Bone & Joint Research

Supplementary Material

10.1302/2046-3758.1210.BJR-2023-0109.R1

Supplementary Methods

Transmission electron microscopy

Extracellular vesicle (EV) samples were mixed 1:1 with 4% formaldehyde (MilliporeSigma, USA) and fixed on copper Formvar/Carbon coated grids (Ted Pella, USA) for 20 minutes. The grids were further processed as previously described.[1] To contrast samples, grids were stained with 2% uranyl acetate for one minute, washed three times with deionized water, and then air dried. Images of EVs were taken with transmission electron microscope Hitachi H600 (Hitachi, Japan) at 50 kV.

Western blot

Transmembrane EV markers from platelet lysates (PL), PL-derived extracellular vesicles (pEVs), mesenchymal stromal cell (MSC) lysate (CL), and human umbilical cord MSC-derived EVs (cEVs) were immunodetected as follows: CD9, CD63, and CD81 were detected using 2.5 µg of protein prepared with non-reducing loading buffer (without β-mercaptoethanol), loaded in a 12% SDS-PAGE gel for protein separation by electrophoresis, and transferred onto nitrocellulose membrane (GE Healthcare, USA) by wet transference. Lysed EVs were used to detect HSC70 as cytosolic proteins recovered in EVs, and cytochrome C as a protein associated with other intracellular compartments than endosomes in 10 µg of protein prepared with reducing loading buffer loaded in a 10% and 12% SDS-PAGE gel, respectively. Apolipoprotein A (ApoA) was evaluated as non-EV co-isolated structures in 5 µg of protein prepared with reducing loading buffer, loaded in a 10% and 12% SDS-PAGE gel. Antibodies were used as follows: anti-human CD63 monoclonal antibody (clone Ts63, diluted 1:5,000; Thermo Fisher Scientific, USA), anti-human CD9 monoclonal antibody (clone Ts9, diluted 1:2,000; Thermo Fisher Scientific), anti-human CD81 monoclonal antibody (clone M38, diluted 1:2,000; Thermo Fisher Scientific), anti-human HSC70 (clone B-6, diluted 1:1,500; Santa Cruz Biotechnology, USA), anti-human cytochrome C monoclonal antibody (clone 37BA11, diluted 1:1,000; Abcam, UK), anti-human ApoA (clone 513, diluted 1:4,000; Thermo Fisher Scientific), and

HRP-coupled secondary antibody (diluted 1:2,000; Thermo Fisher Scientific). Membranes were exposed to Amersham Hyperfilm ECL (GE Healthcare) and revealed with Kodak Dental Readymatic developer and fixer reagents (Carestream Health, USA).

