Induced Chondrocyte-Derived Small Extracellular Vesicles as a Cell-Free Regenerative Strategy for Osteoarthritis

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AIM OF THE STUDY

This study aimed to assess whether small extracellular vesicles derived from iPSC-induced chondrocytes can modulate inflammatory responses in IL-1α-stimulated primary human articular chondrocytes and represent a cartilage-oriented cell-free therapeutic strategy for osteoarthritis.

INTRODUCTION

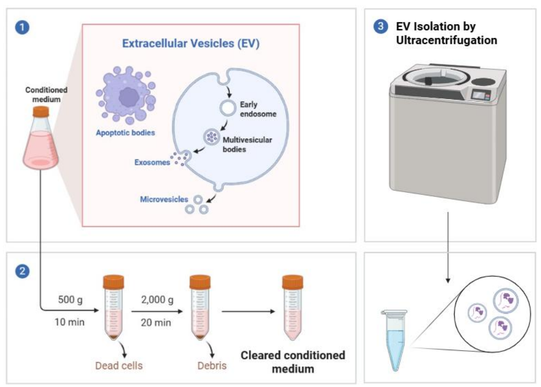
Osteoarthritis (OA) is the most prevalent degenerative joint disease and a leading cause of disability worldwide. It is characterized by progressive articular cartilage degradation, chronic low-grade inflammation, and oxidative stress, ultimately resulting in pain and impaired joint function. Despite its high socioeconomic burden, current clinical management remains largely symptomatic, highlighting the urgent need for disease-modifying and regenerative therapeutic strategies.

Extracellular vesicles (EVs) have emerged as promising cell-free therapeutic tools due to their ability to regulate intercellular communication and modulate inflammatory pathways. EVs derived from mesenchymal stromal cells (MSCs) have shown encouraging anti-inflammatory effects and represent a safer and more scalable alternative to cell-based therapies.

Since the molecular cargo of EVs reflects the identity of their parental cells, vesicles produced by cartilage-like cells may provide a more tissue-relevant and targeted strategy for osteoarthritis. Induced pluripotent stem cell (iPSC)-derived chondrocytes closely resemble native articular chondrocytes and represent a standardized source of cartilage-specific cells, potentially generating vesicles capable of more precisely modulating osteoarthritic inflammation.

