

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: 2/3/17 – 2/9/17

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:

- Hopefully biomaterials will be delivered and we can start experimentation
- Bioreactor design matrix, finalize design, discuss materials
- Continue working on midsemester paper, start working on presentation

Summary of Individual/Team Role Accomplishments:

- **Taylor Marohl:** Wrote progress report.
- **Veronica Porubsky:** N/A
- **Michelle Tong:** N/A
- **Maddie Meier:** Updated website.

Summary of Design Accomplishments/Literature Search:

- After meeting with Dr. Nimunkar last week, we have been given the reports from the freshman design group that created a hypoxic device similar to ours. The main differences are that their device did not also control CO₂, and did not need to be sterilized.
 - We met with Dr. Li to discuss this idea. His incubator has tubing out the back already installed. No other lab personnel will be using the incubator, so we will have good access.
 - He said we will need to purchase gas tanks, because each incubator has their own tank. He encouraged the combination of gasses to conserve space, but this seems difficult if each gas needs to be regulated independently.
 - We have contacted the Li lab's instrumentation specialist, Ron McCabe, and he has offered his guidance on the technical aspects of the project.

- New materials for the biomaterial: 1 still has not been delivered
 - We should be able to start fabrication next week
 - The Li lab has a lyophilizer that we can use
- Dr. Li recommended we tailor our paper for submission to Tissue Engineering Part C, Tissue Engineering Part A, or Integrative Biology.
 - We aren't sure which to choose, but I am leaning towards Tissue Engineering Part C or Integrative Biology.
 - We would like you input.

Activities:

Person	Task	Time	Weekly Total	Sem. Total
Taylor	-Progress report	-1hr	4.5 hr	12.5 hr
	-Bioreactor design	-2hr		
	-Journal paper	-1.5hr		
Veronica	-Biomaterial work	-2hr	2 hr	11 hr
Michelle	-Client meeting	-1hr	3.5 hr	11 hr
	-Bioreactor research	-2hr		
	-Purchasing Materials			
	-Lipocalin-2 & prolactin Journal Article	-0.5hr		
Maddie	-Client meeting	-1.5hr	3.5 hr	9.5 hr
	-Bioreactor mechanical components research	-1hr		
	-bioreactor block diagrams & schematics	-1hr		
Team	-Team Meeting	-1.5 hr	2hr	9.5hr
	-Advisor Meeting	-0.5 hr		

Goals for This Week:

- Start biomaterial experimentation
- Finalize bioreactor design and order all materials
- Create a SolidWorks sketch of the bioreactor
- Finish Midsemester Presentation for Friday
- Work on Midsemester Paper

Schedule for Upcoming Week :

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

Difficulties:

- Dr. Li has a commercially available hypoxic device that goes inside an incubator. He really wants something that can be set to 3 O₂ tensions by the researcher. He suggests a serial dilution design, where the highest O₂ tension is set in the first chamber, and we "dilute" the oxygen in the subsequent chambers.

- If we flow air from one chamber to another, we would have to prevent back-flow and also have an input to each new chamber with nitrogen (and possibly CO₂) delivery. This adds extreme complications to the design, and we aren't sure how feasible it is, especially when combining it with the workload of the biomaterial.
- We have ordered some materials that we know we will need, but we are hoping to finish the design this week.

Project Schedule/Timeline:

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

Mon 1/16 - Sun 1/22

- Discuss O₂ bioreactor ideas (Maddie)
 - Take the week to research/plan any updates
- Update on biomaterial
 - Need to formulate a rationale for choosing our stiffness goal (100-200 Pa) so it can be included in our paper.
- Ask Dr. Puccinelli about format of midsemester report (scientific publication?)
- Plan Outreach project

Mon 1/23 - Sun 1/29

- Plan a client meeting
- Meet with Dr. Nimunkar/Bioinstrumentation TA to discuss bioreactor ideas/difficulties
- Design matrix for bioreactor
 - Decide on design, start discussing/researching materials
- Order new biomaterial materials, hopefully they arrive this week
- Finish planning Outreach project

Fri 1/27 - Thurs 2/2

- Conduct outreach at Bayview Community Center on Tues (1/31) from 3:45-4:45pm
- Michelle - start MSC cell training in Li lab
- Finalize biomaterial protocol
- Meet with Dr. Nimunkar/Bioinstrumentation TA to discuss bioreactor ideas/difficulties
- PDS due Friday 2/3
- Begin preparing midsemester paper

Fri 2/3 - Thurs 2/9 (VERONICA GONE 2/8-2/10)

- Biomaterials delivered, start experimentation
- Bioreactor design matrix, finalize bioreactor design, discuss materials
- Continue working on midsemester paper, start working on presentation

Fri 2/10 - Thurs 2/16 (MADDIE OUT 2/15-2/16)

- Biomaterial experimentation
- Order bioreactor materials
- Bioreactor Soliworks
- **MIDSEMESTER PRESENTATION Friday 2/17**
- Finish midsemester paper

Fri 2/17 - Thurs 2/23 (VERONICA GONE 2/21-2/25)

- Fabricate multiple biomaterial stiffnesses, freeze
- Begin bioreactor fabrication
- **MIDSEMESTER PAPER DUE Wednesday 2/22**

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

- Start cell evaluation on biomaterial
- Continue bioreactor fabrication

Fri 3/3 - Thurs 3/9

- Continue cell evaluation on biomaterial
- Finish bioreactor fabrication

Fri 3/10 - Thurs 3/16

- Finish cell evaluation on biomaterial, analyze data

Fri 3/17 - Thurs 3/23

- **SPRING BREAK**

Fri 3/24 - Thurs 3/30

- Begin cell evaluation on bioreactor
- Begin final cell evaluation on bioreactor + substrate

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

- Continue cell evaluation on bioreactor
- Continue final cell evaluation on bioreactor + substrate

Fri 4/7 - Thurs 4/13

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

Fri 4/14 - Thurs 4/20

- 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**