Supplementary Results

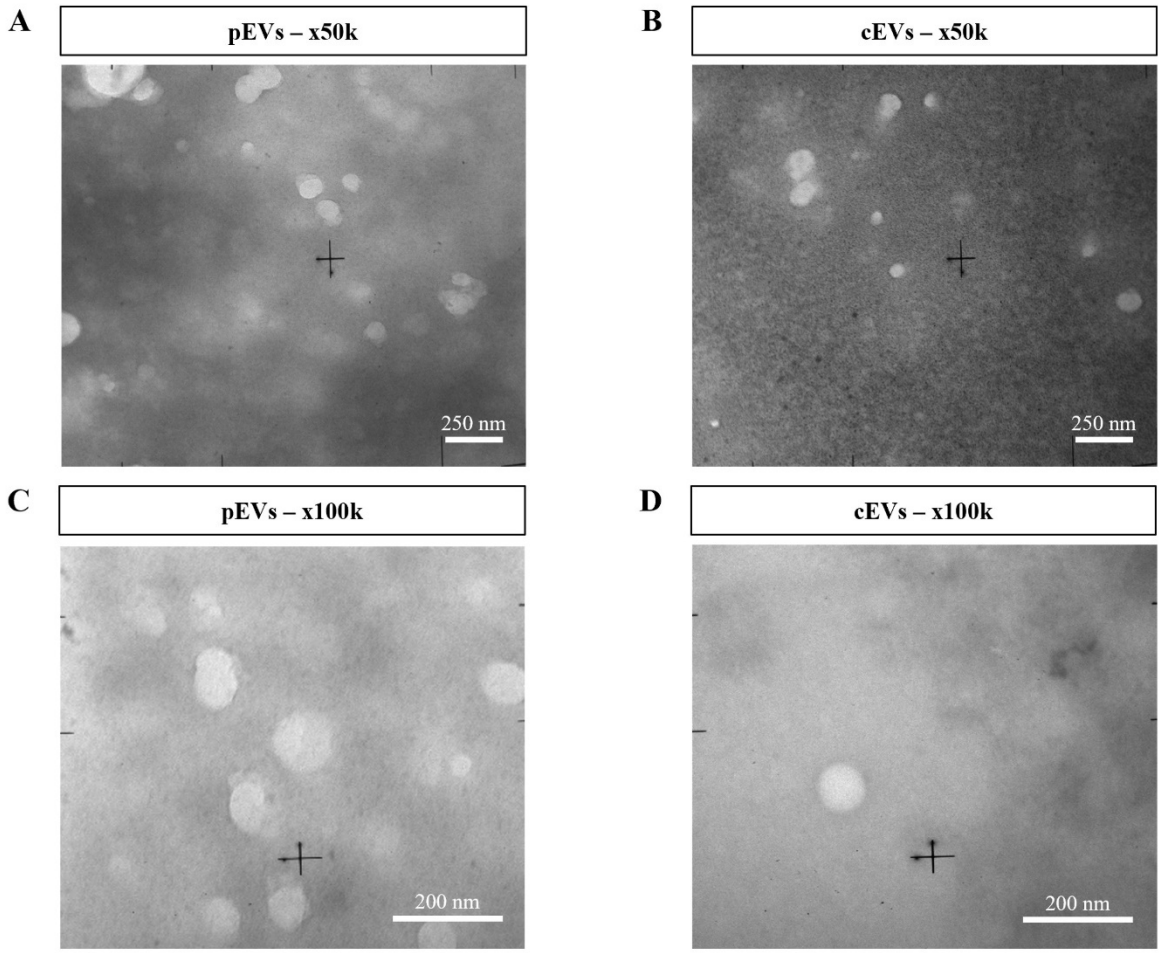

Fig a. Extracellular vesicle (EV) characterization by transmission electron microscopy (TEM). Representative TEM images for a) platelet lysate-derived extracellular vesicles (pEVs) and b) human umbilical cord mesenchymal stromal cell-derived extracellular vesicles (cEVs) at ×50k, and c) pEVs and d) cEVs at ×100k with their respective scale bars.

