

Comparative characteristics of bovine-derived, nanosized synthetic, and micro-sized synthetic hydroxyapatite in polylactic acid-based bone scaffolds

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ABSTRACT

Bone defects resulting from trauma, degenerative diseases, or tumor resection remain a significant clinical challenge, often requiring scaffold implantation to support bone regeneration while enduring physiological mechanical loads. Polylactic acid (PLA) is widely used for scaffold fabrication due to its biodegradability and excellent printability, yet it lacks osteoconductive properties. To address this limitation, PLA is commonly combined with hydroxyapatite (HA), a calcium phosphate ceramic that mimics bone's mineral phase. This study investigated the effect of three distinct HA types – synthetic micro-HA (HA), synthetic nano-HA (nHA), and natural bovine-derived HA (BHA) – in PLA-based three-dimensionally printed scaffolds. All scaffolds exhibited comparable porosity (21–23%), pore size (564–601 μm), and fibre diameter (439–470 μm). However, PLA-BHA scaffolds demonstrated higher mechanical performance, with compressive strength values up to 3.6- and 4.6-fold greater than those of PLA-HA and PLA-nHA, respectively. Thermal analysis showed that BHA slightly reduced PLA's degradation onset but improved high-temperature residue retention. The *in vitro* cytocompatibility assessment using SaOS-2 osteoblasts showed satisfactory cell viability and adhesion across all scaffolds, with the lowest early viability observed on PLA-HA. These *in vitro* findings suggest that BHA has potential for use as a scaffold in weight-bearing sites; however, additional verification in large animal models is needed.

Keywords:

Polylactic acid; Hydroxyapatite; Bovine hydroxyapatite; Synthetic hydroxyapatite; Nano-hydroxyapatite; Three-dimensional printing; Mechanical strength

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1. Introduction

The orthopedic and dental fields continue to face significant challenges in managing bone defects caused by trauma, tumor excision, or degenerative or iatrogenic conditions, such as osteoporosis or medication-related osteonecrosis of the jaw.¹ Although autografts remain the clinical gold standard for bone repair, this approach has several limitations, including donor-site morbidity, risk of immunological rejection, risk of disease transmission, and limited donor availability.² Consequently, advances in materials science have spurred growing

interest in developing mechanically appropriate, biocompatible, and biodegradable synthetic bone scaffolds for various clinical applications.

Polylactic acid (PLA), a wide family of polymers, has emerged as a promising material for synthetic bone scaffolds due to its biodegradability and regulatory acceptance for biomedical purposes.³ Its excellent processability via additive manufacturing further supports its use in this field. Notably, three-dimensional (3D) printing has become a key technique in bone tissue engineering because it enables precise control over both the external and internal

architecture of bone scaffolds. For instance, pore size, porosity, interconnectivity, and geometry can be precisely tuned. Regarding external architecture, digital data obtained from 3D imaging, combined with computer-aided design/computer-aided manufacturing technologies, enable precise shaping of the scaffold before surgical implantation.^{4,5} Technological advancements therefore enable the design of optimized 3D scaffolds that combine functional and mechanical performance, support cellular viability, and provide multiscale customization tailored to surgical and biological requirements.

Despite these benefits, PLA alone lacks the osteoconductive properties essential for effective bone regeneration, representing a significant limitation that warrants further investigation. One strategy is to incorporate bioactive ceramics (e.g., hydroxyapatite [HA]) into PLA to improve its biological performance and functional durability.³ The Weibull modulus of dense HA ceramics typically ranges from 5 to 18, reflecting the characteristic behavior of brittle materials.⁶ Dense HA ceramics have a tensile strength of 40–100 MPa, a bending strength of 20–80 MPa, and a compressive strength of 100–900 MPa, while HA bone grafts have lower mechanical strength due to increased porosity.^{7,8} Therefore, combining PLA with HA is advantageous, as PLA enhances the mechanical integrity and offers excellent printability, making it suitable for fabricating customized scaffolds for clinical applications. This synergy enables the development of bone grafts that meet the mechanical demands of the implantation site while maintaining high biocompatibility. To achieve this, it is essential to use materials whose physical characteristics – such as strength, structural stability, and printability – can be tailored to meet clinical needs without compromising their mechanical or biological performance, including cell adhesion and osteoconductivity.

HA, as a calcium (Ca) phosphate ceramic that mimics the mineral phase of bone, has garnered significant attention in scaffold design. Notably, the literature underscores the importance of distinguishing HA by origin and particle size. Both synthetic and natural HA sources are available, and recent evidence demonstrates that these factors critically influence the physicochemical and biological properties of bone scaffolds. Due to its enormous surface area and ultrafine structure, nanosized HA (nHA) has been shown to enhance cell–material interactions and mechanical strength.⁹ In contrast, natural HA, such as bovine-derived HA (BHA), contains trace elements such as magnesium ions (Mg^{2+}) and sodium ions (Na^+). These elements are thought to enhance both osteoinductive properties and mechanical stability.^{10–12} Despite the diversity of HA sources, rigorous comparative analyses of their performance in PLA-based 3D-printed scaffolds remain largely absent, underscoring the need for further investigation.

In this study, we fabricated porous bone scaffolds using PLA composites incorporating three distinct HA types: Synthetic micro-HA, BHA, and nHA. Using an optimized extrusion-based 3D printing (robocasting) technique, we ensured consistent geometric fidelity across all groups. We systematically evaluated each scaffold's physicochemical, mechanical, and thermal properties, as well as its biological performance using SaOS-2 osteoblasts. Our findings highlight BHA's potential to enhance mechanical strength and biological compatibility in tissue engineering applications and offer recommendations for HA selection in PLA-based bone graft manufacturing.

