Efficient Loading of MSC-EVs into Platelet Gels

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INTRODUCTION: Mesenchymal stromal cell-derived extracellular vesicles (MSC-EVs) have shown strong regenerative potential and are under investigation in clinical trials for treating cartilage lesions. Conventional cartilage repair techniques often result in incomplete or suboptimal tissue regeneration, contributing to long-term joint degeneration. Despite the therapeutic promise of MSC-EVs, a major limitation lies in their rapid dispersion throughout the joint cavity, which reduces their effective concentration at the lesion site. To overcome this challenge, this study aimed to develop a biologically enhanced, platelet-derived fibrin gel capable of efficiently incorporating adipose-derived MSC-EVs (ASC-EVs) to improve targeted delivery.

METHODS: ASC-EVs were characterized by flow cytometry, NTA and TEM. ASC-EVs fingerprint was evaluated by qRT-PCR for embedded miRNAs. ASC-EVs were incorporated into platelet gels (clots) using a modified one-step protocol adapted from standard clinical procedures for PRP preparation. Incorporation efficiency, release kinetics and uptake by osteoarthritic (OA) chondrocytes and cartilage explants were evaluated using flow cytometry, microfluidic platforms, SEM and real-time multimodal nonlinear optics imaging. Functional effects on OA chondrocytes were assessed through qRT-PCR profiling. All human samples were obtained from surgical waste material and this study was approved by the Ethics Committee. Adipose: healthy donors (43 ± 15 yo, 3 females and 2 males); cartilage: OA (KL III–IV) patients (73 ± 11 yo, 2f/3m); PRP: patients undergoing regenerative procedures (55 ± 11 yo, 6f/16m). Parametric or non-parametric tests were applied based on data normality. Significance was set at $p \leq 0.05$.

RESULTS: ASCs were characterized as highly positive for mesenchymal markers CD73 (100 ± 0%), CD90 (98 ± 1%), and moderately positive for CD105 (14 ± 6%), with minimal hematopoietic contamination (CD45: 2 ± 1%) (n=5). Cell viability was 92 ± 3%. EVs isolated from ASCs had consistent size profiles among donors, with a mean diameter of 182 ± 26 nm and mode size of 117 ± 12 nm (n=5). EVs expressed typical markers CD63 and CD81 at 100%, and CD9 at 42 ± 9% (n=5). Mesenchymal markers CD29, CD44, CD49e, and CD105 were also detected at moderate levels (15 to 30%). Electron microscopy confirmed the characteristic cup-shaped morphology of EVs. Analysis of miRNA cargo in pooled EVs identified 364 miRNAs in total, with the 20 most abundant representing 81% of the genetic message; notably, miRNAs with protective functions for cartilage and anti-inflammatory macrophage polarization predominated, suggesting a therapeutic potential for cartilage protection and inflammation resolution. PRP gels incorporated 60 ± 8% of initially added EVs (10×10^9 per 1 ml PRP, n=6), equating to $64\times10^6 \pm 27$ EVs per mg of gel. EV release was sustained over four weeks, with an initial 5 ± 2% released in 24 hours, followed by gradual release totaling 20% by week 4. Released EVs maintained effective uptake by both *ex-vivo* cartilage explants (Figure 1A) and osteoarthritic chondrocytes in 2D and 3D microfluidic cultures, with no significant difference compared to purified EVs (MFI: 17 ± 1 vs. 17 ± 3; p=0.96; n=3). Functional assays showed that while PRP gel factors alone induced pro-inflammatory *IL8* expression, EVs (both free and gel-released) mitigated this effect and further reduced matrix-degrading enzyme *MMP3* expression, with EV-loaded gels exhibiting additive protective effects (*vs* OA chondrocytes: gels 0.44 ± 0.14, EVs 0.76 ± 0.16 and EV-gels 0.20 ± 0.08; p<0.0001 for EV-gels *vs* EVs, n=5) (Figure 1B).

DISCUSSION: This study demonstrates that a clinically translatable, EV-loaded platelet gel can be produced with high efficiency and sustained release capability. The enhanced bioactivity observed supports its use as a local delivery system to modulate chondrocyte homeostasis and improve the regenerative microenvironment. Limitations include *in vitro* and *ex vivo* assessment, warranting *in vivo* validation that is in progress using clinical grade manufactured EV-gels in a pig model of cartilage damage and is expected to end by 2026.

SIGNIFICANCE: This work provides a clinically applicable strategy to enhance MSC-EV retention and activity at cartilage lesion sites, addressing a key translational barrier in regenerative joint therapies and supporting the development of next-generation biologically augmented scaffolds for osteoarthritis treatment.

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IMAGE: Figure 1. Released EVs uptake and functional effects. A) 3D imaging reveals ECM structure (red, CARS) and EV distribution (blue, TPEF) in cartilage treated with platelet gels with EVs (clot+EV). Spatial references are provided via micrometer-scale measurements. B) *MMP3* expression modulation in inflamed (IL1B) chondrocytes exposed to EVs, to platelet gel release (clot), and to the release of combined platelet gel + EVs (clot+EV). Data show fold changes (mean ± SD, n = 5). ** p<0.01; *** p<0.001; **** p<0.0001.