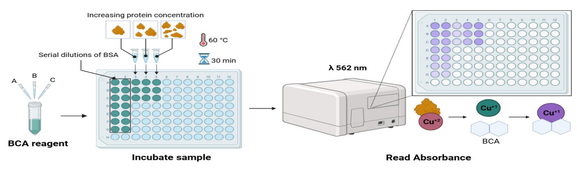
EXPERIMENTAL DESIGN

1 Vesicles Purification

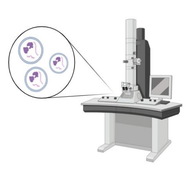


2 Vesicles Characterization

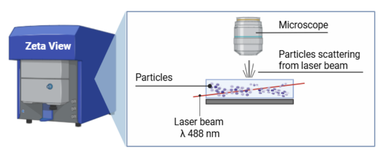
BCA assay



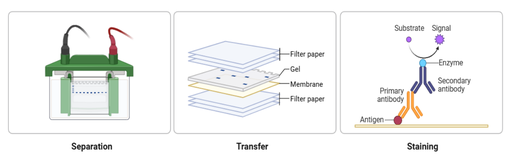
TEM



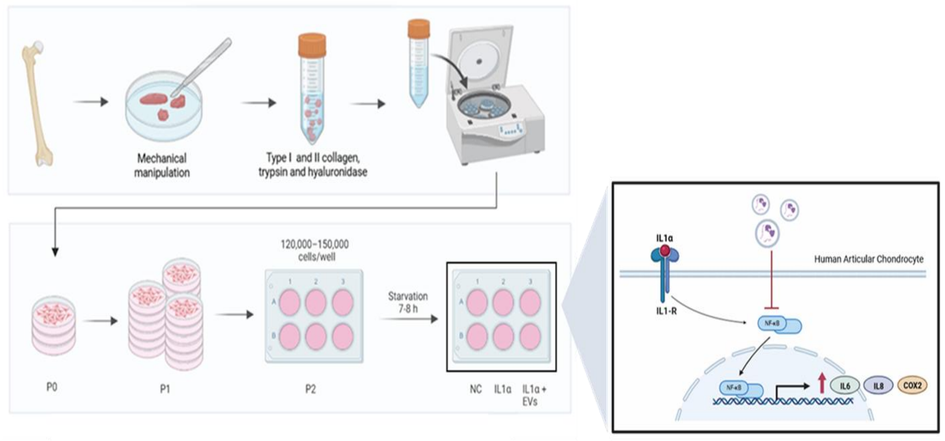
Nanoparticles Tracking Analysis



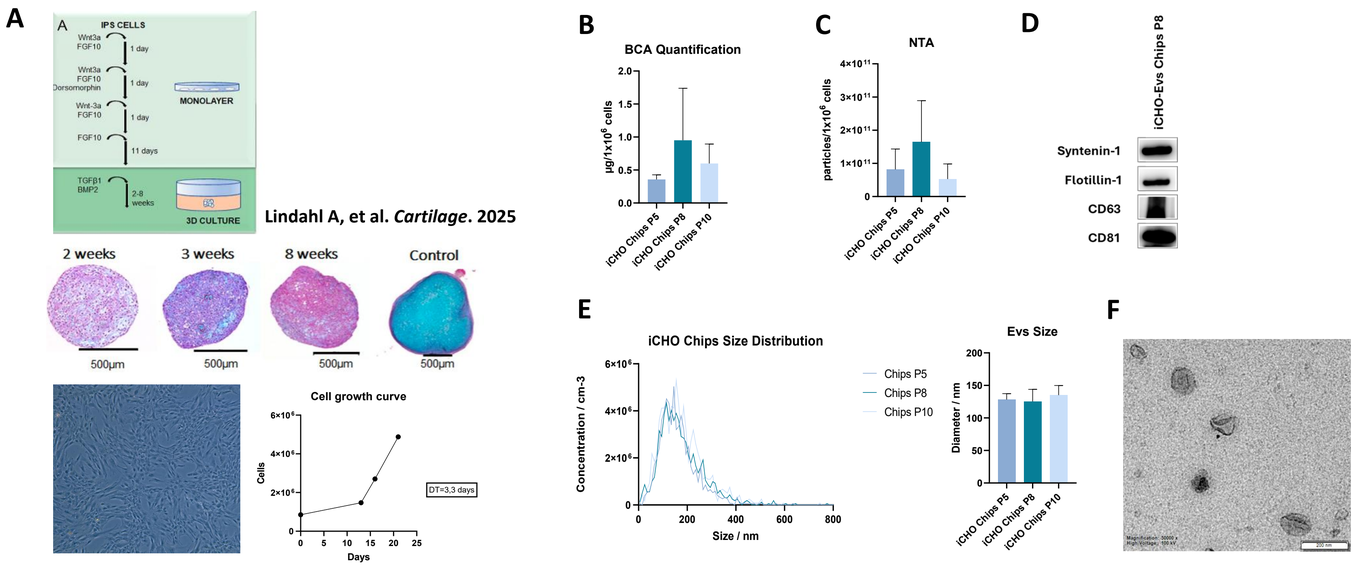
Western Blot



3 Anti-inflammatory & Anti-ROS Activity Assessment

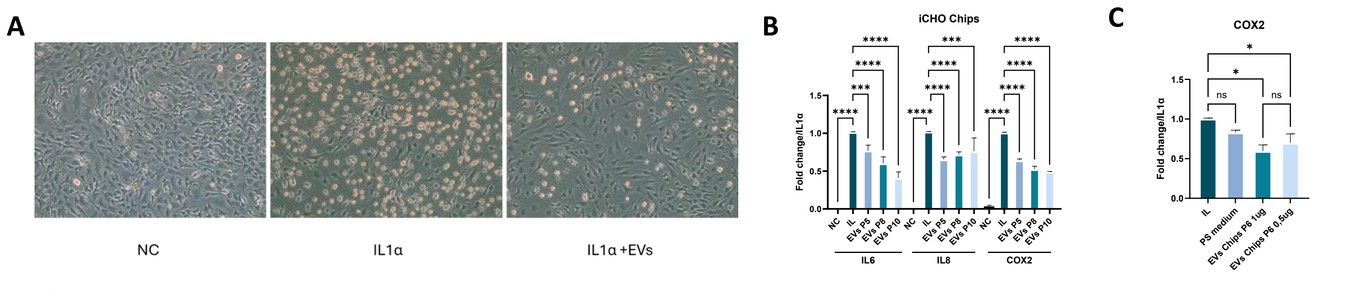


RESULTS 1: Characterization of iCHO-derived sEVs isolated by differential ultracentrifugation



