An innovative approach to neuro-bone engineering: role of microglia-derived extracellular vesicles on osteoblastic differentiation within stem cell-loaded composite bioinks

Bengi Lina COŞKUN[1,2], Zehra Gül MORÇIMEN-GULICLI[1], Zalike KESKIN-ERDOĞAN[1,2], Aylin ŞENDEMIR[1,2]

[1]Ege University, Faculty of Engineering, Department of Bioengineering, Izmir, Türkiye

[2]Ege University, Institute of Natural and Applied Sciences, Department of Bioengineering, Izmir, Türkiye

[3]Ege University, Institute of Natural and Applied Sciences, Department of Biomedical Technologies, Izmir, Türkiye

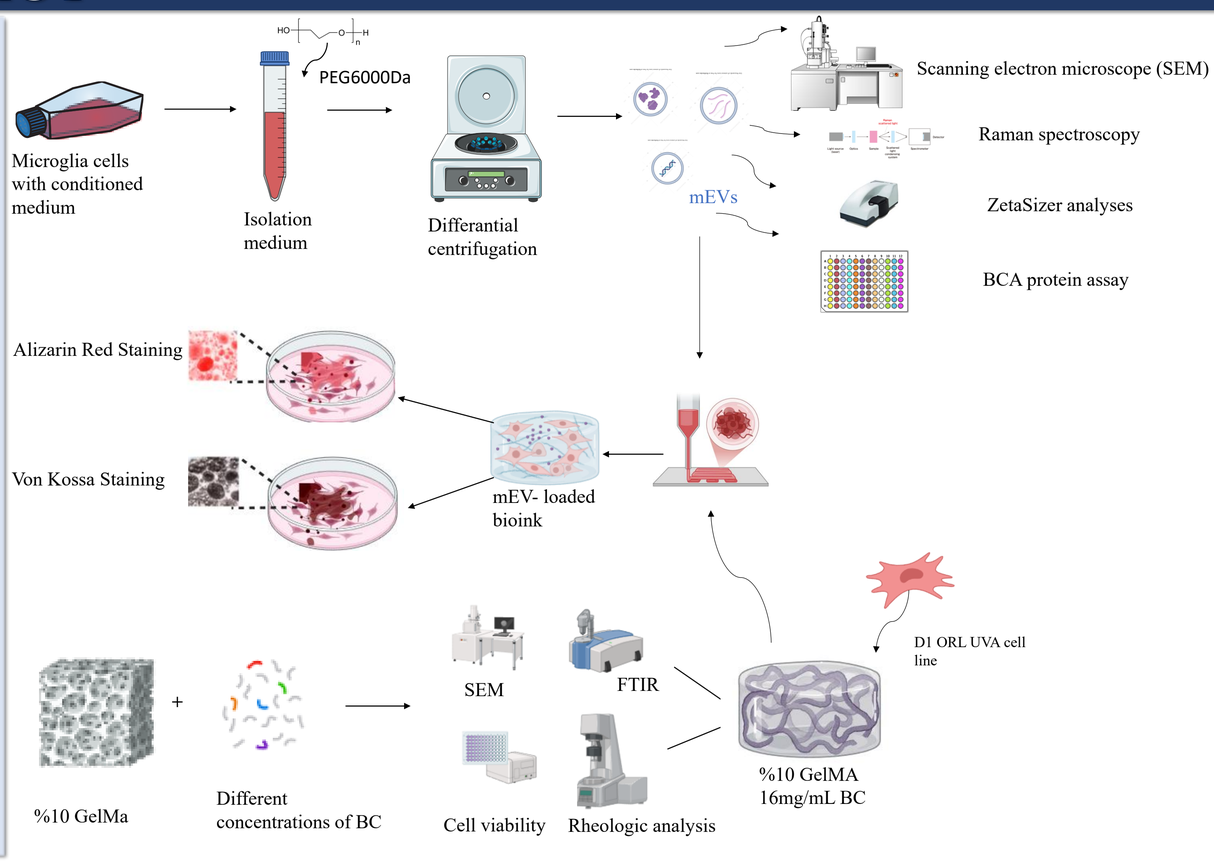
EBIPHASE — EGE BIOMATERIALS AND 3D BIOINTERPHASES RESEARCH GROUP

ABSTRACT

Neuro-bone interactions involve complex communication between the nervous and skeletal systems, including CNS activities and hormonal pathways that regulate bone physiology (Sun et al., 2023). Microglia, CNS immune cells, indirectly influence this process by releasing immune mediators and growth factors such as BDNF and GDNF, which contribute to bone development and repair (Wei et al., 2025). Additionally, extracellular vesicles (EVs) derived from neural tissues can directly promote osteogenesis by transferring bioactive molecules such as microRNAs to bone cells (Xia et al., 2021). However, the role of microglia-derived EVs (mEVs) in bone tissue engineering remains largely unexplored.

