

DEVELOPMENT OF 3D-PRINTED NANO-HYDROXYAPATITE COMPOSITE SCAFFOLDS FOR BONE TISSUE REGENERATION

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ABSTRACT

Large and critical-sized bone defects remain difficult to treat because natural healing is often insufficient. Although autografts and allografts are widely used, their application is limited by donor-site injury, restricted availability, immune complications and the risk of disease transmission. Bone-tissue engineering offers a promising alternative through the development of temporary scaffolds capable of supporting cell attachment, tissue formation and gradual bone replacement. The present study proposes the development of three-dimensional printed nano-hydroxyapatite and polycaprolactone composite scaffolds for bone regeneration. Nano-hydroxyapatite is selected because of its chemical similarity to the mineral phase of natural bone, while polycaprolactone provides flexibility, processability and structural stability. Different concentrations of nano-hydroxyapatite will be incorporated into the polymer matrix and fabricated through extrusion-based 3D printing.

The scaffolds will be evaluated for morphology, pore architecture, chemical composition, crystallinity, wettability, compressive strength, biodegradation and apatite-forming ability. Their biological performance will be assessed through cell viability, adhesion, proliferation, alkaline-phosphatase activity and mineralisation studies.

The central innovation of the study lies in optimising scaffold composition and printing architecture together rather than treating them as separate variables. The proposed scaffold is expected to provide interconnected porosity, improved surface bioactivity, suitable mechanical strength and controlled degradation. The study may contribute to the development of patient-specific and biologically active scaffolds for repairing complex bone defects.

KEYWORDS: Nano-hydroxyapatite, 3D printing, composite scaffold, bone regeneration, polycaprolactone, osteogenesis, biodegradation.

1. INTRODUCTION

Bone possesses a natural capacity for repair, but this ability becomes inadequate in large defects caused by trauma, tumour removal, infection, congenital abnormalities and degenerative disease. Such defects frequently require grafting or implantation.

Autologous bone grafts are biologically effective because they contain living cells and natural growth factors. However, they are limited by donor-site pain, restricted tissue availability and surgical complications. Allografts eliminate the need for a second surgical site but may produce immune reactions and carry a small risk of disease transmission.

Bone-tissue engineering aims to overcome these limitations by combining biomaterials, cells and biological signals. In this approach, the scaffold acts as a temporary framework for cell attachment, migration, proliferation and extracellular-matrix formation.

An ideal bone scaffold should possess:

- biocompatibility;
- interconnected porosity;
- suitable mechanical strength;
- osteoconductivity;
- controlled biodegradation;
- dimensional stability; and
- reproducible fabrication.

Hydroxyapatite is widely used in bone regeneration because it resembles the mineral component of human bone. At the nanoscale, hydroxyapatite offers greater surface area and stronger interaction with proteins and cells. However, pure hydroxyapatite is brittle and difficult to use as an independent load-bearing structure.

Polycaprolactone is a biodegradable polymer with favourable printability and mechanical continuity. Its major limitations are hydrophobicity and limited biological activity. Combining nano-hydroxyapatite with polycaprolactone can create a composite scaffold in which the polymer provides flexibility and processability, while the ceramic phase promotes mineralisation and osteogenic behaviour.

Three-dimensional printing further improves scaffold design by allowing control over pore size, filament orientation, internal geometry and external shape. It also creates the possibility of patient-specific scaffolds based on medical-imaging data.

The proposed research therefore focuses on developing 3D-printed nano-hydroxyapatite composite scaffolds that balance mechanical performance, biological activity and biodegradation.

2. Research Problem

Existing bone substitutes often fail to achieve an appropriate balance between structural strength and biological activity. Highly porous scaffolds may support cell penetration but exhibit poor mechanical stability. Dense scaffolds may be stronger but restrict nutrient transport, vascularisation and tissue ingrowth.

Pure hydroxyapatite scaffolds are osteoconductive but brittle, whereas pure polymeric scaffolds are mechanically processable but biologically less active. Furthermore, excessive addition of nano-hydroxyapatite can reduce printability, cause particle agglomeration and weaken interlayer bonding.

