelMA stiffness, cell viability, and cell spreading to create the best environment for trophoblast movement.
- The results show that GelMA concentration and UV crosslinking time significantly impact cell viability, cell morphology, and the degree of cell spreading.
- An optimization matrix is used to identify the best printing parameters, with 3% GelMA and 45 seconds of UV crosslinking time being the optimal combination.
- Researchers utilize this bioprinting system to create a variety of 3D constructs. These include single-layer sheets, lattice structures, double-ring structures, and tubular structures.
- To overcome challenges associated with low GelMA viscosity, especially at lower concentrations, the study introduces an ice bed that is used underneath the collector to allow thermal gelation of GelMA bio-ink during printing, before UV crosslinking. This approach enables the printing of GelMA at low concentrations (e.g., 3% w/v) and maintains the structural integrity of the constructs.
- By optimizing GelMA-based printing parameters, the research paves the way for more accurate and practical studies of trophoblast invasion and cell interactions within a 3D environment.

Source: Ding, H., Illsley, N.P. & Chang, R.C. 3D Bioprinted GelMA Based Models for the Study of Trophoblast Cell Invasion. Sci Rep 9, 18854 (2019). https://doi.org/10.1038/s41598-019-55052-7

Conclusions/action items:

Consider the impact of UV cross-linking time along with revising the conc. of GelMA currently in use. Also, try to implement the ice bed technique to fix the gelation issue with the current 3D printed scaffold.

09/16/2023_GelMA Bioprinting Applications in TE

ANURAAG SHREEKANTH BELAVADI - Sep 25, 2023, 12:02 PM CDT

Title: 3D-Printed Gelatin Methacryloyl-Based Scaffolds with Potential Application in Tissue Engineering

Date: 09/16/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Understand current methods of 3D printing GelMA and possible improvements to be applied to the scope of the project.

Content:

- The study focused on optimizing GelMA hydrogel ink formulations for 3D printing by varying GelMA methacrylation degrees, concentrations, and photoinitiator levels.
- Characterization methods included FTIR and 1H-NMR analysis to confirm methacrylation, isoelectric point determination, circular dichroism analysis to assess the secondary structure, and mechanical tests to evaluate properties.
- Swelling studies and contact angle measurements were conducted to assess hydrophilicity.
- GelMA was successfully synthesized with varying methacrylation degrees (63%, 64%, 66%) and concentrations (10%, 20%, 30%).
- Methacrylation reduced the isoelectric point, making the material more acidic.
- Circular dichroism analysis showed that the secondary structure of gelatin remained suitable for biological applications after methacrylation.
- GelMA hydrogel formulations with 20% GelMA and 1% photoinitiator exhibited optimal printability and stability for 3D printing.
- Scanning electron microscopy (SEM) revealed high porosity and interconnected macropores in the non-mineralized GelMA hydrogels.
- Hydrogels maintained their 3D microstructure after mineralization, indicating good adherence.
- Swelling behavior decreased with increasing GelMA and photoinitiator concentration, leading to a more compacted structure.
- Hydrophilicity decreased with higher concentrations of GelMA and photoinitiator.
- GelMA-based scaffolds exhibited hydrophilic characteristics with contact angles ranging from 55.45° to 78.84°.

Source:

Leu Alexa R, Iovu H, Ghitman J, Serafim A, Stavarache C, Marin MM, Ianchis R. 3D-Printed Gelatin Methacryloyl-Based Scaffolds with Potential Application in Tissue Engineering. Polymers (Basel). 2021 Feb 27;13(5):727. doi: 10.3390/polym13050727. PMID: 33673486; PMCID: PMC7956788. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7956788/

Conclusions/action items:

Try different concentrations of GelMA for 3D printing and evaluate results to best match concentration and function with respect to culturing fibroblasts in our project,

10/08/2023_Protocols of 3D Bioprinting of Gelatin Methacryloyl Hydrogel Based Bioinks

ANURAAG SHREEKANTH BELAVADI - Oct 11, 2023, 9:01 PM CDT

Title: Protocols of 3D Bioprinting of Gelatin Methacryloyl Hydrogel-Based Bioinks

Date: 10/08/2023 (Added 10/11)

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Understand existing and functional GelMA bioprinting protocol to aid in troubleshooting the team's bioprinting issues and to incorporate when synthesizing the scaffold

Content:

Protocol:

1. Preset printhead temperature to 32 °C and cool the printbed (if using Bio-X) and allow stabilization.
2. Remove CELLINK GelMA Cartridge from storage.
3. Cap it with a sterile nozzle and place it in the INKREDIBLE+ or Bio-X bioprinter printhead.
4. Allow the cartridge to heat for 10 minutes.
5. Verify that the GelMA has achieved a liquid state by removing the cartridge from the printhead and tilting it to observe if air bubbles move.
6. If air bubbles move, recap the cartridge. Change the printhead temperature to 26°C.
7. Place the cartridge on the counter at room temperature for 12 minutes to cool down.
8. Return the cartridge to the printhead and cap it with the nozzle.
9. Wait for 3 minutes and set the pressure to 15-20 kPa.
10. Print test lines.
11. If filament characteristics are sufficient, replace the nozzle, and proceed with the planned printing.
12. If filament characteristics are non-ideal due to low viscosity (high temperature), wait another minute for additional cooling and retest.
13. If filament characteristics are non-ideal due to high viscosity (low temperature), increase pressure or reheat, and repeat steps 1-10. Adjust cooling time as necessary.

https://www.cellink.com/wp-content/uploads/2018/04/GelMAPrintingProtocolmerged.pdf

Conclusions/action items:

The protocol referred by the field agent associated with the bioprinter does not provide significant information for troubleshooting. However, implementing a preheating procedure involving a water bath may be beneficial in bringing he bioink to the appropriate consistency for printing.

10/11/2023_Gelatin-Methacryloyl (GelMA) Hydrogels for 3D Cell Culture and Extrusion Bioprinting

ANURAAG SHREEKANTH BELAVADI - Oct 11, 2023, 9:14 PM CDT

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

Date: 10/11/2023

Content by: Anuraag

Present: N/A

Goals: Understand current protocols for GelMA extrusion and bioprinting and improve current methodology of UV crosslinking.

Content:

1. Synthesis and Properties of GelMA Hydrogels: GelMA hydrogels are known for their biocompatibility, supporting cell adhesion and proliferation. These hydrogels have tunable properties, including stiffness, porosity, and degradation rate, making them suitable for various tissue engineering applications. They can be processed using techniques like 3D printing, electrospinning, and microfluidics.
2. 3D Cell Culture Applications: GelMA hydrogels are particularly well-suited for 3D cell culture, offering a more realistic environment for cell growth compared to traditional 2D culture. Their properties can be tailored to mimic different tissue types in the body. Additionally, GelMA hydrogels can be functionalized with growth factors and adhesion peptides to promote cell growth and differentiation.
3. Extrusion Bioprinting: GelMA hydrogels are ideal for extrusion bioprinting due to their high shear-thinning property. This means they can be easily extruded through a nozzle and solidify rapidly after printing. They have been used to create various 3D printed tissue constructs, including blood vessels, skin, cartilage, and bone scaffolds.

UV-Crosslinking

1. Initiation: GelMA hydrogels are made by introducing methacrylate groups into gelatin, which act as crosslinking sites. When exposed to UV light, these methacrylate groups initiate a photochemical reaction, leading to the formation of covalent bonds between neighboring methacrylate groups.
2. Crosslink Formation: The UV light activates the methacrylate groups, causing them to form strong covalent bonds, or crosslinks, between polymer chains. This crosslinking process transforms the initially liquid or gel-like GelMA solution into a solid hydrogel structure.
3. Tunable Properties: One of the advantages of UV crosslinking in GelMA hydrogels is that it allows for precise control over the degree of crosslinking. The exposure time and intensity of UV light can be adjusted to tailor the hydrogel's properties, such as stiffness, porosity, and degradation rate. This tunability is essential for customizing GelMA hydrogels for specific tissue engineering applications.
4. Biocompatibility: UV crosslinking is a biocompatible method, which means it does not introduce harmful chemicals or byproducts into the hydrogel. This makes GelMA hydrogels suitable for supporting cell adhesion and proliferation, a crucial aspect of tissue engineering and regenerative medicine.

https://www.mdpi.com/2306-5354/5/3/55

Conclusions/action items:

Increase UV wavelength to/and increase crosslink duration. Consider decreasing the distance between light and the base plate. Needs to be tested with varying wavelengths.

