Conjugated linoleic acid promotes anti-inflammatory and chondrogenic MSC phenotype through lipid metabolic remodeling in 2D and 3D collagen scaffolds for cartilage repair

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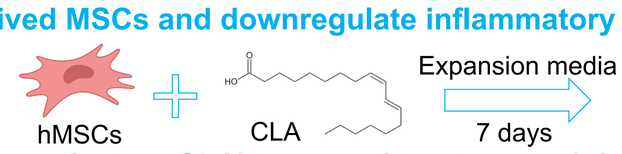
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INTRODUCTION

- **Articular cartilage** damage from trauma or osteoarthritis (OA) remains a major challenge due to **limited regenerative capacity and chronic inflammation**[1].
- OA is a **whole joint degenerative disease** with progressive cartilage deterioration (**500 million people**). Traditional treatments limited success to 5 years; thus, **there is still the clinical need to develop more efficient treatments**[1].
- Our lab has previously demonstrated that the **Conjugated Linoleic Acid (CLA)**, a fatty acid (FA) enriched in grass-fed dairy, **exhibits neuro-anti-inflammatory activity** through PPAR-α, a key regulator of mesenchymal stem cell (MSC) differentiation[2].
- To explore CLA's therapeutic potential for OA and cartilage repair, this study investigates CLA's capability to **modulate inflammation** and **enhance MSC chondrogenesis** in 2D and 3D biomimetic highly porous collagen type I/II-hyaluronic acid (CI/II-HyA) scaffolds previously engineered for stable, hyaline-like cartilage repair[3,4].

OBJECTIVES AND METHODS

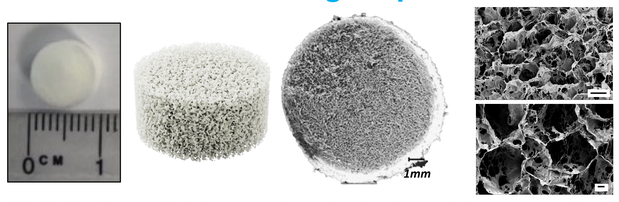
1. **To initially assess CLA's ability to be effectively incorporated into human-derived MSCs and downregulate inflammatory pathways in a 2D culture system.**



Lipid metabolism analysis

Gene expression analysis

2. **To evaluate CLA's capacity to modulate inflammation and enhance chondrogenesis of hMSCs cultured on previously designed 3D collagen-based scaffolds for cartilage repair[3,4].**



*CI/II-HyA scaffolds previously designed by our collaborators at the **Tissue Engineering Research Group (TERG), RCSI**[3,4].*

hMSCs chondrogenic induction ± IL-1β

28 days

Lipid metabolism analysis

Biochemical assays

Gene expression analysis

Protein expression analysis

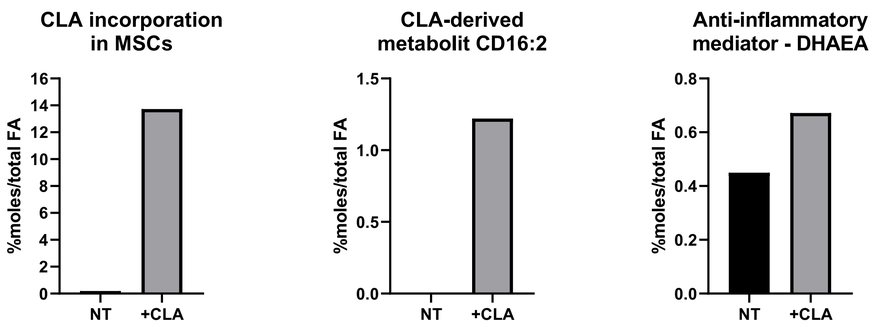
MAIN AIM

To investigate the therapeutic potential of Conjugated Linoleic Acid (CLA), a fatty acid (FA) enriched in grass-fed dairy, as possible target for MSC- and biomaterial-based strategies for osteoarthritis (OA) and cartilage repair

