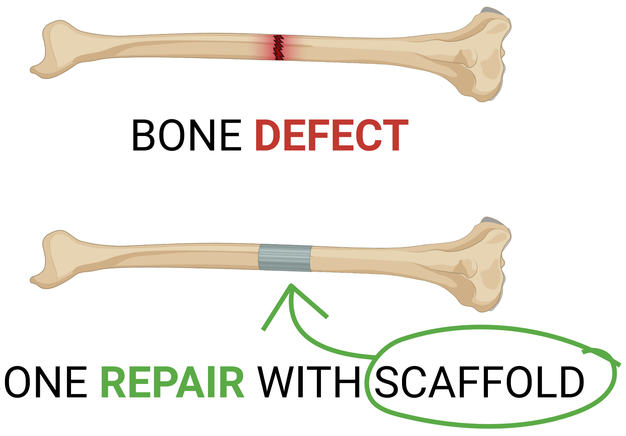
Engineering Bone: Hydroxyapatite-Enriched Nanofibrous Scaffolds Promote Stem Cell Osteogenesis



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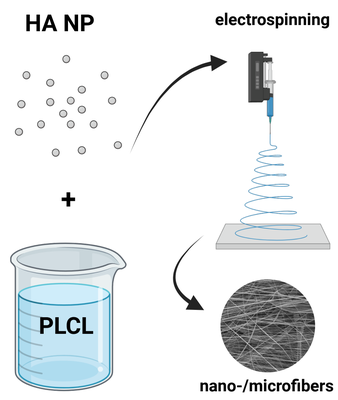
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INTRODUCTION



Electrospun nanofibrous scaffolds enriched with hydroxyapatite nanoparticles (HA NPs) provide a biomimetic environment favorable for bone regeneration. This study aimed to evaluate the physicochemical properties and osteoinductive potential of HA-functionalized electrospun poly(L-lactide-co-caprolactone) (PLCL) scaffolds seeded with bone marrow-derived mesenchymal stem cells (BM MSCs).

METHODS



Schematic illustration of electrospun PLCL and PLCL/HAp fiber scaffold preparation.

Fibrous PLCL (**P**) and PLCL scaffolds enriched with 7 % of HA NPs (**HAp**) were prepared by electrospinning. Scaffolds morphology, chemical composition, and thermal stability were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA). BM MSCs were seeded at 20,000 cells per scaffold for metabolic activity (MTS), dsDNA quantification, alkaline phosphatase (ALP) activity, and mineralization (DioC) assays over 35 days. For gene expression and immunostaining, 25,000 cells per specimen were analyzed for nine osteogenic markers: COL1, ALP, OCN, OPG, BMP, RUNX2, SP1, RANKL, SPP7, and BMP2. Confocal microscopy was used to detect osteocalcin protein.

RESULTS

Metabolic assays revealed differences between scaffolds and tissue culture plastic (TCP), with cell metabolic activity maintained throughout culture, showing a dip at day 21 followed by recovery—potentially due to transient overgrowth and reorganization on scaffolds. ALP activity was not enhanced by scaffolds compared to TCP; ALP gene expression was lowest on HA scaffolds, suggesting distinct osteogenic stages. Osteocalcin and osteopontin, increased on HA scaffolds, are linked to later matrix formation phases and confirmed by immunostaining. HA incorporation significantly enhanced early BMP2 expression and later OPN, OCN, and COL1 compared to PL-CL scaffolds and TCP controls.

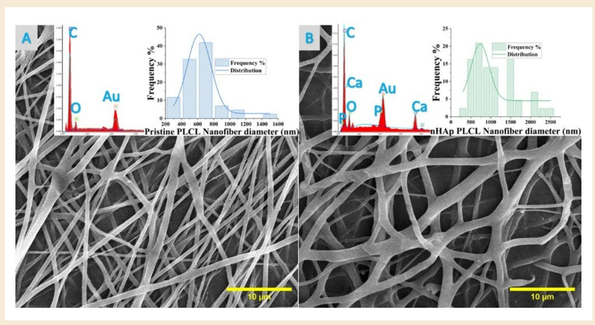


Figure 1. *SEM micrographs (scale bar = 10 µm) with corresponding EDS spectra and fiber diameter distribution histograms of electrospun scaffolds: (A) PLCL and (B) PLCL/hydroxyapatite (HAp). Fiber diameters range from 0 to 2500 nm.*

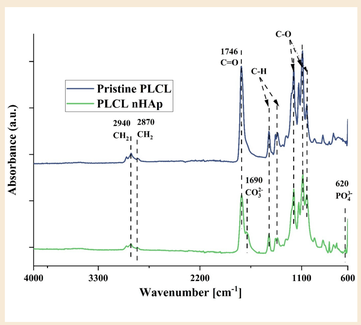


Figure 2. *FTIR spectra of PLCL and PLCL/HAp scaffolds recorded in the 600–4000 cm^{-1} range. Characteristic PO_4^{3-} absorption bands are indicated on the PLCL/HAp spectrum.*

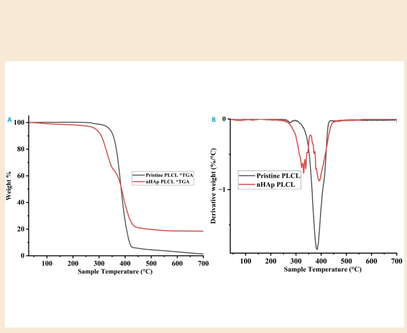


Figure 3. *TGA curves of PLCL and PLCL/HAp scaffolds: (A) weight loss (%) and (B) derivative weight (%·°C⁻¹) as a function of temperature.*

