Extracellular vesicles derived from microfragmented adipose tissue modulate osteoclast differentiation and may enhance fat tissue transplant efficacy in osteoarthritis

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INTRODUCTION

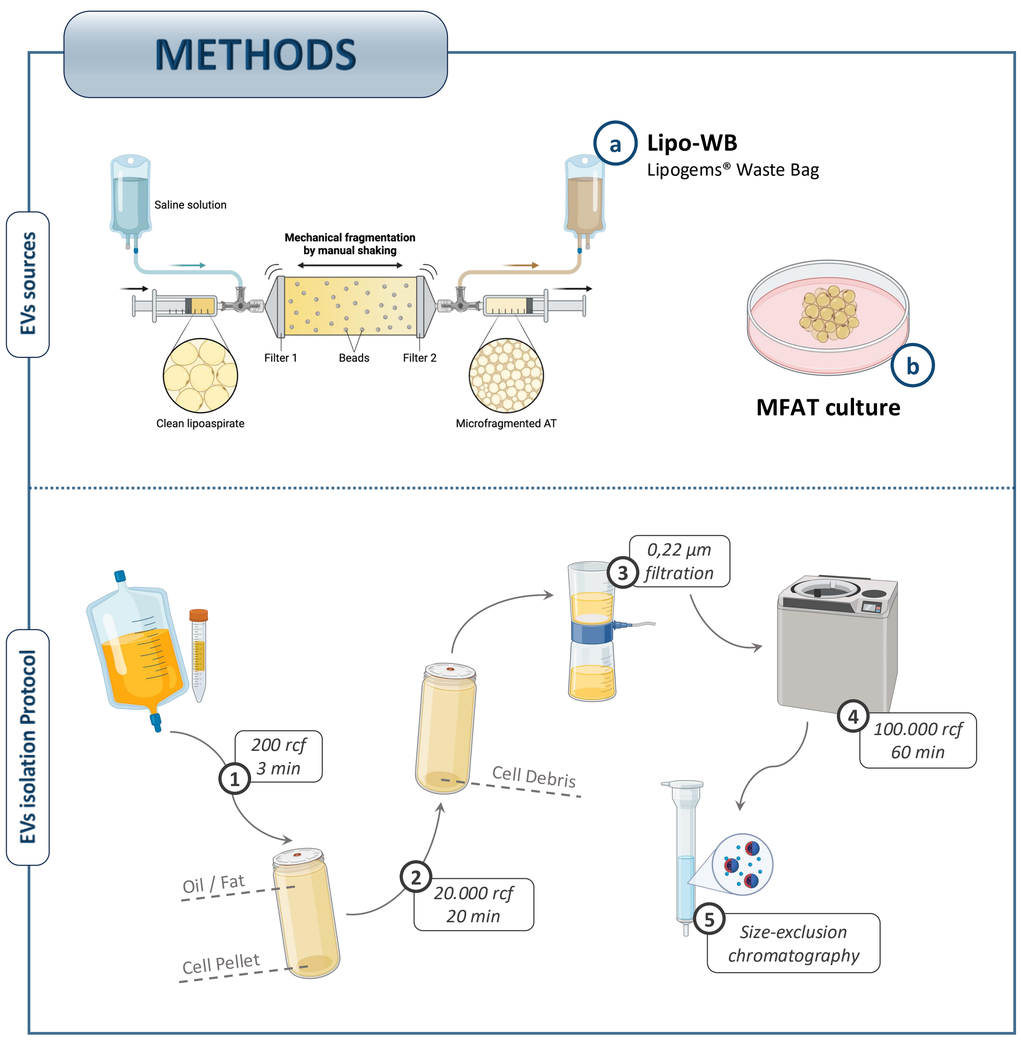
Extracellular vesicles (EVs) are emerging as key mediators of cell–cell communication, with increasing evidence supporting their anti-inflammatory and pro-regenerative roles in musculoskeletal disorders such as osteoarthritis (OA). To date, most EV-based approaches rely on vesicles derived from mesenchymal stem cell (MSC) cultures. However, their clinical translation is limited by low yield, batch variability, and scalability issues.