2. Materials and methods

2.1. Fabrication of 3D scaffolds

The ceramics evaluated in this study included BHA, synthetic HA (Sigma-Aldrich, United States of America [USA]), and nHA (Sigma-Aldrich, USA). BHA was extracted from bovine bone as described in our previous study.¹³ Briefly, we cut bovine bones, boiled them in an open chamber, then boiled them in a pressurized tank, and soaked them in 95% ethanol. The bones were then calcined at 1,000°C for 2 h, ground, and sieved through an 80-mesh screen. Subsequently, 15% (w/w) of each ceramic was combined with PLA (Resinex, United Kingdom [UK]) and mixed vigorously overnight using a planetary mixer (Inversina Bioengineering, USA). The 3D scaffolds were modeled using 3D Builder software. The stereolithography file generated from the 3D model was then transferred to the slicer program (UltiMaker Cura, UltiMaker, Netherlands). Each scaffold was fabricated using a 3D Bioplotter Manufacturer Series (Envisiontec, Germany) with the following parameters: 0.4 mm needle diameter, 8 mm/s printing speed, 1 bar pressure, 1 mm spacing, 190°C syringe temperature, and 60°C platform temperature. The resulting scaffolds measured 18 mm × 18 mm × 4.5 mm (length × width × height).

2.2. Ceramics and scaffold characterizations

The powdered forms of BHA, HA, and nHA were systematically characterised. The concentrations of Ca and phosphorus (P) in each ceramic were measured using an inductively coupled plasma optical emission spectroscopy (ICP-OES) spectrometer (#iCAP 7000; Thermo Fisher Scientific Inc., USA). Each ceramic was dispersed in 0.02% (w/v) nitric acid, incubated at 40°C overnight, and sonicated for 5 min. The dispersion was then diluted with deionized water to obtain a 2% acid solution. The element concentrations were then calculated using calibration curves for the Ca and P standards. Functional substitution groups in the ceramic powders were characterised at room temperature on potassium bromide pellets containing the sample using Fourier transform infrared spectroscopy (FTIR) on an Alpha Bruker spectrometer (ALPHA FT-IR

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spectrometer; Bruker Optics GmbH & Co. KG, Germany) with a resolution of 1 cm^{-1} . Particle size was measured by dynamic light scattering (DLS) with a Zetasizer Nano Series instrument (ZEN3600; Malvern Panalytical Ltd., UK), and particle size and morphology were observed using a transmission electron microscope (TEM; TEM HT7700, Hitachi, Japan). Elemental distribution and particle morphology were also observed using a scanning electron microscope (SEM; SEM SU3500, Hitachi, Japan).

The 3D-printed scaffolds containing BHA, HA, and nHA were characterised, with morphological data acquired via the 3D Bioplotter's integrated camera (0.009 mm/pixel XY resolution). The images were used to determine the scaffold porosity, pore size, and fibre diameter in ImageJ (NIH, USA).

X-ray diffraction (XRD) analysis was performed using a Panalytical X'Pert Pro diffractometer (X'Pert Pro; PANalytical B.V., Netherlands) to assess the crystallinity of both the 3D-printed scaffolds and the ceramic powders (PIXcel 1D detector, 40 kV, 40 mA, $\lambda = 1.5418\text{ \AA}$, L3 radiation, and Cu-L2). With a step size of 0.0131° and a scan period of 200 s per step, data were collected at room temperature over the 2θ range of $5\text{--}120^\circ$.

The melting behavior and temperature of 3D scaffolds were compared using a thermogravimetric analyzer/differential scanning calorimeter (TG/DSC; LABSYS 1600 TG-DTA/DSC; Labsys, France). Briefly, 5 mg of each sample was evaluated using two heating and cooling cycles in a nitrogen environment (flow rate 200 mL/min), with heating and cooling rates of 10°C/min and 20°C/min , respectively.

The mechanical behavior of the 3D scaffolds was evaluated using a compressive strength test on a Lloyd Instruments LR 50K machine (Lloyd Instruments, USA) at a crosshead speed of 1.0 mm/min . The surface area of the samples was previously measured with a digital calliper. After breaking one scaffold layer, the compressive strength test was terminated. Stress-strain curves were calculated from load-displacement data using:

$$\alpha = \frac{F}{A} \quad (1)$$

$$\varepsilon = \frac{\Delta L}{L_0} \quad (2)$$

where σ is stress (MPa), F is load (N), A is cross-sectional area (mm^2), ε is strain (mm), ΔL is displacement (mm), and L_0 is initial scaffold height (4.5 mm).

2.3. Cell culture

SaOS-2 (ATCC, USA), osteosarcoma cells with an osteoblast-like phenotype, were cultured in Dulbecco's modified Eagle medium (DMEM; Biosera, France), supplemented with 10% fetal bovine serum (Gibco, Life Technologies, UK), 2 mmol/L glutamine (Biosera, France), 20 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Biosera, France), 100 U/L penicillin and 100 $\mu\text{g/mL}$ streptomycin (Biosera, France) in a humidified incubator at 37°C with 5% carbon dioxide (CO_2).

They were maintained in exponential growth by subculturing twice weekly with trypsin/ethylenediaminetetraacetic acid (Biosera, France) at 80% confluence.

