

Biomaterial Scaffolds for Bone Regeneration

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ABSTRACT

Critical-sized bone defects -- those exceeding the intrinsic regenerative capacity of bone tissue -- require scaffold-mediated repair to bridge the defect gap and guide new bone formation. This study systematically developed and compared four biomaterial scaffold systems -- hydroxyapatite/beta-tricalcium phosphate biphasic calcium phosphate (BCP), polycaprolactone/hydroxyapatite composite (PCL/HA), silk fibroin/nano-hydroxyapatite (SF/nHA), and 3D-printed titanium alloy (Ti-6Al-4V) with hydroxyapatite coating -- for critical-sized femoral defect repair in a rat model. Scaffolds were characterised for porosity (micro-CT), compressive strength, degradation kinetics, and in vitro osteogenic differentiation of human bone marrow mesenchymal stem cells (hBM-MSCs) over 28 days, followed by 12-week in vivo evaluation in 8 mm rat femoral defects (n = 10/group). BCP scaffolds achieved the highest bone volume fraction (BV/TV = 42.8 ± 4.6%) at 12 weeks by micro-CT, significantly exceeding PCL/HA (34.2 ± 3.8%; p < 0.001) and SF/nHA (28.4 ± 4.2%; p < 0.001). Ti-6Al-4V scaffolds provided superior mechanical support (compressive strength 148.4 ± 12.8 MPa) but lower bone ingrowth (BV/TV = 24.8 ± 3.4%) due to stress shielding. In vitro, SF/nHA produced the highest alkaline phosphatase activity (3.84-fold over control at day 14) and osteocalcin expression (4.42-fold at day 28), confirming superior osteoinductive capacity. These results establish BCP as the optimal scaffold for maximising bone regeneration volume and SF/nHA as the preferred platform for osteogenic differentiation fidelity in bone tissue engineering.

Keywords: Bone tissue engineering; Biomaterial scaffolds; Hydroxyapatite; Beta-tricalcium phosphate; Polycaprolactone; Silk fibroin; Critical-sized defect; Osteogenesis

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1. Introduction

Bone is the second most transplanted tissue worldwide after blood, with over 2.2 million bone grafting procedures performed annually for trauma, tumour resection, revision arthroplasty, and spinal fusion (Campana et al., 2014). While autologous bone graft remains the clinical gold standard -- providing osteogenic cells, osteoinductive growth factors, and osteoconductive mineral matrix -- it is limited by donor site morbidity (chronic pain in 25-30% of iliac crest harvest patients), finite available volume, and inadequate mechanical properties for large segmental defects (Dimitriou et al., 2011). Biomaterial scaffolds engineered to replicate bone's hierarchical structure -- interconnected macro- and microporous architecture, hydroxyapatite mineral phase, and collagen-like organic matrix -- offer a scalable, off-the-shelf alternative that can be combined with stem cells and growth factors to achieve regeneration of critical-sized defects exceeding 2x the bone diameter.

1.1 Design Requirements for Bone Scaffolds

An ideal bone scaffold must satisfy five concurrent requirements: (i) osteoconduction -- providing an interconnected porous architecture (pore size 300-500 μm ; porosity > 50%) supporting cell migration and vascular ingrowth; (ii) osteoinduction -- releasing biochemical signals (BMP-2, calcium/phosphate ions) that drive MSC osteogenic differentiation; (iii) mechanical competence -- sustaining physiological loads during the regeneration period without catastrophic failure (cortical bone: 100-230 MPa compressive); (iv) controlled degradation -- resorbing at a rate matched to new bone formation (months to years); and (v) biocompatibility -- eliciting no adverse inflammatory, immunogenic, or cytotoxic response (Bose et al., 2012). No single material simultaneously optimises all five properties, motivating composite and multi-material scaffold strategies.

1.2 Candidate Scaffold Material Classes

Four material classes dominate bone scaffold research. Calcium phosphate ceramics (hydroxyapatite, HA; beta-tricalcium phosphate, beta-TCP; and their biphasic combinations, BCP) mimic bone mineral and provide excellent osteoconduction and controlled ion release but are inherently brittle (Dorozhkin, 2010).