Micro-fragmented adipose tissue (MFAT), already widely used in intra-articular regenerative therapies, represents a promising and clinically relevant alternative source of EVs. Notably, MFAT spontaneously releases EVs, which may significantly contribute to its therapeutic effects.

This study aims to:

- Identify MFAT as a novel, clinically relevant source of EVs enabling rapid and direct isolation;
- Evaluate the effects of MFAT-derived EVs on osteoclast (OCs) differentiation, a key process in subchondral bone remodeling and osteoarthritis progression.

METHODS



RESULTS

Characterization

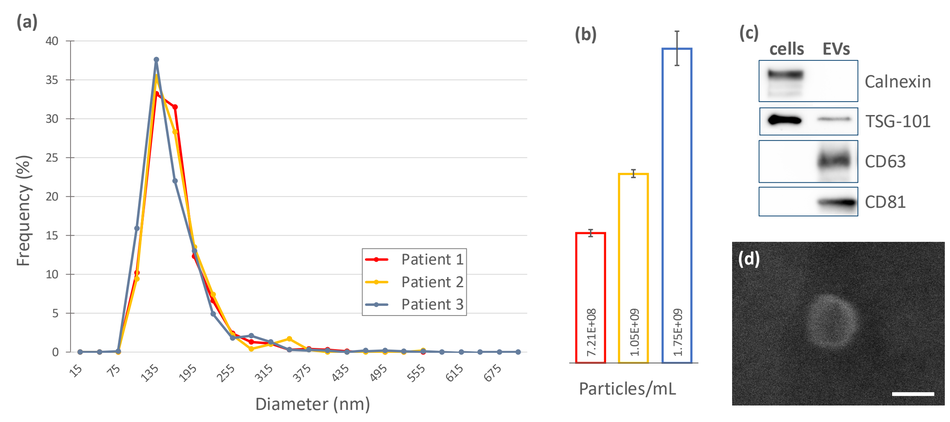


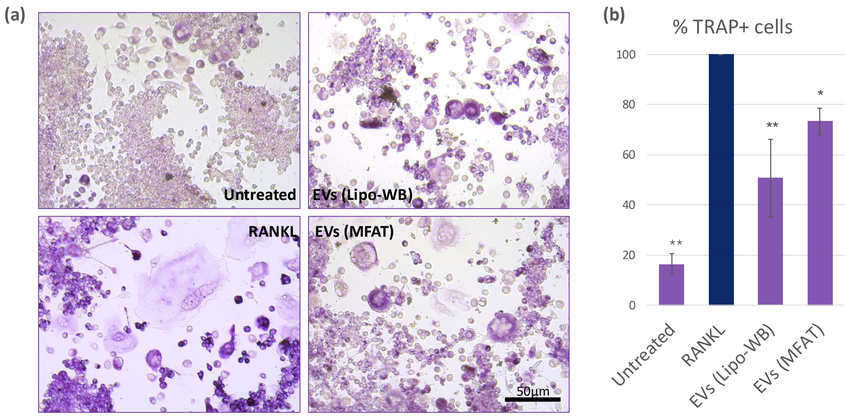
Figure 1. Biophysical and biochemical characterization confirms the EV nature of Lipo-WB particles.

(a) EVs particle size distribution measured by NTA analysis; **(b)** EVs yield expressed as part/mL; **(c)** Western blot analysis showing intracellular (Calnexin) and EVs markers (TSG101, CD63, CD81) in cells and EVs; **(d)** Representative SEM image of EVs (scale bar = 100nm).

Effects on OCs differentiation

Figure 2. EVs inhibit RANKL-induced osteoclast differentiation as assessed by TRAP assay.

(a) Representative images of TRAP staining (tartrate-resistant acid phosphatase) at 20× magnification. **(b)** Quantification of TRAP-positive OCs, defined as cells containing ≥3 nuclei. Data are presented as mean ± SD. Statistical analysis was performed using one-way ANOVA followed by Sidak post-hoc test (*p < 0.05, **p < 0.01 vs. RANKL).