2.4. Acid phosphatase viability assay

Cell viability was assessed using SaOS-2 osteosarcoma cells, a standard osteoblastic cell line model. While SaOS-2 cells serve as a robust model for osteoblastic behavior, they differ from primary human osteoblasts; therefore, future studies should validate these findings with primary cells. The scaffolds were sterilized in 70% ethanol for 30 min, rinsed twice with sterile distilled water and twice with phosphate-buffered saline (PBS), and then preincubated in complete DMEM for 3 days at 37°C in 5% CO_2 . After preincubation, each scaffold was transferred to a 96-well plate and covered with 100 μL of fresh complete DMEM. After 1 h of incubation at 37°C in 5% CO_2 , the scaffolds were seeded with 100 μL of SaOS-2 cell suspension ($1 \times 10^5\text{ cells/mL}$). To standardize the results, an acid phosphatase test was performed on SaOS-2 cells 24 h after seeding (control group). In addition, SaOS-2 cells were deposited on the scaffolds for 4 and 7 days (D4 and D7, respectively). On D4 and D7, the supernatant was removed. The scaffolds were then rinsed with PBS. After that, 100 μL of 4-nitrophenyl phosphate, diluted 1/100 in acetate-Triton buffer, was incubated for 3 h at 37°C . The reaction was stopped by adding 10 μL of a 1 N sodium hydroxide solution. Optical density (Sunrise™ Microplate Absorbance Reader; Tecan, Switzerland) was read at 405 nm after gentle shaking for 10 min. SaOS-2 cells cultured on tissue culture plastic were used as the reference control.

2.5. Phalloidin and Hoechst staining

A suspension of $1.5 \times 10^5\text{ cells/mL}$ of SaOS-2 was prepared in complete DMEM. A 200- μL aliquot of the cell suspension ($\sim 30,000$ cells) was seeded onto each scaffold. The culture medium was changed on day 3. Plates were incubated at 37°C in a humidified incubator with 5% CO_2 . Cells were fixed on the scaffolds on day 8 with 3.7% formaldehyde (Sigma-Aldrich, USA) in PBS, then incubated for 10 min in 0.5% Triton X-100 (Sigma-Aldrich, USA). Incubation with phalloidin AF488 (1:200 dilution according to the supplier's recommendations; Cohésion Biosciences) and Hoechst (5 $\mu\text{g/mL}$; Sigma-Aldrich, USA) was carried out for 1 h. We then observed the samples using a NIKON AZ100 (Nikon Corporation, Japan) with 4 $\times$, 2 $\times$, 4 $\times$, 6 $\times$, and 8 $\times$ zoom lenses. Actin fibers appeared in green (phalloidin AF488) and nuclei in blue (Hoechst).

2.6. Statistical analysis

We assessed data normality using the Shapiro-Wilk test. All data followed a normal distribution and exhibited homogeneity of variances. Consequently, we compared groups using one-way analysis of variance followed by Tukey's *post hoc* test. No non-parametric tests were required for the primary datasets analysed in this study. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software, USA).

3. Results

In this study, we first characterised the ceramics (BHA, HA, and nHA) using ICP-OES, FTIR, DLS, TEM, and SEM coupled with energy-dispersive X-ray spectroscopy (EDX) (Figure 1). Ca and P were the dominant elements, while Mg, Na, and O were also detected in the materials (Figure 1A; Table 1). Essential phosphate ion (PO_4^{3-}) and carbonate ion (CO_3^{2-}) bands of HA were detected on all ceramics, including symmetric stretching of $\nu_3 \text{PO}_4^{3-}$ ($\sim 1,090 \text{ cm}^{-1}$), asymmetric stretching of $\nu_3 \text{PO}_4^{3-}$ ($\sim 1,049 \text{ cm}^{-1}$), symmetric stretching of $\nu_1 \text{PO}_4^{3-}$ ($\sim 961 \text{ cm}^{-1}$), and bending mode of $\nu_2 \text{CO}_3^{2-}$ ($\sim 876 \text{ cm}^{-1}$) (Figure 1B). DLS analysis showed that HA had the largest particle size ($7,367 \pm 875 \text{ nm}$), followed by BHA ($4,074 \pm 623 \text{ nm}$), and nHA ($1,231 \pm 522 \text{ nm}$) (Figure 1C). DLS also showed agglomeration in the particle size distribution. This agglomeration explains the larger effective hydrodynamic diameters detected by DLS, as DLS measures aggregate size in suspension rather than individual primary particles. Therefore, TEM analysis was

performed. TEM analysis qualitatively indicated that BHA particles were larger than those of HA and nHA, whereas HA exhibited a greater tendency to agglomerate, consistent with the DLS observations (Figure 1D-G). Furthermore, elemental mapping (Figure 1H) and EDX-based compositional analysis revealed that BHA contained higher concentrations of Mg and Na than the other materials.

Scaffolds were fabricated under identical conditions (method, ceramic-to-PLA ratio, and geometry) (Figure 2). Post-robotocasting, we first characterised the physicochemical properties of all formulations (PLA, PLA-BHA, PLA-HA, PLA-nHA). The scaffold architectural parameters (porosity, pore size, and fibre diameter) were estimated from two-dimensional image analysis. Moreover, scaffold characterization was performed using XRD patterns, thermal properties, and mechanical properties (compressive strength). The scaffolds exhibited smooth surfaces with uniformly distributed fibers in layered patterns (Figure 2A and B).