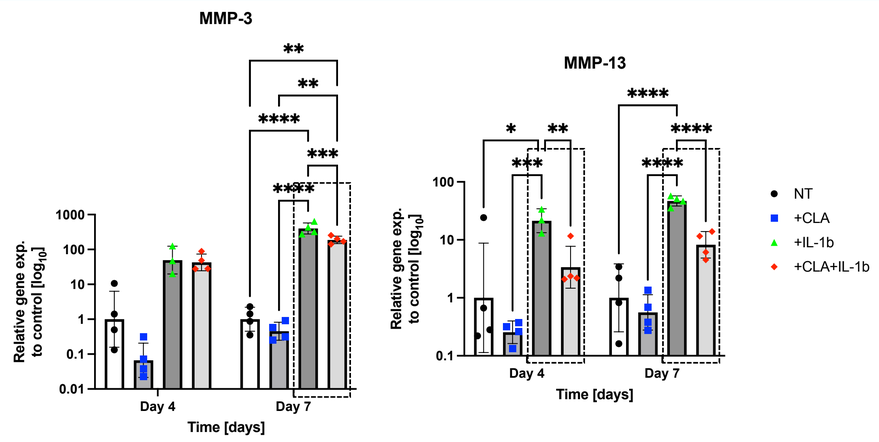
RESULTS

Assessment in 2D

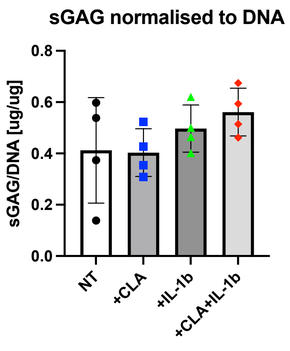
CLA incorporated and metabolised into hMSCs (2D) with indication to shift lipid metabolism toward an anti-inflammatory profile at day 7

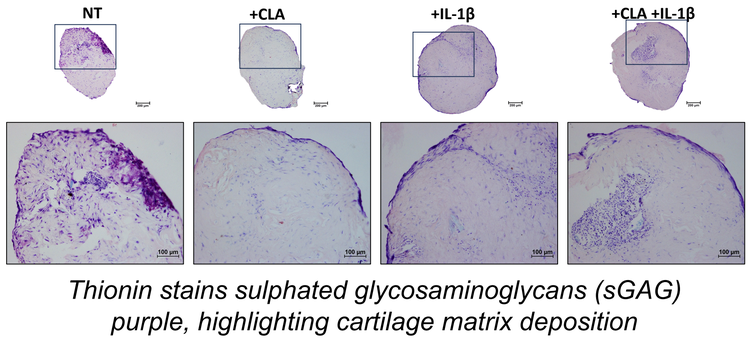


CLA incorporation reduces pro-catabolic proteases (MMP3, MMP13) gene-expression in hMSCs under inflammatory challenge (2D)

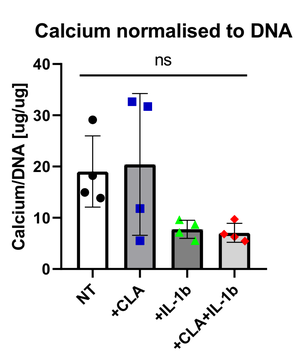


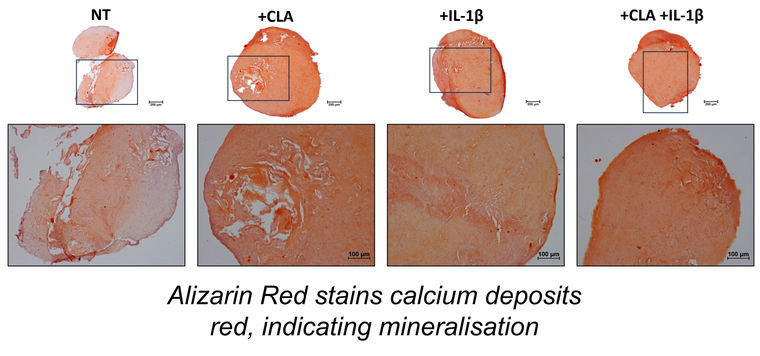
CLA incorporation does not affect sGAG deposition and does not increase mineralisation in the scaffold at day 28





Thionin stains sulphated glycosaminoglycans (sGAG) purple, highlighting cartilage matrix deposition

