Human Breast Milk-Derived Extracellular Vesicles Enhance Osteoblast Activation via BMP2/MAPK Signaling Pathways

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Background

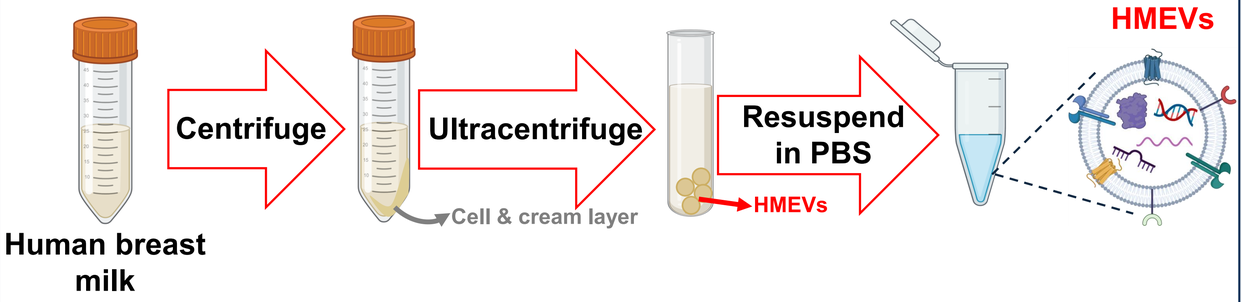
- **Human breast milk** contains diverse **bioactive components** that contribute to **immune regulation, tissue development, and physiological homeostasis.**
- **Human milk-derived extracellular vesicles (EVs)** are nanosized vesicles that carry **proteins and miRNAs** capable of modulating **cellular functions.**
- However, despite their biological relevance, the role of human milk-derived EVs in **osteoblast activation and bone formation remains unclear.**

Objective

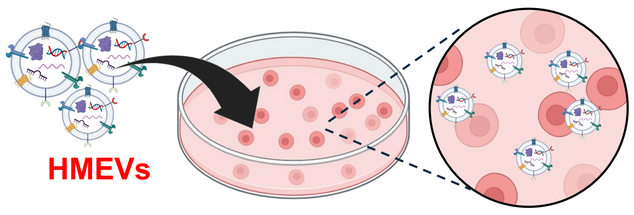
- **To characterize human breast milk-derived extracellular vesicles (HMEVs).**
- **To compare molecular profiles between human and bovine milk EVs.**
- **To evaluate the effects of HMEVs on osteoblast differentiation, mineralization, and bone formation-related marker expression.**
- **To investigate whether BMP2/SMAD and MAPK-related signaling pathways are involved in HMEV-mediated osteoblast activation.**

Methods

HMEVs Isolation



HMEVs treatment in MC3T3-E1 cells



HMEVs were isolated by ultracentrifugation and administered to osteoblasts to evaluate cell proliferation, osteogenic differentiation, mineralization, and the involvement of BMP2/MAPK signaling pathways.