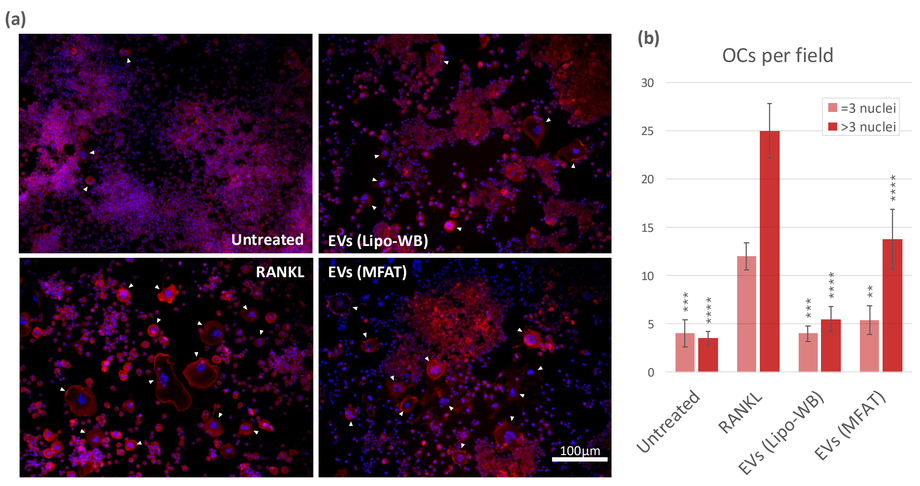


Figure 3. EVs reduce osteoclast maturation and multinucleation as shown by Phalloidin/DAPI staining.

(a) Representative fluorescent images of mature osteoclasts (red:phalloidin; blue:DAPI). **(b)** Quantification of osteoclasts per field (10×), categorized as cells with 3 nuclei (=3) or more than 3 nuclei (>3). Data are presented as mean ± SD. Statistical analysis was performed using two-way ANOVA followed by Tukey's post-hoc test (**p<0.01, ***p< 0.001, ****p < 0.0001 vs. RANKL).

CONCLUSION

Overall, we demonstrate that Lipogems-derived waste bag (Lipo-WB) and MFAT represent novel sources of EVs. In particular, **Lipo-WB is a clinically relevant source**, **enabling rapid and direct EV isolation in a surgical setting**, with yields reaching up to 10^9 EVs/mL. Importantly, EVs derived from these sources exhibit a significant inhibitory effect on osteoclast differentiation, suggesting a potential role in modulating subchondral bone remodeling in osteoarthritis.

These findings support the hypothesis that EVs contribute to the therapeutic efficacy of MFAT-based treatments and highlight their potential for the development of EV-based, cell-free regenerative strategies for OA, including both autologous and allogeneic approaches.

Acknowledgments:
This work was partially funded by the PRIN 2022 program (Grant No. 2022HB7PNP) and by PNRR-M4C2 (Investment 3.3).
Lipogems® MFAT and waste bags were provided by IMAGE Regenerative Clinic (Milan) with informed consent obtained from participants, in accordance with the Council of Europe Recommendation CM/Rec(2006) on research.

DISCUSSION

We established an efficient and reproducible protocol for EV isolation starting from two distinct biological sources, Lipo-WB and MFAT-conditioned medium.

NTA analysis confirmed the reproducibility of the method across donors, with EVs ranging from 75 to 255 nm (mean: 160–170 nm) (Fig.1a) and yields above 7×10^8 EVs/mL (Fig.1b). Western blot and electron microscopy further validated their EV identity, showing expression of canonical markers (CD63, CD81, TSG101) and absence of calnexin (Fig.1c), together with a typical spherical morphology and size around 100 nm (Fig.1d).

Functionally, EVs inhibited RAW 264.7 differentiation into osteoclasts.
Co-treatment with RANKL and EVs significantly reduced the number of TRAP+ multinucleated cells (≥3 nuclei) compared to RANKL alone (Fig.2a–b). Lipo-WB-derived EVs showed a stronger inhibitory effect ($p<0.01$).

These results were confirmed by Phalloidin/DAPI staining (Fig.3a), which better highlights osteoclast structure and actin ring organization. Notably, EV treatment reduced larger, highly multinucleated osteoclasts, typically associated with increased osteolytic activity (Fig.3b).
Overall, these findings suggest that EVs from Lipo-WB and MFAT may contribute to modulating osteoclastogenesis, supporting their potential role in OA-related bone remodeling.