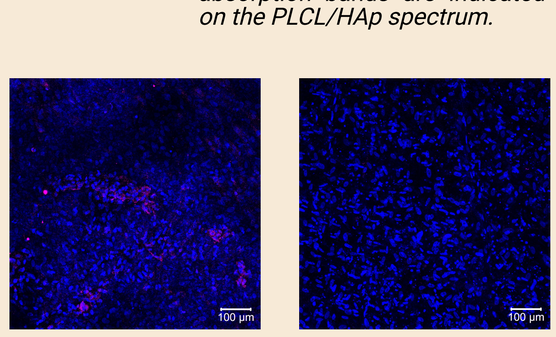


Figure 4. *Osteocalcin (OCN, magenta) and nuclei (Hoechst, blue) staining of cells at day 28 (scale bar = 100 µm) on (left) PLCL/HAp and (right) PLCL scaffolds. OCN expression is detected only in cells on PLCL/HAp scaffold.*

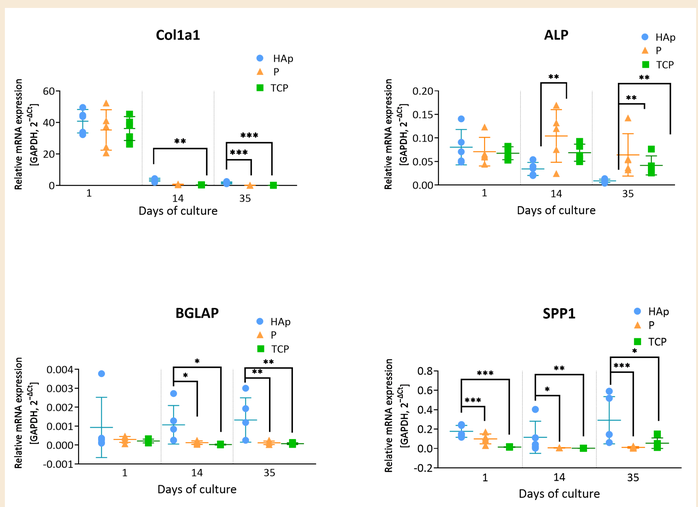


Figure 5. *Relative gene expression of matrix production and mineralization markers (Col1a1, BGLAP, ALP, and SPP1) normalized to GAPDH in cells cultured on PLCL, PLCL/HAp, and tissue culture plastic (TCP) scaffolds at days 1, 14, and 35 (n≥3). Individual data points are shown with mean and SD. Statistical significance is indicated as *p<0.05, **p<0.01, ***p<0.001.*

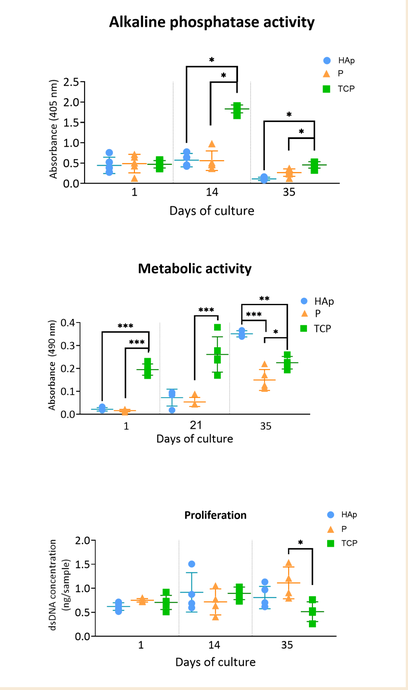


Figure 6. *Alkaline phosphatase activity, metabolic activity, and proliferation (dsDNA content) of cells cultured on PLCL, PLCL/HAp, and tissue culture plastic (TCP) at days 1, 14, and 35; metabolic activity at days 1, 21, and 35 (n≥3). Individual data points are shown with mean and SD. Statistical significance is indicated as *p<0.05, **p<0.01, ***p<0.001.*

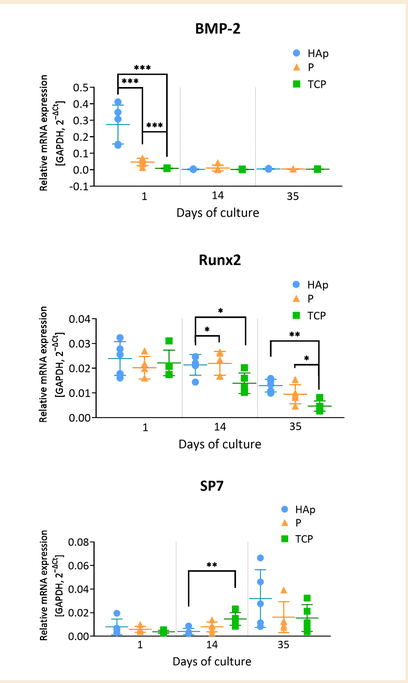


Figure 7. *Relative gene expression of transcription factors and osteogenic signaling markers (BMP-2, RUNX2, and SP7) normalized to GAPDH in cells cultured on PLCL, PLCL/HAp, and tissue culture plastic (TCP) at days 1, 14, and 35 (n≥3). Individual data points are shown with mean and SD. Statistical significance is indicated as *p<0.05, **p<0.01, ***p<0.001.*

CONCLUSION

Hydroxyapatite nanoparticles enriched electrospun scaffolds **support** bone-marrow mesenchymal **stem cells osteogenic differentiation** and bone matrix maturation, offering promising implantable biomaterials for **bone defect repair**.

ACKNOWLEDGEMENT

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