Result 1

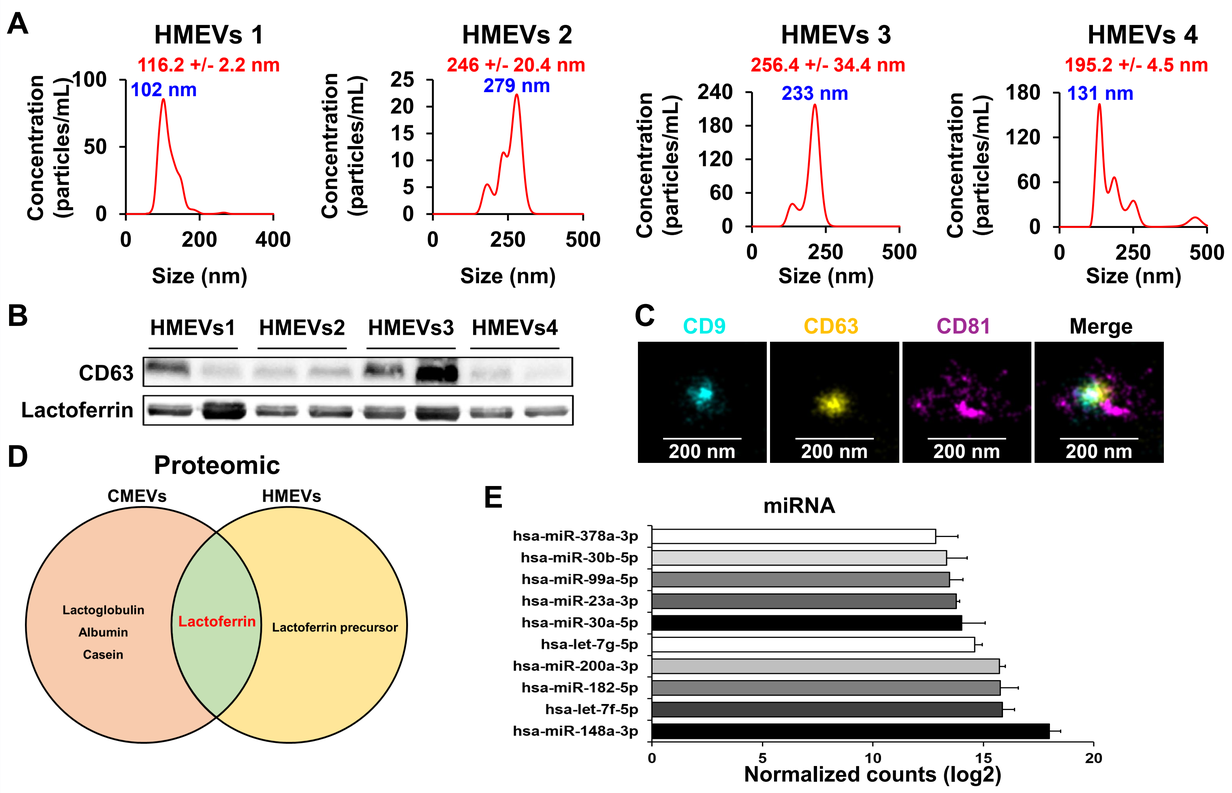


Fig 1. Characterization of HMEVs. (A) Particle size distribution of HMEVs measured using NanoSight NS300. The average particle size and size distribution of HMEVs were analyzed.(B) Western blot analysis of exosome markers (CD9, CD63, and CD81) and lactoferrin in HMEVs (n = 2).(C) Representative super-resolution microscopy images demonstrating the expression of exosome markers CD9, CD63, and CD81 in HMEVs.

