Mechano-Hypoxia Conditioning of Human Stem Cell-Based Cartilage with Transient TGF-β3 Stimulation

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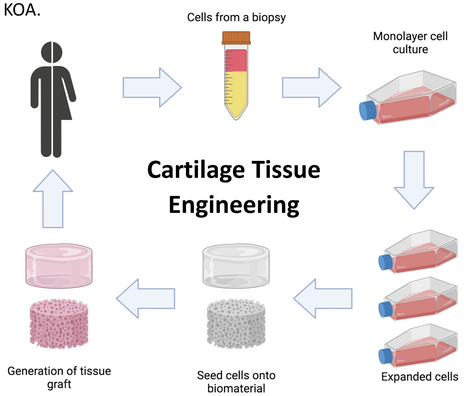
Introduction

Clinical Need

- Knee osteoarthritis (KOA) affects > 650M people globally.
- Injured articular cartilage (AC) increase the risk of KOA.
- AC is avascular and has a limited self-repair capacity, making injuries difficult to treat.
- Cartilage tissue engineering using cell-based methods aims to generate functional replacements to treat cartilage defects and KOA.



Cell Selection

- Human bone marrow mesenchymal stem cells (hBM-MSC) can differentiate into chondrocytes.
- However, they are prone to hypertrophic differentiation upon stimulation with growth factors like TGF-β1/3.

Mechano-Hypoxia Conditioning

- Simulates physiologically relevant conditions of the knee joint (mechanical loading and hypoxia)
- Mechano-hypoxia conditioning of engineered meniscus [1]:
  - ✓ increased the gene expression of hyaline cartilage markers, *SOX9* and *COL2A1*.
  - ✓ inhibited hypertrophic marker *COL10A1*.
  - ✓ promoted mechanical property development.

Hypothesis

We hypothesize that **mechano-hypoxia conditioning** will promote **non-hypertrophic** differentiation of **hBM-MSC** embedded in an **hyaluronan (HA)** hydrogel to **chondrocytes** with **transient TGF-β3** stimulation.

1. Szojka et al. (2021) Mechano-Hypoxia Conditioning of Engineered Human Meniscus. Front. Bioeng. Biotechnol. 9:739438. doi: 10.3389/fbioe.2021.739438

Methods

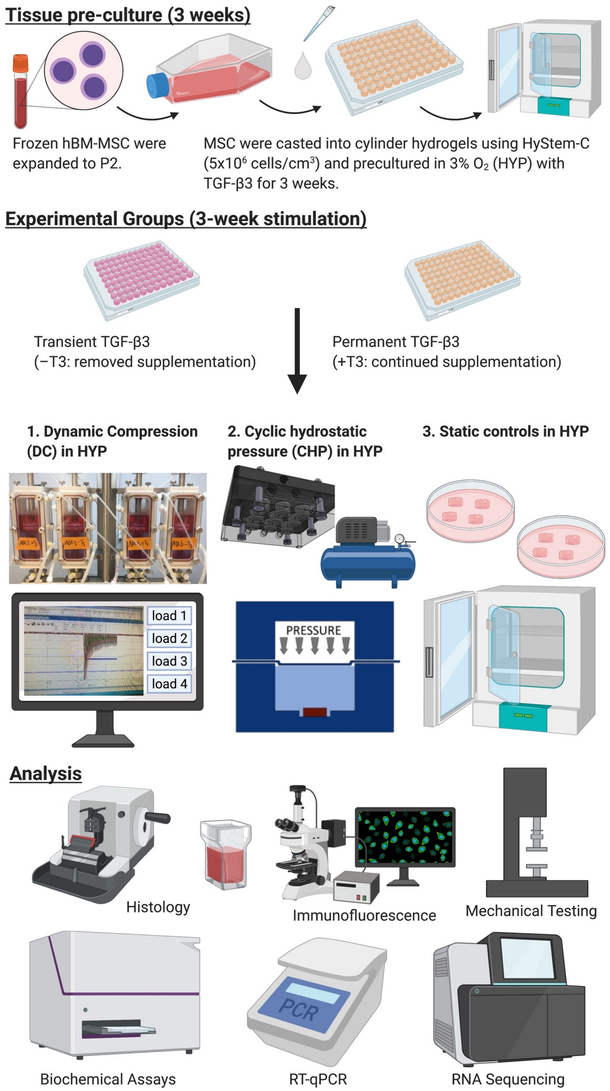


Figure 1. Experimental overview. Made with Biorender.com (2022)

Results

A). Histological and biochemical analysis confirmed that engineered tissues after treatments contained main cartilage components.

