Bone marrow microenvironment culturing system for mesenchymal stem cells

msc_culture

Client: Dr. Wan-Ju Li
Advisor: Dr. Tracy Puccinelli
Team: Taylor Marohl [tmarohl@wisc.edu] (920) 412-8765 (Leader)
Veronica Porubsky [vporubsky@wisc.edu] (262) 720-5818 (Communicator, BPAG)
Michelle Tong [mtong2@wisc.edu] (414) 791-3509 (BSAC)
Maddie Meier [mgmeier2@wisc.edu] (920) 220-1121 (BWIG)

Date: 3/31/17 – 4/6/17

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 cell evaluation plan with Li lab
    - Plan to set up a meeting with Dr. Li and the grad student we'll be working with to discuss
- Fabricate multiple biomaterial stiffnesses, freeze
    - Include RGD
- Finish bioreactor fabrication
    - Validate gas sensors
- Bioreactor validation
    - Gas concentration maintenance over 24 hours
    - Return to set concentration after disturbance (door opening)

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
    - Gels are in the fabrication process – currently in the lyophilizer
- Cell culture evaluation
    - MSC population is being expanded by the Li lab

- Met with Dr. Li to discuss experimental setup
    - Plan to evaluate cell senescence (p21 and p16 via RT-PCR) and quiescence (surface markers via FACS)
    - Will have 5 conditions in culture: tissue culture plastic (- control), tissue culture plastic with layer of gel on top (no pores – to evaluate 2D stiffness), 2 stiffnesses of 3D gel with pores (one of which has same stiffness as 2D gel)
        - Each condition will be done in triplicate
    - 6^{th} condition in assays will be MSCs with CD271 (+ control since these MSCs are fresh from bone marrow)

- Bioreactor
    - Arduino code is complete and functional
    - Holes have been drilled in containers for gas lines
    - Circuit (sensors and solenoids) for 1 chamber is complete
        - Plan to hook up to gas chambers Friday to validate functionality

Activities:

Person	Task	Time	Weekly Total	Sem. Total
Taylor	-Progress report	-0.5hr	8.5 hr	49 hr
	-Arduino code	-1hr		
	-Cell seeding density research	-1hr		
	-Bioreactor assembly with Ron	-1hr		
	-Biomaterial reagent prep	-4hr		
	-Client meeting	-1hr		
Veronica	-Chemical research and protocol chemical calculations	-3hr	14 hr	50 hr
	-Researching cell density and emailing Kevin about timeline and protocol for the final experiments	-2.5hr		
	-Researching quiescent markers	-0.5hr		
	-Answering questions about fabrication and materials/protocol	-0.5hr		
	-RGD prep and methacrylation	-4hr		
	-Dialysis bath and bath changes	-0.5hr		
	-Remove RGD from dialysis and prepare for/start lyophilization	-2hr		
	-Client meeting	-1hr		
Michelle	-Circuit set-up with Ron	-3hr	11 hr	43 hr
	-Arduino code	-1hr		
	-Quiescence markers research	-1.5hr		
	-Fabricating chamber holes	-1.75hr		
	-Finishing circuit set-up with Ron	-1.75hr		
	-Arduino code troubleshooting	-2hr		
Maddie	-Bioreactor assembly/Arduino code	-3hr	3 hr	25.5 hr
Team	-Advisor Meeting	-0.5hr	0.5 hr	18 hr

Goals for This Week:

- Bioreactor validation
    - Validate gas sensors (O2 in ambient conditions, CO2 in cell culture incubator)
    - Gas concentration maintenance over 24 hours
    - Return to set concentration after disturbance (door opening)
- Finish biomaterial fabrication and begin cell culture evaluation
- Update paper draft for review by Dr. Puccinelli

Schedule for Upcoming Week:

- **Friday 9am** Advisor Meeting
- **Thursday 6:00pm** Team Meeting

Difficulties:

- Limited number of cells available for cell culture evaluation
- Biomaterial fabrication process is time-consuming
- May be difficult to match the stiffness of a 2D scaffold with the stiffness of a 3D scaffold for cell culture evaluation

Project Schedule/Timeline:

Color Key: Deliverables Bioreactor Biomaterial Outreach Questions

Fri 4/7 - Thurs 4/13

- Cell evaluation on biomaterial
- Bioreactor validation
    - Gas concentration maintenance over 24 hours
    - Return to set concentration after disturbance
- Begin cell evaluation on bioreactor
- Update paper draft

Fri 4/14 - Thurs 4/20

- Cell evaluation assays, analyze data
- Finish cell evaluation on bioreactor, analyze data
- Paper draft to Dr. Puccinelli for review
- Begin working on poster

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**