11/27/2023 Updated GelMA synthesis Protocol

ANURAAG SHREEKANTH BELAVADI - Nov 27, 2023, 12:33 PM CST

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

Date: 11/27/2023

Content by: Anuraag Shreekanth Belavadi

Present: N/A

Goals: Find an Updated Protocol for GelMA synthesis

Content:

- Soak gelatin in demineralized or UltraPure water to achieve a final concentration of 10% (wt/vol) in a round-bottom flask with a magnetic stir bar, stirring moderately for 10–60 min for dissolution.
- While stirring moderately, heat the mixture to and maintain it at 50 °C in a water bath until gelatin is fully dissolved and the solution becomes clear.
- While stirring vigorously, add 0.6 g of methacrylic anhydride per 1 g of dissolved gelatin for high methacryloyl functionalization. Stir vigorously for 60 min, ensuring homogeneous opacity.
- Ensure sufficient stirring during GelMA functionalization to prevent phase separation.
- Use a glass pipette for methacrylic anhydride handling.
- After the reaction, transfer the solution to 50-ml tubes, remove unreacted methacrylic anhydride by centrifugation, and collect GelMA-containing supernatant.
- Dilute the supernatant with two volumes of preheated water (40 °C).
- Dialyze the solution against water using a 12-kDa MWCO dialysis membrane at 40 °C for 5–7 d, changing water daily.
- Ensure complete removal of contaminants by dialysis.
- Adjust the pH of the GelMA solution to 7.4 using 1 M $NaHCO_3$.
- In a biological safety cabinet, filter-sterilize the GelMA solution using 0.2-μm syringe filter units.
- Treat GelMA and solutions as sterile post-filtration.
- Divide the GelMA solution into aliquots, snap-freeze in liquid nitrogen, and store at −80 °C.
- Samples can be stored at −80 °C for at least 1 month.
- Transfer aliquots to a freeze-dryer, lyophilize until fully dehydrated (typically 4–7 d), and store lyophilized GelMA at −20 °C, protected from light and moisture.
- Maintain a sterile barrier during lyophilization and storage.

Source:

Loessner, D., Meinert, C., Kaemmerer, E. et al. Functionalization, preparation and use of cell-laden gelatin methacryloyl–based hydrogels as modular tissue culture platforms. Nat Protoc 11, 727–746 (2016). https://doi.org/10.1038/nprot.2016.037

Conclusions/action items:

Utilize one of the protocols (Master's/Aforementioned) to synthesize GelMA to be used to make gels and/or used in bioink cartridges for printing

09/24/2023 Training Documentation

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

WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

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

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

Data Last Imported: 02/01/2023 09:31 PM

Download

LabSafetyTrainings.pdf (183 kB)

09/09/2023 Lung Extracellular Matrix Background Research

Nick Herbst - Sep 09, 2023, 5:21 PM CDT

Title: Lung Extracellular Matrix Background Research

Date: 09/09/2023

Content by: Nick Herbst

Goals: Understand the composition of the lung's ECM

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

Content:

- ECM gives structure and provides cues to cells
  - increased stiffness of ECM leads to increased fibroblast activity
    - positive feedback loop
- ECM of lung is split into 2 domains
  - basement membrane
    - thin layer under endothelial and epithelial cell layers
  - interstitial space
    - where the fibroblasts are located
- Composition
  - collagen, elastin, fibronectin, laminin, GAGs, PGs, MMPs, fibroblasts
- Lung ECM has an elastic modulus between 0.44 and 7.5 kPa
  - heterogenous due to varying tissues in the region (ex: alveoli vs bronchial)
  - The fibroblasts in the lung ECM experience an elastic modulus of ~1kPa
    - We are aiming for around 2-5 kPa for the healthy tissue condition
- See attachment for the full journal article

Conclusions:

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

Nick Herbst - Sep 09, 2023, 5:21 PM CDT


ROGER S. MITCHELL LECTURE

Lung Extracellular Matrix and Fibroblast Function

Eric S. White

Abstract

Keywords:

Download

AnnalsATS.201406-240MG.pdf (461 kB)

09/09/2023 Bioprinting Lung Tissue

Nick Herbst - Sep 10, 2023, 3:44 PM CDT

Title: Bioprinting Lung Tissue

Date: 09/09/2023

Content by: Nick Herbst

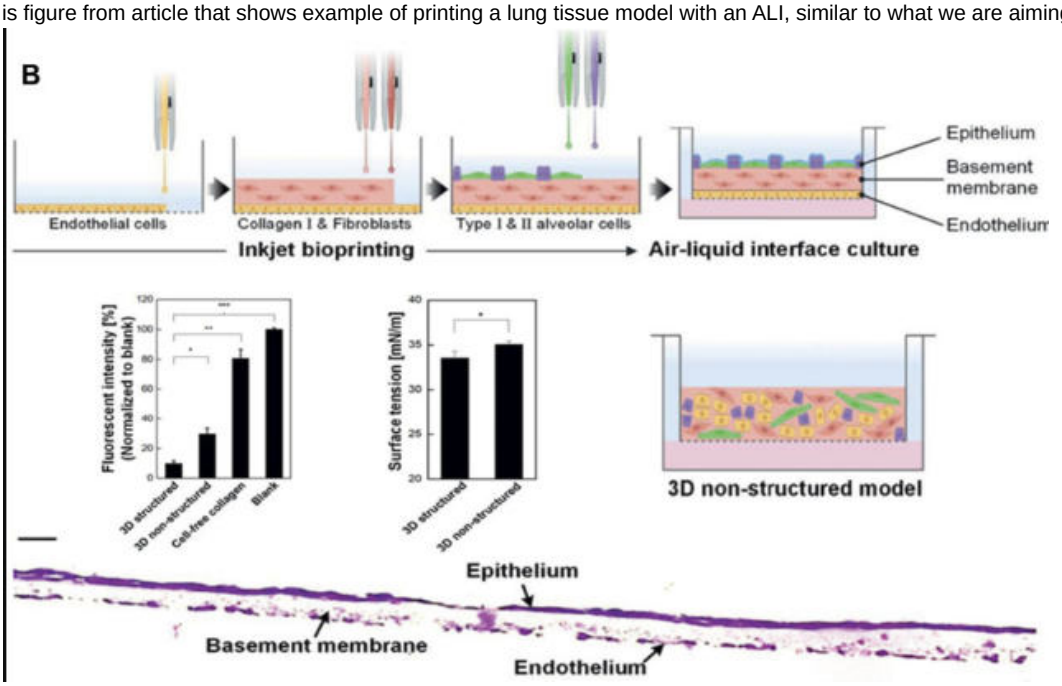
Goals: Understand the current state of bioprinting with a focus on lung tissue

Source: M. Barreiro Carpio, M. Dabaghi, J. Ungureanu, M. R. Kolb, J. A. Hirota, and J. M. Moran-Mirabal, “3D Bioprinting Strategies, Challenges, and Opportunities to Model the Lung Tissue Microenvironment and Its Function,” *Frontiers in Bioengineering and Biotechnology*, vol. 9, 2021, Accessed: Sep. 09, 2023. [Online].

