

## MORPHO-FUNCTIONAL CHARACTERIZATION AND HIGH-RESOLUTION PROTEOMIC ANALYSIS OF PLATELET-DERIVED EXTRACELLULAR VESICLES: NATURAL BIOLOGIC FOR THE TREATMENT OF OSTEOARTHRITIC CHONDROCYTES

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### INTRODUCTION:

An adjuvant therapy based on platelet-derived extracellular vesicles (PEVs), is a natural biologic in which concentrated with bioactive proteins. It has recently been seen as a promising approach to assist cartilage repair in osteoarthritis (OA). However, PEVs have rarely been studied, yet it is unclear which bioactive proteins directly affect the regeneration of damaged cartilage under various knee OA conditions. Therefore, this study investigated the effect of PEVs in treating osteoarthritic chondrocytes.

### METHODS:

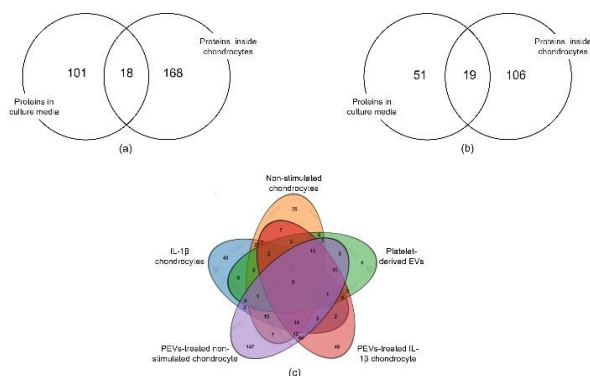
Platelet-derived EVs were isolated from healthy donors after informed consent and subjected to morpho-functional analysis for characterization. Th osteoarthritic chondrocytes *in vitro* model was created by prestimulating human chondrocytes with interleukin-1 beta (IL-1 $\beta$ ) to mimic osteoarthritic environment and subsequently the culture was supplemented with PEVs. Non-stimulated chondrocytes served as comparison. After 48 hours, chondrocytes and culture media in each condition were collected and were used to analyze the major bioactive proteins using high-resolution liquid chromatography-tandem mass spectrometry.

### RESULTS:

The result from ultra-structural and nanoparticle tracking analysis demonstrated a highly heterogeneous size and number of isolated PEVs, with a particle size range of 80-500 nm. *In vitro*, PEVs are found to stimulate chondrocyte proliferation. Pre-stimulation with IL-1 $\beta$  induced distinct shrinkage of chondrocytes, but not compromising the cell number. The condition is reversed by PEVs that induce chondroprotection and promote proliferation. Proteomic analysis of PEVs-treated non-stimulated chondrocytes and PEVs-treated IL-1 $\beta$  chondrocytes identified 287

and 176 proteins, respectively (Figure 1). Those secreted proteins were closely related to chondrocyte proliferation. Protein-protein interaction networks were changed depending on environmental differences and were found to enhance the secretion of bioactive proteins related to cartilage regeneration.

Figure 1: Venn diagram of identified protein (a) in PEVs-treated non-stimulated chondrocytes, (b) in PEVs-treated IL-1 $\beta$  chondrocytes and (c) in all experiment's groups.



### DISCUSSION:

Platelet-derived EVs are natural mediators of different physiological processes and contribute to many regenerative processes [1]. The bioactive proteins in PEVs rich in growth factors promote chondroprotective [2] and anti-inflammatory effects [3] as demonstrated by proteomic profiling.

### CONCLUSION:

Platelet-derived EVs diminished inflammatory IL-1 $\beta$  mediated effects on human osteoarthritic chondrocyte by modulating the protein profiles in which related to cartilage regeneration.

### REFERENCES:

- [1] Antich-Rossello M et al. Int J Mol Sci 2021
- [2] Dai Z et al. Materials Today Bio 2023
- [3] Ma Q et al. et al. J Control Release 2021