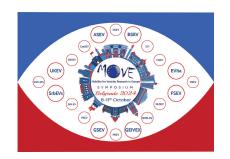


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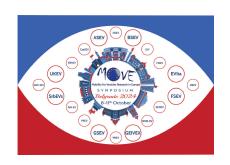








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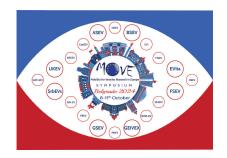


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Collagen Hydrogel Embedded SHED-EVs for Enhanced Osteogenesis

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Introduction: Mesenchymal stem/stromal cells (MSCs) from the dental pulp of human exfoliated deciduous teeth (SHED cells) have shown promising results in bone tissue regeneration. The application of SHED-derived extracellular vesicles (EVs) to bone defects can be achieved using biomaterial scaffolds. Collagen scaffolds, which are natural polymers with slow degradation times, are particularly suitable for EVs entrapment. This study aimed to examine the release rate of embedded SHED-EVs from collagen scaffolds and to evaluate their osteogenic capacity when combined with collagen and gradually released.

Methods: SHED-EVs were isolated using differential ultracentrifugation and characterized using nanotracking particle analysis (NTA), Western Blot (WB), and scanning electron microscopy (SEM). The SHED-EVs were labeled with PKH67 fluorescent lipophilic dye and embedded in a collagen hydrogel matrix (0.3% collagen in PBS). The cumulative release of the fluorescently labeled EVs was monitored for 35 days using NTA. The osteogenic potential of the collagen scaffolds with EVs was assessed by analyzing the relative expression of key osteogenic genes in treated SHED cells using RT-PCR.

Results: The isolated SHED-EVs exhibited a uniform size distribution, as confirmed by NTA and SEM analyses, and were positive for CD63 as shown by WB analysis. The release of EVs from the collagen matrix was gradual, with half of the entrapped EVs being released within the first 10 days and an additional 10% released over the subsequent 10 days, followed by a plateau phase. The SHED-EVs embedded within the collagen hydrogel influenced the expression of osteogenic genes.

Conclusion: The use of a collagen matrix for embedding and gradually releasing SHED-EVs provides a promising strategy for enhancing the osteogenic potential of these vesicles in regenerative therapies.

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