Result 2

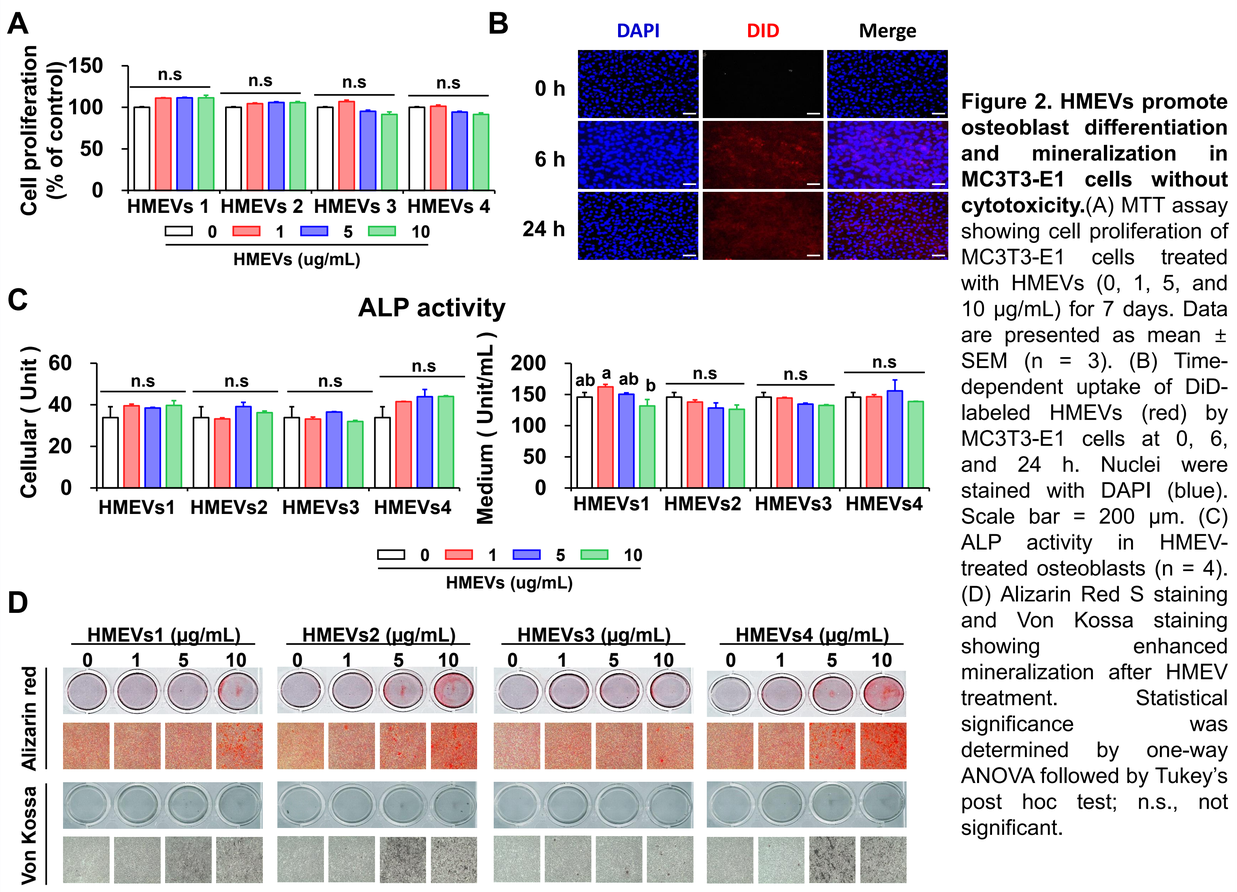


Figure 2. HMEVs promote osteoblast differentiation and mineralization in MC3T3-E1 cells without cytotoxicity.(A) MTT assay showing cell proliferation of MC3T3-E1 cells treated with HMEVs (0, 1, 5, and 10 μg/mL) for 7 days. Data are presented as mean ± SEM (n = 3). (B) Time-dependent uptake of DiD-labeled HMEVs (red) by MC3T3-E1 cells at 0, 6, and 24 h. Nuclei were stained with DAPI (blue). Scale bar = 200 μm. (C) ALP activity in HMEV-treated osteoblasts (n = 4). (D) Alizarin Red S staining and Von Kossa staining showing enhanced mineralization after HMEV treatment. Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test; n.s., not significant.

Result 3

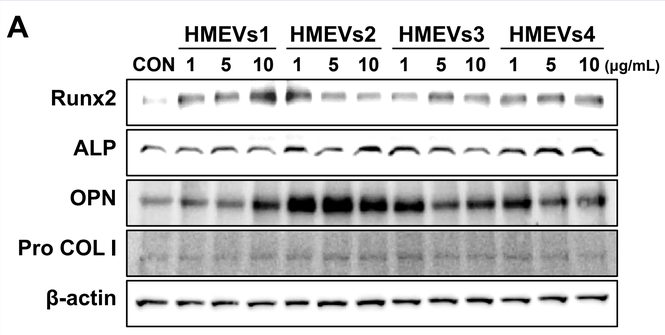


Figure 3. Western blot and qRT-PCR analysis of bone-related markers in HMEV-treated MC3T3-E1 cells. (A) Representative western blot bands of bone-related proteins in MC3T3-E1 cells treated with HMEVs. (BRelative mRNA expression levels of bone-related genes measured by qRT-PCR in HMEV-treated MC3T3-E1 cells. Data are presented as mean ± SEM (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Differences with $p < 0.05$ were considered statistically significant; non-significant results are indicated as "n.s."

