Department of Biomedical Engineering
The Chinese University of Hong Kong

BMEG4998 – Final Year Project (Thesis I) 2024/2025

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Project Title: Role of hypoxic conditions in cartilage tissue engineering

Project Supervisor: Prof Alan Li

Monthly Progress Report for **BMEG4998**
For the 2nd meeting with the Project Supervisor in Oct 2024

1. Project Objectives

To investigate the effectiveness of hypoxic condition of cartilage differentiation from mesenchymal stem cells by comparing the number of differentiated cartilage cells against cultures in normoxic condition.

2. Project Plan and Proposed Methodology

Refer to progress report #1 for other parts of the project methodology.

Making cell pellets in well plate:

1. Let all reagents come to room temperature before using.
2. Retrieve four P6 cell cultures in T75 flasks from the incubator. Make sure that they reach ~80% cell convergence by observing under the microscope.
3. Wipe the exterior of the flasks clean with 75% alcohol.
4. Slowly suck the culture medium out of the flasks and discard it. Be careful not to disturb the bottom of the flasks since the cells are adhered to the bottom.
5. Wash each flask with 4mL of PBS to remove the remaining medium. Gently rock the flasks back and forth. Suck it out and discard it afterwards.
6. Squirt 1mL of Trypsin to the bottom of each flask to re-suspend the cells. Knock on the bottom of the flasks to help re-suspend.
7. Incubate the flasks at 37°C for 3-5 mins until ~80% are re-suspended as observed under the microscope. Add trypsin as necessary.
    - Trypsin is an enzyme that can digest the cells and help re-suspend them, but it will kill the cells if exposed for too long.
8. Add culture medium equivalent to 2.5 times of the volume of Trypsin used to each flask to stop the digestion.
9. Squirt the medium onto the bottom of each flask to detach as many cells as possible.
10. Mix the contents of each flask together and transfer to one falcon tube. Use more falcon tubes if necessary.
11. Centrifuge the falcon tube(s) at 300*g* for 5 mins.
12. Remove the supernatant without disturbing the cells pellets.
13. Re-suspend the pellets with 2mL of PBS total and mix them together.
14. Isolate 10µL of the mixture to a centrifuge tube and add 10µL Trypan blue stain.
15. Load 10µL of the stained mixture to each window of an A/B slide.
16. Count the cell numbers. *(We got 6,400,000 cells total.)*
    - It is expected to have 3,000,000 cells per flask.

17. Calculate how many wells can be filled with each well having at least 200,000 cells. *(We got 30 wells.)*
    - Make sure that at least 21 wells minimum for each of hypoxia and normoxia. 7 wells are necessary each week, with 3 for staining and 4 for RNA extraction.
18. Centrifuge the master mixture at 300*g* for 5 mins. Remove the supernatant.
19. Re-suspend with 1mL of chondrogenic medium.
20. Add more chondrogenic medium until the total amount is sufficient for 150µL per well.
21. Warm ascorbate and TGF-β1 to room temperature. Add 1µL of each for every 1mL of medium.
22. Pipette 150µL of mixture of each well. Use one well plate for each of hypoxia and normoxia.
23. Centrifuge the well plates at 300*g* for 1 min.
24. Incubate at 37°C for the appropriate oxygen levels.