In this study, a gelatin methacryloyl (GelMA)/bacterial cellulose (BC) composite hydrogel was developed as a biomimetic scaffold and loaded with mEVs to evaluate their osteogenic potential. GelMA offers excellent biocompatibility and supports cell adhesion and tissue remodeling, while BC provides the mechanical reinforcement and ECM-like nanofiber architecture that GelMA alone lacks. The hydrogel was characterized for its chemical and rheological properties, and mEVs were analyzed for size distribution and protein content. Bone marrow-derived stem cell line (D1-ORL-UVA) was cultured within the scaffold, and cell viability and osteogenic differentiation were assessed over time.

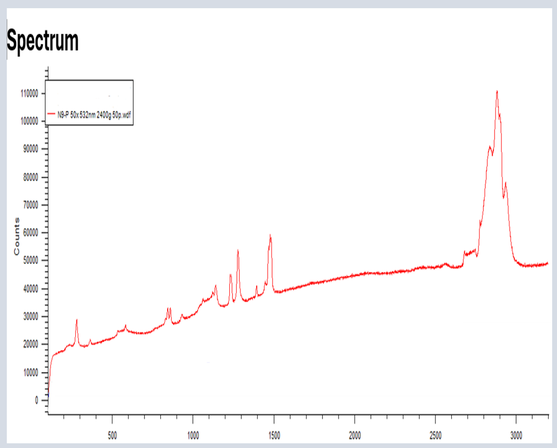
The mEV-loaded hydrogels significantly enhanced osteogenic differentiation in a dose-dependent manner, with early mineralization observed at day 7 and progressive calcium-phosphate matrix formation at later time points. These findings suggest that incorporating mEVs into GelMA/BC composite hydrogels represents a promising strategy for advancing bone regeneration.



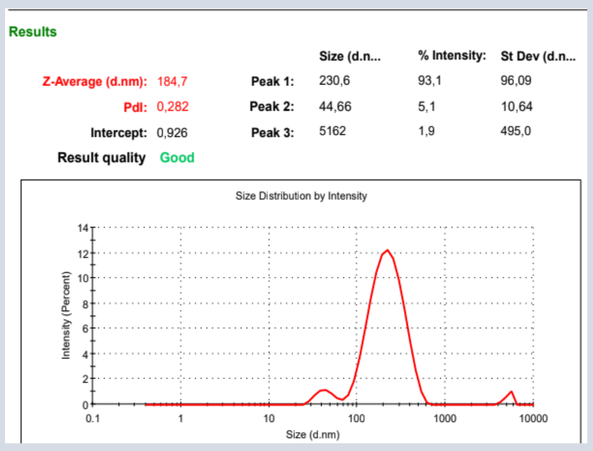
RESULTS

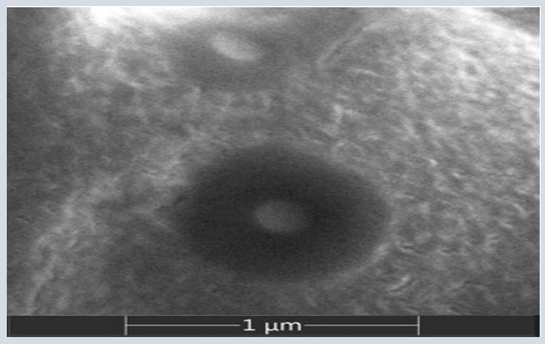
EVs Characterization

Raman Spectroscopy Analysis



mEV Size Distribution (Zeta)





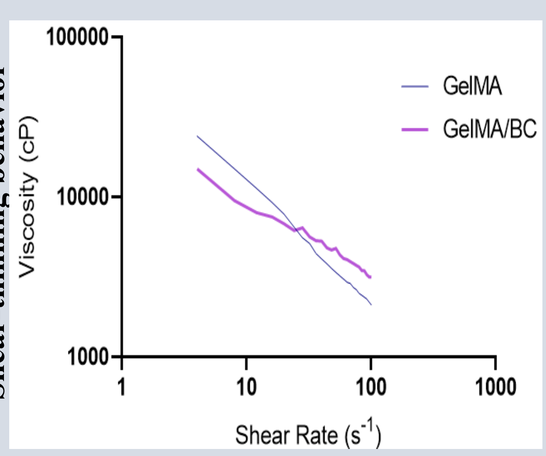
Nanostructural Architecture of EVs (SEM)