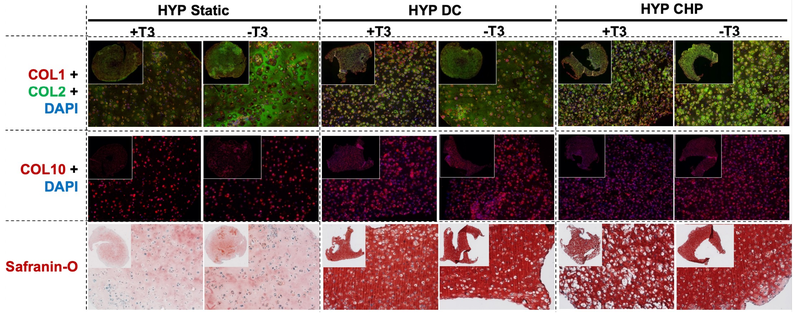


Figure 2. Histological and immunofluorescence staining of proteoglycans and collagen type I, II and X.

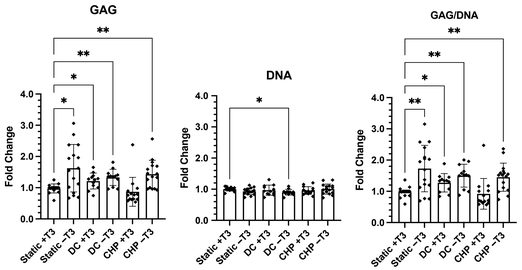


Figure 3. GAG/DNA analysis to quantify proteoglycan content. *p<0.05, **p<0.01

B). RNA sequencing shows a significant (q < 0.05) modulation of select cartilage– and skeletogenesis–regulatory gene markers from treatments.

Gene	Description	Fold Changes DC –T3 vs Static +T3	Fold Changes CHP –T3 vs Static +T3
ASPN	Asporin	13.38	5.61
CHADL	Chondroadherin Like	11.26	9.81
CNMD	Chondromodulin	71.43	54.94
COL2A1	Collagen Type II Alpha 1 Chain	7.78	6.99
COL9A1	Collagen Type IX Alpha 1 Chain	20.93	14.17
COL9A2	Collagen Type IX Alpha 2 Chain	7.10	5.92
COL9A3	Collagen Type IX Alpha 3 Chain	9.38	8.04
COL11A2	Collagen Type XI Alpha 2 Chain	12.17	9.62
EPYC	Epiphycan	13.44	7.69
FRZB	Frizzled Related Protein	23.91	42.05
GDF10	Growth Differentiation Factor 10	100.04	68.32
GPM6B	Glycoprotein M6B	10.52	10.98
HAPLN1	Hyaluronan And Proteoglycan Link Protein 1	6.24	6.26
HAPLN3	Hyaluronan And Proteoglycan Link Protein 3	-25.80	-7.15
HAS2	Hyaluronan Synthase 2	5.05	5.33
MATN3	Matrilin 3	9.48	9.68
MATN4	Matrilin 4	27.95	24.62
MMP7	Matrix Metallopeptidase 7	18.54	19.60
MMP13	Matrix Metallopeptidase 13	16.84	21.51
PAPPA2	Pappalysin 2	-6.68	-11.74
PKD2	Polycystin 2, Transient Receptor Potential Cation Channel	-41.85	-6.26
PHOSPHO1	Phosphoethanolamine/Phosphocholine Phosphatase 1	-28.54	-7.44
PPARG	Peroxisome Proliferator Activated Receptor Gamma	5.32	6.09
PTX3	Pentraxin 3	5.92	7.29
SLC26A2	Solute Carrier Family 26 Member 2	-20.12	-6.67
SNORC	Secondary Ossification Center Associated Regulator Of Chondrocyte Maturation	5.14	5.51
SPARCL1	SPARC Like 1	27.24	37.84
TNC	Tenascin C	6.91	7.69

C). Dynamic Compression (DC) promoted mechanical properties development

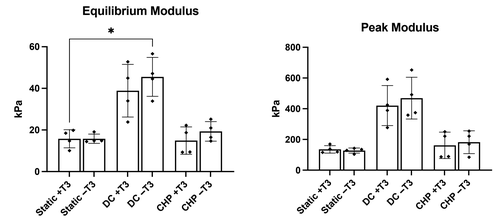


Figure 4. Measurement of equilibrium and peak modulus from mechanical testing. *p<0.05

Conclusions

- Mechano-hypoxia conditioning with transient TGF-β3 stimulation promoted non-hypertrophic differentiation of hBM-MSC to chondrocytes.
- RNA seq data showed that the modality of loading treatment (DC vs CHP) impacts the level of gene expression which eventually consequents to different mechanical properties.
- The mechanical property development in the 3-week treatment shows promise toward generating functional engineered cartilage through more optimized and longer culture conditions.

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