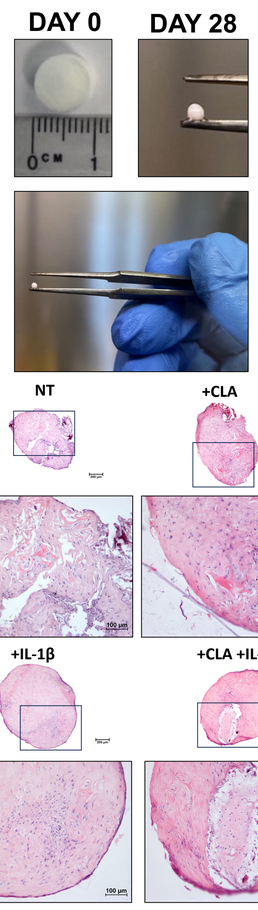




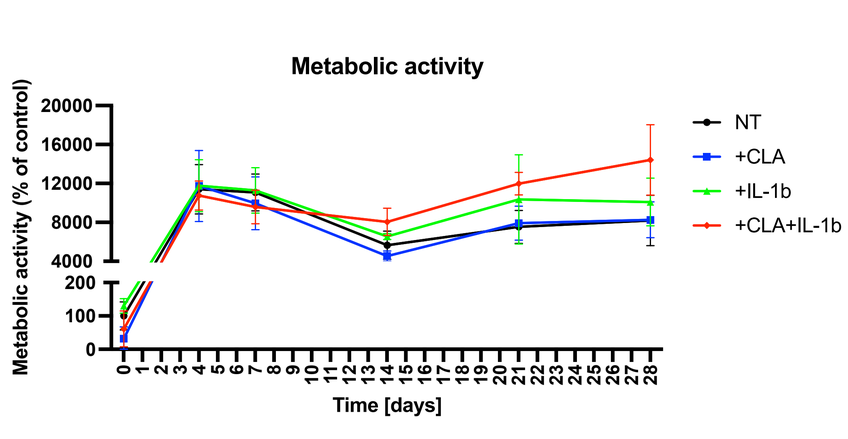
Alizarin Red stains calcium deposits red, indicating mineralisation

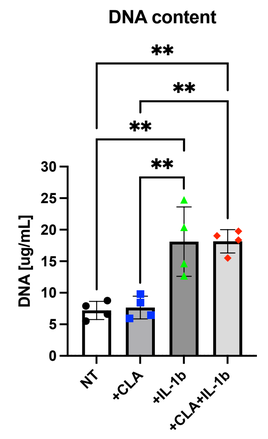
Assessment in 3D

CLA does not alter hMSC viability and distribution in a 3D collagen-based scaffold platform for cartilage repair at day 28

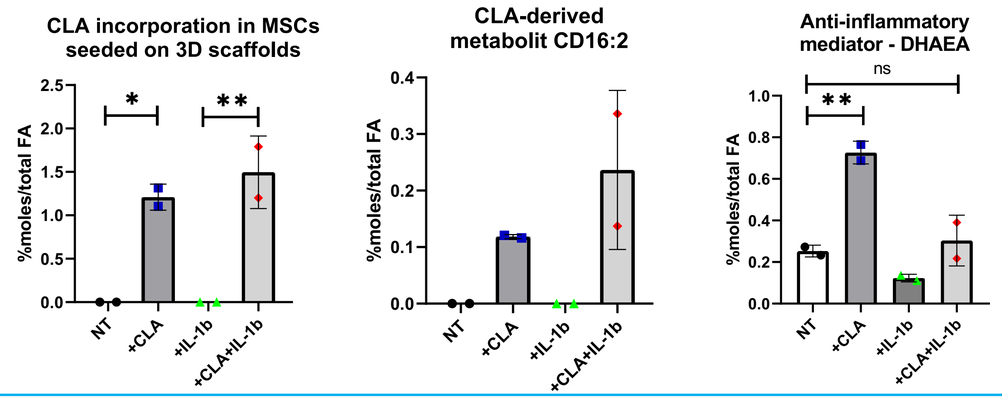


H&E stains nuclei blue/purple and cytoplasm/extracellular matrix pink





CLA incorporated into hMSCs cultured on 3D scaffolds with indication to shift lipid metabolism toward an anti-inflammatory profile at day 28



DISCUSSION

- **CLA showed promising anti-inflammatory/-catabolic effects in 2D hMSC culture**, reducing MMP-3/-13 and shifting lipid metabolism to anti-inflammatory mediators.
- **CLA incorporation** in hMSCs seeded into **3D collagen-based scaffolds** for cartilage repair **showed indications to shift lipid metabolism to an anti-inflammatory profile** while supporting cartilage-like tissue formation following 28 days chondrogenic culture.
- **CLA did not increase mineralisation and calcium-like tissue formation in 3D**, indicating that does not promote osteogenic drift under chondrogenic conditions.
- Further studies are underway to confirm CLA's anti-inflammatory action in 2D and 3D through time-course RT-qPCR and lipidomic analyses.

SIGNIFICANCE

These results identify CLA as a bioactive FA capable of modulating inflammation and metabolism during MSC chondrogenesis, highlighting its therapeutic promise for MSC- and biomaterial-based cartilage repair strategies in inflammatory joint environments

ACKNOWLEDGEMENTS

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