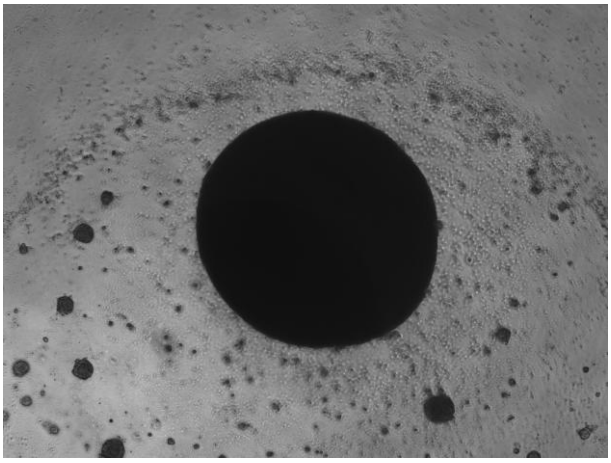

Figure 1
MSC in well after 2 days of culturing under hypoxia, 1250µm in diameter

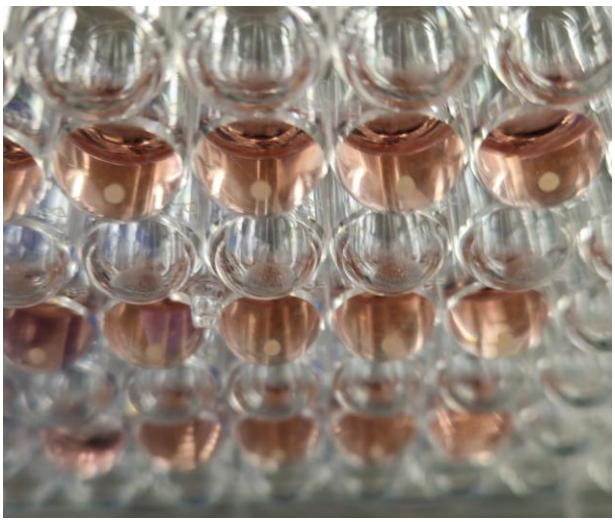

Figure 2
Bottom view of the well plate after 2 days of culturing under hypoxia

3. Activities and progress in relation to the project objectives up to the submission of this report

Shadowed the process of transferring MSC from T75 flasks to well plates, procedures as described above.

Investigated the reason for using ultra-low bending well plate. Ultra-low bending well plates promote the formation of cell balls, and the hydrophobic material deters the cells from adhering to the bottom. See ***F*** in *Figure 3* below.

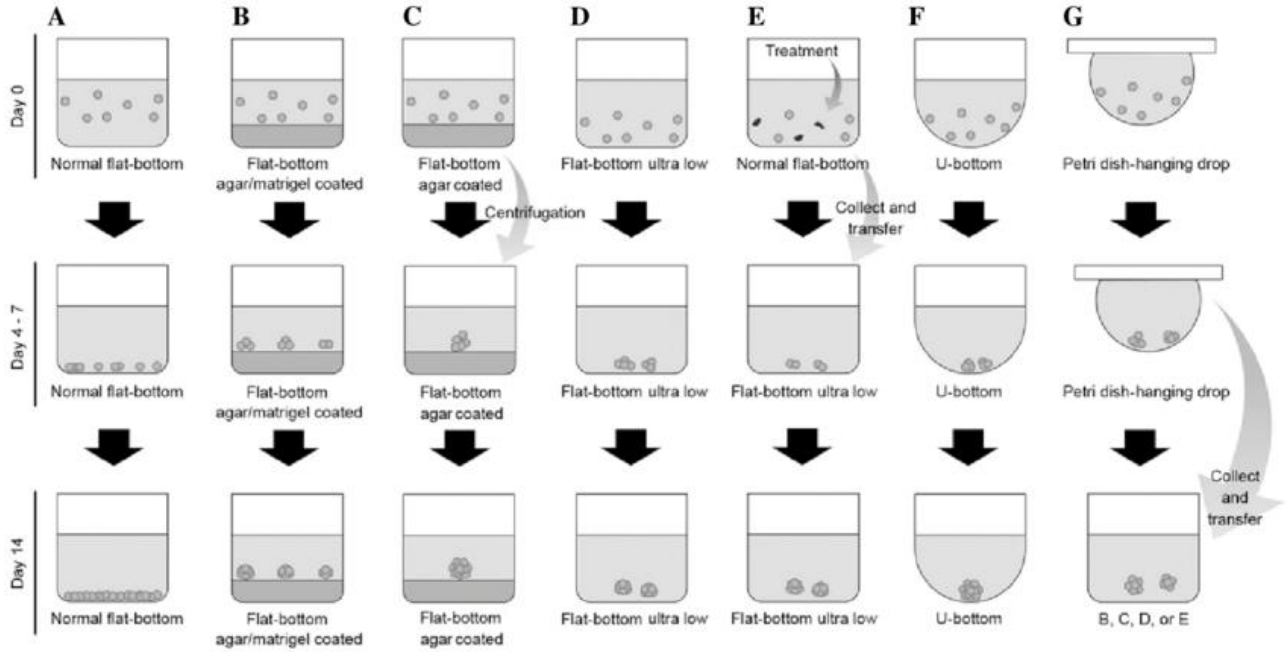

Figure 3
Comparison between various methods and plate types used for the generation of 3D spheroids (Siva, et al., 2017)

4. Problems encountered

N/A

5. Solutions investigated

N/A

6. Milestones achieved

N/A

7. Areas to be addressed and results expected in the next four weeks

Continue research and shadowing.

8. References

Siva, P. S., Firdaus, M. C., Muniandy, K., Lian, B. S., Su, P. L., Ling, S. L., . . . Mohana-Kumaran, N. (2017, February 8). Modeling nasopharyngeal carcinoma in three dimensions (Review). *Oncology Letters*, 2034-2044. doi:10.3892/ol.2017.5697