Content:

- Lung Biology
  - different kinds of epithelial cells
    - ciliated
    - club: cuboidal, becomes more dominant as get downstream
    - goblet: produce surfactant/mucus
  - 2 types of alveolar cells
    - AT1: thin, flat, gas exchange
    - AT2: produce surfactant, can act as alveolar stem cells by differentiating into AT1
  - lung ECM
    - collagen, elastin, fibronectin, GAGs, laminin
    - collage I and III primary ECM
    - basement membranes are laminin and collagen IV
    - healthy lung tissue is ~2 kPa Young's Modulus
    - fibrotic lung tissue is ~17 kPa Young's Modulus
- Bioprinting Basics
  - can import a CAD file to direct the print
  - stiffness gradients can be achieved
    - HOW???
  - biomaterial ink = ink without cells, needs post-print processing to seed cells
  - bioink = ink with cells, can be done in all one step since cells embedded
  - photocrosslinkable bioinks are good b/c can modulate stiffness by varying crosslinking density by varying UV time and/or intensity
    - can even further crosslink *after* printing
  - natural inks: alginate, collagen, gelatin, cellulose, HA, ECM
    - functionalized naturals like GelMA
  - synthetic inks: PEG, PCL
  - natural inks have high biocompatibility, biodegradability, and biochemical cues
    - but they have poor mech props and high variability (so low reproducibility)
  - synthetic inks are highly tunable, good mech props, and high reproducibility
    - but they don't have the biochemical cues so those need to be introduced
  - Mixing different hydrogels could be a good idea
    - alginate + gelatin is given as example, but maybe we could look into using a combo of the GelMA inks that our printer can use
- Bioprinting Techniques
  - inkjet printing
    - bioink droplets deposited without direct contact between nozzle and substrate
    - can use multiple print heads
    - precise control over droplet deposition and thus over final location of cells
    - Issues
      - cells in bioink subjected to thermal/mech stress during deposition
      - nozzle is small so can only use smaller cells and also there is frequent clogging
      - inks must be low viscosity
  - light-based printing
    - SLA, DLP, DLW, LIFT, volumetric
    - laser polymerizes vat of ink in shape of construct
    - no nozzle
    - can use higher viscosity inks
    - higher resolution but longer print time
    - Issues
      - laser light and heat can damage cells

        - bad control of cell location
        - possible cytotoxic photoinitiatiors or small molecules
        - restricted to only one cell type since coming out of a vat
    - direct extrusion printing
        - continuous stream of highly viscous ink pushed out by mech force through a nozzle
        - printed layer-by-layer
        - most popular b/c simplest
        - issues
            - limited resolution
            - need good structure so layers don't collapse
    - assisted extrusion printing
        - uses sacrificial ink as a support material or void creator
        - print construct in a vat of *different* material and then remove/melt/dissolve the other material
        - print construct in a vat of *different* material and then remove/melt/dissolve the printed material to get void space
- Lung Bioprinting Considerations
    - Cells
        - Client wants us to embed fibroblasts and then seed epithelial cells on top after printing
        - A549 and NCI-H441 lung epithelial cell lines are common (need to find out what client uses)
        - Need to figure out what fibroblasts client uses and then get specifics on our bioprinter to figure out how/if the fibroblasts can be suspended in the GelMA ink cartridge
    - Dynamic
        - native lung tissue undergoes constant oscillations due to breathing, which is why lung tissue has elastin and is elastic
        - Ideal tissue model would incorporate this dynamic mechanical environment
            - need to ask client why he only wants static model
    - need high resolution if you want to model alveoli or bronchioles
    - need printing material suitable for gas exchange
    - Below is figure from article that shows example of printing a lung tissue model with an ALI, similar to what we are aiming for

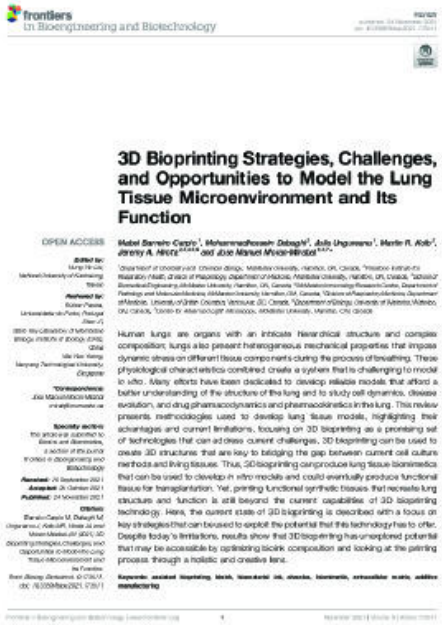


- See attachment for the full journal article

Conclusions:

Since we are switching directions from fabricating the GelMA tissue model by hand to instead bioprinting it, it is really important to understand the basics of bioprinting. Additionally, this review article goes over several considerations for bioprinting lung tissue, which I plan to bring up to the rest of the team.

Nick Herbst - Sep 09, 2023, 5:24 PM CDT



frontiers in Bioengineering and Biotechnology

3D Bioprinting Strategies, Challenges, and Opportunities to Model the Lung Tissue Microenvironment and Its Function

OPEN ACCESS

Human lungs are organs with an intricate hierarchical structure and complex composition; lungs also present heterogeneous mechanical properties that impose dynamic stress on different tissue components during the process of breathing. These physiological characteristics combined create a system that is challenging to model in vitro. Many efforts have been dedicated to develop reliable models that afford a better understanding of the structure of the lung and to study cell dynamics, disease evolution, and drug pharmacodynamics and pharmacokinetics in the lung. This review presents methodologies used to develop lung tissue models, highlighting their advantages and current limitations, focusing on 3D bioprinting as a promising set of technologies that can address current challenges. 3D bioprinting can be used to create 3D structures that are key to bridging the gap between current cell culture methods and living tissues. Thus, 3D bioprinting can produce lung tissue biomimetics that can be used to develop in vitro models and could eventually produce functional tissue for transplantation. Yet, printing functional synthetic tissues that recreate lung structure and function is still beyond the current capabilities of 3D bioprinting technology. Here, the current state of 3D bioprinting is described with a focus on key strategies that can be used to exploit the potential that this technology has to offer. Despite today's limitations, results show that 3D bioprinting has unexplored potential that may be accessible by optimizing bioink composition and looking at the printing process through a holistic and creative lens.

Download

fbioe-09-773511.pdf (4.71 MB)

09/16/2023 CELLINK Bioprinter

Nick Herbst - Sep 16, 2023, 9:27 PM CDT

Title: CELLINK Bioprinter

Date: 09/16/2023

Content by: Nick Herbst

Goals: Understand how the client's bioprinter operates

Source: CELLINK, "Bioprinting Basics - BIO X Gen 2 Tutorial," *YouTube*, (Mar. 02, 2022). Accessed: Sep. 16, 2023. [Online Video]. Available: https://www.youtube.com/watch?v=rn8IB3plNsQ

Content:

- This is a tutorial video provided to us by Dr. Brasier from the company he bought his bioprinter from
    - the bioprinter is from CELLINK, and the model is a BIO X Gen 2
    - it is an extrusion bioprinter with 3 printheads/nozzles, temperature-controlled printbed, germicidal UV lamp for sterilization, HEPA filter, and exchangeable photocuring modules for photopolymerizable bioinks (4 wavelength options: 365 nm, 405 nm, 485 nm, and 520 nm)
    - it can take STL files, so SolidWorks WILL work for making the print structure
- The video covers hardware, settings, single-material printing, multi-material printing, and droplet printing
    - I will only be focusing on the settings and single-material printing sections
- Settings - Utilities menu
    - accessed through top right gear icon
    - can home it in the move tab
    - can control bed temp, HEPA-filtered fan, and chamber lights in the printer tab
    - can control print head settings like ink cartridge temp, extrusion time, and extrusion pressure in the tools tab
        - can set pressure to 20 kPa and then hit test flow in pressure tab in order to see when ink becomes extrudable
    - can turn on and off the photocrosslinking modules in the tools tab
    - can sterilize chamber in clean chamber tab
- Single-material printing
    - workflow: 3D model, STL file, sliced into Gcode by printer slicer (so can add an STL via USB drive), print
    - click bioprint
    - in model page, click 3D models then select STL file
    - in surface page, can choose to print on petri dish, glass slide, or well plate (any well plate, and can select which wells)
    - in printer page
        - on printer tab can modify hardware like HEPA fan, extra pump, bed temp, and photocrosslinking module
            - can have layer by layer OR bulk at end photocrosslinking
            - can have photocrosslinking occur in center of print or do a line scan
        - on tool tabs can enable the tool, save settings for an ink profile, turn on photocrosslinking (again but different settings), nozzle diameter (can add different nozzles), print head temperature, extrusion speed (speed of the print head's movement), and extrusion pressure
            - extrusion speed and pressure can be varied to change the filament diameter
                - increased pressure = increased diameter
                - increased speed = decreased diameter
            - viscous materials need slower speeds since come out slower
            - need to find balance of pressure, speed, and temperature to avoid both discontinuous filament and over-extrusion
            - it's a pneumatic extrusion system, so there is slight lag between print head movement and when ink extrudes, so you can adjust the preflow delay and postflow stop time
                - preflow delay is so you don't miss a getting ink at starting spot of the layer
                - postflow stop time is so you don't "drag" excess ink over to the starting spot of the next layer
            - photocrosslinking menu can select which wavelength module, how long the light should be on for when you crosslink in seconds, and how far the light should be from print surface/bed in centimeters
                - CONTROL THE EXTENT OF CROSSLINKING
    - in layers page, can see toolpath of print, infill pattern (honeycomb, grid, concentric, rectilinear (orthogonal alternating)), infill density (affects pore size, over 50% = solid object for hydrogels b/c viscoelastic), and layer height
        - printed ink sags and merges, doesn't stay as cylinders, so set layer height to slightly lower than nozzle diameter (ideally it would be