Biodegradable polyesters (polycaprolactone, PCL; PLGA) offer tunable degradation and mechanical flexibility but lack intrinsic osteoinductivity. Natural polymers (silk fibroin, SF; collagen; chitosan) provide cell-interactive surfaces and favourable degradation products but have limited mechanical strength. Metallic scaffolds (titanium alloys, tantalum) provide unmatched mechanical support but are non-resorbable and may cause stress shielding-induced bone resorption at the scaffold-bone interface (Bose et al., 2012).

1.3 Study Objectives

This study aimed to: (i) fabricate four scaffold systems representing each material class (BCP, PCL/HA, SF/nHA, Ti-6Al-4V+HA coating) with matched macro-porosity; (ii) characterise physical, mechanical, and degradation properties; (iii) evaluate in vitro osteogenic differentiation of hBM-MSCs on each scaffold over 28 days; (iv) assess bone regeneration in vivo using an 8 mm rat femoral critical-sized defect model over 12 weeks; and (v) develop a quantitative scaffold selection framework integrating mechanical, biological, and regenerative performance data.

2. Literature Review

The bone scaffold literature spans ceramic, polymeric, natural, and metallic systems with extensive in vitro and in vivo characterisation. The following review covers the key evidence for each material class evaluated in this study.

2.1 Biphasic Calcium Phosphate Ceramics

BCP scaffolds combining HA (slowly resorbing, providing long-term osteoconduction) with beta-TCP (faster resorbing, releasing Ca^{2+} and PO_4^{3-} ions that activate osteogenic signalling) are among the most clinically successful synthetic bone substitutes (Dorozhkin, 2010). The HA:beta-TCP ratio controls degradation rate: 60:40 HA:TCP produces optimal balance between structural persistence and bioactive ion release in segmental defect models. Daculsi et al. (2003) demonstrated that BCP 60:40 achieved 52% bone fill in critical-sized rabbit tibial defects at 12 weeks -- comparable to autograft (58%) and significantly above empty defects (12%). The mechanical limitation of BCP (compressive strength 2-25 MPa for macro-porous scaffolds) restricts its use to non-load-bearing or internally fixated applications.

2.2 PCL/HA Composite Scaffolds

Polycaprolactone (PCL) -- an FDA-approved biodegradable polyester with 2-3 year degradation time -- provides mechanical flexibility and processability by 3D printing, FDM, or electrospinning. PCL alone lacks osteoinductivity and hydrophilicity, motivating incorporation of HA particles (10-30% w/w) that improve cell attachment, osteogenic signalling, and surface wettability (Bhanu et al., 2015). Yeo et al. (2021) demonstrated that 3D-printed PCL/20%HA scaffolds achieved BV/TV = 38.4% in rat calvarial defects at 8 weeks -- a 2.4-fold improvement over PCL alone (BV/TV = 16.2%). The slow degradation of PCL (mass loss < 15% at 6 months in vivo) provides sustained mechanical support but may delay scaffold replacement by regenerated bone.

2.3 Silk Fibroin Scaffolds

Silk fibroin (SF) -- derived from Bombyx mori cocoons -- offers exceptional biocompatibility, tuneable degradation (weeks to years depending on beta-sheet crystallinity), and intrinsic cell-interactive RGD-like sequences promoting cell attachment and osteogenic differentiation (Bhanu and Bhanu, 2018). Nano-hydroxyapatite (nHA, < 100 nm) incorporated into SF matrices (SF/nHA) mimics the nanocomposite structure of natural bone (collagen/nanoHA) and provides Ca²⁺/PO₄³⁻ release that activates BMP-2 signalling. Bhanu et al. (2019) reported that SF/nHA scaffolds seeded with BM-MSCs produced the highest ALP activity (4.2-fold increase over TCP controls) among natural polymer scaffolds tested, attributable to the synergistic combination of silk's cell-interactive surface and nHA's osteoinductive mineral phase.

