Role of Hypoxic Conditions in Cartilage Tissue Engineering

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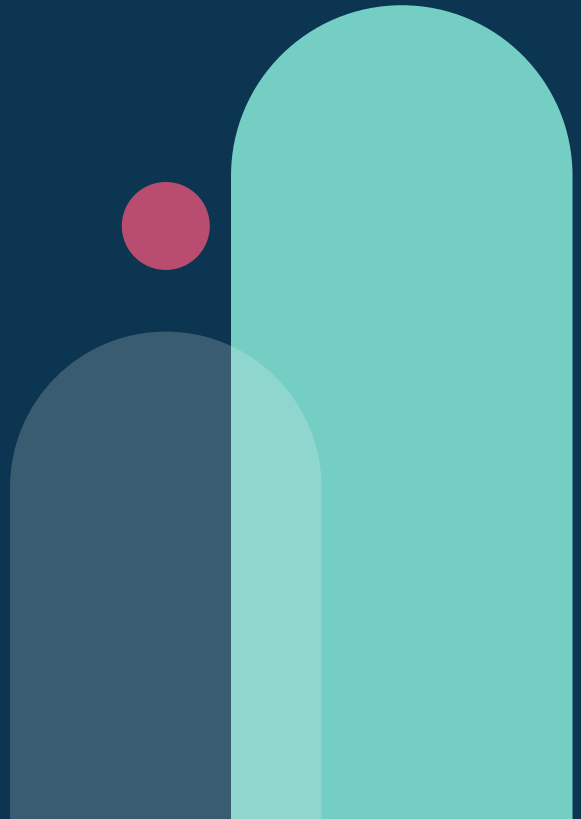
Background

Problem statement



Cartilage Damage

Current situation

Cartilage damage is a very common ailment affecting many people worldwide

[1]

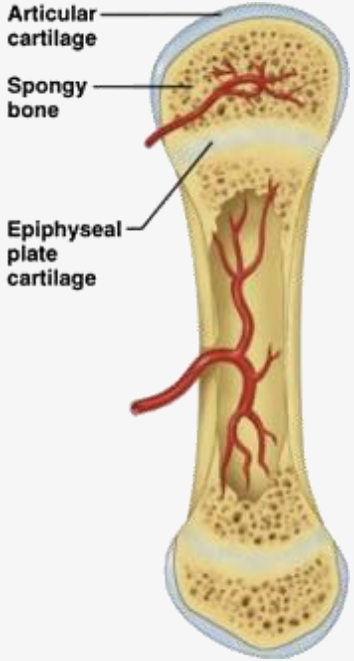
Limited Regeneration Capacity [2]

Avascular

Lacks direct oxygen supply

Aneural

Lacks stimulation



[3]