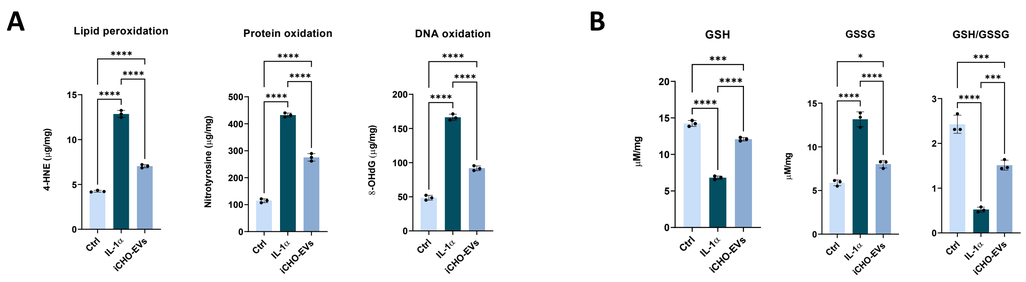
(A) Schematic overview of the differentiation protocol from iPSCs to human induced chondrocyte progenitors (iCHO). Histological and immunohistochemical characterization of iCHO cultured in 3D. Representative image of cells in 2D culture and corresponding proliferation curve. **(B-F)** Charaxcterization of iCHO-derived sEvs : **(B)** BCA assay showed variable total protein content across passages in EV-enriched preparations; **(C)** Particle quantification by Nanoparticle Tracking Analysis (NTA); **(D)** Western blot confirmed the presence of canonical sEV markers (Syntenin-1, Flotillin-1, CD63, CD81); **(E)** NTA size distribution showed a homogeneous sEV population with a main peak between 120–150 nm; **(F)** TEM imaging revealed vesicles with the typical cup-shaped morphology and confirmed the structural integrity of isolated sEVs.

RESULTS 2: Anti-inflammatory effects of iCHO-derived sEVs in 2D osteoarthritic model



(A-C) Anti-inflammatory activity of iCHO-derived sEVs in a 2D osteoarthritic model: **(A)** Phase-contrast microscopy showed morphological recovery in IL-1α-stimulated chondrocytes treated with sEVs compared to the positive control; **(B)** RT-qPCR analysis demonstrated significant downregulation of IL6, IL8, and COX2 expression following treatment with iCHO-derived sEVs; **(C)** Increasing sEV concentration did not further enhance COX2 downregulation. PurStem alone had no significant effect on inflammatory gene expression, supporting the conclusion that the anti-inflammatory response is specifically mediated by sEVs.

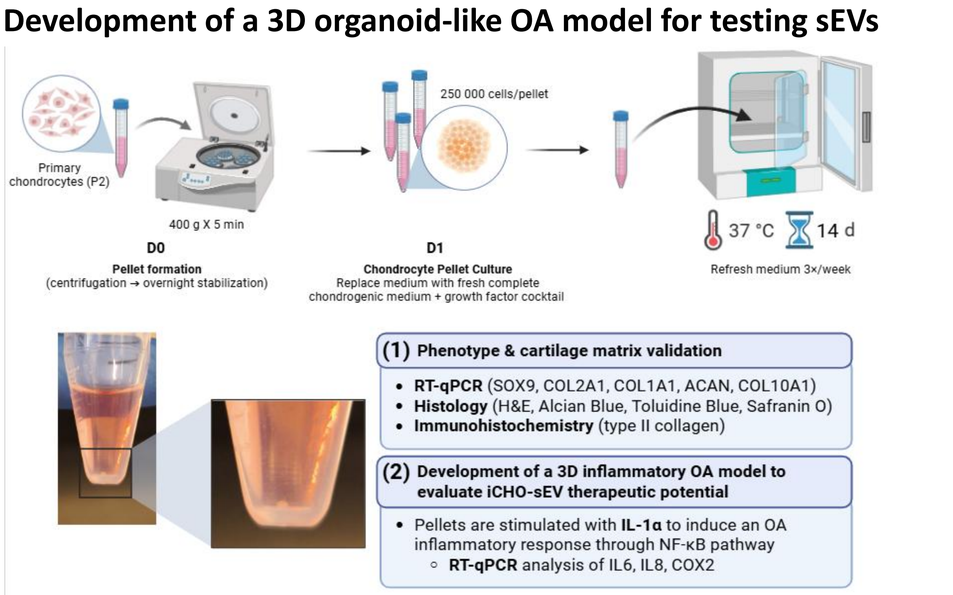
RESULTS 3: Anti-ROS effects of iCHO-derived sEVs in 2D osteoarthritic model