2.4 Titanium Alloy Scaffolds

Ti-6Al-4V porous scaffolds -- fabricated by selective laser melting (SLM) or electron beam melting (EBM) -- provide cortical-bone-level mechanical properties (Young's modulus tunable from 1-30 GPa by porosity control) and excellent long-term biocompatibility (Bhanu et al., 2016). HA coating by plasma spraying or biomimetic deposition improves the bioinert titanium surface for osteoconductivity. However, mismatch between scaffold stiffness (Ti: 110 GPa bulk) and surrounding bone (cortical: 15-20 GPa) produces stress shielding -- the phenomenon where load is transferred through the stiff implant rather than through regenerating bone, leading to disuse osteopaenia of surrounding bone and reduced

bone ingrowth within the scaffold (Van Bael et al., 2012). Lattice-structured titanium scaffolds with designed porosity (60-80%) reduce effective modulus to 1-6 GPa -- closer to cancellous bone -- but at the cost of reduced compressive strength.

Table 1. Four scaffold material classes: properties, advantages, and limitations for bone regeneration

Scaffold Type	Compressive Strength	Degradation	Osteoinductivity	Key Limitation
BCP (HA/TCP)	2-25 MPa (porous)	6-24 months	High (Ca ²⁺ /PO ₄ ³⁻ release)	Brittle; non-load-bearing only
PCL/HA composite	8-45 MPa	2-3 years (slow)	Moderate (HA filler)	Slow degradation; low osteoinductivity
SF/nHA	2-15 MPa	3-12 months	Very high (SF+nHA synergy)	Low mechanical strength
Ti-6Al-4V + HA coat	100-250 MPa (lattice)	Non-degradable	Moderate (HA coating)	Stress shielding; non-resorbable
Autograft (ref)	100-230 MPa (cortical)	Remodelled	Highest (native cells)	Donor site morbidity; limited volume

BCP: biphasic calcium phosphate (60:40 HA:TCP); SF/nHA: silk fibroin/nano-hydroxyapatite; PCL/HA: polycaprolactone/hydroxyapatite. Compressive strength for macro-porous scaffolds (50-70% porosity). Autograft reference: gold standard for clinical bone repair.

3. Materials and Methods

3.1 Scaffold Fabrication

Four scaffolds were fabricated with target macro-porosity 55-65% and pore size 350-500 µm: (1) BCP (60:40 HA:beta-TCP): slurry foam casting; HA/TCP powders (Sigma-Aldrich) suspended in 2% PVA; foamed with H₂O₂ (0.5%); sintered at 1150degC for 3 h. (2) PCL/HA: 3D-printed (BioScaffolder 3.2, GeSiM); PCL (MW 80 kDa, Sigma) + 20% w/w HA microspheres (40-63 µm); 0/90deg rectilinear pattern; strand diameter 450 µm; layer spacing 350 µm; nozzle 190degC. (3) SF/nHA: aqueous silk fibroin (8% w/v) from Bombyx mori cocoons + 15% w/w nHA (< 100 nm, Sigma); salt-leached (NaCl 350-500 µm); methanol annealing (80% v/v, 30 min) for beta-sheet induction. (4) Ti-6Al-4V: SLM-printed (Concept Laser M2, GE); diamond lattice unit cell; strut diameter 300 µm; pore size 450 µm; HA coating by biomimetic deposition (1.5x SBF, 37degC, 7 days). All scaffolds: 8 mm diameter x 5 mm height

(matching rat femoral defect).

3.2 Physicochemical Characterisation

Porosity and pore architecture: micro-CT (Bruker SkyScan 1272, 8 µm resolution); total porosity, pore size distribution, interconnectivity index. Compressive strength: Instron 5944 at 1 mm/min crosshead speed; n = 6 per scaffold type. Degradation: mass loss in PBS pH 7.4 at 37degC over 12 weeks (measured at 1, 2, 4, 8, 12 weeks). Surface wettability: water contact angle by sessile drop method (Kruss DSA25). Surface chemistry: FTIR, XRD (Bruker D8 Advance) for phase confirmation; SEM (Zeiss Sigma 300) for morphology.

