Good day everyone, I am Chan Cheuk Ka, and today I am here to present about my final year project on the role of hypoxic conditions in cartilage tissue engineering.

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Here is my table of contents.

First, I will introduce the background of the topic.
Then, I will discuss the current techniques and their limitations.
Next, I will talk about the experimental design of my project.
After that, I will detail the experimental protocols and methods.
Finally, I will present the results and conclusions of my study.

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Now, for the background of the topic,

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let's look into the problem with cartilage damage.

Cartilage damage is a very common ailment plaguing many individuals worldwide, especially the elderly. It is a common effect of trauma, arthritis, and joint injuries and can lead to severe pain and even disability.

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One of the major reasons for this is that, cartilage, by its nature, lacks vasculature, neural connection, lymphatic system, and progenitor cells. This means that it has a very limited capacity for self-repair.

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The cartilage that can be found normally in joints are called hyaline cartilage. This type of cartilage is rich in type 2 collagen and is very smooth, which allows for low friction between bones to help joint articulation.

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However, when this cartilage is damaged, especially when the damage is severe, it is usually replaced by fibrocartilage scar tissue, which is rich in type 1 collagen. But this type of cartilage is not as smooth and has inferior biomechanical properties and cannot help joint articulation. Moreover, it can release enzymes to remodel the extra-cellular matrix, which can lead to further damage. We can see that cartilage damage can, therefore, very easily lead to cartilage breakdown and eventually other diseases like osteoarthritis.

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While there are currently some treatments available, such as microfracture surgery and joint replacement, they all have their limitations and they often fail to produce satisfactory therapeutic outcomes, in addition to introducing side effects like immunogenicity, allergenicity, and invasiveness.

And therefore, we can see that there is currently a medical need for cartilage regeneration.

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Now, let me briefly talk about the current cartilage regeneration techniques.

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Currently, some of the most recent and promising treatment options involve the in vitro cultivation of cartilage tissue, before implanting it into the patient. For example, stem cells can be obtained from the patient, and then differentiated into chondrocytes within an injectable hydrogel. The hydrogel can then be injected intra-articularly into the patient, where it will form a new cartilage tissue.

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Cartilage cultivation usually involves the use of mesenchymal stem cells, MSCs, since they are easy to obtain. MSCs can differentiate into chondrocytes in our application.

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However, the major obstacle in these techniques is the inherent phenotypic instability of chondrocytes. When chondrocytes are cultured in vitro, they tend to dedifferentiate into fibroblasts, which can lead to the formation of fibrocartilage instead of hyaline cartilage. This exhibition of an hypertrophic phenotype is similar to than found in osteoarthritis.

To cultivate chondrocytes in vitro, current techniques often have to involve a cocktail of growth factors and hormones to maintain the phenotypic stability. However, these techniques are often complicated and expensive with limited efficacy.

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For the design rationale of my project,

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considering the avascular nature of cartilage, its native oxygen concentration in vivo is 2-7%.
And incubating chondrocytes in a hypoxic condition can better mimic the in vivo environment.

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In fact, a hypoxic condition is actually essential for chondrogenesis. Under a normoxic condition, which is 21% oxygen, a transcription factor called HIF-1α only have a half-life of 5-10 minutes. But it is important for chondrogenesis since it regulates differentiation and stabilises the chondrocyte phenotype.

For this study, I have decided to use a 5% oxygen concentration for the hypoxic condition.

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For the next design choice, a 3D culture technique was chosen over a conventional 2D technique.
Despite being more convenient, 2D monolayer culturing techniques cannot mimic in vivo environments since there are more cell-plastic and cell-medium interactions than cell-cell interactions.
In contrast, 3D techniques can promote cell-cell and cell-ECM interactions, and encourage a more natural morphology by allowing cell aggregation and the subsequent formation of micro-environments. Studies have shown that 3D techniques can in fact induce more chondrogenic markers and proteins and reduce hypertrophic markers.

For this study, I have decided to use a scaffold-free 3D culture technique, specifically a cell spheroid culture technique.

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To further encourage cell aggregation, an ultra-low attachment well plate was chosen, so that cells cannot adhere to the surface. A round bottom well plate, such as the one highlight in the illustration, was chosen to funnel the cells to the center to form one big spheroid.

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In summary, the experiment was designed to incubate the MSCs at a hypoxic condition of 5% oxygen compared to a normoxic condition of 21% oxygen to facilitate chondrogenesis. A 3D spheroid culturing technique was chosen to better mimic in vivo environments, and an ultra-low attachment well plate was chosen to encourage cell aggregation.

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Next, allow to briefly discuss the experimental protocols and methods.

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Here is the general flow of the experiment.

First, P5 MSCs will be passaged into P6 MSCs in T75 flasks under normal conditions.
Then, the cultures are transferred into two well plates, each with 21 occupied wells, and they are incubated in a hypoxic and normoxic incubator respectively.
Every 7 days, 4 samples are collected from each plate for qPCR, and 3 samples are collected for IHC.

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Here is the proposed well plate occupancy.

Since we have to collect at least 7 wells for testing each week, we will need a total of 21 wells for each plate.

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Quantitative polymerase chain reaction, qPCR, will be used to measure Col1a1, Col2a1, Col10a1, Acan, Hif1α, Sox9, and Mmp13 gene expression levels, while immunohistochemistry, IHC, will be used to measure COL2, COL10, and HIF-1α protein expression levels.
It is expected that type 2 collagen, aggrecan, HIF-1α and Sox9 levels, which are the ones shaded in green, will be higher in the hypoxic culture, while types 1 and 10 collagen and Mmp13 levels, which are the ones shaded in red, will be lower.

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Let's move on to the results.

Sadly, as of currently, no experiments were done due to restrictions. Hence, the results shown here are from my advising PhD student, Cai Run Xuan.

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Here are the microscopic images of the MSCs at days 1 and 4 of passaging. We can observe the increase in cell count in the image on the right, at approximately 80% cell convergence.

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Here is an image of the well plate after 2 days of hypoxic chondrogenic incubation. We can see that the cells have aggregated into spheroids.

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Here are the microscopic images of the MSCs at days 4 and 21 of chondrogenic incubation.
We can clearly observe that those in hypoxic conditions have a more pronounced spheroid growth, with an average of a 66% increase in diameter, while those in normoxic conditions have only a 2% increase.

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No qPCR and IHC were done yet due to testing failures.
So, finally, let us conclude this presentation.

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Firstly, the preliminary microscopic observations showed more pronounced cell spheroid growth in hypoxia versus normoxia, which confirms the results in other studies.
Next, qPCR and IHC will be done in the future to provide quantitative evidence to further support this claim.

These preliminary results along with previous studies imply that hypoxic culturing of cartilage tissue is a viable way to do cartilage tissue engineering. If this technique is mastered, it can be an effective and efficient

method of controlling the chondrogenic phenotype of chondrocytes, which is an improvement over current techniques.

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Here are a list of the references that I used □ throughout this presentation.

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This is the end of my presentation.
Thank you for your listening.