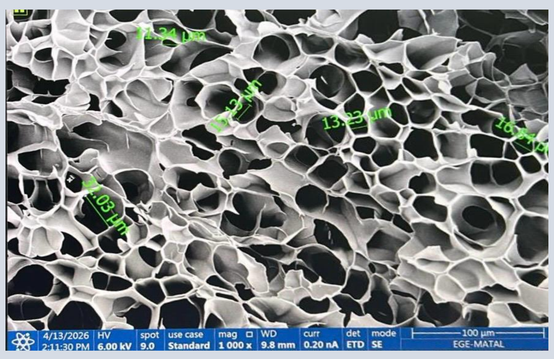
- **mEVs**: ~100–120 nm, PDI 0.28, 256 µg/mL protein; Raman showed lipid-rich membrane (CH stretching ~2800–3000 cm⁻¹)
- **Bioink:** Shear-thinning, printable
- **GelMA/BC FTIR**: BC incorporation confirmed.
- **Viability:** ↑ (mEV-30, Day 21, $p < 0.0001$)

Osteogenesis: ↑ mineralization & differentiation

Hydrogel Characterization

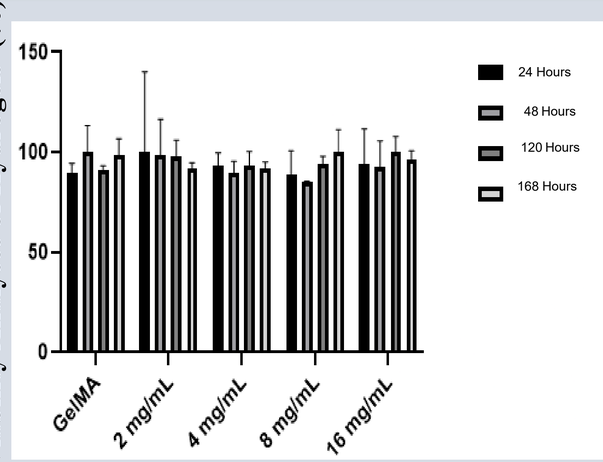
Shear-thinning behavior

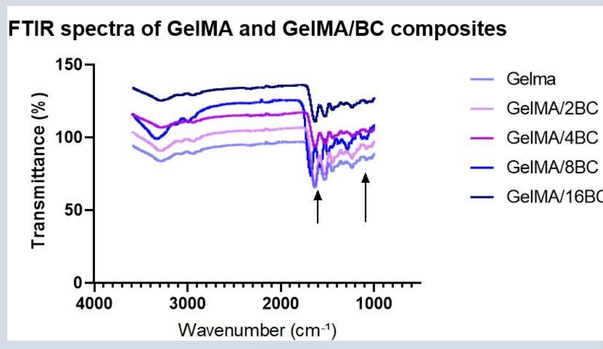




Hydrogel Porosity Structure (SEM)

Viability Analyses of Hydrogels (%)