Result 4

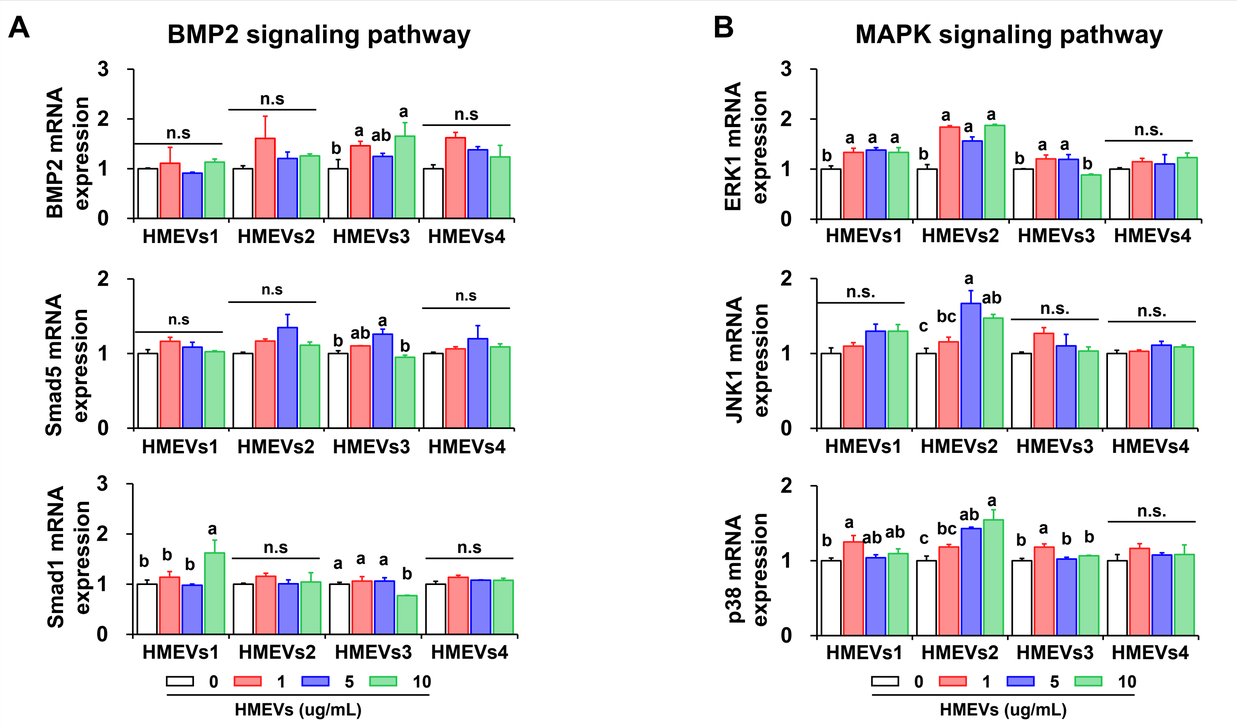


Figure 4. Gene expression analysis of BMP2 and MAPK signaling pathways in HMEV-treated MC3T3-E1 cells. (A) Relative mRNA expression levels of BMP2 signaling pathway-related genes. (B) Relative mRNA expression levels of MAPK signaling pathway-related genes. Data are presented as mean ± SEM (n = 3). $p < 0.05$ was considered statistically significant; n.s., not significant.

Conclusion

HMEVs were characterized, showed distinct molecular profiles from bovine milk EVs, and were internalized by MC3T3-E1 cells without cytotoxicity. HMEVs promoted osteoblast differentiation and mineralization, potentially through BMP2/SMAD and MAPK-related signaling, suggesting their potential as bioactive materials for bone health research.

Significance

- **Demonstrates the osteogenic potential of human breast milk-derived EVs.**
- **Suggests HMEVs as bioactive vesicle-based materials for bone health research.**

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References

(1) Kwon JH et al. J Korean Soc Food Sci Nutr. 2022:1158-1165.
(2) Kwon JH et al. J Med Food. 2025:1047-1059.