        - can set the height of first layer as percentage which ensures it is adhered to print surface (think semi cylinder filament for first layer)
    - in print page, gives overview of print protocol
        - can save protocol
        - hit and hold droplet icon to prime nozzle with material
            - we would have to be careful doing this because that means the ink would leave the heated cartridge and could gel in the nozzle, so we would need to start the print right away
    - calibration
        - auto calibration is a beta feature, don't try it
        - auto bed leveling should be done right away at beginning of print session
            - put surface probe in probe holder on underside of print head
            - system will auto level by touching probe to whatever you print on (i.e. petri dish)
        - manual calibration positions nozzle *just* above print surface, and the center of the print/3D model must be in the back half of the print surface so that the UV light can reach
            - that means that our print can only be as big as half of our print surface (i.e. petri dish)
            - use manual controls to get nozzle to the (x,y) you want the origin of the print to be
            - use up and down controls to get the nozzle close to the print surface, starting in 10mm increments then switch to 1mm and finally 0.1mm
                - up/down moves the print BED, not the print HEAD
                - move bed until nozzle and its reflection in print surface are barely touching
                - print surface should still be able to move freely under the nozzle
            - press calibrate
    - you can change the settings as it is printing
        - this is probably how you can print a gradient??
- See link for the full video: https://www.youtube.com/watch?v=rn8IB3plNsQ

Conclusions:

Since we are bioprinting our tissue model, it is critical to understand how the bioprinter used in the Brasier Lab works. On 09/17/2023, the team will meet to learn the basics from Carley, who has worked with it before during her time this summer in the Brasier Lab. I believe that the photocrosslinking settings we should first try are layer by layer and line scan. I am not fully sure if layer by layer will result in a photocrosslinking gradient with stiffer GelMA on the bottom, but if this happens then we can try the "once at the end" setting. Also, I don't know if line scan will be worth it due to our print size, so we possibly can just do center.

09/21/2023 Bioprinting GelMA

Nick Herbst - Sep 29, 2023, 1:14 PM CDT

Title: Bioprinting GelMA

Date: 09/23/2023

Content by: Nick Herbst

Goals: Understand the how GelMA is bioprinted by looking at a the protocol provided by CELLINK

Source: CELLINK, “Protocols,” CELLINK. Accessed: Sep. 20, 2023. [Online]. Available: https://www.cellink.com/protocols/

Content:

- CELLINK gave a protocol for printing with their GelMA bioink
    - protocol is optimized for 10% w/w GelMA photocrosslinked with 0.25% LAP and uses the temperature-controlled printhead with **thermal nozzle cover** and a cooled print bed
    - I am summarizing critical steps of the protocol and adding notes
- Heat bioink to 37 C until liquid, and set printhead to 25 C (25 C is the absolute minimum printing temp)
- When embedding cells in the bioink (which we will do with fibroblasts) mix bioink and cells in 10:1 ratio
- Print with 22-27 gauge nozzles
- equilibrate bioink to 25 C and cool print bed to 15 C
- Example settings
    - 25 C, 25 gauge nozzle, 5mm/ss print speed, 300ms pre-flow delay, 23-33 kPa print pressure
    - adjust print pressure by 1 kPa to fine-tune
- too low viscosity = decrease printhead temp by 1 C
- too high viscosity = increase printhead temp by 1 C
- GelMA can be photocrosslinked at 405 nm OR 365 nm, but 405 nm is recommended
    - recommended 10 sec per mm layer height
    - recommended distance of 5 cm
- if print goes longer than 20 min, then increase printhead temp by 0.5 C after 20 min
- See attachment for the full protocol

Conclusions:

The biggest thing I noticed in this protocol was the mention of a thermal nozzle cover/insulator in order to keep the bioink from gelling in the nozzle. I do Carley ever mentioned the use of one, and I don't remember seeing her take it off during our training on the bioprinter using the basic starter ink. I will bring this up to the team when we next meet.

Nick Herbst - Sep 20, 2023, 2:34 PM CDT

BIOPRINTING PROTOCOL

GelMA Bioink

This is a suggested procedure, please adjust according to your experimental needs.
To maintain the sterility of the product work under sterile conditions.

Protocol aim

Materials needed

Download

Bioprinting-Protocol-GelMA-Bioink_7-Feb-2023.pdf (104 kB)

09/29/2023 GelMA Bioink SDS and Specifications

Nick Herbst - Sep 29, 2023, 1:34 PM CDT

Title: GelMA Bioink SDS and Specifications

Date: 09/29/2023

Content by: Nick Herbst

Goals: Determine whether or not the bioink is affected by regular heating/cooling cycles by looking at the safety data sheet and specifications

Source: CELLINK, “GelMA Bioink LAP 0.25%,” CELLINK. Accessed: Sep. 29, 2023. [Online]. Available: https://www.cellink.com/product/gelma-bioink-lap-0-25/

Content:

- SDS Highlights
  - not a hazardous substance or mixture
  - first aid: flush eyes, wash hands, rinse mouth, get fresh air (minimal measures)
  - store in dry, well-ventilated place
  - 1 g/mL density (since hydrogel)
  - BP is over 100 C
  - Viscosity is 1-1000 Pa*s (depends on temperature)
  - Avoid freezing
- Specifications Highlights
  - derived from porcine gelatin
  - 365 nm or 405 nm crosslinking of methacrylate groups via LAP photonitiator
  - print with temperature controlled printhead and cooled print bed
  - biocompatible
  - 3 life minimum shelf life
  - store in fridge (4 C)
  - protect from light and **avoid temperature fluctuations**
  - clear amber gel
  - sterile (when it arrives, not necessarily always)
  - 6.5-7.4 pH
  - 24.5-27 C gelation temperature
- See attachments for full SDS and specifications

Conclusions:

The GelMA bioink cartridge is heated and cooled regularly each time it is used to print. During the 09/29 advisor meeting, it was brought up that it is important to see if this thermal stress will affect the bioink over time. I thought that it shouldn't since it's a physical issue of viscosity change, but after researching the issue I found that CELLINK recommends avoiding temperature fluctuations. However, I do not know how exactly this affects the bioink over time, so I believe it would be a good idea to call CELLINK and ask them about it along with our other questions on GelMA bioprinting.

Nick Herbst - Sep 29, 2023, 1:34 PM CDT

Ref No: SPS-IK-305102
Date: 08-APR2021 Author: MG, JB Version: 4

CELLINK

Specification Sheet

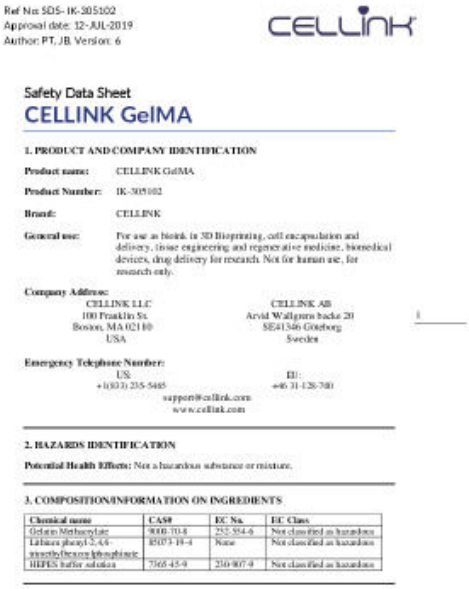
GelMA Bioink LAP 0.25%

Product description	This bioink is derived from porcine gelatin with enhanced printability and crosslinking through the incorporation of methacrylate groups. Provided with LAP as a photoinitiator, GelMA is easily photocrosslinked with 365 nm or 405 nm modules. Recommended for use with BIO X Temperature-controlled Printhead and cooled print bed or for use with INKREDIBLE+ and heated printhead. For description on how to mix with cells, bioprint and crosslink, follow the Bioprinting Protocol.
Intended use	Biocompatible material for 3D bioprinting. Research Grade. For research use ONLY. Not intended for in vitro diagnostics or in vivo uses. Not intended for administration in humans or animals. Produced under sterile and aseptic conditions.
Product number	IK305102
Shelf life	Minimum 3 months, expiration date stated on package.
Storage and handling	Store at 4-8°C. DO NOT FREEZE. Protect from light and avoid temperature fluctuations. Ensure that the bioink container is capped prior to storage to prevent drying.
Safety	Handle in accordance with good hygiene and laboratory safety practices. Read Safety Data Sheet for more information regarding ingredients and potential hazardous compounds.
Related documents	Bioprinting Protocol as well as Safety Data Sheet can be downloaded from our website https://cellink.com/product/cellink-gelma or scan the QR code below.

