Bone marrow microenvironment culturing system for mesenchymal stem cells

msc_culture

Client: Dr. Wan-Ju Li
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Date: 4/7/17 – 4/20/17

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:

- 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

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
    - RGD-conjugated gels failed a brief cell adhesion experiment
    - Plan to switch to some variation of Gelatin gels
        - If don't use glutaraldehyde to crosslink, may not remain stable at 37C
        - If use glutaraldehyde, stiffness is increased and thus less relevant to our experimental question
- Cell culture evaluation

- MSC population is being expanded by the Li lab – currently have plenty of cells for experiment
- Experimental setup
  - Evaluate cell senescence (p21 and p16 via RT-PCR) and quiescence (Ki-67 via FACS)
  - 3-4 wells/condition will give enough cells for assays
  - Conditions:
    - Normoxia:
      - Tissue culture plastic
      - Gels of 4 stiffnesses
    - Hypoxia:
      - Tissue culture plastic
      - Gel of 1 stiffness
- Opting to do a longer cell culture experiment in favor of better results for the paper, rather than rushed results for the poster

- Bioreactor
  - System is set up in the Li lab incubator with gas tanks and computer to monitor gas output concentrations
  - Oxygen sensor works well, oxygen can be maintained within 1% of set tension
  - CO2 sensor is non-functional at CO2% higher than 4.5% (must be 5% for cell culture) – emailed the company and they are sending a new sensor with rush shipping, but we're not sure it will get here in time
    - Wondering if there are any alternatives we can use?

Activities:

Person	Task	Time	Weekly Total	Sem. Total
Taylor	-Progress report	-0.5hr	11 hr	60 hr
	-Lyophilizer updates	-0.5hr		
	-Gelatin fabrication	-3hr		
	-Gelatin rheometer testing	-1hr		
	-Bioreactor setup	-1.5hr		
	-Gelatin fabrication	-1hr		
	-Meet with Kevin	-0.5hr		
	-Pick up materials at WIMR	-1hr		
	-Bioreactor setup	-2hr		
Veronica	-Lyophilizer troubleshooting	-1hr	18 hr	68 hr
	-Emails (to update and discuss experimental design with Dr. Puccinelli, Tony Berger, Kevin Wang, and Dr. Li)	-3hr		
	-GRGDS prep	-2hr		
	-GRGDS + coating + gelatin research	-2hr		
	-GRGDS + PEGDA dilutions prep	-3hr		
	-Gel prep and fabrication + changing dialysis on GRGDS	-1.5hr		
	-Second gel prep and fabrication + changing dialysis on GRGDS (rushed)	-1.5hr		

	-Third gel prep and fabrication -Flow quiescence antibodies research -Seeding cells (fallopian tube epithelial and ovarian surface epithelial cells) and evaluating adhesion	-2hr -1hr -1hr		
Michelle	-Hypoxia chamber troubleshooting -Executive summary edits -Hypoxia chamber validation w/ Maddie -Planning hypoxia chamber set-up for cell culture -Rheometer troubleshooting -Gelatin rheometry -Machining second chamber -Setting up and troubleshooting hypoxia chamber	-3.5hr -0.5hr -3hr -1hr -2.5hr -2.5hr -1hr -2hr	16 hr	59 hr
Maddie	-Bioreactor assembly	-4hr	4 hr	29.5 hr
Team	-Advisor Meeting	-0.5hr	0.5 hr	18.5 hr

Goals for This Week:

- Start cell evaluation
- Create poster
- Turn in final report draft to Dr. Puccinelli for review

Schedule for Upcoming Week:

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

Difficulties:

- Can't get cell adhesion to the gels
- CO2 sensor non-functional
- Deciding on our poster title:
  - "We Just Can't Gel"
  - "To Gel or Not to Gel, It's Not a Question… It's Not Going to Gel"
  - "1000 Ways Not To Make a Gel"
  - "10 Things I Hate About Gels (feat. Hypoxia Chambers)"

Project Schedule/Timeline:

Fri 4/21 - Thurs 4/27

- Work on poster
- **Fri 4/28 FINAL POSTER PRESENTATION Friday 4/28**
- Finish cell evaluation, analyze data
- Final report draft to Dr. Puccinelli 4/26

Fri 4/28 - Thurs 5/4

- Finish final report
- **Wed 5/3 FINAL REPORT DUE Wednesday 5/3**