Role of Hypoxic Conditions in Cartilage Tissue Engineering

Chan Cheuk Ka
1155174356

Supervisor: Prof Li Zhong Alan
Associate Examiner: Prof CHAN Pui Barbara

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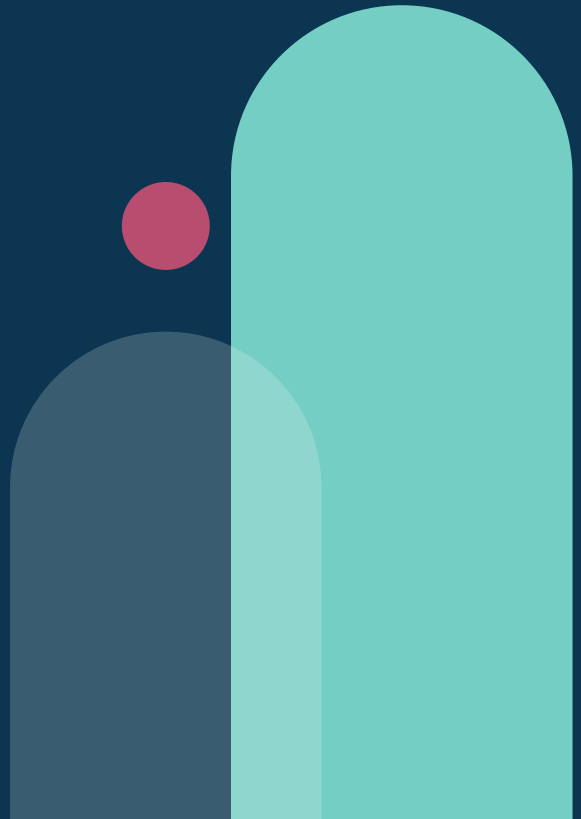
Background

Problem statement



Cartilage Damage

Current situation

Cartilage damage is a very common ailment affecting many people worldwide

[1] M. Liu *et al.*, *Bone Research*, 2017

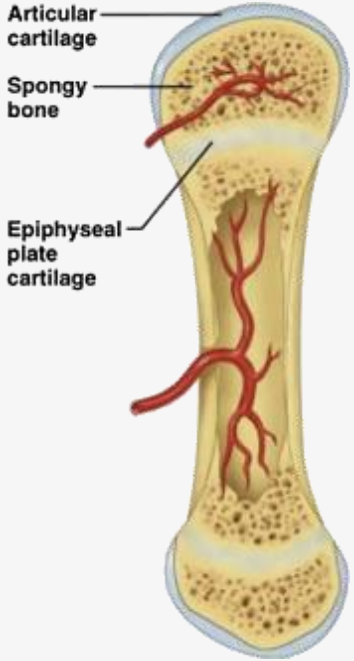
Limited Regeneration Capacity [2]

Avascular

Lacks direct oxygen supply

Aneural

Lacks stimulation



[3]

Alymphatic

Slow metabolism

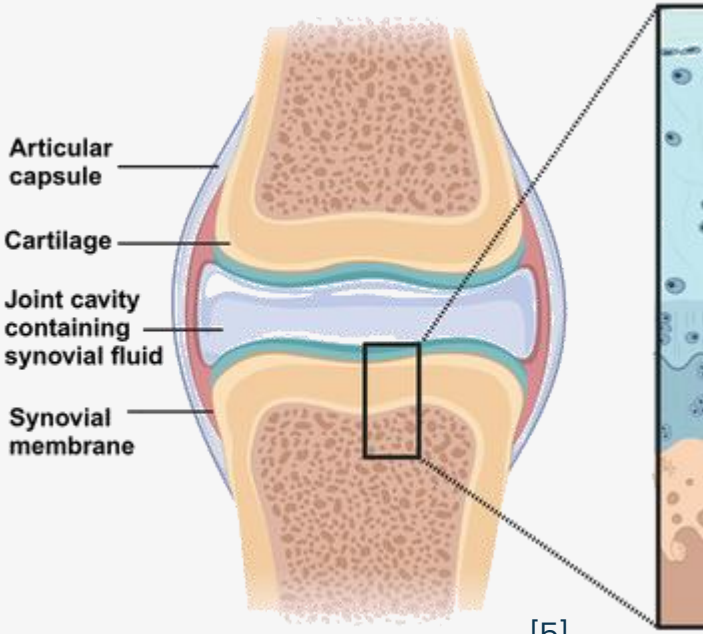
Lack Progenitors

Slow healing

[2] C. A. Vilela *et al.*, *ACS biomaterials science & engineering*, 2015
[3] A. Lindahl *et al.*, *Tissue Engineering*, 2023