Download

Specification-Sheet-GelMA-Bioink-LAP-0.25_08-Apr-2021-1-4.pdf (107 kB)

Nick Herbst - Sep 29, 2023, 1:34 PM CDT



Ref No: SDS- IK-305102
Approval date: 12-JUL-2019
Author: PT, JB Version: 6

CELLINK

Safety Data Sheet

CELLINK GelMA

1. PRODUCT AND COMPANY IDENTIFICATION

Product name: CELLINK GelMA

Product Number: IK-305102

Brand: CELLINK

General use: For use as bioink in 3D Bioprinting, cell encapsulation and delivery, tissue engineering and regenerative medicine, biomedical devices, drug delivery for research. Not for human use, for research only.

Company Address:

CELLINK LLC
100 Franklin St.
Boston, MA 02110
USA

CELLINK AB
Arvid Wallgrens backe 20
SE41346 Göteborg
Sweden

Emergency Telephone Number:

US:
+1(833) 235-5465

EU:
+46 31-126-780

support@cellink.com
www.cellink.com

2. HAZARDS IDENTIFICATION

Potential Health Effects: Not a hazardous substance or mixture.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Chemical name	CAS#	EC No.	EC Class
Gelatin Methacrylate	9000-70-8	232-554-6	Not classified as hazardous
Lithium phenyl-2,4,6-trimethylbenzoylphosphinate	85073-19-4	None	Not classified as hazardous
HEPES buffer solution	7365-45-9	230-907-9	Not classified as hazardous

Download

CELLINK-GelMA-SDS-12-July-2019-4.pdf (471 kB)

10/17/2023 Cell Viability Assays in 3D Hydrogels

Nick Herbst - Oct 17, 2023, 1:23 PM CDT

Title: Cell Viability Assays in 3D Hydrogels

Date: 10/17/2023

Content by: Nick Herbst

Goals: Learn if commercial cell viability assays work for 3D hydrogel systems

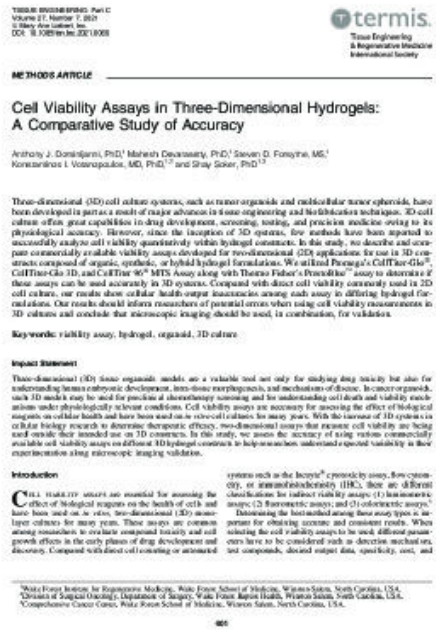
Source: A. J. Dominijanni, M. Devarasetty, S. D. Forsythe, K. I. Votanopoulos, and S. Soker, “Cell Viability Assays in Three-Dimensional Hydrogels: A Comparative Study of Accuracy,” *Tissue Eng Part C Methods*, vol. 27, no. 7, pp. 401–410, Jul. 2021, doi: 10.1089/ten.tec.2021.0060.

Content:

- This study used 4 different commercial cell viability assays to quantify the cell viability of CRC cells within collagen, HA/Gelatin, and HA/ColMA hydrogels
    - CellTiter-Glo, CellTiter-Glo 3D, and CellTiter 96 from Promega
    - PrestoBlue from Thermo Fisher
- Assays
    - CellTiter-Glo and CellTiter-Glo 3D
        - measures ATP levels as indicator of viability
            - requires cell lysis (they differ in degree of lysing, 3D has harsher lysing agent)
        - gives chemiluminescent readout
            - well plate reader (luminescence)
    - CellTiter 96
        - identifies metabolically active cells
        - gives colorimetric readout
            - well plate reader (absorbance)
    - PrestoBlue
        - reagent is taken up by metabolically active cells and reduced to a fluorescent form
        - gives fluorescence readout
            - well plate reader (fluorescence)
- Assays are for indirectly detecting cell viability, but you can directly detect cell viability via immunofluorescence microscopy
    - IF was used to get true viabilities to compare what the assays said the viabilities were
    - stained with DAPI for nuclei and used a caspase-3 antibody
        - cleaved caspase-3 is a marker for cell death/apoptosis
    - VisioPharm software takes IF images and ID's cells without caspase-3 and intact nuclei as alive and makes them green and ID's cells with caspase-3 and fragmented nuclei as dead and makes them red
- Conclusion: assays all had inaccuracies/discrepancies due to 3D nature of system
- NOTE: authors mention that used "calcein AM with ethidium homodimer-1 staining" such as "Live/Dead®" would work for hydrogels
    - need confocal microscope
- See attachment for full article

Conclusions:

Originally, we were considering a commercial cell viability assay to quantify cell viability. However, our advisor pointed out that this may not work because we have a 3D hydrogel, not a 2D surface. Because of this, the team began looking into cell viability assays for 3D cultures. This paper looked at how different cell viability assays worked in hydrogels, and revealed that there are significant inaccuracies (as predicted by our advisor). **However**, the authors state that a Live/Dead® stain would work for hydrogels because the reagents have high penetration and you can use a confocal microscope to get a Z-stack. I plan on bringing this up to Dr. Brasier during the client meeting this Thursday.



TISSUE ENGINEERING: Part C
Volume 27, Number 7, 2021
© Mary Ann Liebert, Inc.
DOI: 10.1089/ten.tec.2021.0060

termis.
Tissue Engineering & Regenerative Medicine International Society

METHODS ARTICLE

Cell Viability Assays in Three-Dimensional Hydrogels: A Comparative Study of Accuracy

Anthony J. Domínguez, PhD,[1] Mahesh Devarasetty, PhD,[1] Steven D. Forsythe, MS,[1] Konstantinos I. Votanopoulos, MD, PhD,[1,2] and Shay Soker, PhD[1,3]

Three-dimensional (3D) cell culture systems, such as tumor organoids and multicellular tumor spheroids, have been developed in part as a result of major advances in tissue engineering and biofabrication techniques. 3D cell culture offers great capabilities in drug development, screening, testing, and precision medicine owing to its physiological accuracy. However, since the inception of 3D systems, few methods have been reported to successfully analyze cell viability quantitatively within hydrogel constructs. In this study, we describe and compare commercially available viability assays developed for two-dimensional (2D) applications for use in 3D constructs composed of organic, synthetic, or hybrid hydrogel formulations. We utilized Promega's CellTiter-Glo®, CellTiter-Glo 3D, and CellTiter 96® MTS Assay along with Thermo Fisher's PrestoBlue™ assay to determine if these assays can be used accurately in 3D systems. Compared with direct cell viability commonly used in 2D cell culture, our results show cellular health output inaccuracies among each assay in differing hydrogel formulations. Our results should inform researchers of potential errors when using cell viability measurements in 3D cultures and conclude that microscopic imaging should be used, in combination, for validation.