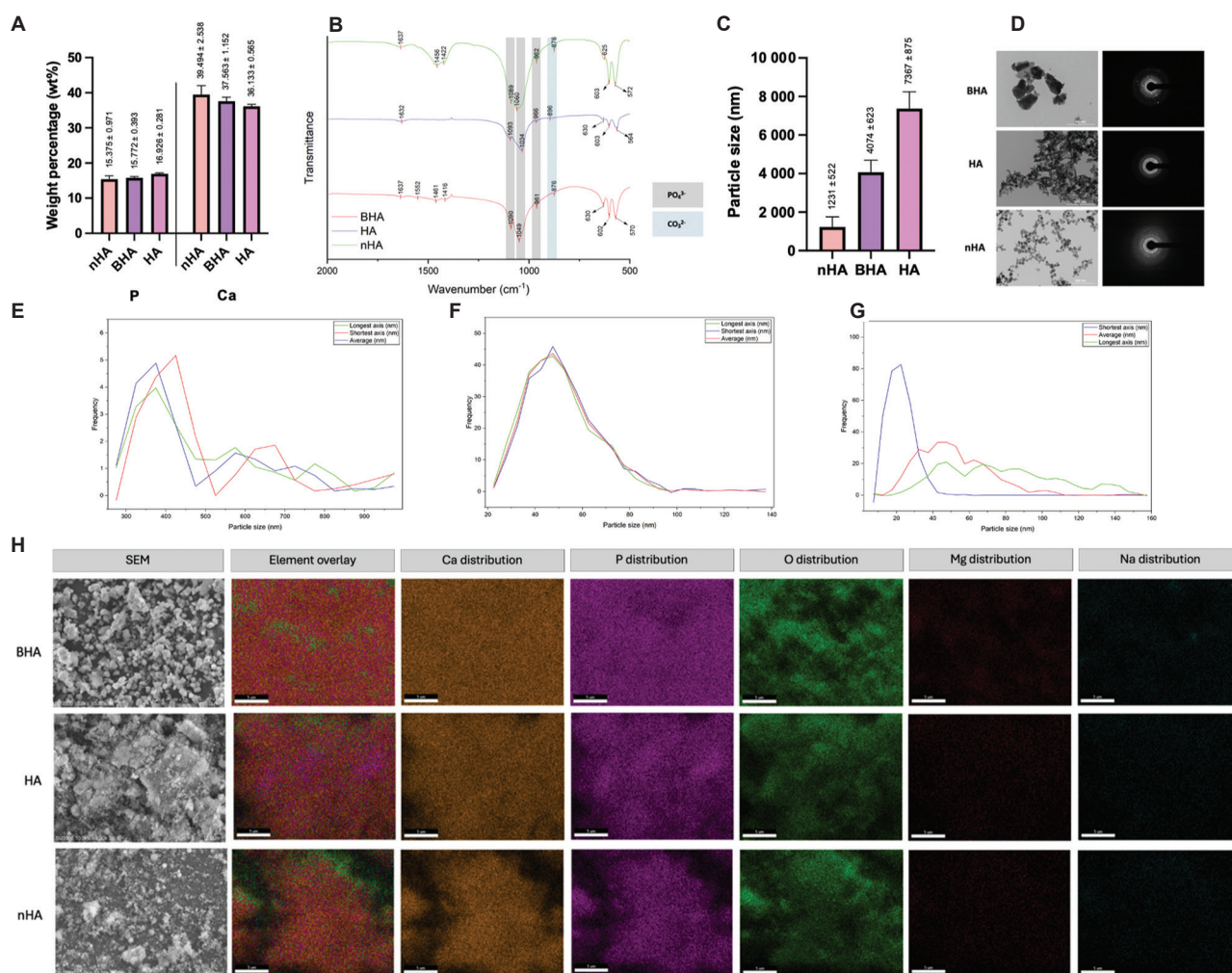


Figure 1. Characteristics of the BHA, HA, and nHA ceramics. Weight percentage of P and Ca by ICP-OES (A), FTIR peaks (B), particle size (C), TEM and SAED pattern (D), TEM-derived particle-size distributions for BHA (E), nHA (F), and HA (G), and SEM and elemental distribution (H) of the ceramics. Data are presented as mean $\pm$ SD. Scale bar: 5 μm ; magnification: (D) $\times 20\text{k}$ and (H) $\times 5\text{k}$.

Abbreviations: BHA: Natural bovine-derived HA; Ca: Calcium; CO_3^{2-} : Carbonate ion; FTIR: Fourier transform infrared spectroscopy; HA: Hydroxyapatite; ICP-OES: Inductively coupled plasma optical emission spectroscopy; nHA: Synthetic nano-HA; P: Phosphorus; PO_4^{3-} : Phosphate ion; SAED: Selected-area electron diffraction; SEM: Scanning electron microscopy; TEM: Transmission electron microscopy.