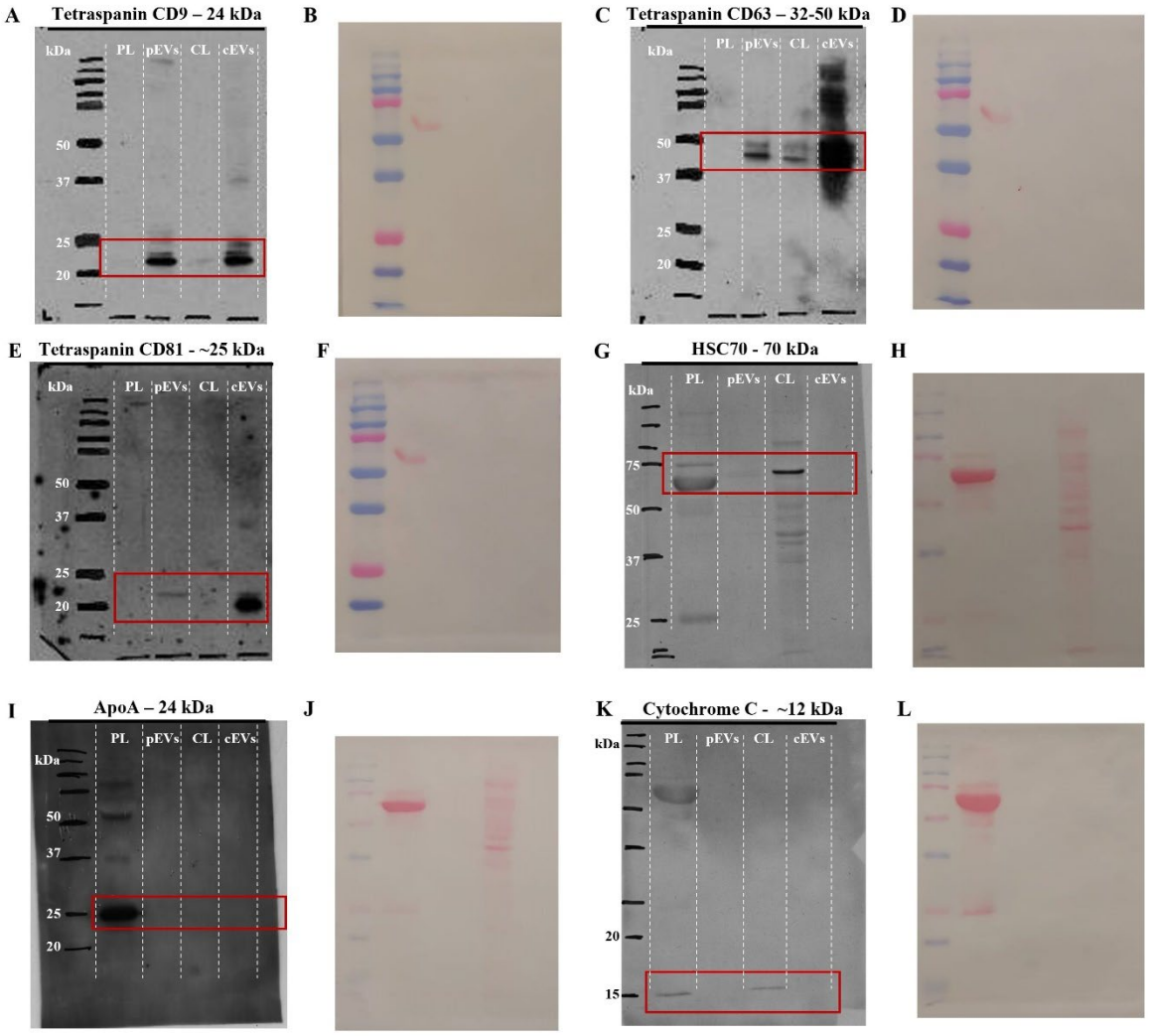

Fig b. Full-length blots for extracellular vesicle (EV) characterization by western blot (WB) and respective Ponceau membrane stains in platelet lysates (PL), platelet lysate-derived extracellular vesicles (pEVs), human umbilical cord mesenchymal stromal cell (hUC-MSC) lysate (CL), and hUC-MSC-derived extracellular vesicles (cEVs). Protein content in EVs was characterized in terms of transmembrane proteins associated with the plasma membrane and endosomes as: a) to b) CD9, c) to d) CD63, and e) to f) CD81; g) to h) cytosolic proteins recovered in EVs as HSC70; i) to j) major components of non-EV co-isolated structures; and k) to l) proteins associated to other intracellular compartments than endosomes as cytochrome C.

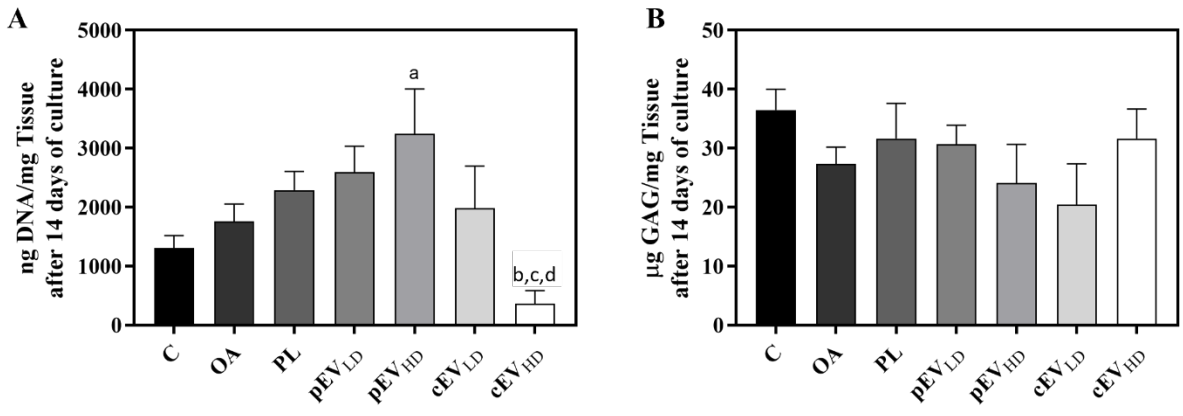

Fig c. Cartilage explants under osteoarthritis (OA)-like conditions after 14 days of treatment with platelet lysate-derived extracellular vesicles (pEVs) or human umbilical cord mesenchymal stromal cell-derived extracellular vesicles (cEVs). a) DNA and b) glycosaminoglycans (GAG) quantification of cartilage explants after 14 days of in vitro culture under inflammatory stimulus to induce OA-like conditions and treated with 1×10^8 or 1×10^9 particles of pEVs or cEVs (low dose (LD) and high dose (HD), respectively). Nine different donors were used and the experiments were performed in triplicate. Results were statistically compared using Kruskal-Wallis test with Mann-Whitney U test for DNA and GAG in digested explants. $^{a}p < 0.05$ vs control, $^{b}p < 0.05$ vs PL, $^{c}p < 0.05$ vs $pEVs_{-LD}$, $^{d}p < 0.05$ vs $pEVs_{-HD}$. C, control.

References

1. **Antich-Rosselló M, Munar-Bestard M, Forteza-Genestra MA, et al.** Evaluation of platelet-derived extracellular vesicles in gingival fibroblasts and keratinocytes for periodontal applications. *Int J Mol Sci*. 2022;23(14):7668.