

Bone marrow microenvironment culturing system for mesenchymal stem cells

msc_culture

Client: Dr. Wan-Ju Li

Advisor: Dr. Tracy Puccinelli

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Date: 3/3/17 – 3/9/17

Problem Statement:

Mesenchymal stem cells (MSCs) are widely studied for their valuable multipotent character that could enable tissue regeneration, especially in orthopedic injuries. Unfortunately, the yield of MSCs through extraction from bone marrow is low, and cells must be expanded in culture without the risk of spontaneous differentiation. Current culture methods are variable in their ability to maintain MSCs in a multipotent state, and do not adequately attempt to recreate the physiological conditions that prevent differentiation. For this reason, there is a need for a culture system that allows researchers to sustain multipotency in their cells by mimicking the bone marrow microenvironment through substrate stiffness and oxygen concentration.

Last Week's Goals:

- Biomaterial
 - Begin fabrication and complete MTS testing on resulting gels
 - Initial fabrication will be done without RGD sequences until we achieve gels of appropriate stiffness, in order to save materials
- Bioreactor
 - Drill holes in containers for gas lines
 - Order remaining circuit/gas line components
 - Begin circuit/gas line assembly
 - Order gas tanks if necessary (talk to Li lab about availability)

Summary of Individual/Team Role Accomplishments:

- **Taylor Marohl:** Wrote progress report.
- **Veronica Porubsky:** Ordered materials and communicated with the client.
- **Michelle Tong:** Attended BSAC meeting.
- **Maddie Meier:** Updated website.

Summary of Design Accomplishments/Literature Search:

- Biomaterial
 - Have cell strainer, waiting on mortar and pestle
 - Have been trained on lyophilizer by Dr. P
 - Reached out to Tony (Masters lab) and received advice on lyophilizer use

- In terms of using MTS vs rheometer for stiffness evaluation, Tony said we can use the rheometer and convert G' to E because PEGDA is a linear elastic material (but we will need to know the material's Poisson's ratio). He said that the gels we create would likely be too small for MTS testing. He typically uses the TA dynamic mechanical analyzer (DMA) in the Soft Materials Lab, but there is a fee to use this instrument.
- Bioreactor
 - Identified some components that still need to be ordered, and possible sources

Activities:

Person	Task	Time	Weekly Total	Sem. Total
Taylor	-Progress report -MTS/Rheometer protocols -Lyophilizer training -Other notebook updates	-0.5hr -3hr -0.5hr -1hr	5 hr	31.5 hr
Veronica	-Scaffold mechanical/chemical research -Scaffold protocol update -Transfer materials/prep materials -Research + purchase materials	-2hr -1hr -1hr -0.5hr	4.5 hr	23.5 hr
Michelle	-MTS protocol -Lyophilizer training	-0.5hr -0.5hr	1 hr	21.5 hr
Maddie	-Bioreactor work	-1hr	1 hr	17.5 hr
Team	-Advisor Meeting	-0.5hr	0.5 hr	14.5 hr

Goals for This Week:

- Fabricate multiple PEGDA gels, test mechanical properties
- Finalize the cell evaluation plan, run by Dr. Li
- Bioreactor fabrication
 - Drill holes in containers
 - Get rubber stoppers and tubing
 - Build circuit and Arduino code
 - Order solenoid valves and remaining elements

Schedule for Upcoming Week:

- **Friday 2:30pm** Advisor Meeting
- **Thursday 6:00pm** Team Meeting

Difficulties:

- Still waiting on mortar and pestle to arrive so we can grind up the NaCl for the biomaterial
 - Once this arrives, we should be set to get started with fabrication
- Weren't able to measure incubator hole earlier this week (team members who are regularly at WIMR didn't know where the incubator was) – this prevented reaching out to the Student Shop to drill holes in the containers, and prevented ordering rubber stoppers and gas tubes
- May need to do an ACD conversion to translate the data from the sensor

- Inconsistencies in power supply for Arduino and solenoid valves – probably need to talk to Dr. Nimunkar about how to solve this

Project Schedule/Timeline:

Color Key: Deliverables Bioreactor Biomaterial Outreach Questions

Fri 3/3 - Thurs 3/9

- Biomaterial experimentation
 - Start without RGD
- Bioreactor fabrication
 - Circuit, Arduino code, chamber containers

Fri 3/10 - Thurs 3/16

- Fabricate multiple biomaterial stiffnesses, freeze
 - Include RGD
- Finalize cell evaluation plan with Li lab
- Bioreactor fabrication
 - Circuit, Arduino code, chamber containers
 - Mechanical elements, gas

Fri 3/17 - Thurs 3/23

- **SPRING BREAK**

Fri 3/24 - Thurs 3/30

- Cell validation of biomaterial
- Write up Outreach deliverables
- Bioreactor validation
 - Gas concentration maintenance over 24 hours
 - Return to set concentration after disturbance

Fri 3/31 - Thurs 4/6 (MADDIE GONE THIS WEEK)

- Start cell evaluation on biomaterial
- Begin cell evaluation on bioreactor

Fri 4/7 - Thurs 4/13

- Continue cell evaluation on biomaterial
- Continue cell evaluation on bioreactor
- Begin working on poster, final report

Fri 4/14 - Thurs 4/20

- Finish cell evaluation on biomaterial, analyze data
- Finish cell evaluation on bioreactor, analyze data
- Finish poster, continue working on final report

Fri 4/21 - Thurs 4/27

- **Fri 4/28 FINAL POSTER PRESENTATION Friday 4/28**
- Finish final report

Fri 4/28 - Thurs 5/4

- **Wed 5/3 FINAL REPORT DUE Wednesday 5/3**