Keywords: viability assay, hydrogel, organoid, 3D culture

Impact Statement

Three-dimensional (3D) tissue organoids models are a valuable tool not only for studying drug toxicity but also for understanding human embryonic development, inter-tissue morphogenesis, and mechanisms of disease. In cancer organoids, such 3D models may be used for precision chemotherapy screening and for understanding cell death and viability mechanisms under physiologically relevant conditions. Cell viability assays are necessary for assessing the effect of biological reagents on cellular health and have been used on in vitro cell cultures for many years. With the increase of 3D systems in cellular biology research to determine therapeutic efficacy, two-dimensional assays that measure cell viability are being used outside their intended use on 3D constructs. In this study, we assess the accuracy of using various commercially available cell viability assays on different 3D hydrogel constructs to help researchers understand expected variability in their experimentation along microscopic imaging validation.

Introduction

CELL VIABILITY ASSAYS are essential for assessing the effect of biological reagents on the health of cells and have been used on *in vitro*, two-dimensional (2D) monolayer cultures for many years. These assays are common among researchers to evaluate compound toxicity and cell growth effects in the early phases of drug development and discovery. Compared with direct cell counting or automated systems such as the Incyte® cytotoxicity assay, flow cytometry, or immunohistochemistry (IHC), there are different classifications for indirect viability assays: (1) luminometric assays; (2) fluorometric assays; and (3) colorimetric assays.[1]

Determining the best method among these assay types is important for obtaining accurate and consistent results. When selecting the cell viability assays to be used, different parameters have to be considered such as detection mechanism, test compounds, desired output data, specificity, cost, and

[1]Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA.
[2]Division of Surgical Oncology, Department of Surgery, Wake Forest Baptist Health, Winston-Salem, North Carolina, USA.
[3]Comprehensive Cancer Center, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA.

Download

ten.tec.2021.0060.pdf (891 kB)

10/30/2023 Bioprinting With Fibroblasts

Nick Herbst - Oct 30, 2023, 3:49 PM CDT

Title: Bioprinting With Fibroblasts

Date: 10/30/2023

Content by: Nick Herbst

Goals: Further understand the details of bioprinting with fibroblasts within the bioink

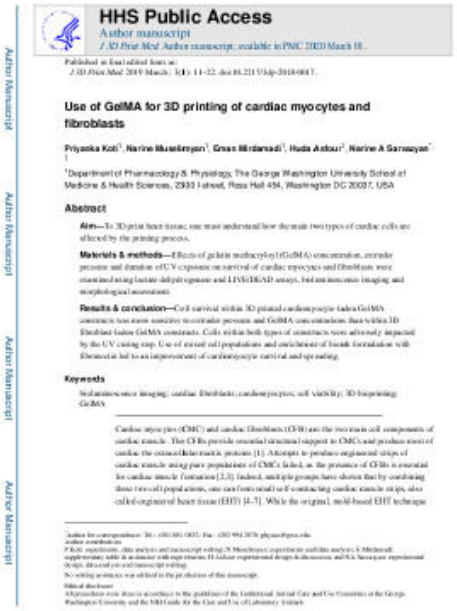
Source: P. Koti, N. Muselimyan, E. Mirdamadi, H. Asfour, and N. A. Sarvazyan, “Use of GelMA for 3D printing of cardiac myocytes and fibroblasts,” *J 3D Print Med*, vol. 3, no. 1, pp. 11–22, Mar. 2019, doi: 10.2217/3dp-2018-0017.

Content:

- This study used GelMA bioink with encapsulated cardiomyocytes and cardiac fibroblasts
    - Looked at how printing affected cell viability using bioluminescent imaging (transfected cells with luciferase), LIVE/DEAD fluorescent assays, and LDH assays
- The authors noted that the GelMA bioink could provide fibroblasts with a degree of protection against shear stresses from printing
- Characterized minimum pressure needed to extrude different w/v% GelMA bioinks
    - 10% needs ~17 psi
    - 15% needs ~23 psi
    - 20% needs ~32 psi
    - 25% needs ~ 37 psi
- Extruding pressure impacts cardiomyocytes more than fibroblasts, but generally speaking higher pressure means decrease in viability
- UV time greater than 6 minutes killed the fibroblasts, even though it is a non cytotoxic wavelength
- GelMA is gelatin so it absorbs most dyes/stains, which makes imaging harder
- non-polymerized bioink can negatively affect cell viability if exposed for an extended period of time
    - which is why we need to do cell viability testing
    - allegedly, using LAP instead of an igacure photoinitiator has a negligible effect on cell viability
- See attachment for full article

Conclusions:

This paper had more information about cell viability assays for printing with cells, which is helpful. Also, the paper claims that adding cells should not significantly affect the printability of the bioink. Additionally, the paper mentions that non-polymerized bioink can negatively impact the fibroblasts if they are left in solution for a long time. This is the critical reason why we need to test cell viability, as mentioned by our advisor. We need to figure out how long we have to print once we add the fibroblasts. This also means that we can't make a big batch of cell-laden bioink; we need to only mix as much as we need at a time. This will be a critical challenge that I plan to bring up to my teammates and the client during the next respective meetings.



HHS Public Access

Author manuscript

J 3D Print Med. Author manuscript; available in PMC 2020 March 01.

Published in final edited form as:

J 3D Print Med. 2019 March ; 3(1): 11–22. doi:10.2217/3dp-2018-0017.

Use of GelMA for 3D printing of cardiac myocytes and fibroblasts

Priyanka Koti[1], Narine Muselimyan[1], Eman Mirdamadi[1], Huda Askour[1], Narine A Sarvazyan[1]

[1]Department of Pharmacology & Physiology, The George Washington University School of Medicine & Health Sciences, 2300 I street, Ross Hall 454, Washington DC 20037, USA

Abstract

Aim—To 3D-print heart tissue, one must understand how the main two types of cardiac cells are affected by the printing process.

Materials & methods—Effects of gelatin methacryloyl (GelMA) concentration, extruder pressure and duration of UV exposure on survival of cardiac myocytes and fibroblasts were examined using lactate dehydrogenase and LIVE/DEAD assays, fluorescence imaging and morphological assessment.

Results & conclusion—Cell survival within 3D printed cardiomyocyte-laden GelMA constructs was more sensitive to extruder pressure and GelMA concentration than within 3D fibroblast-laden GelMA constructs. Cells within both types of constructs were adversely impacted by the UV curing step. Use of mixed cell populations and enrichment of bioink formulation with fibronectin led to an improvement of cardiomyocyte survival and spreading.

Keywords

bioluminescence imaging; cardiac fibroblasts; cardiomyocytes; cell viability; 3D bioprinting; GelMA

Cardiac myocytes (CMC) and cardiac fibroblasts (CFB) are the two main cell components of cardiac muscle. The CFBs provide essential structural support to CMCs and produce most of cardiac the extracellular matrix proteins [1]. Attempts to produce engineered strips of cardiac muscle using pure populations of CMCs failed, as the presence of CFBs is essential for cardiac muscle formation [2,3]. Indeed, multiple groups have shown that by combining these two cell populations, one can form small scale contracting cardiac muscle strips, also called engineered heart tissue (EHT) [4–7]. While the original, mold-based EHT technique

Author for correspondence: Tel.: +1 202 994 3507; Fax: +1 202 994 2870; physns@gwu.edu

Author contributions

P Koti: experiments, data analysis and manuscript writing; N Muselimyan: experiments and data analysis; E Mirdamadi: supplementary table & assistance with experiments; H Askour: experimental design & discussion; and NA Sarvazyan: experimental design, data analysis and manuscript writing.

No writing assistance was utilized in the production of this manuscript.

Ethical disclosure

All procedures were done in accordance to the guidelines of the Institutional Animal Care and Use Committee of the George Washington University and the NIH Guide for the Care and Use of Laboratory Animals.

