

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: 2/24/17 – 3/2/17

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:

- Finalize protocols for biomaterial fabrication and evaluation
- Learn how to use lyophilizer and plan rheometer stiffness characterization
- Begin bioreactor fabrication

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
 - Fabrication protocol is complete – we plan to begin fabrication early next week if all materials have arrived
 - Waiting on mortar and pestle (Dr. P ordered for us) and cell strainer (Veronica ordered)
 - Have all other materials (PEGDA, etc)
 - Plan to get trained on Lyophilizer tomorrow or early next week
 - Will potentially use MTS for stiffness evaluation like we did last semester. Rheometer testing would require conversion from storage modulus (G) to elastic modulus (E); we have a relationship for this from a paper but I'm not sure we want to rely on this since it's not a widely-used conversion.

- Bioreactor
 - Purchased chamber containers
 - Will need to drill holes for gas lines in/out – plan to talk to Student Shop about this next week
 - Will need rubber gasket to seal holes and prevent leakage
 - We have most materials needed but still need to order Solenoid valves (to control gas flow) and another sensor

Activities:

Person	Task	Time	Weekly Total	Sem. Total
Taylor	-Progress report -Biomaterial mechanical testing plan	-0.5hr -1hr	1.5 hr	26.5 hr
Veronica	-Reagent/equipment search	-1hr	1 hr	19 hr
Michelle	-Container shopping	-1hr	1 hr	20.5 hr
Maddie	-Bioreactor work -Container shopping	-2hr -1hr	3 hr	16.5 hr
Team	-Advisor Meeting	-0.5hr	0.5 hr	14 hr

Goals for This Week:

- 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)

Schedule for Upcoming Week:

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

Difficulties:

- No new difficulties

Project Schedule/Timeline:

Color Key: **Deliverables** **Bioreactor** **Biomaterial** **Outreach** **Questions**

Fri 2/24 - Thurs 3/2 (VERONICA GONE 3/1-3/5)

- Biomaterial experimentation
- Bioreactor fabrication

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
- Finish bioreactor fabrication
 - Mechanical elements, gas

Fri 3/17 - Thurs 3/23

- **SPRING BREAK**

Fri 3/24 - Thurs 3/30

- Write up Outreach deliverables
- Start cell evaluation on biomaterial
- Bioreactor validation
 - Gas concentration maintenance over 24 hours
 - Return to set concentration after disturbance

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

- Continue cell evaluation on biomaterial
- Begin cell evaluation on bioreactor

Fri 4/7 - Thurs 4/13

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

Fri 4/14 - Thurs 4/20

- 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**