Hyaline Cartilage

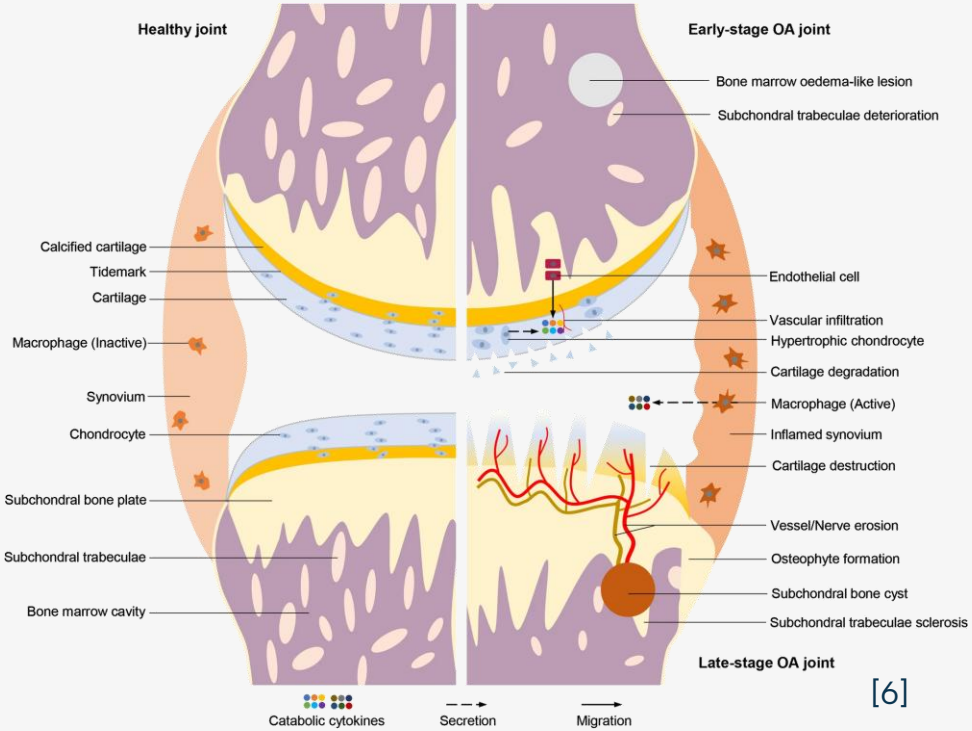
- Smooth in nature
- Rich in type II collagen
- Exhibits low friction
- Aids joint articulation [4]



[5]

[4] J. C. Sherwood *et al.*, *Drug Discovery Today*, 2014 [3] A. Lindahl *et al.*, *Tissue Engineering*, 2023
[5] X. Li *et al.*, *Advanced Healthcare Materials*, 2024

Fibrocartilage Formation

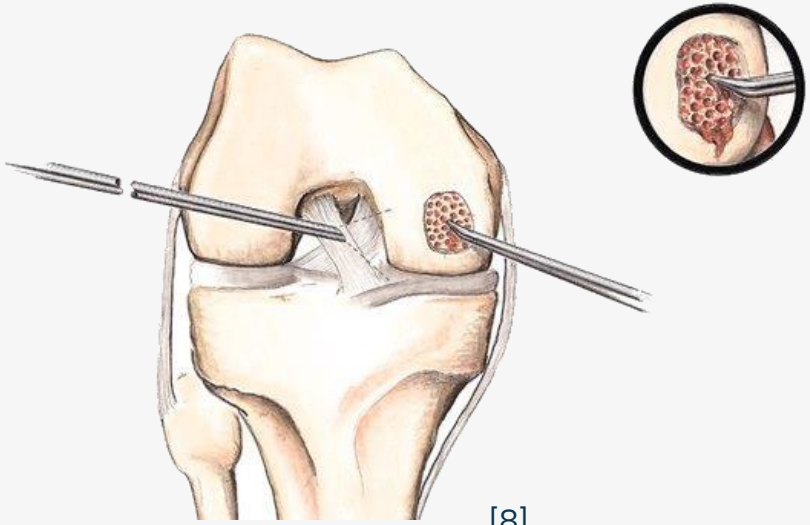


[6]