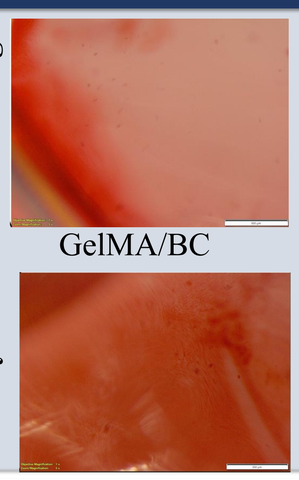




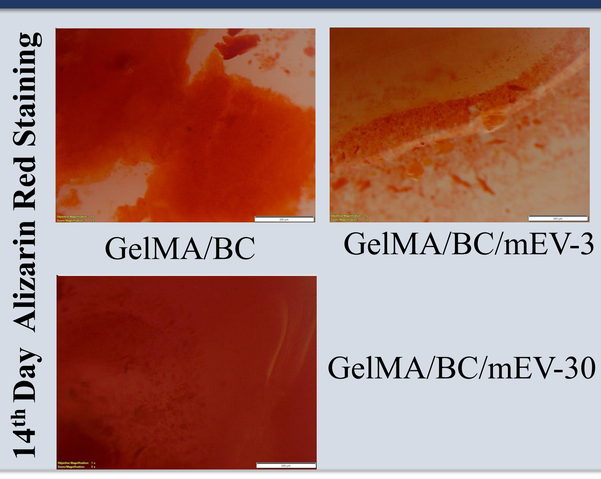
FTIR

Bone Differentiation

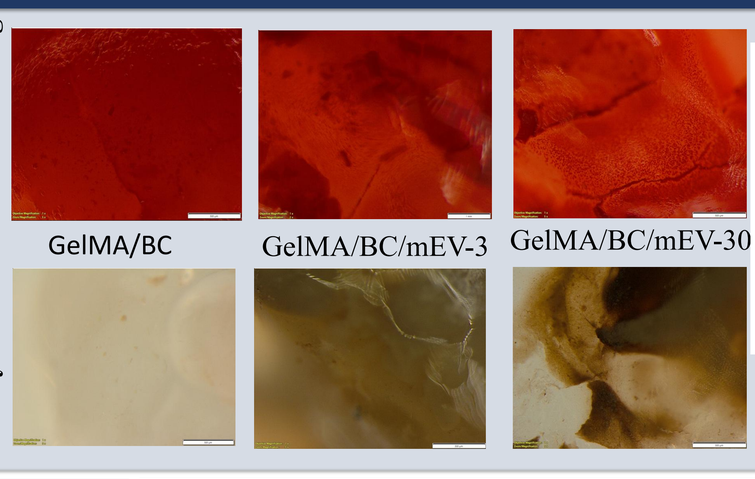
7th Day Alizarin Red Staining

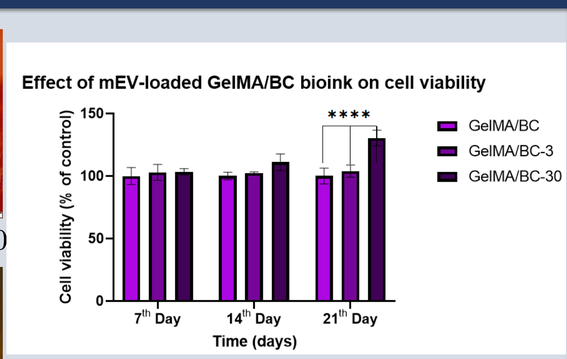


14th Day Alizarin Red Staining



21th Day ALZR/ Von Kossa Staining





DISCUSSION & CONCLUSIONS

This study demonstrates that microglia-derived extracellular vesicle (mEV)-loaded GelMA/BC hydrogels significantly enhance osteogenic differentiation in a dose-dependent manner. The physicochemical characterization confirmed the successful development of a printable and cytocompatible hydrogel system, while EV characterization indicated a relatively homogeneous vesicle population within the expected nanoscale range.

Mineral Staining showed that mEVs accelerate early mineralization and promote increased and more organized calcium-phosphate deposition over time. These findings suggest that mEVs contribute to osteogenesis through bioactive signaling rather than solely increasing cell proliferation. Our results are consistent with previous studies showing that extracellular vesicles derived from neural tissues can promote osteogenesis, further supporting the concept of neuro-bone crosstalk.

Notably, the integration of neuroimmune-derived EVs into a bone tissue engineering platform represents a relatively underexplored approach, highlighting the novelty of this study. However, this study has certain limitations. The molecular mechanisms underlying the observed effects were not directly investigated, and specific EV cargo components were not identified.

REFERENCES

- Sun, W., Ye, B., Chen, S. et al. Bone Res 11, 65 (2023).
- Wei, X., Li, M., You, J., Luo, J., Zhai, J., … Zhou, Y. (2025). International Journal of Nanomedicine, 20, 1263–1284
- Xia, W., Xie, J., Cai, Z. et al. Damaged brain accelerates bone healing by releasing small extracellular vesicles that target osteoprogenitors. Nat Commun 12, 6043 (2021). https://doi.org/10.1038/s41467-021-26302-y

ACKNOWLEDGEMENTS

We thank the Health Institutes of Turkey (TÜSEB) for their support of this research (Grant No. 42719). We also acknowledge Prof. Dr. Özlem Yeşil Çeliktaş for Zeta potential measurements, Dr. Seniha Morsümbül for providing access to rheological analysis facilities, and Prof. Dr. Oğuz Bayraktar for permission use light microscopy and lyophilization.