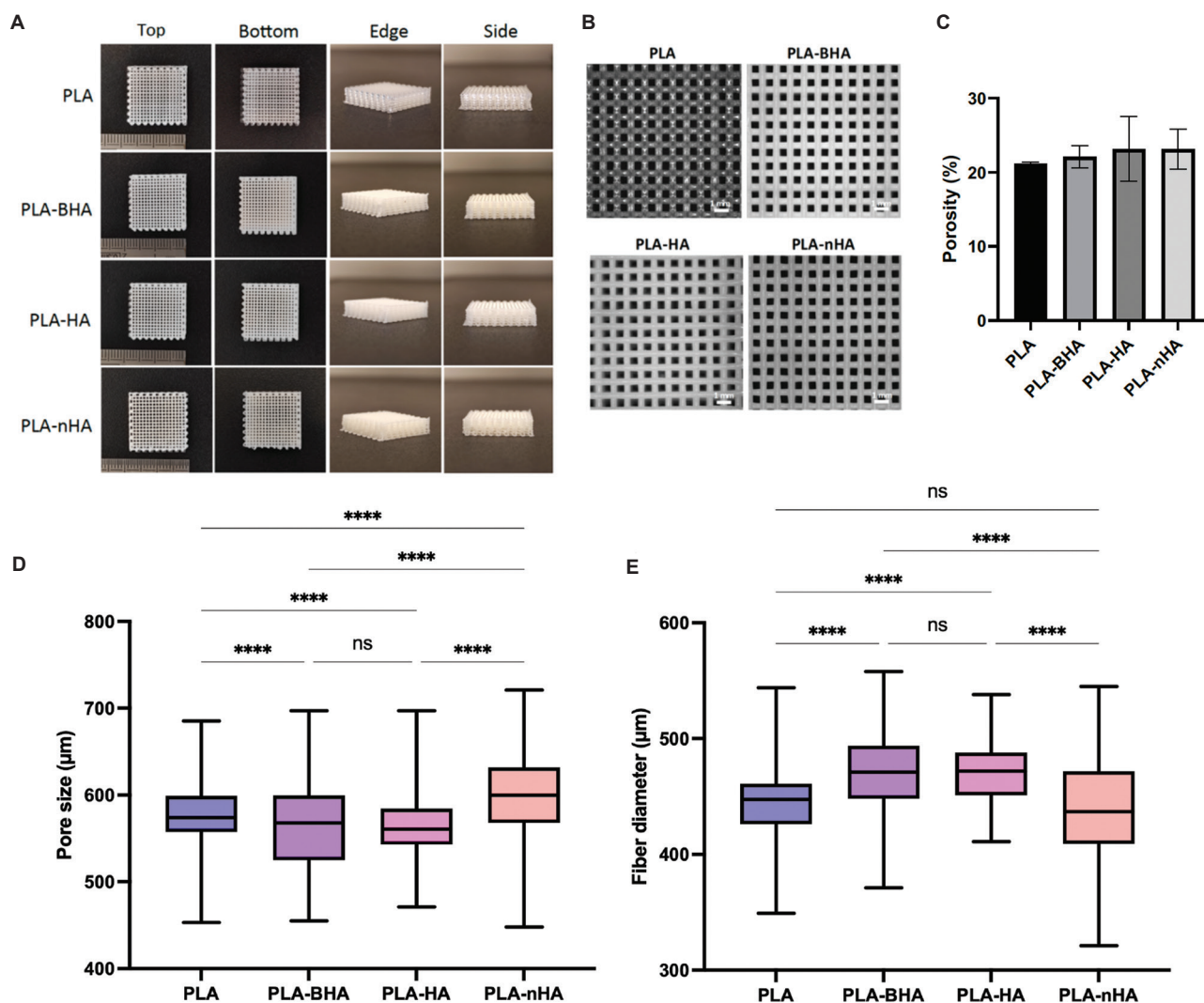


Figure 2. Geometry of the PLA, PLA-BHA, PLA-HA, and PLA-nHA scaffolds. 3D-printed scaffolds with dimensions of $18 \times 18 \times 4.5$ mm (length $\times$ width $\times$ height) (A); representative enlarged scaffold area (B); porosity quantification of the enlarged areas (C); pore size (D); and fibre diameter (E). Porosity shows the mean $\pm$ SD value from four different scaffolds in each group. Pore size and fibre diameter show the mean $\pm$ SD value from 480 counts. ****Denotes $p \leq 0.0001$.

Abbreviations: 3D: Three-dimensional; BHA: Natural bovine-derived HA; HA: Hydroxyapatite; nHA: Synthetic nano-HA; PLA: Polylactic acid.

Porosity analysis confirmed no significant differences among groups (Figure 2C). Pore sizes (564–601 μm ; Figure 2D) and fibre diameters (439–470 μm ; Figure 2E) showed statistically significant differences among several formulations, although the absolute differences were relatively modest, generally on the order of tens of micrometers. Overall, the scaffold geometry remained comparable across formulations despite the addition or type of ceramic component.

The XRD pattern indicates that BHA has higher crystallinity than the other ceramics, as shown by its sharp and intense peaks (Figure 3A). The addition of PLA introduced a new peak at ~ 16 theta (Figure 3B). Moreover, Figure 4A illustrates the thermal transitions of the scaffolds: Glass transition temperature (T_g), onset crystallization temperature (T_c), and melting temperature (T_m). T_m decreased on BHA addition (Figure 4A). TGA data (Figure 4B) showed that 50% weight loss occurred at 363°C (PLA-HA), 347°C (PLA-nHA), 320°C (PLA-BHA), and 356°C (PLA). The PLA scaffold fully degraded

by $\sim 400^\circ\text{C}$, while composites retained ceramic-derived residual mass at 773°C. The remaining weight at high temperatures in the composites was due to the ceramic fillers.

Scaffold characteristics were evident in their mechanical properties (Figure 5). The results clearly showed that the PLA-BHA scaffold exhibited significantly higher mechanical properties than all other scaffolds (Figure 5). The compressive strength of PLA-BHA scaffolds was 3.6-fold and 4.6-fold higher than that of the other bone scaffolds.

We also assessed SaOS-2 osteoblast viability after culturing them in each scaffold. After 4 days of cell culture, the PLA-HA scaffold showed the lowest cell viability compared with the other groups ($p < 0.01$ vs. PLA-BHA, $p < 0.001$ vs. PLA, and $p < 0.001$ vs. PLA-nHA; Figure 6A). In addition, cell viability did not differ significantly among the PLA, PLA-BHA, and PLA-nHA groups ($p > 0.05$). A lower signal at Day 4 on PLA-HA suggests slower initial cell adhesion and metabolic activation compared to BHA and nHA. Furthermore, on day