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

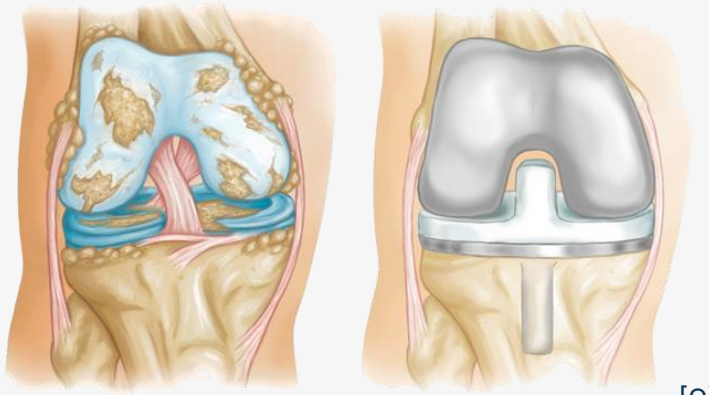
[6] Y. A. Pei *et al.*, *Cellular and Molecular Life Sciences*, 2022
[7] J. Li *et al.*, *Science Advances*, 2022

Current Treatments



[8]

Microfracture

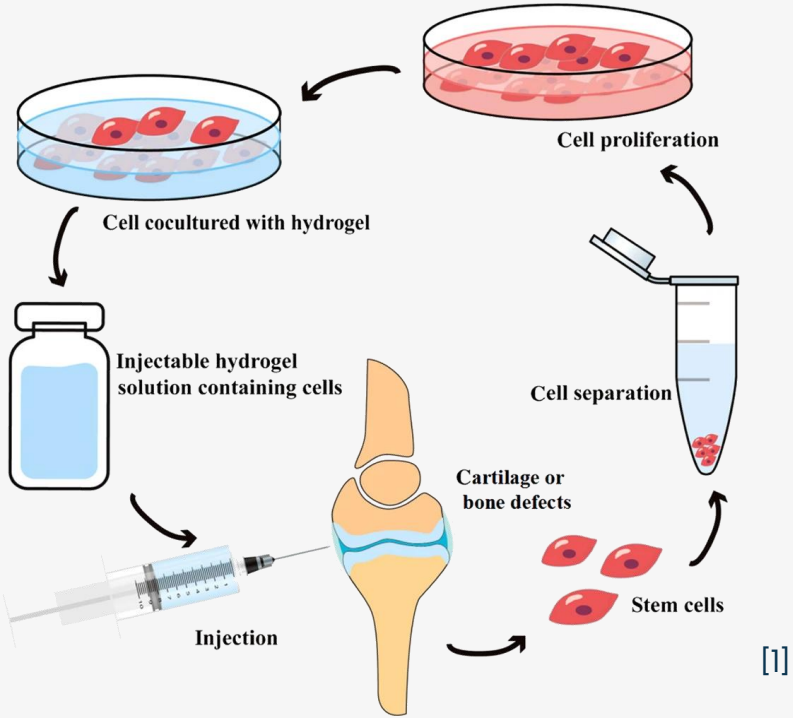


[9]

Joint replacement

[8] K. R. Stone, *www.stoneclinic.com*, 2020
[9] J. R. H. Foran *et al.*, *Aaos.org*, 2016

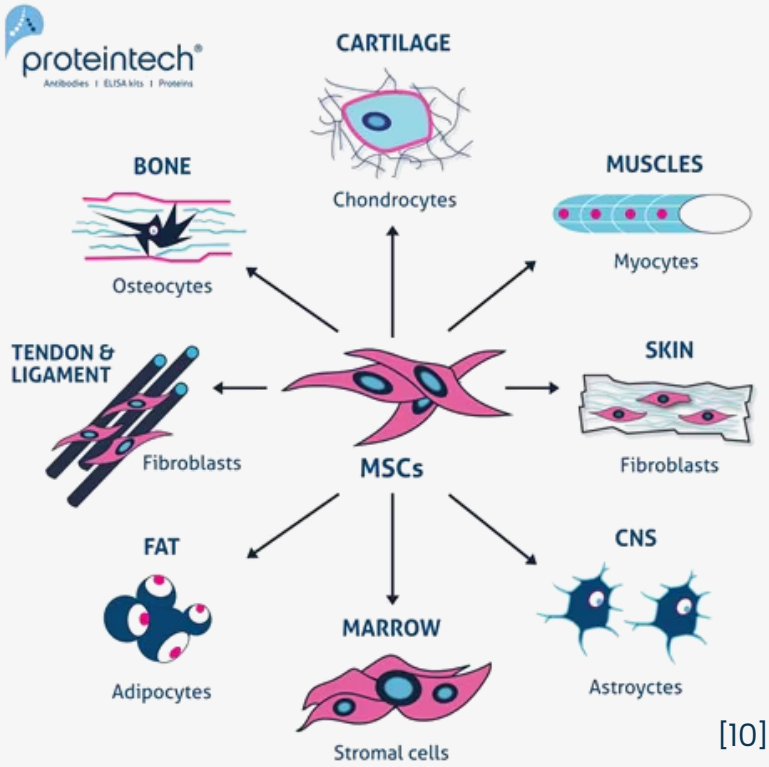
Autologous Chondrocyte Implantation



[1]

