Osteogenic Differentiation Potential of Gingival Mesenchymal Stem Dell-Derived Extracellular Vesicles

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Abstract

Objective:

GMSC-derived EVs are easily accessible in the oral environment and can be found in biological discarded tissue samples. The purpose of this study was to evaluate the osteogenic potential of gingival mesenchymal stem cell (GMSC)-derived extracellular vesicles (EVs).

Results

Our results confirmed successful formation of GMSC-derived EVs with average 110 nm in diameter. Moreover, western blotting analysis confirmed the specificity of our extracted GMSC-derived EVs. Our results revealed that after 4 weeks of differentiation in vitro, GMSC derived EVs exhibited positive xylenol orange staining and high levels of mRNA expression for osteogenic-related gene markers.

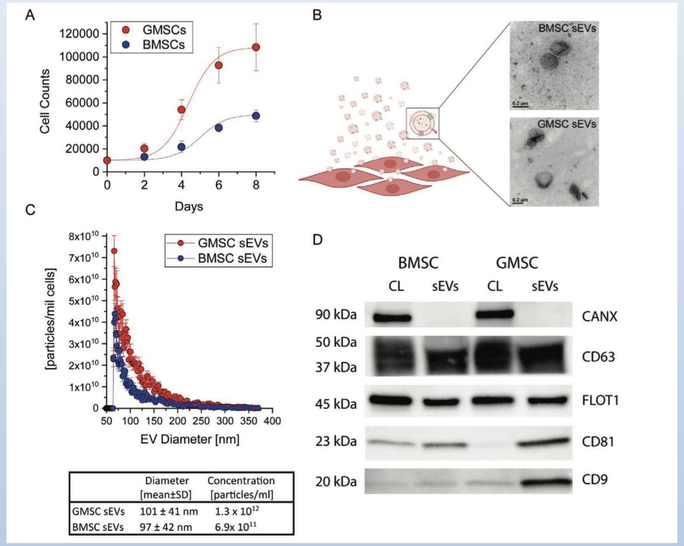
Conclusion

GMSC derived EVs can be produced in large quantities and have enhanced osteogenic differentiation properties, making them a great candidate for craniofacial tissue regeneration applications.

Materials and Methods

- Human gingival mesenchymal stem cell (GMSC) were extracted according to the methods in the literature.
- Extracellular vesicles were extracted using differential ultracentrifugation and analyzed using transmission electron microscopy (TEM) and western blot.
- GMSC-derived EVs were encapsulated in alginate-based hydrogel delivery vehicle and cultured in osteogenic media for 4 weeks.
- The osteogenic differentiation potential of encapsulated GMSC-derived EVs were evaluated using xylenol orange staining and PCR.

Results



	Diameter [mean±SD]	Concentration [particles/ml]
GMSC sEVs	101 ± 41 nm	1.3×10^{12}
BMSC sEVs	97 ± 42 nm	6.9×10^{11}

Fig. 1 – Comparison and characterization of BMSC and GMSC sEVs. A) Growth of BMSCs and GMSCs in medium containing EV-depleted FBS. B) TEM images of BMSC and GMSC sEVs. C) Comparison of size and concentration of BMSC and GMSC sEVs. D) Western blotting analysis of sEVs. All data are presented as mean ± standard deviation (SD) (n = 5).

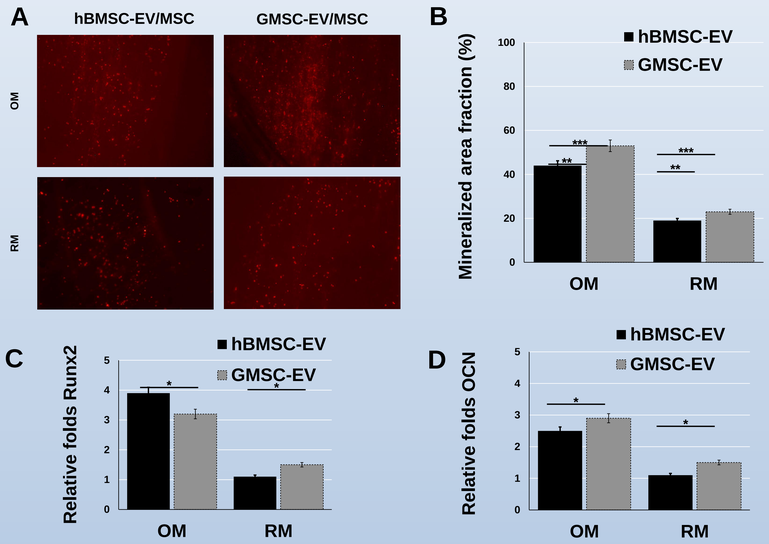


Fig. 2 – In vitro osteogenesis assay. PCR-based expression levels of (A) Runx 2 and (B) OCN. (C) Histochemical staining analysis of mineralization. Mineralization analysis of the hBMMSCs-EV and hGMSCs-EV after four weeks of culturing in osteogenic medium using xylenol orange. (D) Quantification of staining intensity of xylenol orange.

Conclusion

Altogether, the data obtained in the current study shows that GMSC derived EVs are a promising source for stem cell banking and have enhanced osteogenic differentiation properties, making them a great candidate for bone tissue engineering applications.

References

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