Small Extracellular Vesicles From Platelet-Rich and Platelet-Poor Plasma Are Primarily From Platelets and Protect Synoviocytes From IL-1β–Induced Inflammation

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Takeaway: sEVs in Arthrex PRP and PPP can protect synoviocytes from inflammation in vitro by targeting metabolic and inflammatory pathways via their miRNA cargo.

OBJECTIVE

The objective of this study was to characterize small extracellular vesicles (sEVs) from platelet-rich plasma (PRP) and platelet-poor plasma (PPP) by using known sEV markers, quantify sEV miRNA content, and evaluate sEV bioactivity via an in vitro IL-1β–induced synovitis model.

METHODS AND MATERIALS

Blood from 6 healthy donors was processed according to manufacturer instructions by either ACP Max™ or Angel® system to obtain leukocyte-poor PRP (LP-PRP), leukocyte-rich PRP (LR-PRP), LP-PPP, and LR-PPP. sEVs were isolated from all 4 biofluids by precipitation or tangential flow methods. The size and concentration of sEVs were quantified, and their cellular origin was determined via multiplex analysis. The cellular pathways affected by the sEV miRNA cargo were identified through small RNA library preparation (TruSeq, Illumina) and single-end sequencing of 50 base pairs following manufacturer's protocol (HiSeq 2500, Illumina). The effect of LP-PRP, PPP, and their plasma-derived sEVs on synoviocyte proliferation and response to inflammation was determined in an IL-1β–induced synovitis model. sEVs were also fluorescently stained for CD9, CD63, and CD81, and incorporation by cells was visualized through fluorescent imaging.

RESULTS

sEV origin

In addition to CD29 and exosome markers CD9, CD63, and CD81, all groups showed an increase in leukocyte-derived CD8, CD14, and HLA-DRDPDQ, and a high expression of platelet-derived CD41b, CD42a, and CD62P.

sEV targets

A total of 653 unique miRNAs were identified for all biofluids, with 346 miRNAs conserved among all. The most commonly targeted pathways of note were involved in metabolism, cell survival (PI3K-AKT), and inflammation (JAK-STAT).

sEV Bioactivity and Synoviocyte Incorporation

Synoviocytes treated with PPP and its sEVs show greater sEV incorporation than PRP and PRP-derived sEVs, with all 4 conditions showing sEV incorporation vs control (Figure 2). Cell proliferation in IL-1β–treated cells was comparable between the positive control and biofluid- or sEV-isolated groups. Supernatant without sEVs resulted in significantly decreased cell proliferation compared to positive control.

Figure 1. Mean concentration of sEVs in all 4 biofluids. No difference in the particle concentration (P = .331).

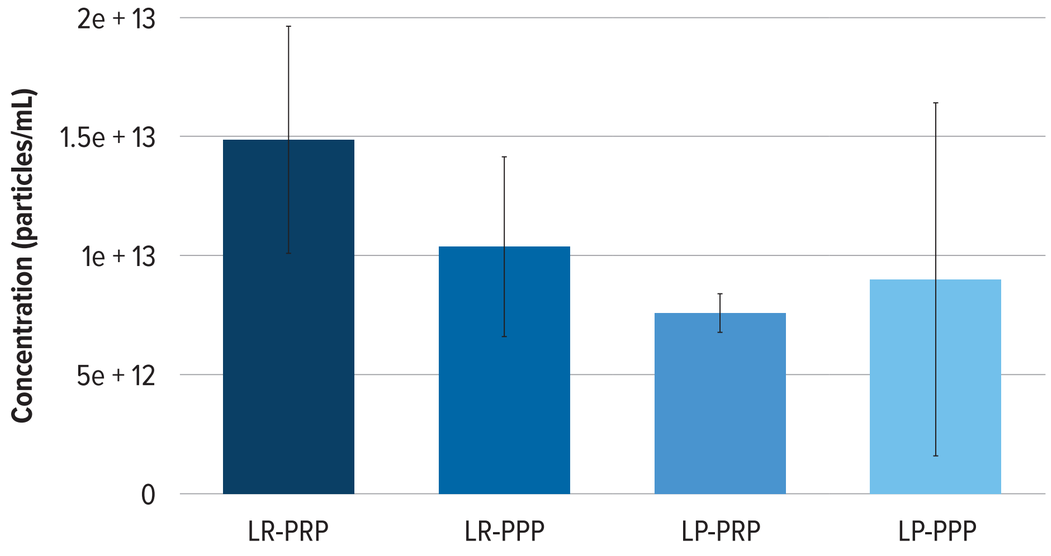
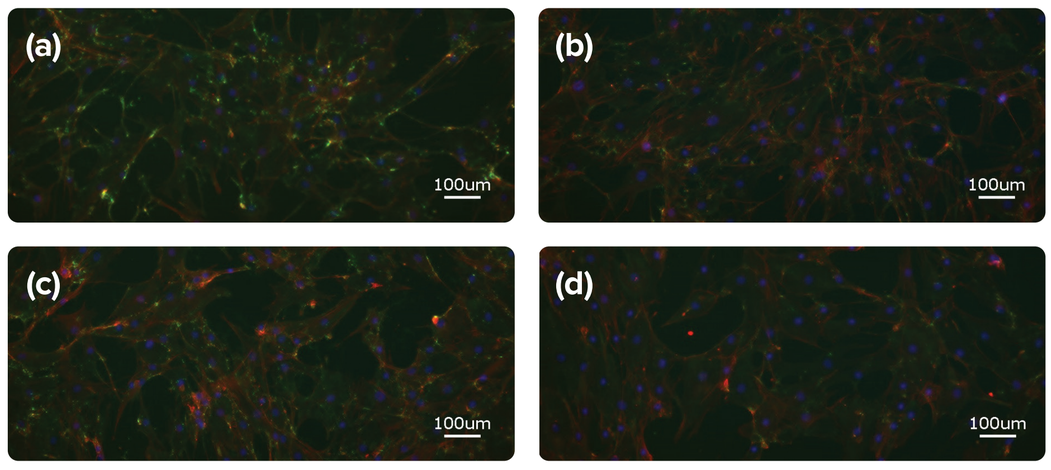


Figure 2. Fluorescent imaging of sEVs (green) incorporation into synoviocytes. sEVs uptake can be seen in all PRP and PPP groups. PPP **(a)** and PPP sEVs **(c)** show bright staining, while PRP **(b)** and PRP EVs **(d)** show dimmer staining, but demonstrated incorporation into synoviocytes. Red = actin, blue = cell nucleus.



CONCLUSION

Most sEVs were derived from platelets, with a small subset from white blood cells. miRNA within the sEV affected various cell signaling pathways associated with inflammation, and plasma sEVs were crucial in protecting synoviocytes from damage due to inflammation.

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