Department of Biomedical Engineering
The Chinese University of Hong Kong

BMEG4999 – Final Year Project (Thesis II) 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 **BMEG4999**
For the 3rd meeting with the Project Supervisor in Feb 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 note that there are substantial modifications in the methodology due to equipment permission restraints.

Replacing hypoxic conditions with $CoCl_2$:
Due to equipment permission restraints, a true 5% O_2 hypoxic condition cannot be produced using a hypoxia incubator. Instead, hypoxia-mimetic agent $CoCl_2$ will be added to the medium to emulate hypoxic conditions in any future experiments. Based on the work of Teti et al. (2018), a $CoCl_2$ concentration of 100µM will be used.

Replacing 3D culture technique with 2D cell culturing:
Due to equipment permission restraints, a centrifuge capable of centrifuging 96-well plates is not available. Hence, it is no longer possible to do cell pellet culturing with round-bottom wells. Flat-bottom 12-well plates will be used in future to do 2D cell culturing instead.

Cancellation of plans to do IHC:
Due to facility access restraints, the plans to perform IHC for condrogenesis evaluation is cancelled. Other methods will possibly be considered in place of IHC.

Addition of Alcian Blue staining:
An Alcian Blue staining step is added to visualise the cell morphology for preliminary observation under the microscope to assess chondrogenesis.

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

After acquiring the $CoCl_2$, a 100mM (1000×) stock solution was made using PBS as the solvent.

We attempted to test for the effects of $CoCl_2$ on MSCs; however, due to problems to be discussed in *4 Problems encountered* below, it was unsuccessful. RunXuan was busy for the subsequent month, and I have not been able to acquire another batch of cells to redo culturing.

4. Problems encountered

Possibly due to poor cell quality from the cell freezing, or cell degradation from the transportation between MMW and ERB, or reagent degradation from the transportation between MMW and ERB, the frozen cells in the first trial did not survive the thawing process, ceasing all signs of metabolism after day 3.

On a sidenote, a fellow FYP student suspected bacterial contamination is his culture recently.

5. Solutions investigated

Waiting for the second batch of cells to be acquired.

Incubator and BSC sterilisation are being considered, pending concurrence from the other facility users.

6. Milestones achieved

N/A

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

Start trial 2 of passaging.
Test the effects of $CoCl_2$ on MSCs.
Start the titular investigation.

8. References

Teti, G., Focaroli, S., Salvatone, V., Mazzotti, E., Ingra', L., Mazzotti, A., & Falconi, M. (2018, March 13). The Hypoxia-Mimetic Agent Cobalt Chloride Differently Affects Human Mesenchymal Stem Cells in Their Chondrogenic Potential. *Stem Cells International, 2018*(1). https://doi.org/10.1155/2018/3237253