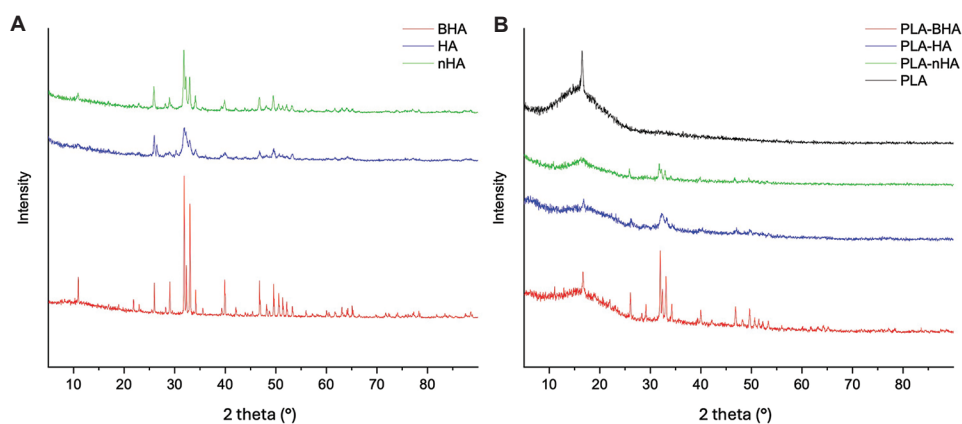


Figure 3. XRD spectra of BHA, HA, and nHA ceramics (A) and PLA, PLA-BHA, PLA-HA, and PLA-nHA scaffolds (B). Abbreviations: BHA: Natural bovine-derived HA; HA: Hydroxyapatite; nHA: Synthetic nano-HA; PLA: Polylactic acid; XRD: X-ray diffraction.

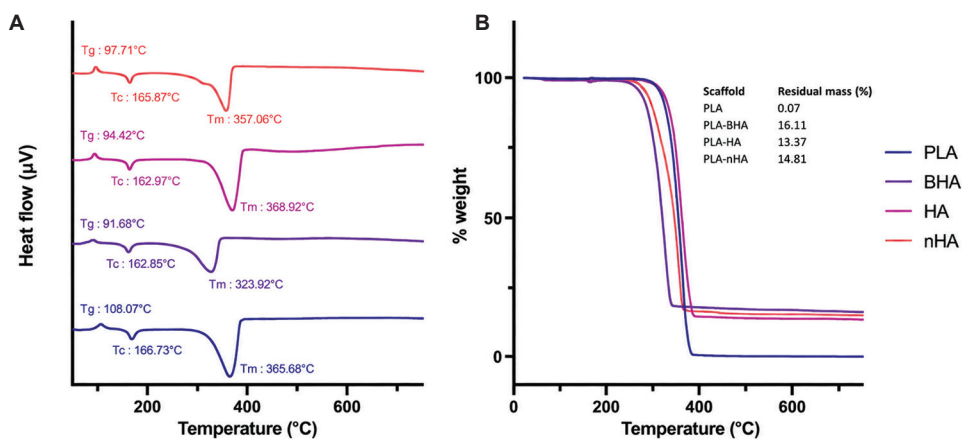


Figure 4. DSC thermograms (A) and residual mass (%) with temperature from TGA analysis (B) of PLA, PLA-BHA, PLA-HA, and PLA-nHA scaffolds.

Abbreviations: BHA: Natural bovine-derived HA; DSC: Differential scanning calorimetry; HA: Hydroxyapatite; nHA: Synthetic nano-HA; PLA: Polylactic acid; Tc: Onset crystallization temperature; Tg: Glass transition temperature; TGA: Thermogravimetric analysis; Tm: Melting temperature.

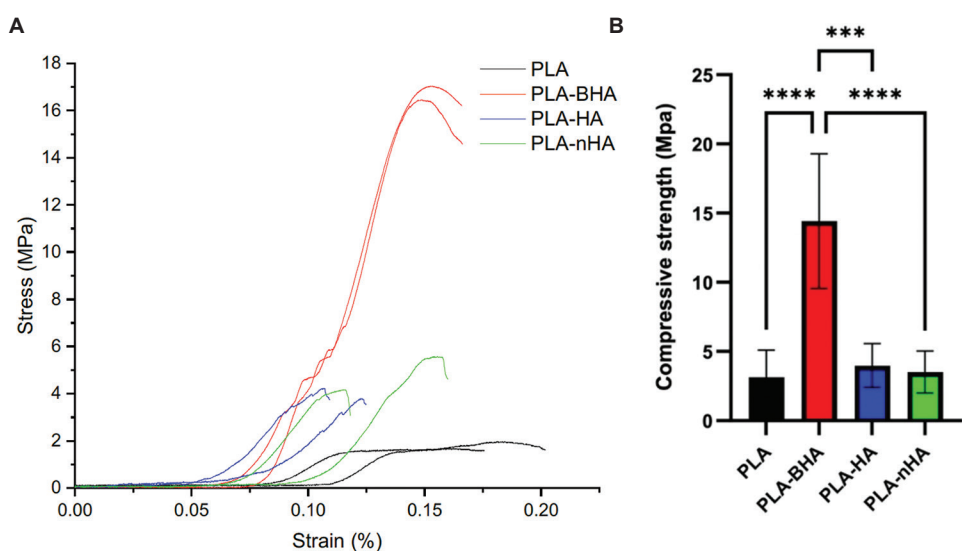


Figure 5. Stress-strain curve (A) and compressive strength (B) of PLA, PLA-BHA, PLA-HA, and PLA-nHA scaffolds. The bar chart was calculated from the stress-strain curves in Figure 5 ($n=5$). ***Denotes $p \leq 0.001$ and ****Denotes $p \leq 0.0001$.

Abbreviations: BHA: Natural bovine-derived HA; HA: Hydroxyapatite; nHA: Synthetic nano-HA; PLA: Polylactic acid.

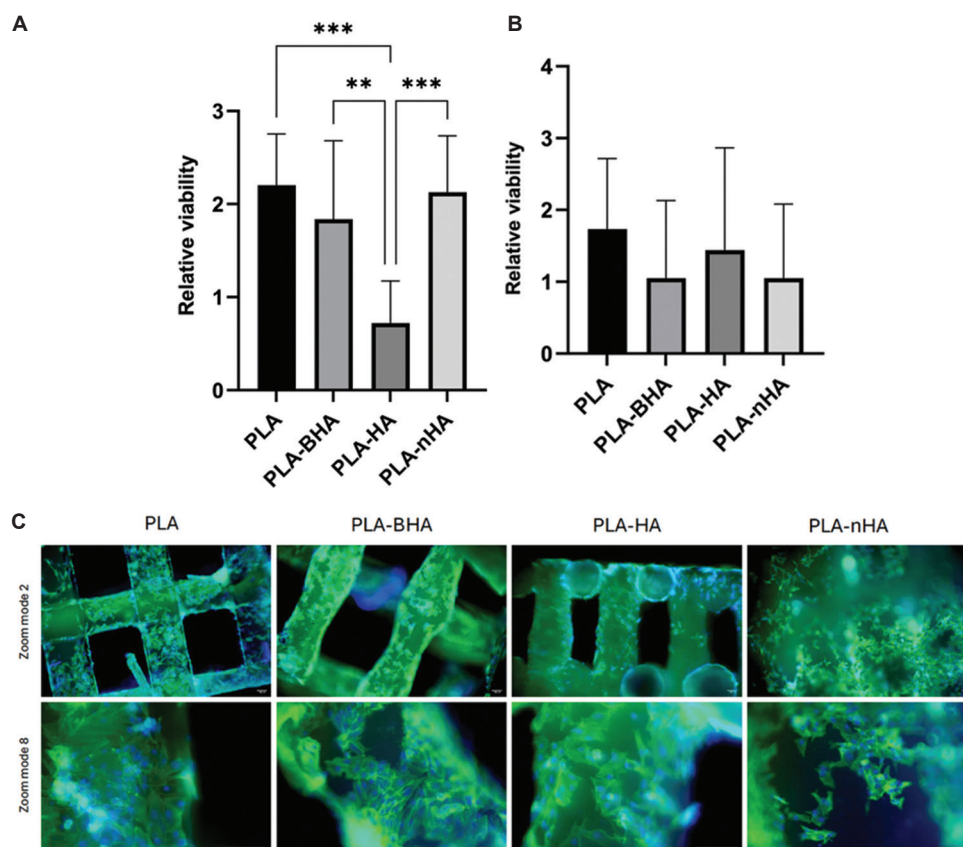


Figure 6. Viability and SaOS-2 osteoblast attachment on PLA, PLA-BHA, PLA-HA, and PLA-nHA scaffolds. Relative viability compared to the control group, by acid phosphatase assay at 4 (A) and 7 days (B). SaOS-2 osteoblasts attached to the scaffolds were visualized with phalloidin and Hoechst staining (C). Relative viability shows the mean $\pm$ SD value from 12 replicates. Green, F-actin filaments; blue, nuclei. **Denotes $p \leq 0.01$ and ***Denotes $p \leq 0.001$. Magnification: Zoom mode 2 = $\times 2$ and zoom mode 8 = $\times 8$.

Abbreviations: BHA: Natural bovine-derived HA; HA: Hydroxyapatite; nHA: Synthetic nano-HA; PLA: Polylactic acid.

Table 1. Elemental composition of the ceramics determined by EDX

Element	BHA		HA		nHA	
	Wt	At	Wt	At	Wt	At
Ca K	43.27	26.48	41.99	25.46	42.50	25.28
P K	17.35	13.74	18.36	14.41	15.09	11.62
Mg K	0.83	0.83	0.10	0.10	0.01	0.01
Na K	0.31	0.33	0.00	0.00	0.00	0.00
O K	38.24	58.62	39.48	59.97	42.25	62.96

Abbreviations: At: Atomic percentage; BHA: Natural bovine-derived HA; Ca: Calcium; EDX: Energy-dispersive X-ray spectroscopy; HA: Hydroxyapatite; Mg: Magnesium; Na: Sodium; nHA: Synthetic nano-HA; O: Oxygen; Wt: Weight percentage.

7, the groups' relative viability did not differ significantly (Figure 6B). This also indicates that on day 7, once adhered, the cells on PLA-HA exhibited robust proliferation and metabolic activity. This delayed but strong response confirms PLA-HA biocompatibility, despite its slower initial attachment kinetics. Subsequently, after 8 days of culture, excellent cell adhesion was observed on the surfaces of all scaffolds (Figure 6C). The result suggests that all scaffolds supported sufficient cell attachment, indicating that—despite initial differences in cell viability—the materials are biocompatible and conducive to osteoblast adhesion and proliferation.