Alymphatic

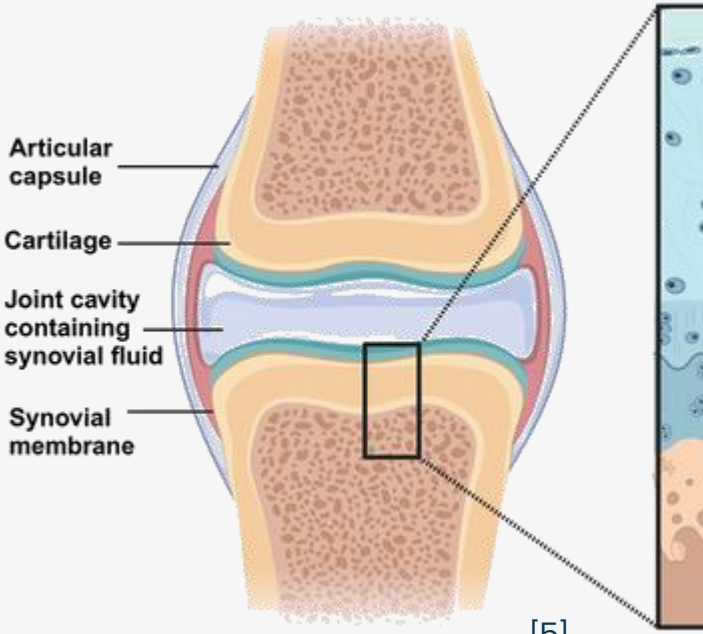
Slow metabolism

Lack Progenitors

Slow healing

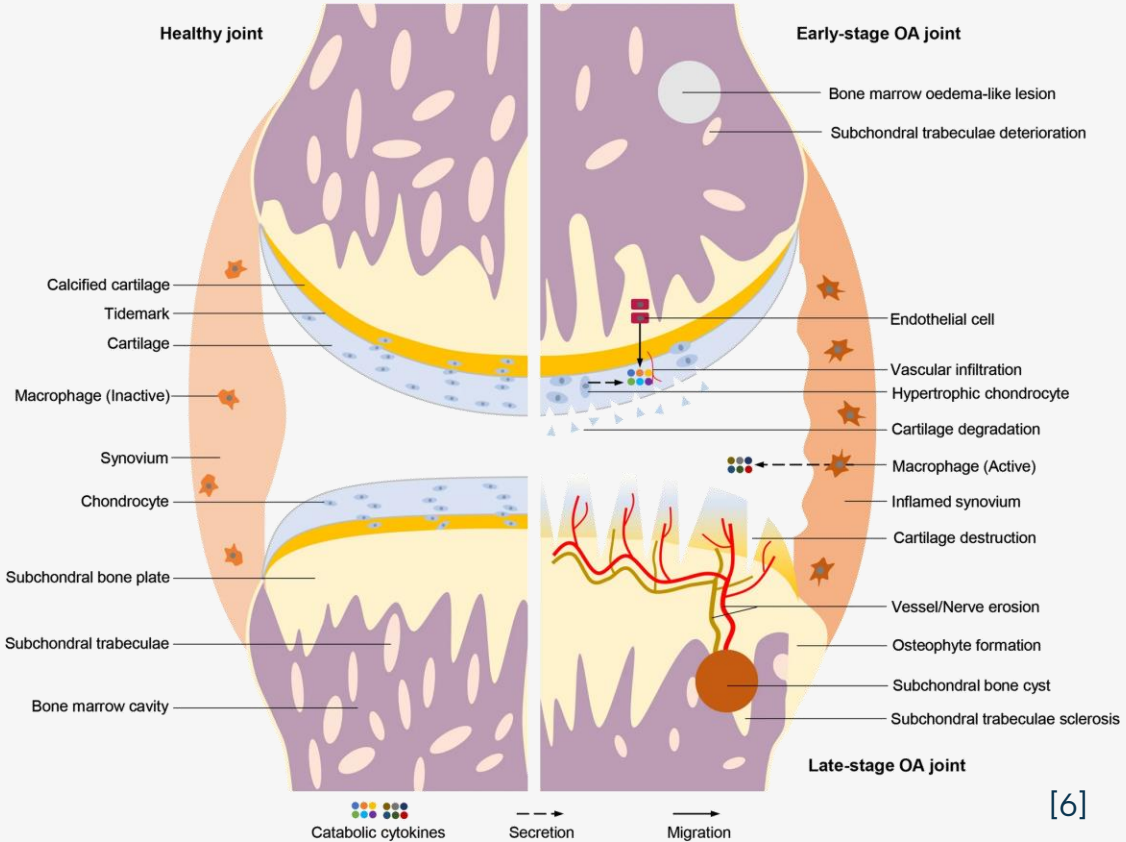
Hyaline Cartilage

- Smooth in nature
- Rich in type II collagen
- Exhibits low friction
- Aids joint articulation [4]



[5]