[1] M. Liu *et al.*, *Bone Research*, 2017

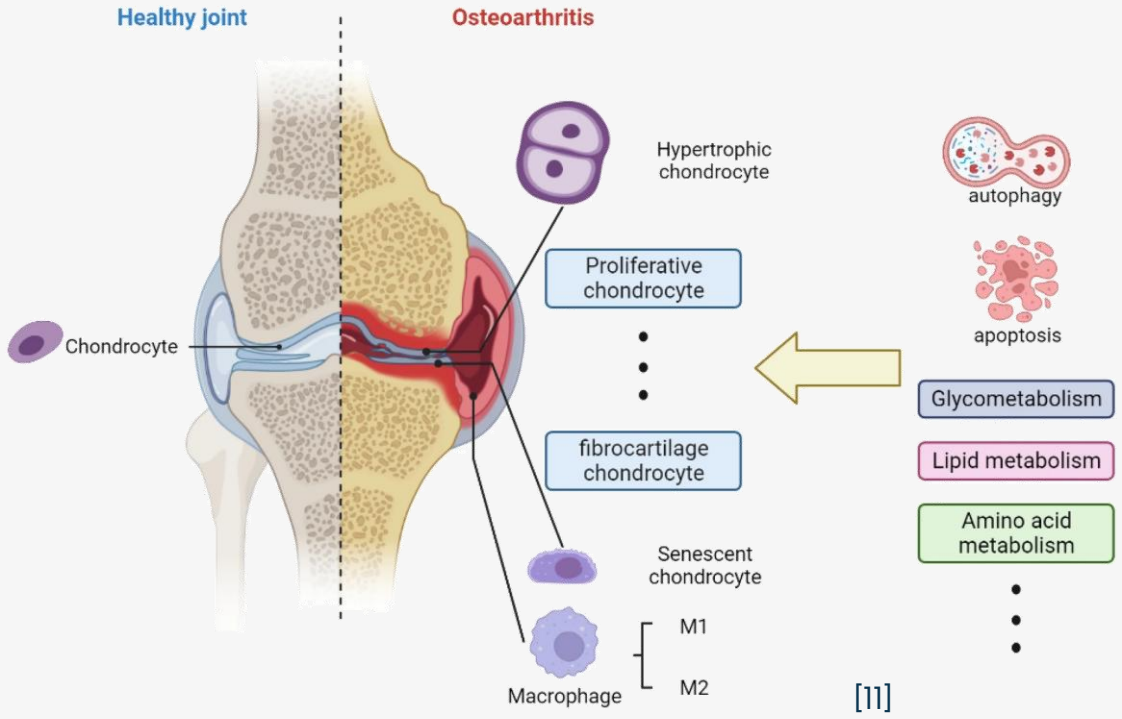
Mesenchymal Stem Cells (MSCs)



[10]

[10] Proteintech Group, *Ptglab.com*, 2018

Chondrocyte Phenotypic Instability



[11] Z. Liu *et al.*, *Human Cell*, 2023

02

Theory

Experimental design rationale

2% – 7% O_2

The native oxygen concentration of cartilage [12]

[12] H. Le *et al.*, *Journal of Tissue Engineering*, 2020

Hypoxia-Inducible Factor-1α

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

Normoxia:
Degraded via prolyl hydroxylation and proteasome degradation [14]

Hypoxia:
Hydroxylases are inhibited [15,16]

[13] S. Salceda *et al.*, *Journal of Biological Chemistry*, 1997
[14] R. Amarilio *et al.*, *Development*, 2007
[15] G. Teti *et al.*, *Stem Cells International*, 2018
[16] M. Y. Koh *et al.*, *Trends in Biochemical Sciences*, 2012

