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 1st meeting with the Project Supervisor in Sep 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

Passaging MSC cells:
Retrieve P4/P5 MSC cells from liquid nitrogen storage and warm them to 37°C with a water bath for the cells to recover.
Dilute the cells and the freezing medium 5 times using growth medium. Centrifuge it at 300×*g* for 5 mins and throw away the supernatant. This is necessary because the cells will die if they stay in the freezing medium.
Resuspend the cell pellet with 1mL of PBS.
Isolate 10µL into a centrifuge tube to mix with 10µL of blue dye and load 10µL into each of the A and B slides of the cell counting machine. Measure and calculate the initial cell count. *(We got ~800000)*
Centrifuge the cells again and remove the supernatant.
Resuspend the cells with 1mL growth medium and transfer them into a T175 flask.
Add 19mL of growth medium and 0.8µL of FGF2 into the flask.
Rock the flask back and forth slightly to spread the medium evenly.
Incubate the cells until there are enough cells to achieve ~50000 cells per well.

Project flow:
Set up MSC cultures in normoxic (20% O_2) and hypoxic (5% O_2) conditions and culture them at 37°C. The medium should be changed every 2 days.
Take samples after 7, 14, and 21 days.
Observe the samples under a microscope to see morphological changes.
Extract RNA from the samples and reverse-translate into cDNA and measure the amount with qPCR. The DNA sequences measured (Col1, Col2, Col10, HIF-1, MMP13, SOX9, ACAN) are found only in cartilage tissue.
Run IHC on the samples to measure the amount of cartilage tissue present. The antigens (Col2, Col10, HIF-1) are only found in cartilage tissue.
Compare the results obtained from qPCR and IHC between normoxic and hypoxic conditions.
The culture in hypoxic condition should more readily differentiate into cartilage tissue.

Changing chondrogenic medium:
Using a pipette, carefully remove the old medium without disturbing the cells.
In a centrifuge tube, mix 1µL of ascorbate and 1µL of TGF-β1 per 1mL of chondrogenic medium.
Slowly add 200µL of medium to each well.

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

Shadowed the process of passaging MSC from liquid nitrogen storage, procedures as described above.
Shadowed the process of changing chondrogenic medium, procedures as described above.

Researched background knowledge.

Learned how to use the EVOS M5000 microscope. Observed the differences of MSC cells in different stages. See the appendix.

4. Problems encountered

Minor theoretical questions about hypertrophy under prolonged hypoxic conditions.

5. Solutions investigated

Asked RunXuan for answers; determined to be negligible for this project.

6. Milestones achieved

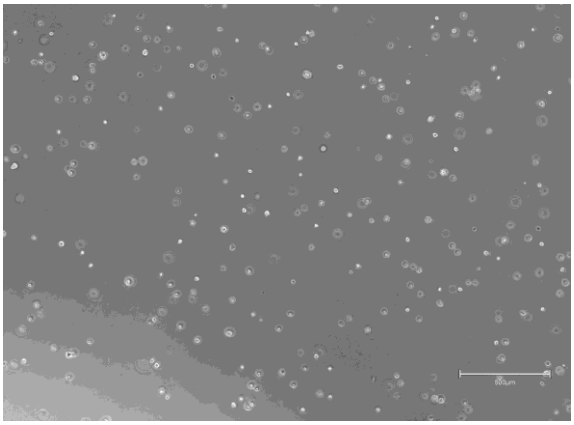
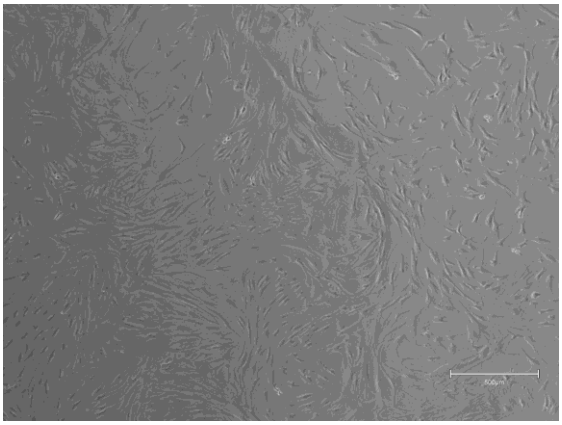
N/A

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

Continue research and shadowing.

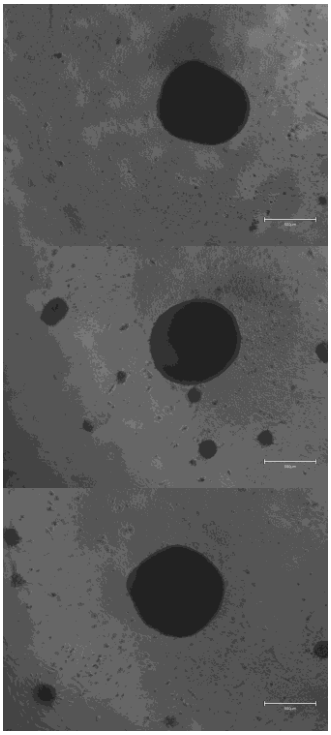
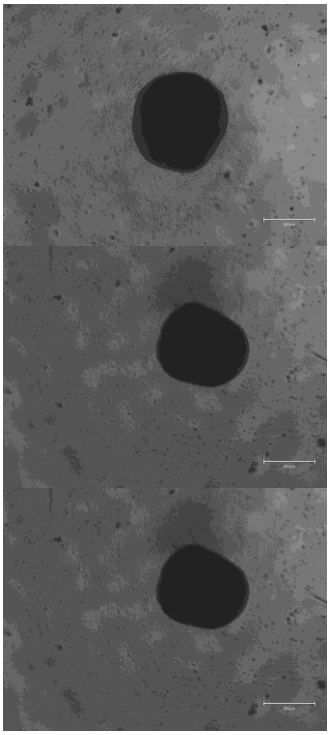
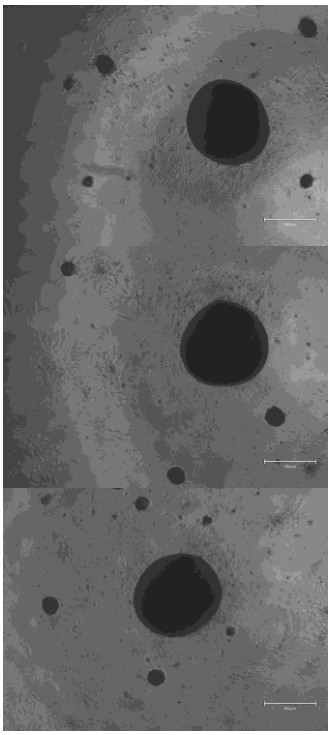
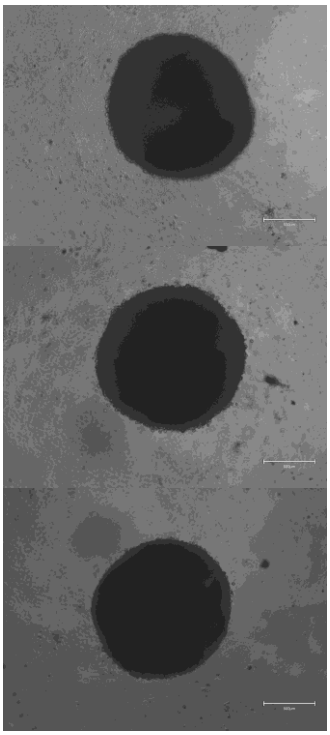
Appendix 1

MSC in T175 flask, regular incubation, 4× magnification

Day 1	Day 4
	 Note the 80% cell convergence degree

Appendix 2

MSC in well plate in chondrogenic medium, 4× magnification

	Day 4	Day 21
Normoxia		
Hypoxia		

Note that the cell pellet growth is more pronounced under hypoxia, which reached ~900µm in diameter.