Download

nihms-1011886.pdf (1.64 MB)

11/11/2023 Mixing Bioink and Cells

Nick Herbst - Nov 11, 2023, 4:19 PM CST

Title: Mixing Bioink and Cells

Date: 11/11/2023

Content by: Nick Herbst

Goals: Understand the protocol we will use for adding fibroblasts to the GelMA bioink

Source: CELLINK, “Mixing cells and bioink Protocol,” CELLINK. Accessed: Nov. 11, 2023. [Online]. Available: https://www.cellink.com/wp-content/uploads/2023/02/Mixing-cells-and-bioink-Protocol_5-Jan-2023-1.pdf

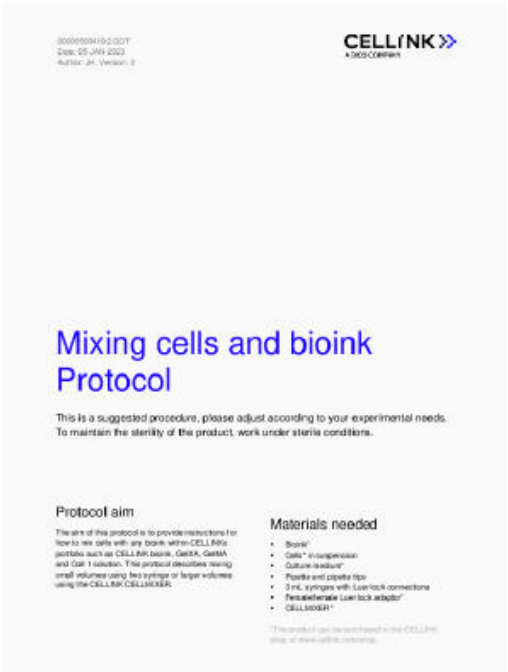
Content:

- Protocol can be adjusted for 1 mL or 3 mL of cell+bioink
    - Bioink:cells should be 10:1, so use 100 uL of cell suspension for 1mL bioink (1/3 of cartridge) or use 300 uL of for 3 mL bioink (whole cartridge)
- Summarized protocol + notes
    - Prepare the bioink
        - Heat the GelMA by heating it up at 37 C until liquid (very low viscosity)
        - Transfer 1 mL of GelMA to a fresh 3 mL syringe via a F/F Luer lock adaptor
    - Prepare the cells
        - Determine desired cell density/concentration
        - Passage the cells and resuspend the calculated amount of cells in 100 uL of media
        - Transfer the suspension to a fresh 3 mL syringe via a F/F Luer lock adaptor
    - Mix the bioink and cells
        - Attach a Luer lock adaptor to the syringe containing the GelMA and pre-fill the adaptor with bioink to minimize air bubles
        - Connect the 2 syringes and mix the bioink and cell suspension by gently pushing the contents back and forth to homogenize
            - NOTE THAT THIS INDUCES SHEAR STRESS SO BE GENTLE
        - Once homogenized, transfer the mixture to a fresh 3 mL bioink cartridge
            - Preferably an amber cartridge over a clear one
            - Use immediately
- See attachment for the protocol

Conclusions:

The cells have a limited viability in uncured bioink, so we can only make as much mixture as we are going to use to print in that session. This means that we should probably make 1 mL of mixture whenever we need to print with fibroblasts. The CELLLINK starter kit provided by the client has the materials needed to make the solution. For a full cartridge, a CELLMIXER is used, but for just 1 mL you can swish back and forth between syringes

Nick Herbst - Nov 11, 2023, 3:28 PM CST



Mixing cells and bioink Protocol

This is a suggested procedure, please adjust according to your experimental needs. To maintain the sterility of the product, work under sterile conditions.

Protocol aim

Materials needed

Download

Mixing-cells-and-bioink-Protocol_5-Jan-2023-1.pdf (105 kB)

12/02/2023 Hydrogel Scaffold Porosity for Lunge Model

Nick Herbst - Dec 02, 2023, 6:00 PM CST

Title: Hydrogel Scaffold Porosity for Lunge Model

Date: 12/02/2023

Content by: Nick Herbst

Goals: Look into the details of porosity necessary for a hydrogel scaffold modeling lung tissue by looking at a case study

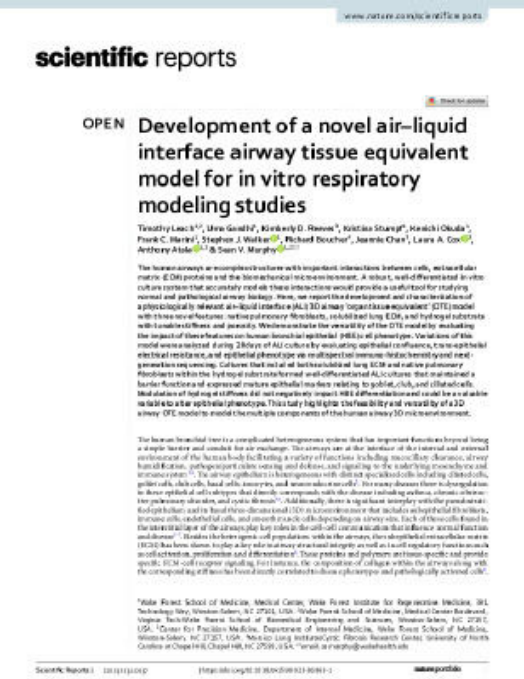
Source: T. Leach et al., “Development of a novel air–liquid interface airway tissue equivalent model for in vitro respiratory modeling studies,” *Sci Rep*, vol. 13, p. 10137, Jun. 2023, doi: 10.1038/s41598-023-36863-1.

Content:

- The authors did a study in which they made an ALI 3D airway organ tissue equivalent that consisted of bronchial epithelial cells, lung fibroblasts, solubilized lung ECM, and a hydrogel substrate in order to model the airway tissue environment
- Their hydrogel was thiolated gelain + thiolated HA crosslinked with PEGDA
- They varied their hydrogel stiffness by changing the amount of PEGDA crosslinker used
    - They had 3 "healthy" airways stiffness conditions (they said that these were all healthy and not yet in diseased range yet)
        - Soft
            - 2.6 kPa elastic modulus
            - 56 µm average pore size
        - Medium
            - 5.1 kPa elastic modulus
            - 20 µm average pore size
        - Stiff
            - 9.8 kPa elastic modulus
            - 15 µm average pore size
    - Increase in stiffness = decrease in porosity
- Adding solubilized lung ECM (slurry of collagen (I/V/VI), elastin, laminin, fibronectin, sulfated GAGs, HA) did not impact the hydrogels' stiffnesses
- All 3 of their tested conditions allowed for successful bronchial epithelial cell culture for 4 weeks
- See attachment for full article

Conclusions:

This paper mirrors our design project almost perfectly. This case study, which I find hard to believe none of us have come across before, further reinforces the idea that one way that hydrogel crosslinking is tuned by crosslinker concentration (other ways are polymer concentration, photocrosslinking time, and photocrosslinking distance). Even though everything in this paper is applicable to our project, I specifically looked at it to see data on hydrogel pores for lung tissue models. This paper gave me what I consider a lower limit for porosity of our hydrogels; I still need to find an upper limit. Additionally, I need to figure out how we will actually quantify our bioprinted hydrogel's porosities since it won't be visible to the unaided eye.



scientific reports

OPEN

Development of a novel air–liquid interface airway tissue equivalent model for in vitro respiratory modeling studies

Download

41598_2023_Article_36863.pdf (5.87 MB)

09/20/2023 GelMA Options

Nick Herbst - Sep 21, 2023, 4:06 PM CDT

Title: GelMA Options

Date: 09/20/2023

Content by: Nick Herbst

Present: Nick Herbst

Goals: Understand the differences in CELLINK's GelMA inks

Content:

- CELLINK has multiple GelMA bioinks and their website provides descriptions for each
- GelMA 95%
  - biodegradable, great cell adhesion, physiologically relevant mech props, can blend with proteins/GFs/other photoinks, kit comes with proprietary Xcite and Xsorb used to initiate
- GelMA LAP 0.25%
  - creates physiologically relevant environments, comes with 0.25% LAP photoinitiator in the bioink, 24.5-27 C gelation temp, 45-55% methacrylated, most general GelMA bioink available
- GelMA A
  - GelMA alginate blend, prints better since softer bioink, prints at low pressure and forms filaments, comes with 0.25% LAP photoinitiator in the bioink, can do additional crosslinking by adding ionic agent, 45-55% methacrylated
- GelMA C
  - GelMA with cellulose nanofibers, comes with 0.25% LAP photoinitiator in the bioink, prints at low pressure so smoother printing, 45-55% methacrylated, nanofibers means still translucent for imaging
- GelXG
  - GelmA with xanthan gum for wider printability window, 23-27 C gelation temp, 45-55% methacrylated, comes with 0.25% LAP photoinitiator in the bioink
- GelXA
  - GelMA with xanthan gum and alginate to enhance printability and stability, can do addtional crosslinking by adding ionic agent, 23-27 C gelation temp, 45-55% methacrylated, used as base for other bioinks (bone/cartilage/skin/laminin/fibrin)

Conclusions:

- From the looks of it, all of the variations of GelMA bioink exist in order to improve printability, which means that GelMA bioprinting is known to have issues. I think we should try to print with GelMA C (since it prints smoother and has cellulose nanofibers for added support)

10/23/2023 Effect of Cells in Bioink

Nick Herbst - Oct 23, 2023, 2:29 PM CDT

Title: Effect of Cells in Bioinks

Date: 10/23/2023

Content by: Nick Herbst

Goals: Understand how adding cells to bioink affects the printed structure

Source: G. J. Gillispie et al., "The Influence of Printing Parameters and Cell Density on Bioink Printing Outcomes," *Tissue Eng Part A*, vol. 26, no. 23–24, pp. 1349–1358, Dec. 2020, doi: 10.1089/ten.tea.2020.0210.