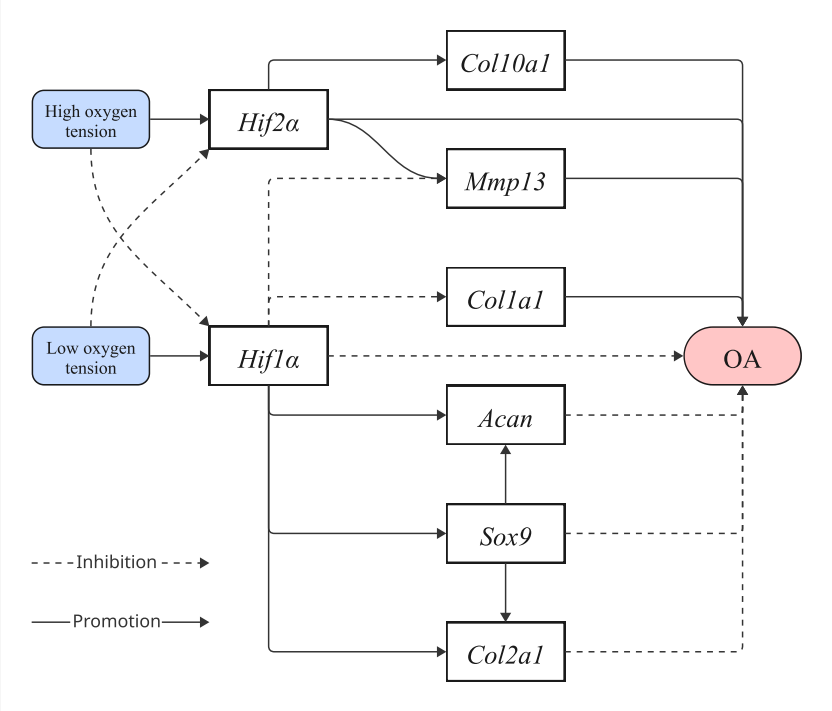
Hypoxia-Inducible Factor-1α

Regulates differentiation [17]

Upregulates related genes like *Sox9*

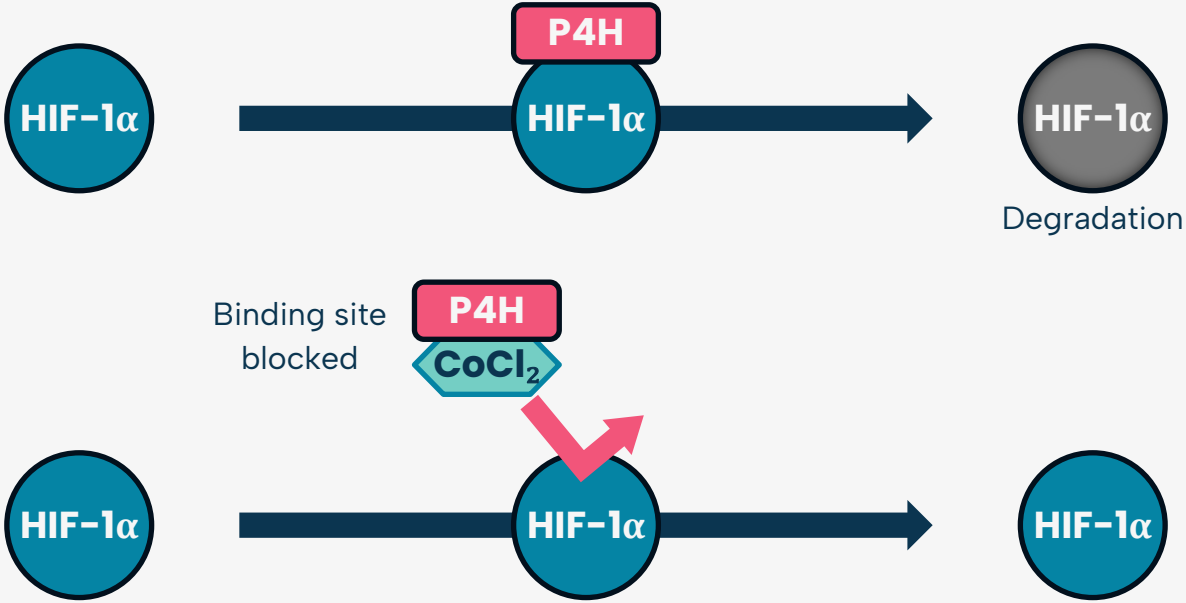
Stabilises phenotype [18]

Epigenetic effects



[17] M. B. Goldring *et al.*, *Annals of the New York Academy of Sciences*, 2010
[18] D. K. Taheem *et al.*, *Tissue Engineering Part B: Reviews*, 2020

Hypoxia mimetic agent $CoCl_2$



3D Culturing

3D

- Promote cell-cell & cell-ECM interactions [19]
- Natural cell morphology by allowing aggregation and micro-environments [19]
- Can induce more chondrogenic markers and proteins[20]

2D

- Convenient
- Easily reproducible
- Cannot mimic *in vivo* environments (mostly cell-plastic & cell-medium interactions) [20,21]