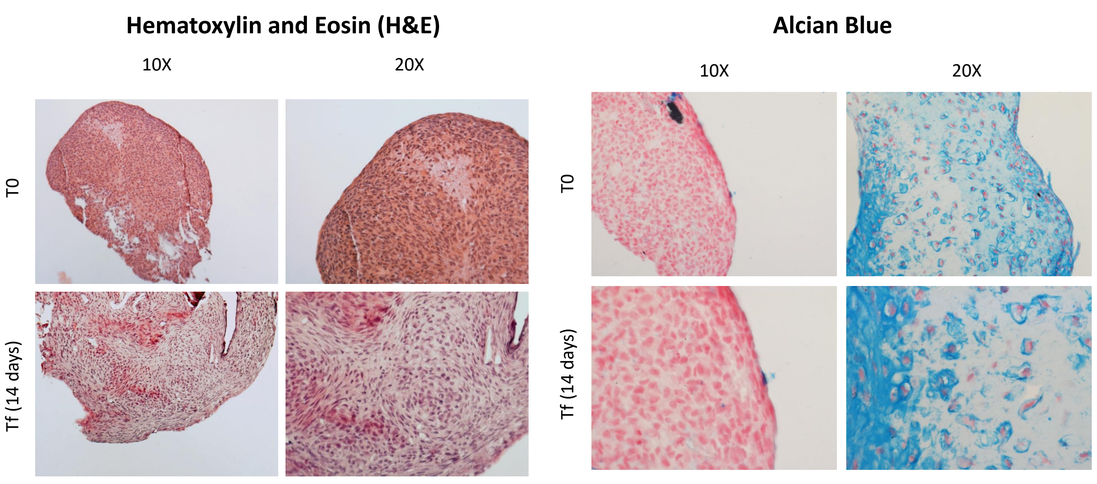


(A-B) Anti-ROS activity of iCHO-derived sEVs in a 2D osteoarthritic primary model. **(A)** Quantification of oxidative damage markers, including lipid peroxidation, protein oxidation, and DNA oxidation, in treated and control conditions. **(B)** Evaluation of intracellular redox balance through measurement of reduced glutathione (GSH), oxidized glutathione (GSSG), and the GSH/GSSG ratio. Treatment with iCHO-derived sEVs modulates oxidative stress by reducing biomolecular oxidation and improving cellular antioxidant capacity.

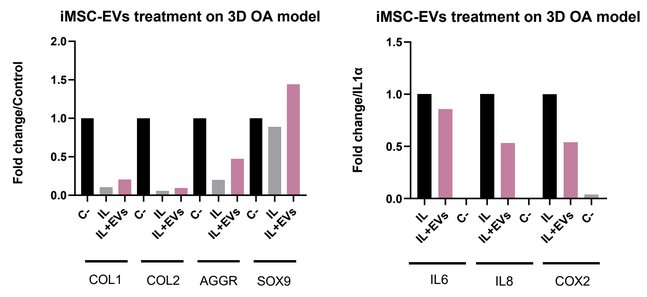
ONGOING

Development of a 3D organoid-like OA model for testing sEVs





Proof-of-concept: iMSC-EVs in 3D OA model



iMSC-derived EVs were used as a preliminary model to validate EV penetration and biological activity in the 3D OA system. **This approach will be translated to iCHO-derived EVs.**

DISCUSSION

iCHO-derived sEVs demonstrate moderate anti-inflammatory effects in primary human chondrocytes, effectively downregulating IL-6, IL-8, and COX-2 expression. Compared to iMSC-sEVs, iCHO-sEVs were particularly effective in modulating chondrocyte inflammation, likely reflecting their phenotypic proximity to target tissue.

While our current 2D model provides robust proof-of-concept, it cannot fully recapitulate the complex architecture and cell–matrix interactions of osteoarthritic cartilage. Ongoing studies are focused on developing a 3D organ-like OA platform using patient-derived chondrocyte pellets to translate our findings into a more physiologically relevant system. Together, these results support the use of iCHO-sEVs as a promising cell-free strategy for cartilage-targeted therapies in osteoarthritis.

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