4. Discussion

These results showed significant differences between PLA-based bone scaffolds containing BHA and those with synthetic HAs. Despite having similar Ca and p contents and identical functional groups, BHA in powder form exhibited sharper XRD peaks, likely due to high-temperature calcination.¹⁴ Furthermore, incorporating ceramics into the PLA scaffold did not significantly alter its geometry, regardless of the ceramic type used. This also indicates successful incorporation of the ceramic fillers into the PLA scaffolds, as evidenced by the appearance of new XRD peaks at approximately 16° (20) (Figure 3B), corresponding to the (200)/(110) planes of the α -form of PLA.¹⁴ Thermal characterization revealed a decrease in the PLA melting temperature upon BHA incorporation, which is likely attributable to trace elements in BHA that disrupt the regular packing of PLA chains,¹⁴ which was also observed in ion-doped PLA composites.¹⁵

A key finding of this study was the significant difference in mechanical performance between BHA-based and synthetic HA-based PLA scaffolds. While porosity remains the primary determinant of bone scaffold mechanical properties,¹⁶ our findings indicate that ceramic filler properties can independently modulate the mechanical behavior of PLA-based scaffolds, even when porosity is held constant.¹⁶

Particle size variations can also affect material strength. For instance, Niaza *et al.*⁹ reported that, in the same formulation and ratio, a composite made from nHA had higher compressive strength than a micro-sized HA composite. However, our results showed that nano-sized and micro-sized synthetic HA did not differ significantly in mechanical properties when incorporated into 3D-printed PLA scaffolds. This suggests that other factors, such as the material synthesis method, may influence the resulting scaffold's strength. In particular, the low sintering temperature of the ceramic decreased its strength.⁸ In this study, high-temperature calcination of BHA powder may have enhanced its mechanical strength as a scaffold and produced sharp XRD peaks in the powder form.¹³ While compressive strength is a critical parameter for bone scaffolds, it is not the sole determinant of clinical success. The elastic modulus is equally important for preventing stress shielding effects. Furthermore, long-term durability under physiological conditions will require fatigue and cyclic compression testing. These parameters will guide our future mechanical and preclinical evaluations to ensure the scaffolds' viability for clinical translation.

One key advantage of BHA over synthetic HAs is the presence of trace elements—such as Na⁺, stannous ion, Mg²⁺, potassium ion, barium ion, and fluoride ion—naturally found in biological grafts such as BHA.^{10,11,17} In general, adding elements to ceramics improves the material's strength. Feng *et al.*¹⁸ reported that doping with zinc oxide to 2.5 wt% increased the scaffolds' fracture toughness from 1.09 to 1.40 MPa and the compressive strength from 3.01 to 17.89 MPa.¹⁸ The superior mechanical performance of the PLA-BHA scaffolds may be attributed to BHA's specific composition, which contains natural trace elements such as Mg, strontium, and zinc.^{10,11,17} Although this study did not perform elemental release analysis, the literature suggests that these trace elements can enhance the interfacial bonding or alter the crystallinity of the ceramic phase.^{19–21} Therefore, the observed mechanical enhancement should be interpreted as a potential effect of the BHA composition, warranting further elemental characterization in future work.

Moreover, the improved mechanical properties may also be attributed to various physical and/or chemical interaction mechanisms between the filler and the PLA matrix. In this study, the sharper XRD peaks of BHA indicate higher crystallinity, which may enhance stress transfer within the PLA matrix. In addition, the reduced PLA melting temperature suggests that BHA influences PLA chain organization during processing. This may also include the formation of a highly interconnected interfacial network between the filler surface and PLA chains, as well as enhanced interfacial stress transfer and mechanical interlocking at the filler–polymer interface.^{22,23} However, the underlying mechanism warrants further exploration.

Trace elements present in natural HA also confer a distinct advantage over synthetic HA in biological performance. For instance, Mg was documented to exert superior activity on a range of osteoblast-derived cells, including promoting cell differentiation,^{17,24} similar to Na.²⁵ While the current study demonstrates excellent cytocompatibility and cell

adhesion for all scaffolds, particularly BHA at early stages, it is important to acknowledge that osteogenic differentiation markers (such as alkaline phosphatase activity, mineralization nodules, or gene expression of *RUNX2* and *BGLAP*) were not evaluated. Future studies should include these specific assays to fully elucidate the osteoinductive potential of the BHA-incorporated scaffolds.

Taken together, these findings support the notion that natural HA sources, such as BHA, can significantly enhance the mechanical resilience of 3D-printed scaffolds without compromising biocompatibility, making them promising candidates for load-bearing applications in bone regeneration. However, further investigations are needed to evaluate the mechanical performance of PLA-BHA and PLA-HA scaffolds under physiological conditions and to comprehensively assess their biological efficacy in promoting bone regeneration through *in vivo* studies.

5. Conclusion

This study successfully fabricated PLA-based scaffolds with different types of HA—PLA-BHA, PLA-HA, and PLA-nHA—using a consistent fabrication method, ceramic-to-PLA ratio, and geometry. The results showed that incorporating HA did not significantly affect scaffold geometry. Among the tested materials, the PLA-BHA scaffold exhibited significantly superior mechanical strength. The *in vitro* biological evaluation revealed that all scaffold formulations supported robust cell adhesion and equivalent osteoblast viability, as demonstrated by SaOS-2 cultures. These findings suggest that while PLA-BHA scaffolds offer superior mechanical properties, all scaffold types are biocompatible and support osteoblast attachment and viability. Taken together, BHA can be prioritized for scaffolds in weight-bearing sites, and future large-animal studies are needed to evaluate the safety, mechanical performance, and bone-regeneration efficacy of these scaffolds *in vivo*.

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Conflicts of interest statement

The authors declare no conflicts of interest.

Author contributions

Conceptualization: MAG and DB; *Data curation:* MAG, DB, TB, ST, AN, and BL; *Formal analysis:* MAG and DB; *Investigation:* MAG, DB, TB, ST, AN, and BL; *Methodology:* MAG, DB, AL, RL, and JK; *Project administration:* MAG; *Resources:* DB and JK; *Supervision:* DB; *Validation:* MAG and AL; *Writing—original draft:* MAG; *Writing—review & editing:* MAG, DB, and AL.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All analysed data are presented in the paper.

Open access statement

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