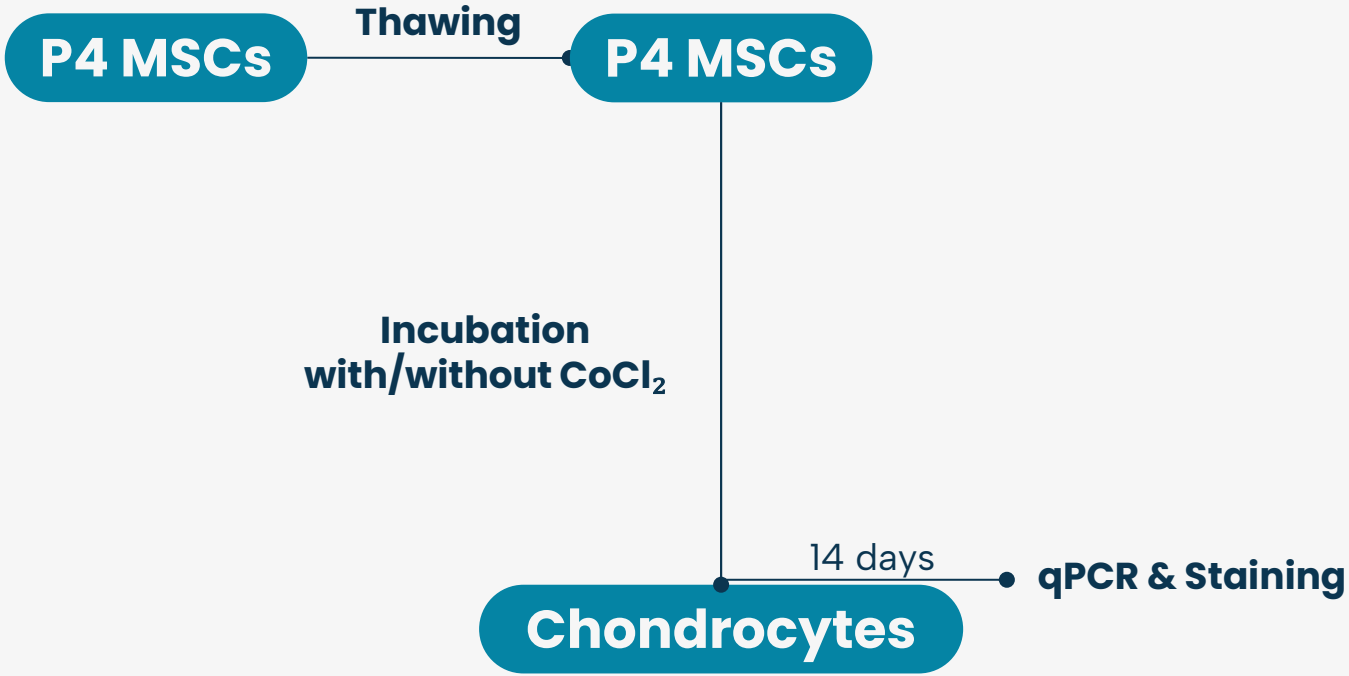
[19] D. Antoni *et al.*, *International Journal of Molecular Sciences*, 2015
[20] M. M. J. Caron *et al.*, *Osteoarthritis and Cartilage*, 2012
[21] J. C. Fontoura *et al.*, *Materials Science and Engineering: C*, 2020

03

Materials & Methods

Experimental protocols

Experimental Flow



Chondrogenesis Evaluation

Gene (qPCR)	Chondrogenic Involvement	Expectation
Hif1α	Regulates chondrogenic differentiation	↑ in hypoxia
Col1a1	Type I collagen	↓ in hypoxia
Col2a1	Type II collagen: major cartilage component	↑ in hypoxia
Col10a1	Type X collagen	↓ in hypoxia
Acan	Aggrecan: major cartilage component	↑ in hypoxia
Sox9	Maintains cartilage homeostasis	↑ in hypoxia
Mmp13	Degrades type II collagen	↓ in hypoxia