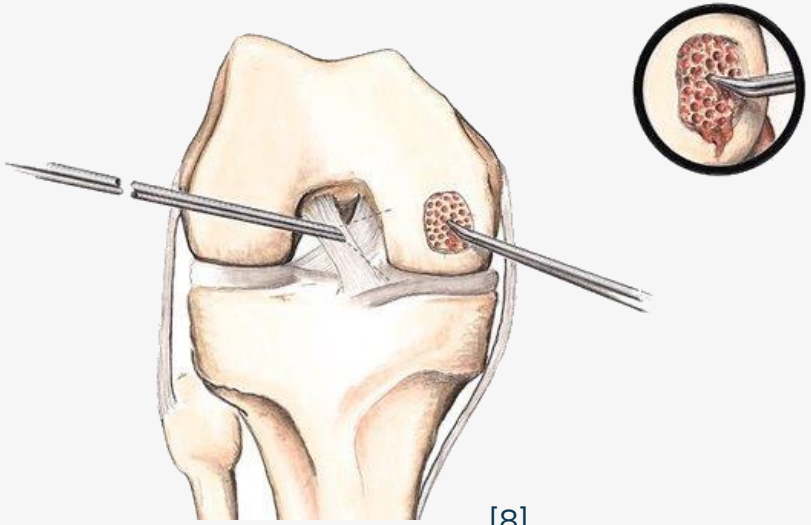
Fibrocartilage Formation



[6]

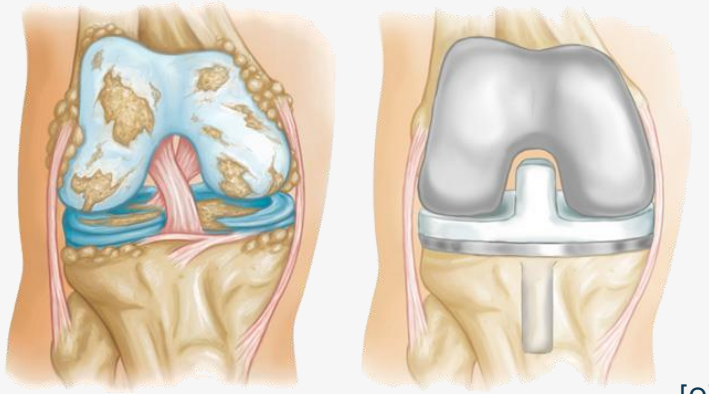
- Form after injuries
- Rich in type I collagen
- Inferior biomechanical properties
- Unsuitable for joint articulation
- Degrades cartilage
- Can lead to osteoarthritis [7]

Current Treatments



[8]

Microfracture



[9]

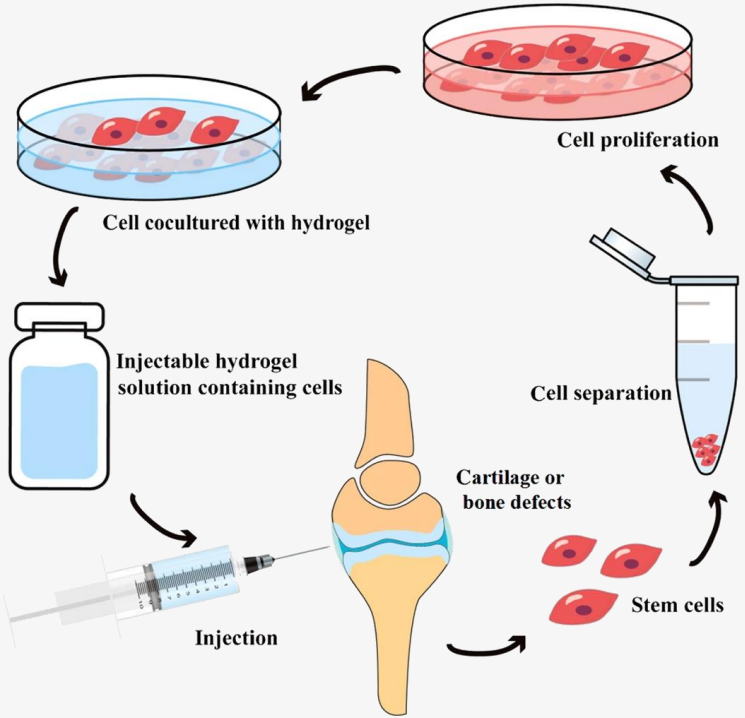
Joint replacement

02

Literature Review

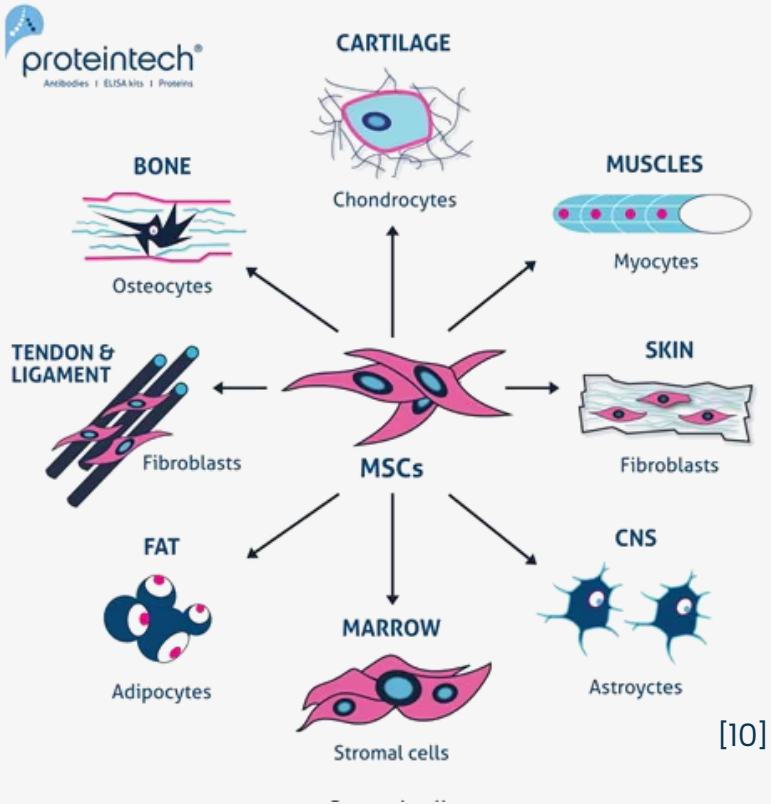
Current techniques

Autologous Chondrocyte Implantation



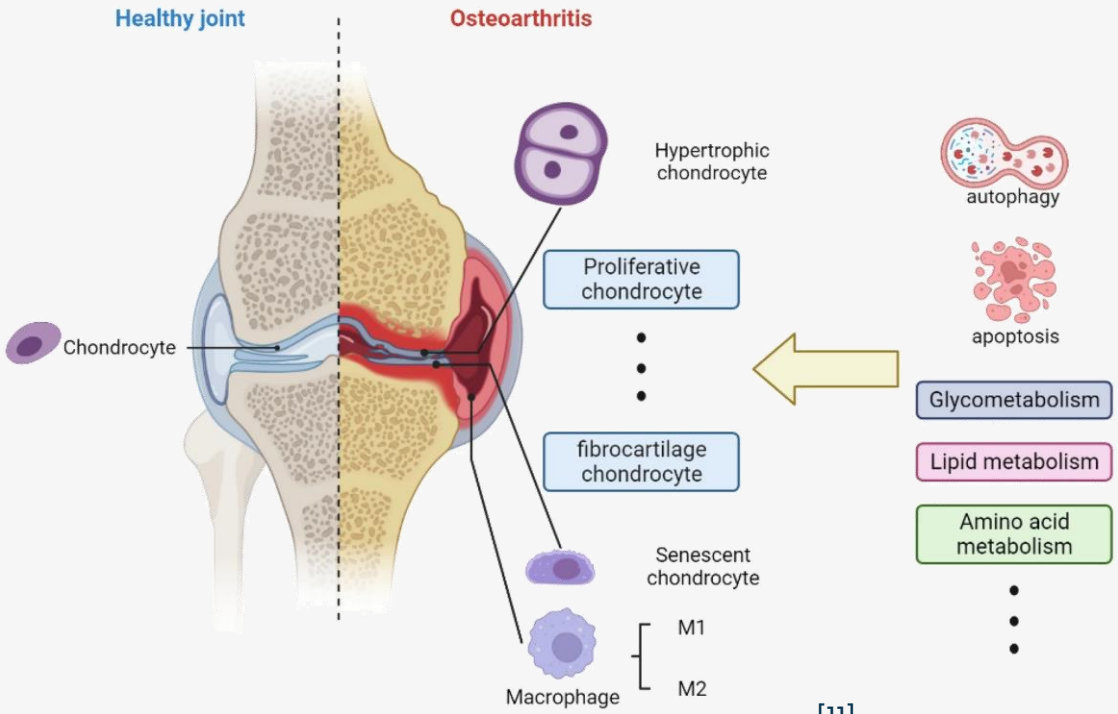
[1]

Mesenchymal Stem Cells (MSCs)



[10]

Chondrocyte Phenotypic Instability



[11]

03

Theory

Experimental design rationale

2% – 7% O_2

The native oxygen concentration of cartilage [12]

HIF-1α

5-10 mins half-life in O_2 [13]

Degraded via prolyl hydroxylation and proteasome degradation [14]

Regulates differentiation [15]

Upregulates related genes like *Sox9*

Stabilises phenotype [16]

Epigenetic effects

3D Culturing

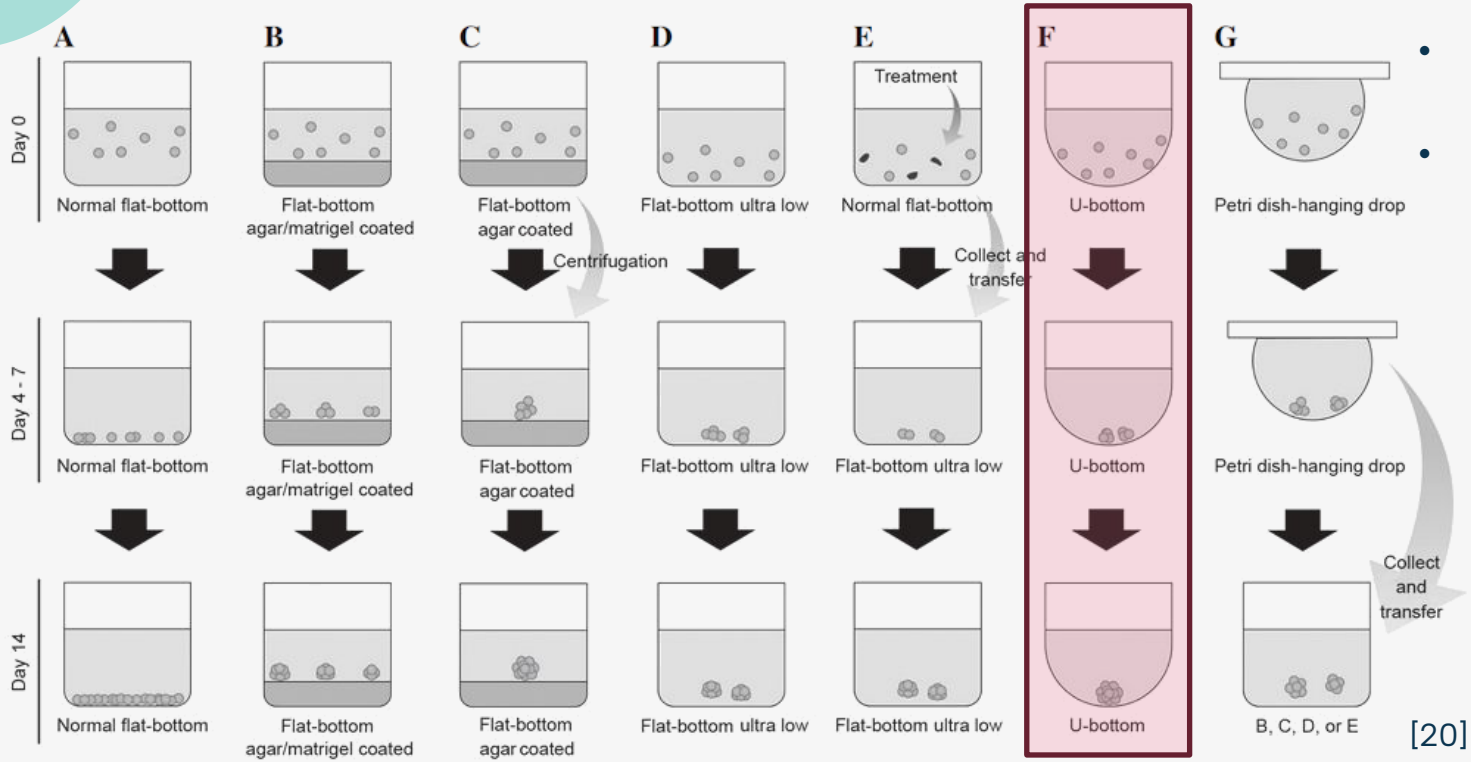
2D

- Convenient
- Easily reproducible
- Cannot mimic *in vivo* environments (mostly cell-plastic & cell-medium interactions) [17,18]

3D

- Promote cell-cell & cell-ECM interactions [18]
- Natural cell morphology by allowing aggregation and micro-environments [18]
- Can induce more chondrogenic markers and proteins [19]

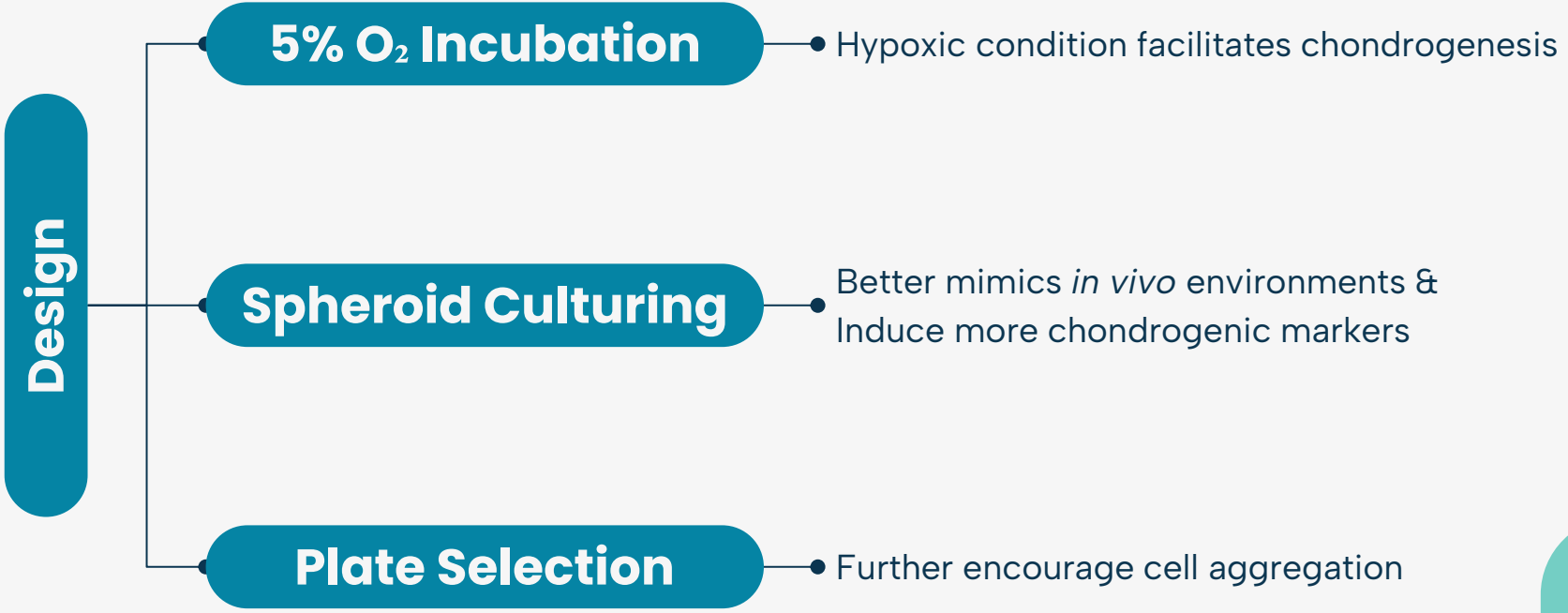
Well Plate Selection



- Ultra-low attachment
- Round bottom

[20]

Experimental Design



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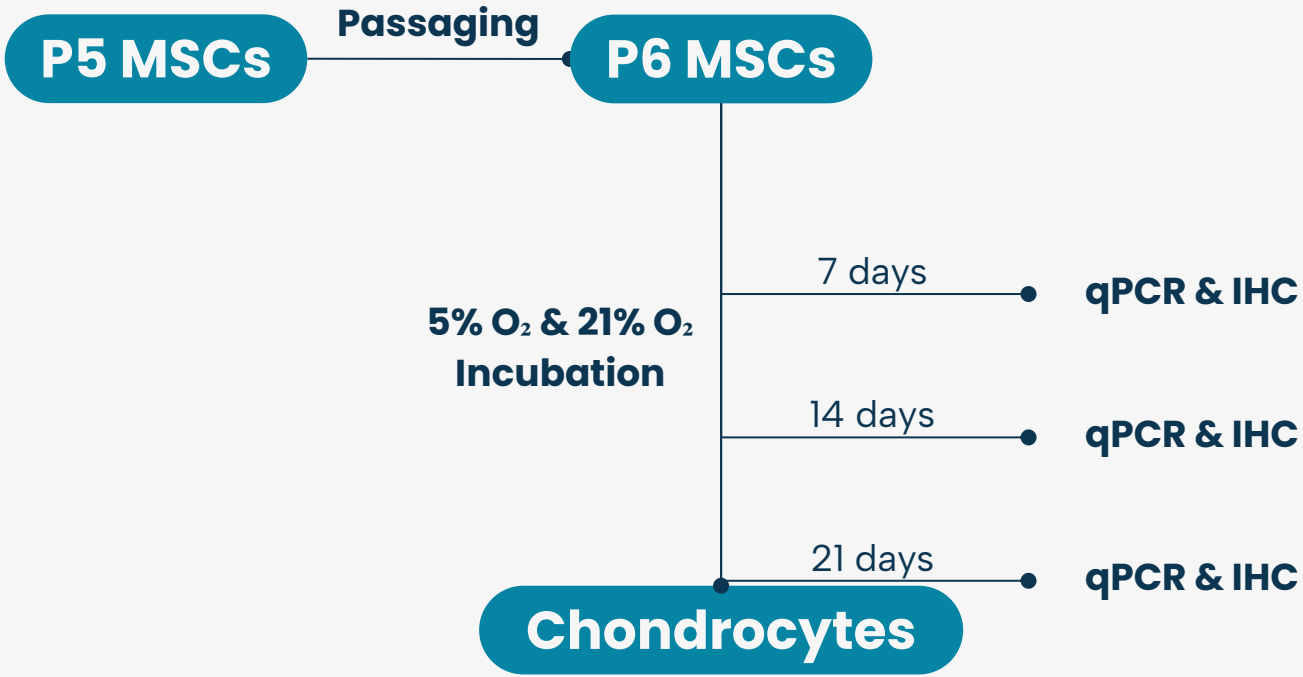
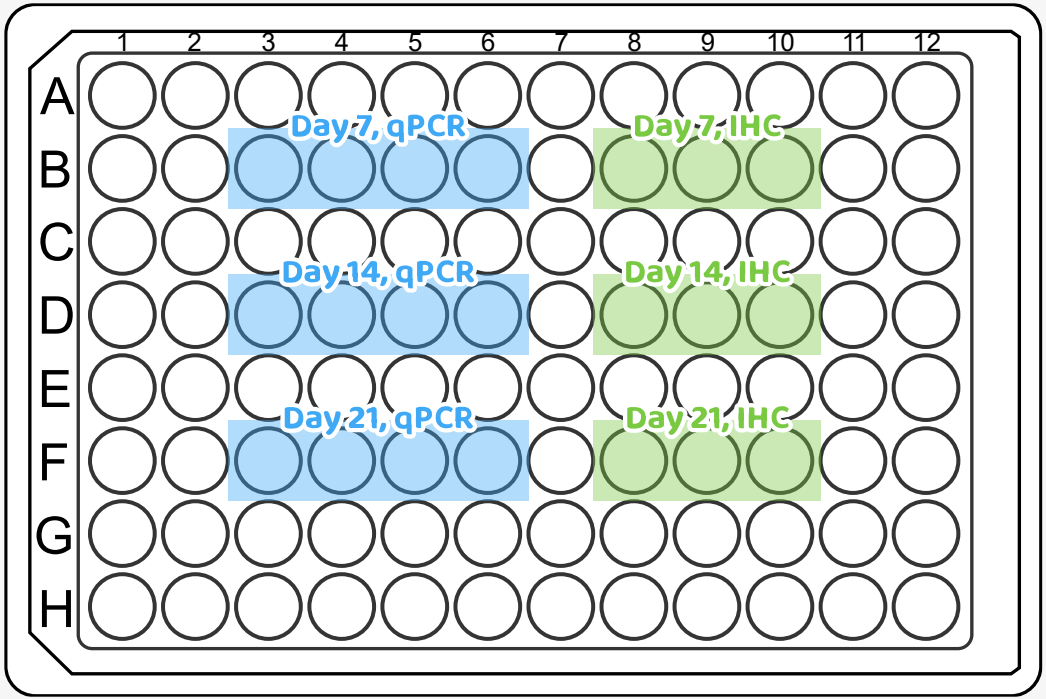
Experiment

Experimental protocols

Experimental Flow



Well Plate Occupancy



Chondrogenesis Evaluation

Gene (qPCR)	Protein (IHC)	Chondrogenic Involvement	Expectation
Col1a1		Type I collagen	↓ in hypoxia
Col2a1	***COL2***	Type II collagen: major cartilage component	↑ in hypoxia
Col10a1	***COL10***	Type X collagen	↓ in hypoxia
Acan		Aggrecan: major cartilage component	↑ in hypoxia
Hif1α	***HIF-1α***	Regulates chondrogenic differentiation	↑ in hypoxia
Sox9		Maintains cartilage homeostasis	↑ in hypoxia
Mmp13		Degrades type II collagen	↓ in hypoxia

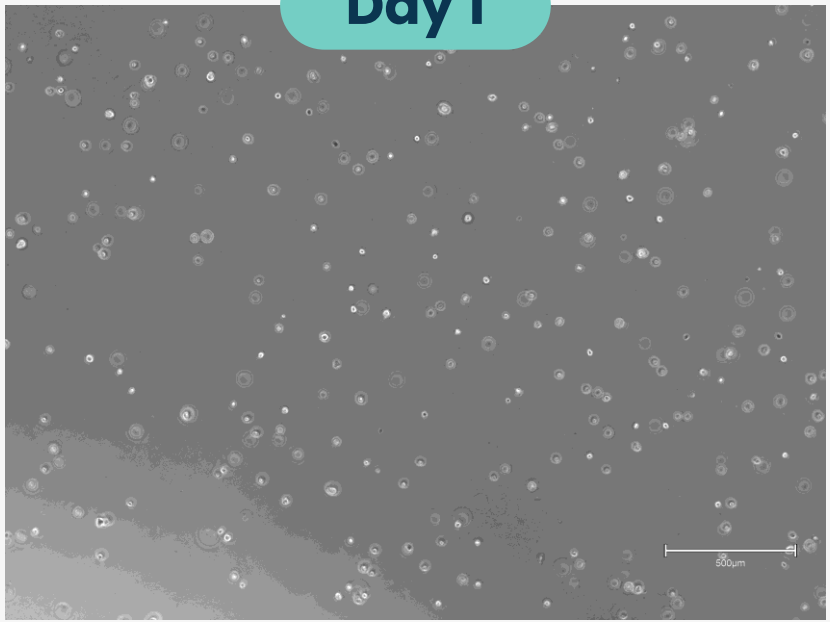
05

Results

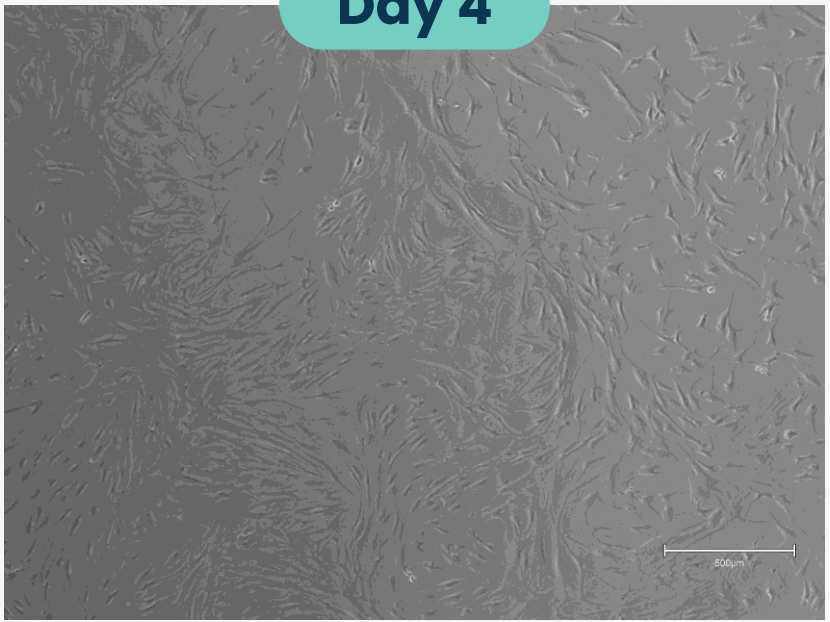
Experimental results

MSC Passaging

Day 1



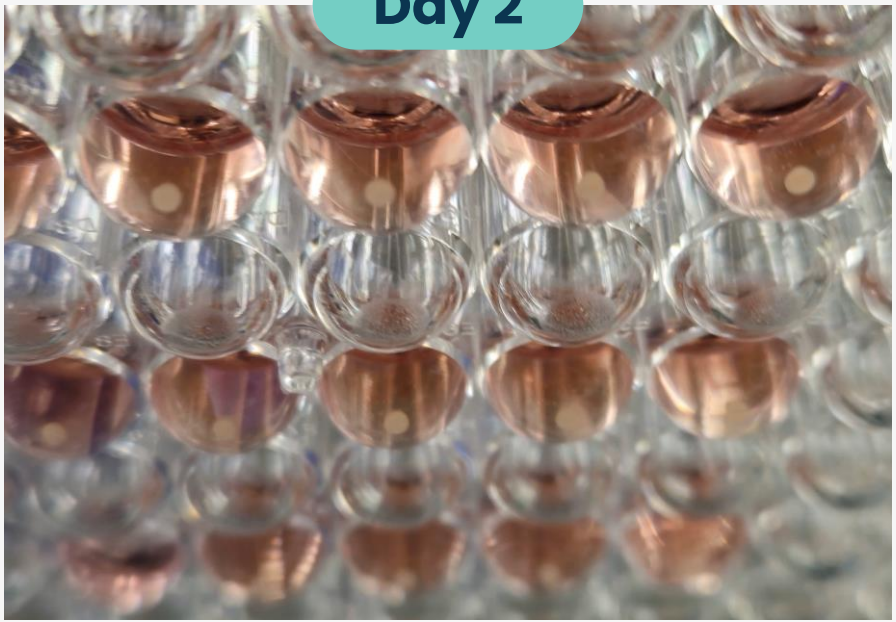
Day 4



MSCs in T75 flask, 4× magnification

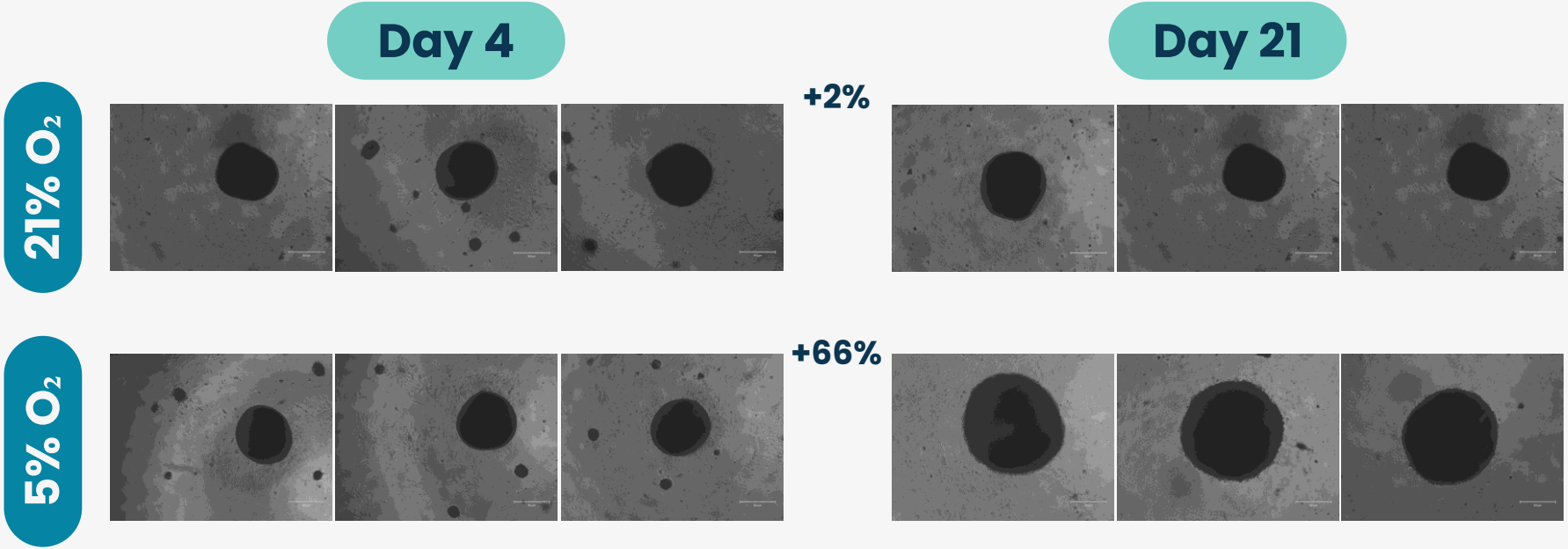
Cell Spheroid Aggregation

Day 2



Bottom view of the well plate

MSC Passaging



MSCs in well plates, 4× magnification

06

Discussion

Conclusion

Discussion

Microscopic Observations

More pronounced cell spheroid growth in hypoxia (+66%) vs normoxia (+2%)

qPCR & IHC

To be done in the future to provide quantitative results

Implications

Hypoxic culturing of cartilage tissue *in vitro* is viable

It is an effective and efficient method of phenotypic control, compared to the use of factor and hormone cocktails

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Thanks!

Guidance and assistance from
supervisor Prof LI Zhong Alan and
PhD student CAI Runxuan
are acknowledged