The principal research problem is therefore to determine an optimum nano-hydroxyapatite concentration and scaffold architecture that can provide:

- reliable 3D printability;
- interconnected porosity;
- adequate compressive strength;
- favourable surface bioactivity;
- controlled degradation; and
- enhanced osteogenic activity.

3. Review of Related Literature

Three-dimensional printing has emerged as an effective technique for fabricating bone-tissue-engineering scaffolds with controlled shape, pore size and internal architecture.

Wang et al. (2020) observed that additive manufacturing allows the production of customised scaffolds with improved structural reproducibility and defect-specific geometry. Their study established that scaffold performance is influenced not only by the selected biomaterial but also by pore arrangement, filament orientation and printing conditions.

Hydroxyapatite is widely used in bone regeneration because its chemical composition resembles the mineral phase of natural bone.

Mo et al. (2023) reported that nano-hydroxyapatite improves scaffold bioactivity and can support the delivery of drugs, growth factors and other therapeutic agents. Its nanoscale structure provides a greater surface area for protein adsorption and cellular interaction. However, hydroxyapatite is brittle when used alone and therefore requires combination with a flexible and biodegradable polymer.

Polycaprolactone is frequently selected for 3D printing because of its favourable processability, low melting temperature and structural stability. Its main limitations are hydrophobicity and weak biological activity.

Ebrahimi et al. (2022) demonstrated that the incorporation of hydroxyapatite and collagen into 3D-printed PCL scaffolds improved stem-cell proliferation, alkaline-phosphatase activity and mineral deposition. Their findings showed that PCL provides a stable framework, whereas hydroxyapatite improves the osteogenic character of the scaffold.

Bernardo et al. (2022) studied PLA/hydroxyapatite composite scaffolds and reported that hydroxyapatite improved mechanical behaviour, reduced the acidic effect of polymer degradation and promoted osteogenic differentiation. This study highlighted that hydroxyapatite can influence scaffold strength, degradation chemistry and biological performance simultaneously. However, the behaviour of PLA-based composites cannot be applied directly to PCL-based systems because the two polymers differ in flexibility, crystallinity and degradation rate.

Zhang et al. (2017) developed 3D-printed PLA–hydroxyapatite scaffolds for vascularised bone formation. Their results demonstrated that polymer–ceramic composites can provide useful biodegradability, biocompatibility and osteoconductivity. The study also indicated that 3D-printed scaffolds can be combined with biological systems to develop anatomically relevant bone substitutes.

The biological effectiveness of a scaffold depends on its ability to support cell attachment, proliferation and differentiation. Meesuk et al. (2022) investigated mesenchymal stem cells on 3D-printed hydroxyapatite scaffolds and found that the cells survived, proliferated and expressed osteogenic markers. Calcium-phosphate surface modification further increased alkaline-phosphatase activity and the expression of bone-related genes. Their findings confirmed that scaffold-surface chemistry plays an important role in directing osteogenic behaviour.

Pore architecture is another critical factor in bone regeneration. Interconnected pores support nutrient movement, cell migration and vascular ingrowth, while excessive porosity may reduce mechanical strength.

Wang et al. (2020) and Ananth et al. (2024) emphasised that successful scaffold design requires a balance between biological accessibility and structural stability. Controlled 3D printing provides an opportunity to maintain consistent pore geometry while investigating the influence of material composition.

Nano-hydroxyapatite concentration also affects mechanical and printing behaviour. Moderate ceramic loading may increase stiffness and surface bioactivity, whereas excessive loading may cause particle aggregation, nozzle blockage and weak interlayer bonding.

Bernardo et al. (2022) reported favourable mechanical properties in hydroxyapatite-rich composite scaffolds, but the optimum concentration depends on polymer type and processing conditions.

Kennedy et al. (2026) observed that nano-hydroxyapatite and polymer molecular weight can influence the degradation and mechanical properties of printable PCL formulations. Their work suggested that scaffold degradation can be adjusted by modifying material composition. This supports the need to assess mass loss, water absorption, pH variation and residual mechanical strength together.

Recent research has also moved toward multifunctional scaffolds. Cao et al. (2022) combined 3D printing and electrospinning to produce PCL/nano-hydroxyapatite composite scaffolds with hierarchical structures. Mo et al. (2023)

described the potential of hydroxyapatite scaffolds for drug delivery and biological-factor incorporation. Rong et al. (2025) further demonstrated that modified 3D-printed scaffolds may support both disease treatment and bone regeneration. Patient-specific fabrication represents another important development. Jeong et al. (2022) and Lee et al. (2020) demonstrated that customised polymer–calcium-phosphate scaffolds can be used for complex craniofacial and mandibular defects. These studies indicate that 3D printing has practical value beyond laboratory-scale scaffold development. Overall, the reviewed literature confirms that nano-hydroxyapatite can improve the osteoconductivity, mineralisation and biological activity of polymeric scaffolds. Polycaprolactone provides printability and structural support, while 3D printing enables control over scaffold architecture. However, no universally accepted PCL/nano-hydroxyapatite ratio has been established. Differences in material composition, particle dispersion, pore design and testing methods make direct comparison difficult. The present study therefore proposes a systematic evaluation of different nano-hydroxyapatite concentrations to identify a scaffold formulation that balances printability, mechanical strength, controlled degradation and osteogenic activity.

3. Research Gap

Previous studies have demonstrated the potential of hydroxyapatite-polymer composites, yet several limitations remain. First, many investigations examine material composition without sufficiently considering printing architecture. Scaffold behaviour is controlled by the interaction of composition, pore size, filament arrangement and porosity. Second, there is no universally suitable nano-hydroxyapatite concentration. Excessive ceramic loading may increase stiffness but reduce flow, ductility and printing accuracy. Third, some studies rely mainly on structural characterisation and do not include adequate biological evaluation. Fourth, degradation behaviour is often assessed separately from mechanical stability and cell response. Fifth, many laboratory formulations are difficult to reproduce at larger scales because of particle agglomeration and inconsistent extrusion. The proposed study addresses these gaps through an integrated optimisation of composition, scaffold architecture and biological performance.

4. Aim of the Study

To develop and evaluate 3D-printed nano-hydroxyapatite composite scaffolds with improved mechanical, biological and degradative properties for bone-tissue regeneration.

5. Objectives

- To synthesise and incorporate nano-hydroxyapatite into a biodegradable polymer matrix for the development of bioactive composite materials suitable for bone-tissue regeneration.
- To fabricate porous nano-hydroxyapatite composite scaffolds through extrusion-based 3D printing and optimise their printability, dimensional accuracy and internal pore architecture.
- To characterise the developed scaffolds in terms of morphology, chemical composition, crystallinity, wettability, compressive strength, water absorption, biodegradation and apatite-forming ability.
- To evaluate the biological performance of the scaffolds through cell viability, adhesion, proliferation and osteogenic differentiation and identify the most suitable formulation for bone regeneration.

6. Research Questions

- How does nano-hydroxyapatite concentration affect scaffold printability?
- Does nano-hydroxyapatite improve surface wettability and bioactivity?
- What relationship exists between porosity and compressive strength?
- Which formulation provides the best balance between strength and biodegradation?
- Can the developed scaffold support osteogenic cell activity and mineralisation?

7. Hypotheses

- H₀₁: Nano-hydroxyapatite concentration has no significant effect on scaffold mechanical strength.
- H₁₁: Nano-hydroxyapatite concentration significantly affects scaffold mechanical strength.
- H₀₂: Nano-hydroxyapatite incorporation does not significantly influence scaffold bioactivity.
- H₁₂: Nano-hydroxyapatite incorporation significantly improves scaffold bioactivity.
- H₀₃: There is no significant difference in cell viability among the scaffold formulations.
- H₁₃: There is a significant difference in cell viability among the scaffold formulations.
- H₀₄: Nano-hydroxyapatite does not significantly enhance osteogenic differentiation.
- H₁₄: Nano-hydroxyapatite significantly enhances osteogenic differentiation.

8. MATERIALS AND METHODS

8.1 Research Design

The study will follow an experimental and comparative design. A pure polycaprolactone scaffold will serve as the control, while composite scaffolds containing different concentrations of nano-hydroxyapatite will form the experimental groups.

8.2 Proposed Scaffold Formulations

Group	Polycaprolactone	Nano-Hydroxyapatite
Control	100%	0%
Group I	95%	5%
Group II	90%	10%

Group III	85%	15%
Group IV	80%	20%

The final concentrations may be adjusted after preliminary printability testing.

8.3 Preparation of Nano-Hydroxyapatite

Nano-hydroxyapatite may be synthesised through wet-chemical precipitation using calcium- and phosphate-containing solutions. The pH will be maintained under alkaline conditions to promote hydroxyapatite formation.

The precipitate will be aged, washed, dried and thermally treated. Its phase purity and particle morphology will be examined before scaffold fabrication.

8.4 Composite Preparation

Polycaprolactone and nano-hydroxyapatite will be blended through melt mixing or solvent-assisted mixing. Uniform particle dispersion will be prioritised to minimise agglomeration.

8.5 Scaffold Design and Printing

Scaffolds will be designed using computer-aided-design software and fabricated through extrusion-based 3D printing. A 0°/90° filament orientation may be used to create regular and interconnected pores.

Important printing variables will include:

- nozzle diameter;
- printing temperature;
- extrusion rate;
- layer height;
- filament spacing;
- printing speed; and
- infill density.

8.6 Physicochemical Characterisation

The scaffolds will be characterised using:

- X-ray diffraction for crystalline-phase identification;
- Fourier-transform infrared spectroscopy for functional groups;
- scanning electron microscopy for morphology;
- energy-dispersive X-ray spectroscopy for elemental composition;
- contact-angle analysis for wettability; and
- micro-computed tomography or image analysis for pore structure.

8.7 Porosity

Porosity will be determined using liquid displacement, density measurement or micro-computed tomography.

Porosity (%) = [(Theoretical density – Apparent density) / Theoretical density] × 100

8.8 Mechanical Testing

Compression testing will be conducted using a universal testing machine. The following parameters will be recorded:

- compressive strength;
- elastic modulus;
- deformation behaviour; and
- structural failure pattern.

8.9 Biodegradation Study

Scaffolds will be immersed in phosphate-buffered saline at 37°C for predetermined periods.

Mass loss will be calculated as:

Mass loss (%) = [(Initial dry weight – Final dry weight) / Initial dry weight] × 100

Changes in pH, morphology and mechanical properties will also be examined.

8.10 Simulated Body Fluid Study

The scaffolds will be immersed in simulated body fluid to assess their ability to develop an apatite-like mineral layer.

Following immersion, surface mineralisation will be examined through microscopy, X-ray diffraction and elemental analysis.

8.11 Biological Evaluation

Osteoblast-like cells or mesenchymal stem cells will be seeded on sterilised scaffolds.

The following tests will be performed:

- cell-viability assay;
- live/dead staining;
- cell-adhesion analysis;
- proliferation assay;
- alkaline-phosphatase activity;
- calcium-mineralisation assay; and
- osteogenic gene-expression analysis, where facilities permit.

8.12 Statistical Analysis

Data will be expressed as mean $\pm$ standard deviation. One-way or two-way analysis of variance will be applied according to the number of variables and time points.

A probability value below 0.05 will be treated as statistically significant.

9. Proposed Experimental Workflow

Material selection

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Nano-hydroxyapatite synthesis

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Composite formulation

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Computer-aided scaffold design

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Three-dimensional printing

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Morphological and chemical characterisation

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Mechanical and degradation testing

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Simulated body fluid assessment

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Cell viability and adhesion studies

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Osteogenic differentiation

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Statistical optimisation

10. Expected Results

The pure polycaprolactone scaffold is expected to show good printability but limited bioactivity. Incorporation of nano-hydroxyapatite is expected to increase surface roughness, hydrophilicity and mineral deposition.

Moderate nano-hydroxyapatite concentrations may improve compressive stiffness and osteogenic response. However, excessive ceramic loading may cause:

- poor extrusion;
- nozzle blockage;
- particle agglomeration;
- reduced flexibility; and
- weak interlayer bonding.
- The optimised scaffold is expected to demonstrate:
 - regular and interconnected pores;
 - improved compressive performance;
 - enhanced apatite formation;
 - controlled mass loss;
 - high cell viability;
 - better cell spreading; and
 - increased osteogenic mineralisation.

11. DISCUSSION

The success of a bone scaffold depends on a balanced combination of structure, chemistry, mechanics and degradation. No single parameter can independently determine regenerative performance.

The proposed composite system imitates the basic organisation of natural bone. Polycaprolactone acts as the continuous polymeric phase, while nano-hydroxyapatite provides mineral reinforcement and osteoconductive surfaces.

The effect of nano-hydroxyapatite is expected to depend strongly on concentration and dispersion. Uniformly distributed particles may improve stiffness and cellular interaction. Agglomerated particles may create weak regions and reduce printing accuracy.

Pore architecture will also influence scaffold performance. Interconnected pores support nutrient movement and tissue invasion, but excessive porosity reduces structural strength. The scaffold must therefore be designed according to the intended anatomical application.

The biological evaluation is important because cell viability alone cannot confirm osteogenic effectiveness. The inclusion of alkaline-phosphatase activity and mineralisation tests will provide stronger evidence of bone-forming potential.

Controlled biodegradation will remain a major consideration. The scaffold should retain sufficient strength during early healing but gradually lose mass as new bone develops.

12. Innovative Contribution

The proposed study is innovative in the following ways:

- It combines material optimisation with architectural optimisation.
- It evaluates mechanical strength, degradation and osteogenic activity within one research framework.
- It applies controlled 3D printing for reproducible scaffold fabrication.
- It identifies an optimum nano-hydroxyapatite concentration rather than assuming that higher ceramic content is always beneficial.
- It creates a platform that can later incorporate drugs, growth factors, therapeutic ions or stem cells.
- It supports the future development of customised scaffolds for irregular bone defects.

13. Significance of the Study

The study may provide a scientifically reproducible scaffold system for bone-tissue engineering. It may also improve understanding of the relationship among nano-hydroxyapatite concentration, pore architecture, mechanical strength and cellular behaviour.

- Potential applications include:
- craniofacial bone reconstruction;
- dental bone regeneration;
- traumatic bone defects;
- tumour-resection cavities;
- non-union fractures;
- spinal-fusion research; and
- patient-specific orthopaedic implants.

14. LIMITATIONS

In-vitro findings may not fully represent in-vivo conditions.

- Simulated body fluid cannot independently confirm clinical bone regeneration.
- Polycaprolactone degrades slowly.
- High nano-hydroxyapatite concentrations may cause agglomeration.
- Cell-line behaviour may differ from primary human-cell behaviour.
- Animal and clinical studies will be required before therapeutic application.

15. Future Scope:

Future studies may explore:

- collagen- or chitosan-modified scaffolds;
- magnesium-, zinc- or strontium-doped hydroxyapatite;
- antibacterial composite scaffolds;
- growth-factor delivery;
- stem-cell-loaded constructs;
- gradient and multilayer scaffolds;
- vascularised scaffold systems;
- triply periodic minimal-surface designs; and
- patient-specific implants produced from medical imaging.

16. CONCLUSION

Three-dimensional printed nano-hydroxyapatite composite scaffolds offer a promising approach for bone-tissue regeneration. Polycaprolactone provides structural continuity and printability, whereas nano-hydroxyapatite contributes mineral similarity, osteoconductivity and surface bioactivity.

The proposed research will evaluate multiple scaffold formulations to determine an optimum balance among printability, porosity, strength, biodegradation and osteogenic activity. Its major contribution lies in treating scaffold composition and architecture as interconnected factors.

An optimised composite scaffold may provide a stable yet gradually degradable framework capable of supporting cell attachment, mineralisation and new-bone formation. With further in-vivo validation, the developed system may become a useful platform for customised bone-regeneration therapies.

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