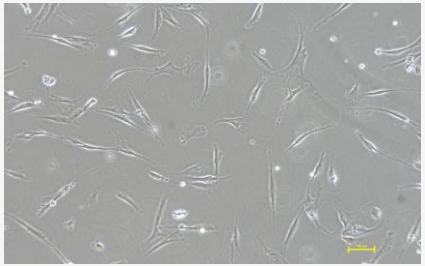
04

Results & Discussion

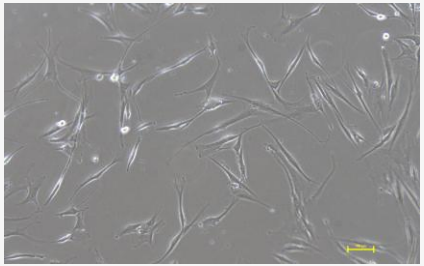
Experimental results

MSC Thawing

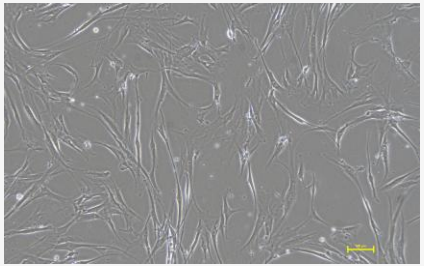
Day 3
14-3-2025



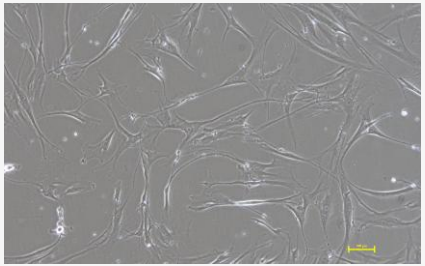
Day 5
16-3-2025



Day 7
18-3-2025



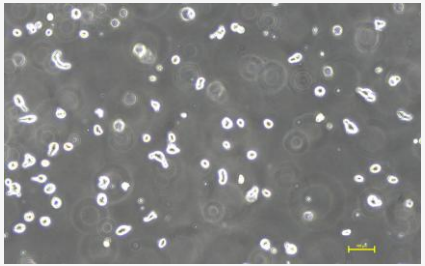
Day 9
20-3-2025



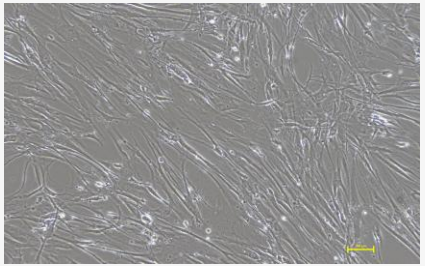
MSCs in T75 flask, 10× magnification

MSC Culturing

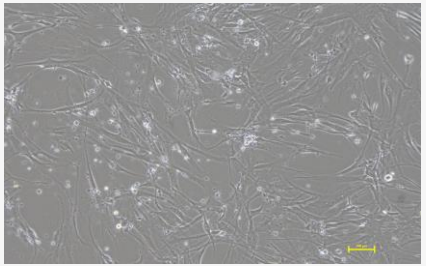
Day 1
20-3-2025



Day 3
22-3-2025

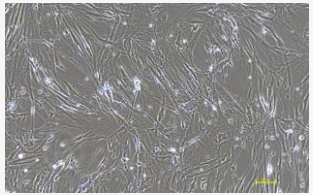
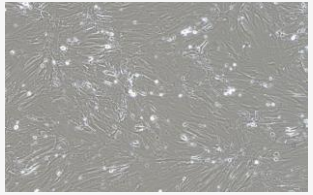
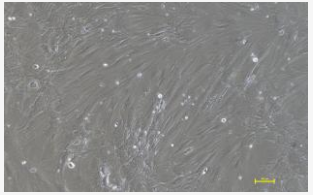
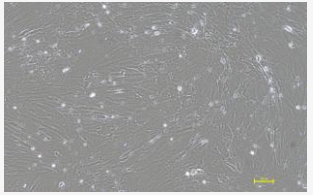
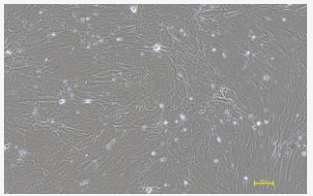
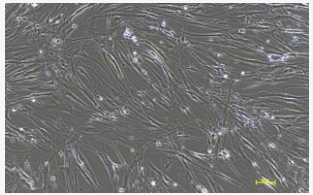
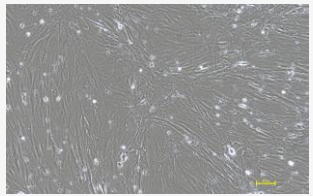
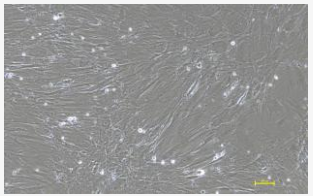
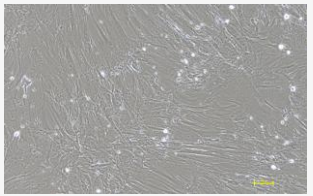
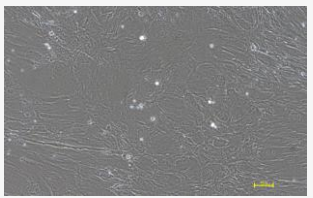


Day 5
24-3-2025



MSCs in 12-well plate, 10× magnification

MSC Chondrogenesis

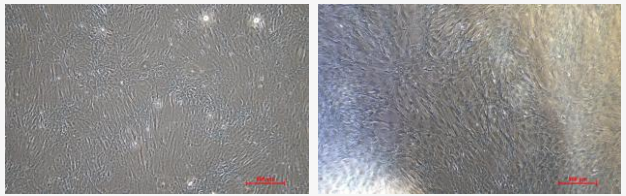
	Day 1 24-3-2025	Day 5 28-3-2025	Day 9 1-4-2025	Day 11 3-4-2025	Day 14 6-4-2025
Control					
$CoCl_2$					