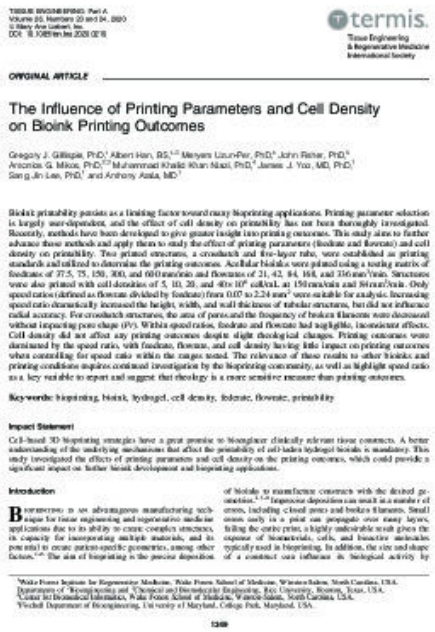
Content:

- In our design, we're adding fibroblasts to the GelMA bioink, so we need to know how adding cells affects the print
- Cell density has no effect on print fidelity
- As cell density increases, storage modulus (and thus elastic modulus) increases and yield strength decreases
    - In terms of elastic modulus, if cell density is doubled then the elastic modulus increases by roughly 0.3 kPa
    - This study only looked at cell densities of 5, 10, 20, and 40 million cells per mL
- See attachment for full article

Conclusions:

After the first client meeting, I began to wonder how incorporating fibroblasts within the GelMA bioink would affect the printability and mechanical properties of the hydrogel scaffold. If we make a CELLINK GelMA crosslinking/stiffness curve, it may not be applicable/correct once we add cells.

Nick Herbst - Oct 17, 2023, 12:20 PM CDT



ORIGINAL ARTICLE

The Influence of Printing Parameters and Cell Density on Bioink Printing Outcomes

Download

ten.tea.2020.0210.pdf (1.03 MB)

11/01/2023 Current State of Affairs

Nick Herbst - Nov 01, 2023, 1:29 PM CDT

Title: Current State of Affairs

Date: 11/01/2023

Content by: Nick Herbst

Goals: Document current project status and thoughts on challenges/issues

Content:

- Overall Goal
    - Make hydrogels of 2 distinctly different stiffness ranges (healthy and fibrotic) which can support fibroblast encapsulation as well as small airway epithelial cell adhesion and culture
- Fall 2023
    - Rest of the team trained on bioprinter operation
    - Discovery of the thermal insulator nozzle cover and additional bioprinting protocol research led to resolution of nozzle clogging issue
    - Advisor states team should divide and conquer to look at cell viability with GelMA and optimize bioprinting
    - Bioprinting status
        - Can semi-reliably do droplet printing
        - Issues/Considerations
            - Running out of GelMA bioink, need to order more ASAP
            - Eventually will need clear set of optimized parameters for our 2 printing conditions to give to client
                - Need to mess with photocrosslinking settings to get a stiffness vs photocrosslinking curve
            - Only have been doing droplet printing, what about printing with a CAD file?
                - Droplets may have more variability (?)
                    - Yes, when I printed (carley) they were all diff sizes and Dianhua didn’t seed cells (frustrated about this) because he thought they shouldn’t have any rounding
                - Only doing droplets removes a degree of design that advisor may want to see in deliverables
                - How will we do rheometry testing with droplets?
                    - The droplets spread and look super similar to the ones formed in molds just not uniform but could for sure do rheometry on them
            - Due to uncrosslinked bioink being cytotoxic for extended periods of time, we can’t make bulk mixture of bioink w/ fibroblasts (can’t do it with entire cartridge)
                - Need to figure out process of adding cells to bioink and only make as much mixture as we need to use to print each time

            - Cell link has protocol of this but I think we need to focus on the successfully printing then putting cells in - really need a CAD file
    - Cell viability status
        - Considering different methods
            - Collagenase > trypan blue > cell counting
            - LIVE/DEAD stain
        - Currently pipetting bioink out of cartridge, putting into molds, and crosslinking for 5 min at a set height with Dr. Masters' old UV light
        - Issues/Considerations
            - Using bioink + molds at the set distance and time gave gels that were very stiff and had very high variability
                - Possibly adjust crosslinking conditions
            - Need to get materials for cell viability methods
                - Reagents
                - LIVE/DEAD stain needs a fluorescent microscope
                - Trypan blue + cell counting needs a cell counter + counter slides
            - As of now, we are only planning to look at fibroblast viability in a printed construct to see how temperature and shear stress of printing affects cells, but we need to consider fibroblast viability in the uncrosslinked bioink
                - Having cells in a solution with methacrylates and LAP for an extended time is cytotoxic, so *how long do we have to print once we add cells to the bioink??*
                    - We will likely need to print immediately as we wouldn't want the cells to become settled due to gravity either (this is in cellink protocol as well)
- Prior Work
    - Summer 2023
        - Switched directions to 3D printing GelMA
        - Carley and Elijah began figuring out how to use the bioprinter
            - Ran into issues with GelMA bioink cooling and clogging the nozzle
    - Spring 2023
        - Switched to GelMA since couldn't get PEG to work
        - Using advisor's GelMA and protocol, fabricated various hydrogels with molds
            - Only variable was "cooling time" which was the amount of time the gels were kept in the fridge before crosslinking
                - This variable wasn't actually appropriate since GelMA stiffness is dependent on polymer concentration, crosslinker concentration, UV duration, and UV distance/intensity
        - Soft hydrogels had poor epithelial cell adhesion

        - Stiff hydrogels were too stiff and had too much variability
    - Fall 2022
        - PEG hydrogels attempted
            - Did not work because could not get to polymerize
        - For deliverable purposes, used gelatin
            - Did mechanical testing via rheometry

Conclusions/action items:

The team has split into 2 subgroups. Carley, Caitriona, and I are focusing on bioprinting while Elijah, Will, and Anuraag are focusing on cell viability. I personally felt like I have not been contributing as much as normal, so I felt it necessary to make a list of the project's status and concretely identify issues so that I could refocus myself on the project.

11/05/2023 Scaffold Design 1

Nick Herbst - Nov 05, 2023, 10:40 AM CST

Title: Scaffold Design 1

Date: 11/05/2023

Content by: Nick

Present: Nick

Goals: Make a SolidWorks model to be used for bioprinting

Content:

- Made a cylindrical model that is 2 mm thick and 9 mm in diameter
- See attached STL file for the full model

Action items:

- Try to print a scaffold using this file
  - Print with concentric infill and infill density of 50% or over

Nick Herbst - Nov 05, 2023, 10:41 AM CST

Download

BME400_GelMA_Trial1.STL (12.1 kB)

09/09/2023 Prior Completed Trainings

Nick Herbst - Sep 09, 2023, 5:16 PM CDT

Title: Prior Completed Trainings

Date: 09/092023

Content by: Nick Herbst

Present: Nick Herbst

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

Content:

WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

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

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

Data Last Imported: 09/18/2022 08:30 PM

You have the following permits and upgrades:

Name	Date
Green Permit	01/29/2022
Lab Orientation	09/26/2020
Red Permit	01/26/2022
Laser 1	10/06/2020

Conclusions/action items:

- I have completed all necessary training for use of the TEAM Lab and the Teaching Lab

- I can get additional qualifications for the TEAM Lab/Makerspace if I want/need to

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: