Department of Biomedical Engineering
The Chinese University of Hong Kong

BMEG4999 – Final Year Project (Thesis II) 2024/2025

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Student ID: 1155174356

Project Title: Role of hypoxic conditions in cartilage tissue engineering

Project Supervisor: Prof Alan Li

Monthly Progress Report for **BMEG4999**
For the 4th meeting with the Project Supervisor in Mar 2025

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

Please refer to progress report #3 for the numerous project modifications.

Well allocation:
A 12-well plate is used for the entire experiment, with 6 wells for the normoxia group and 6 wells for the hypoxia group. For each group, the cells from 2 wells will be used to do Alcian staining while 4 will be used to do qPCR.

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

Trial 4 was started concurrently with trial 3 after the trial 3 culture was confirmed to be in stable and healthy condition.

Trial 3:
A T75 flask of P4 MSC was passaged into P5. The P5 were subsequently allowed to reach confluency on the well plate. Chondrogenic differentiation was started afterwards.

Trial 4:
A T75 flask of P4 MSC was passaged into P5.

See *9 Appendix* below for the microscope images of trials 3 and 4.

4. Problems encountered

Since minimal morphological differences can be observed under the microscope, it is very difficult to track the chondrogenic progress of the cells. Only the health and viability of the cells can be estimated during the differentiation process.

The trial 4 MSC health is not ideal. Confluency was expected on day 5, but the culture exhibited much lower activity than expected.
See *9 Appendix* below for the microscope images of trial 4.

5. Solutions investigated

N/A

6. Milestones achieved

Started steps 1 and 2 of the project.

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

Finish culturing and inducing chondrogenesis of trial 3 and doing staining and qPCR. Continue passaging and culturing trial 4 concurrently as a contingency plan.

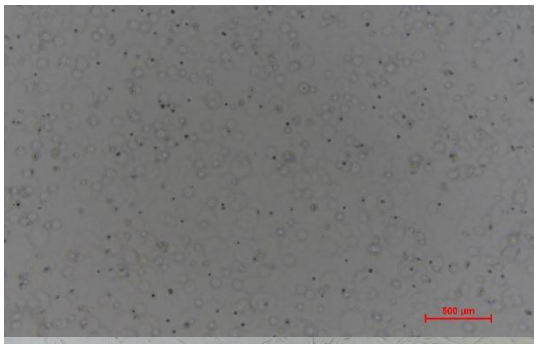
8. References

There are no sources in the current document.

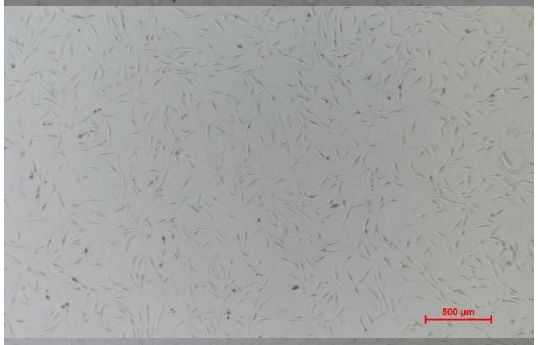
9. Appendix

Trial 3 P5 MSC passaging (T75 flask; growth medium)

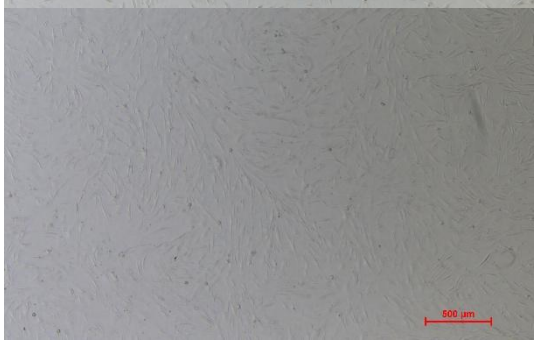
Day 1 (22/2/2025)

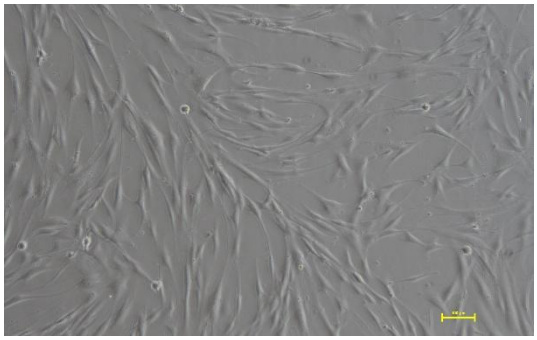


Day 3 (24/2/2025)



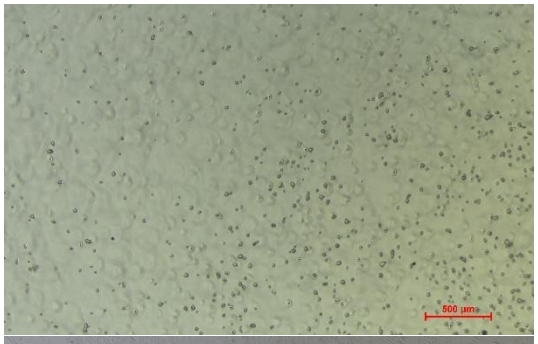
Day 5 (26/2/2025)

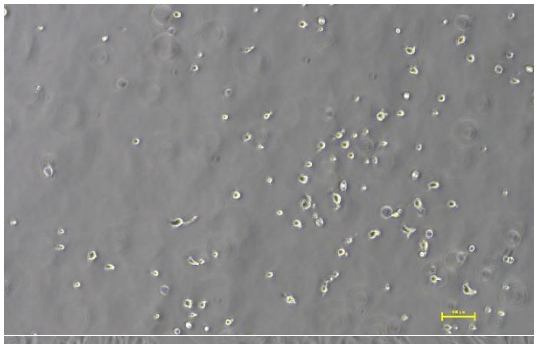




Trial 3 P5 MSC confluency (12-well plate; growth medium)

Day 1 (26/2/2025)





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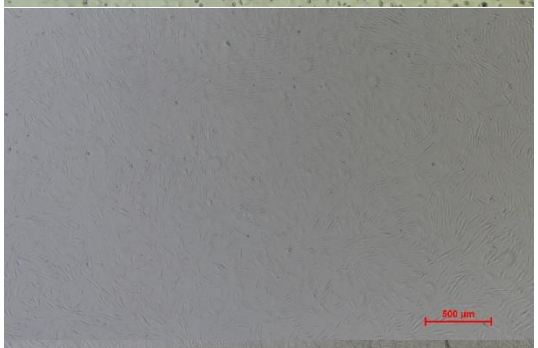
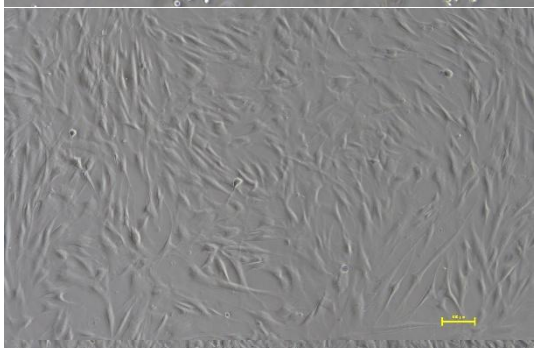
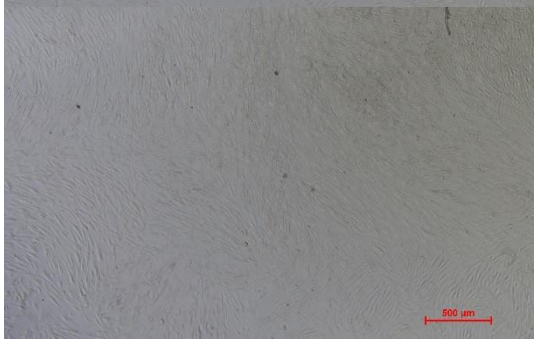
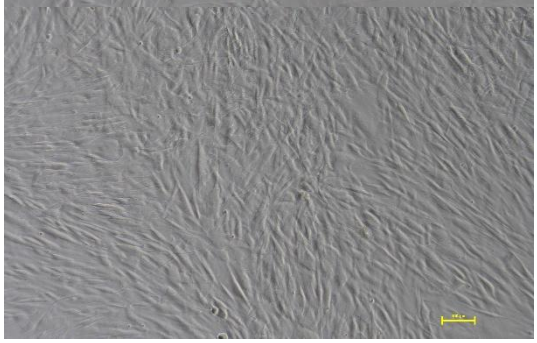


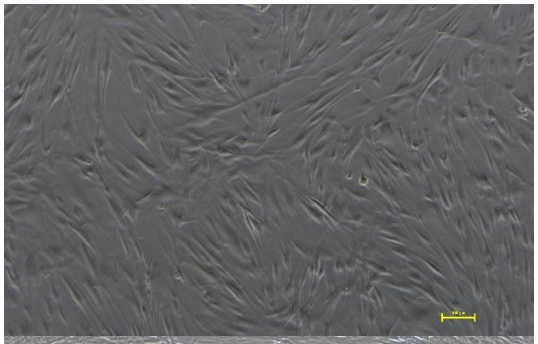
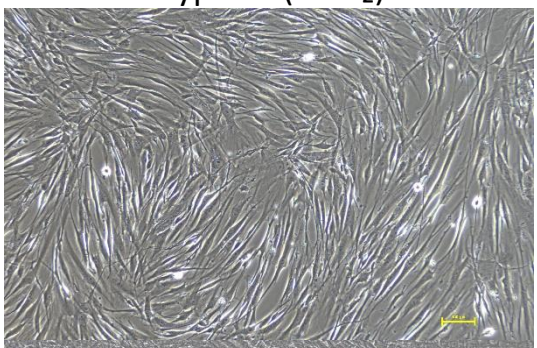
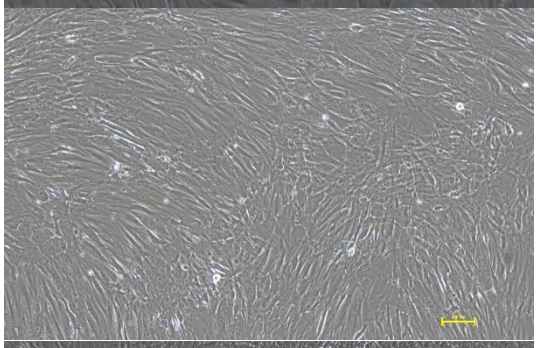
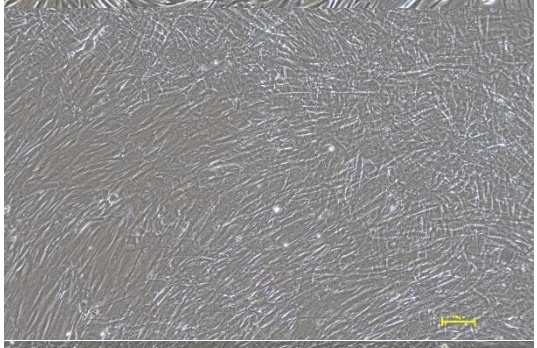
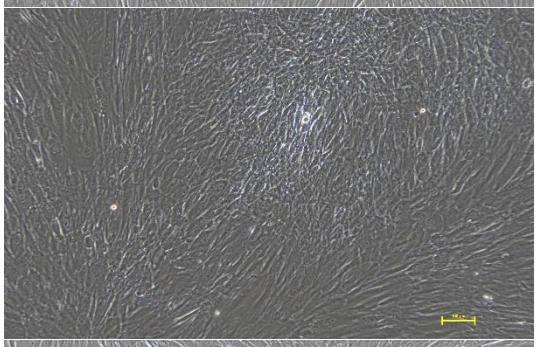
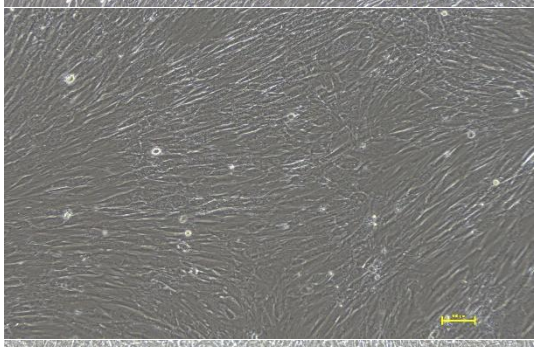
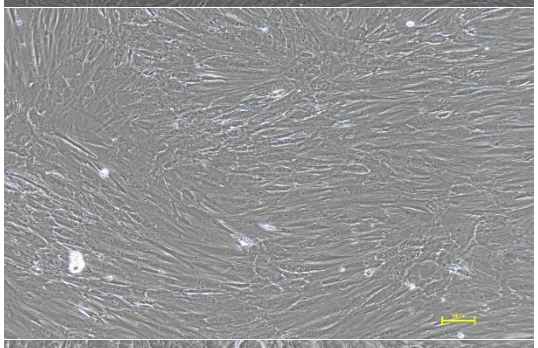
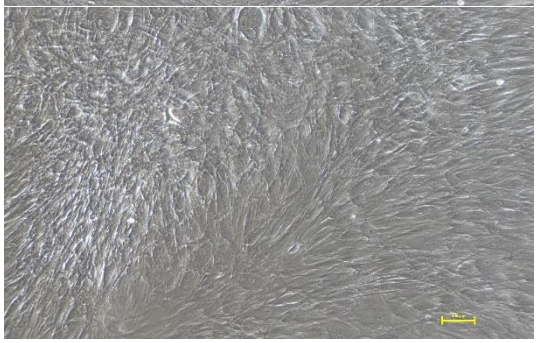
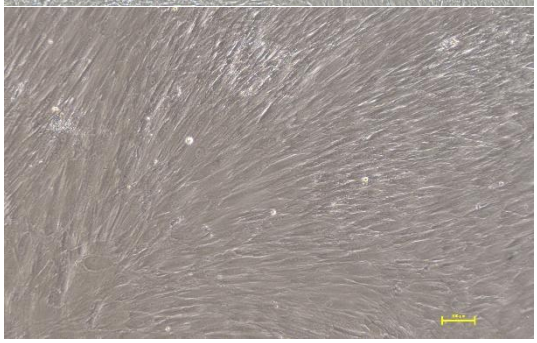


Day 3 (28/2/2025)





Trial 3 P5 MSC chondrogenesis (12-well plate; chondrogenic medium)

	Normoxia	Hypoxia ($CoCl_2$)
Day 1 (28/2/2025)		
Day 3 (2/3/2025)		
Day 5 (4/3/2025)		
Day 7 (6/3/2025)		
Day 9 (8/3/2025)		

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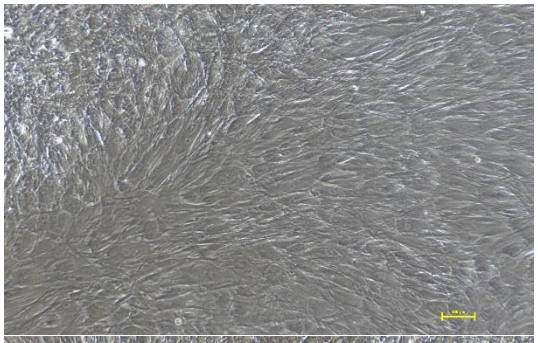
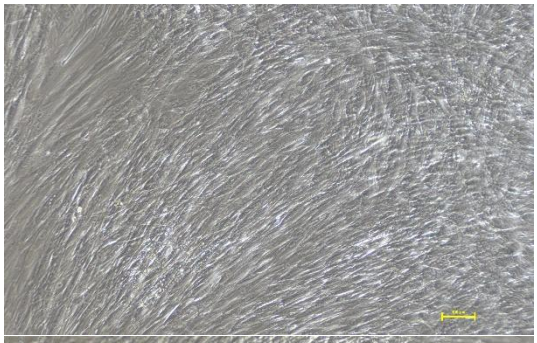
(10/3/2025)





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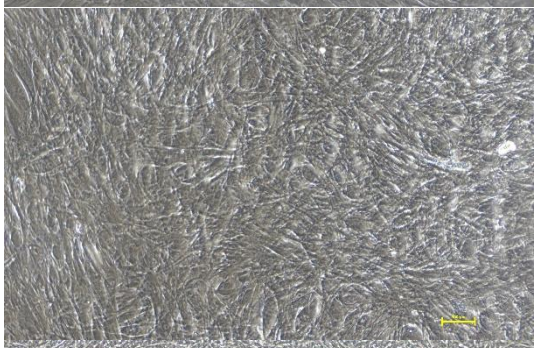
(12/3/2025)



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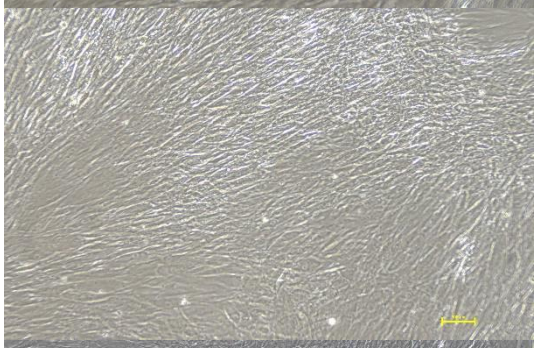
(14/3/2025)



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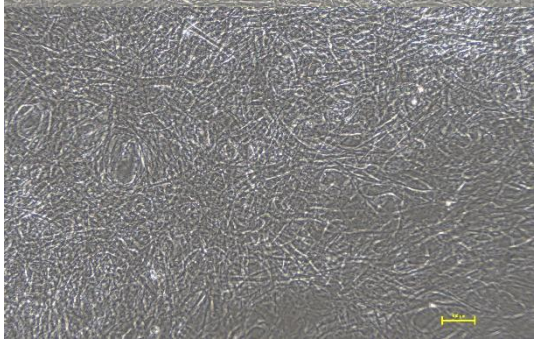

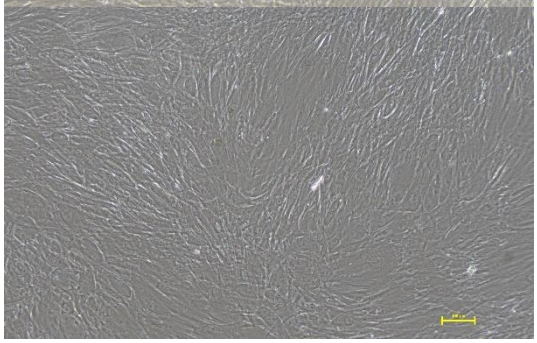

Trial 4 P5 MSC passaging (T75 flask; growth medium)

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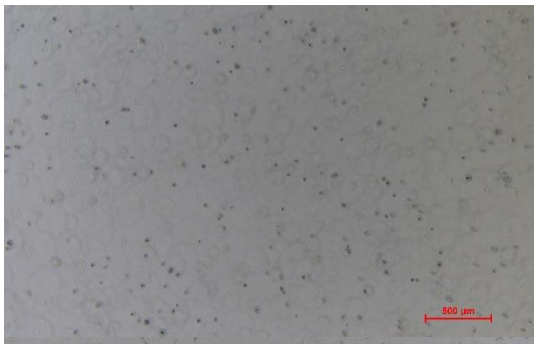
Day 1
(12/3/2025)



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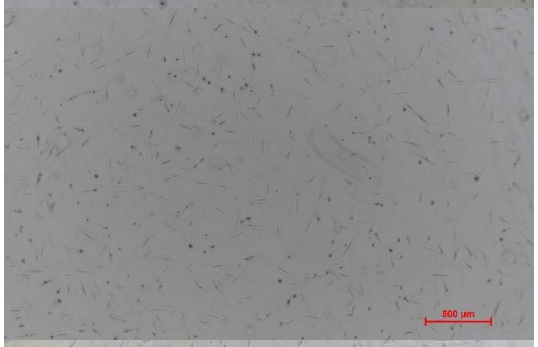
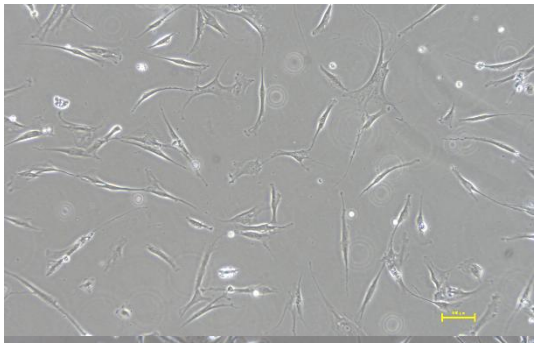
(14/3/2025)





Day 5
(16/3/2025)