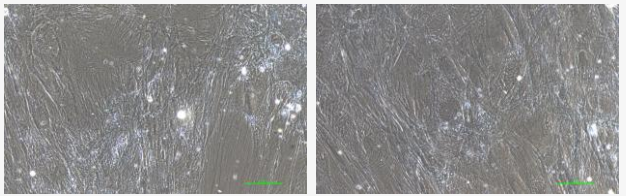
MSCs in 12-well plate, 10× magnification

Alcian Blue Staining

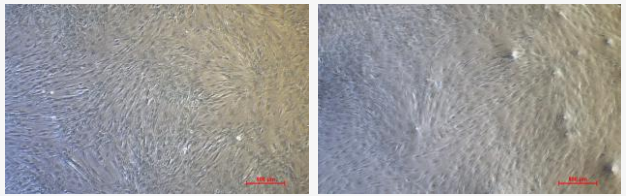
4× **20×**

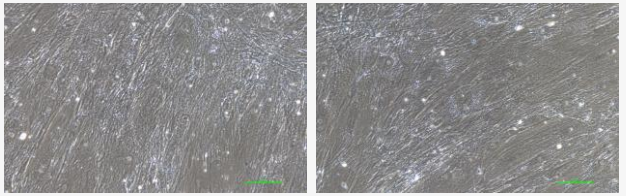
Control





$CoCl_2$



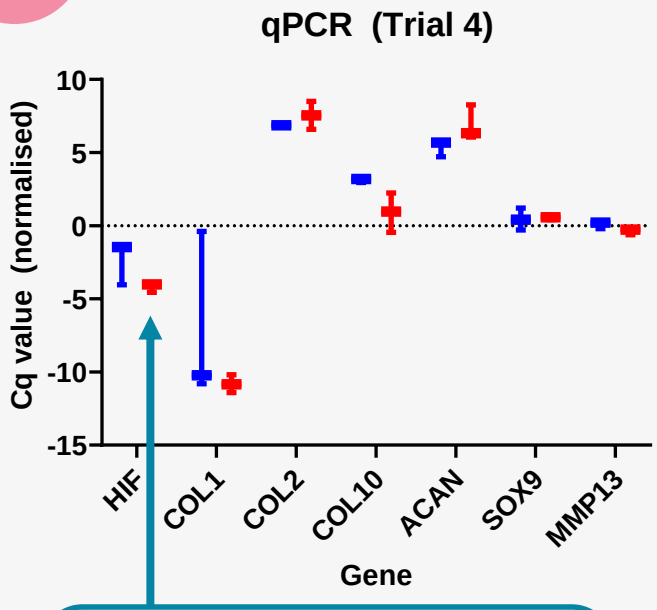


MSCs in 12-well plate (Alcian blue)

RNA extraction

Sample	Concentration (ng/μL)	A260/A280	A260/A230
Norm-1	0.74	1.67	0.09
Norm-2	1.28	1.80	0.18
Norm-3	***1.92***	***2.09***	***3.30***
Hypo-1	***1.98***	***2.98***	***3.09***
Hypo-2	1.70	1.57	0.58
Hypo-3	1.58	3.37	0.01

qPCR



Normoxia
Hypoxia

Gene	Normalised Cq						Mean		SD		P-value
	NORM			HYPO			NORM	HYPO	NORM	HYPO	
Hif1α	-1.46	-4.04	-1.31	-4.56	-4.02	-3.89	-2.27	-4.15	1.54	0.36	0.054
Col1a1	-0.40	-10.8	-10.2	-11.4	-10.8	-10.2	-7.14	-10.8	5.85	0.61	0.170
Col2a1	-	-	+6.87	+8.50	-	+6.59	+6.87	+7.54	1.35	1.35	-
Col10a1	+3.19	+3.22	+2.95	+0.96	+2.24	-0.46	+3.12	+0.91	0.15	1.35	0.024
Acan	+4.71	+5.68	+5.73	+6.06	+8.26	+6.32	+5.37	+6.88	0.57	1.2	0.061
Sox9	-0.30	+1.20	+0.41	+0.42	+0.71	+0.57	+0.44	+0.57	0.75	0.15	0.392
Mmp13	0.21	+0.32	-0.21	-0.62	-0.14	-0.26	+0.11	-0.34	0.28	0.25	0.053

ΔΔCq = -1.88

→ 3.69× higher in hypoxia

p=0.054

Discussion

qPCR & Staining

To be reattempted in the future to acquire more conclusive results

Implications

Based on literature...

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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