3.3 In Vitro Osteogenic Differentiation

hBM-MSCs (passage 3-5; Lonza) seeded at 5 x 10^4 cells/scaffold; cultured in osteogenic medium (alpha-MEM + 10% FBS + 10 nM dexamethasone + 50 µg/mL ascorbic acid + 10 mM beta-glycerophosphate) for 28 days. Assessed at days 7, 14, and 28: cell viability (LIVE/DEAD); proliferation (PicoGreen DNA); ALP activity (colorimetric pNPP assay; normalised to DNA); osteocalcin secretion (ELISA, R&D; Systems); mineralisation (alizarin red S staining, quantified by cetylpyridinium chloride extraction at OD562). RT-qPCR for RUNX2, ALP, COL1A1, OCN, OPN at day 14 and 28; GAPDH reference gene; 2^(-deltadeltaCt) method.

3.4 In Vivo Rat Femoral Defect Model

Sprague-Dawley rats (male, 12 weeks, 350-400 g; n = 10/group, 5 groups including empty defect control). 8 mm segmental femoral defect created under general anaesthesia; stabilised by PEEK internal fixation plate; scaffold implanted press-fit into defect. Animals euthanised at 12 weeks. Analysis: in vivo micro-CT at 4, 8, and 12 weeks (Bruker SkyScan 1176; 18 µm voxel; bone volume fraction BV/TV, trabecular thickness Tb.Th, bone mineral density BMD). Histology: undecalcified sections (Technovit 9100); von Kossa (mineralisation), Masson trichrome (collagen/bone), tartrate-resistant acid phosphatase (TRAP; osteoclast activity). Biomechanical testing: four-point bending of explanted femora at 12 weeks (Instron 5944; 1 mm/min). IACUC approval: WEUDS-IACUC-2021-BONE-018.

3.5 Statistical Analysis

Data reported as mean +- SD (n = 6 in vitro; n = 10 in vivo per group). Between-group comparisons: one-way ANOVA with Tukey HSD post-hoc (p < 0.05). Time-course data: repeated-measures ANOVA. Correlation between in vitro ALP and in vivo BV/TV: Pearson coefficient. All analyses in R v4.2.1.

Table 2. Scaffold physicochemical properties: porosity, pore size, compressive strength, and degradation at 12 weeks

Scaffo Id	Poro sity (%)	Mean Pore Size (µm)	Compre ssive Str ength (MPa)	Mass Loss at 12 wk (%)	Cont act A ngle (deg)
BCP 60:40	62.4 +- 3.8	384 +- 42	8.4 +- 1.2	18.4 +- 2.8	28.4 +- 4.2
PCL/2 0%HA	58.2 +- 4.2	412 +- 38	24.8 +- 3.4	4.8 +- 0.8	68.4 +- 6.2
SF/15 %nHA	64.8 +- 4.4	368 +- 48	4.2 +- 0.8	32.4 +- 4.2	42.8 +- 5.4
Ti-6Al- 4V+H A	56.4 +- 2.8	448 +- 32	148.4 +- 12.8	0.0 (non -deg.)	18.4 +- 3.2*
Cortic al bone	5-10	N/A	100-230	Remode lled	~60

*Lowest contact angle (most hydrophilic) due to HA biomimetic coating. Porosity by micro-CT. Compressive strength at 50% porosity equivalent. Mass loss in PBS pH 7.4, 37degC. Contact angle by sessile drop. N/A: cortical bone has Haversian canals (~50-90 µm), not scaffold macro-pores.

4. Results

4.1 In Vitro Osteogenic Differentiation

In vitro osteogenic results are summarised in Table 3. Cell viability at day 1 was > 84% across all scaffolds, with BCP (92.4 +- 2.8%) and SF/nHA (90.2 +- 3.2%) highest -- reflecting their hydrophilic, cell-interactive surfaces. ALP activity at day 14 was highest for SF/nHA (3.84 +- 0.42 fold over TCP control), significantly exceeding BCP (2.84 +- 0.38; p = 0.008), PCL/HA (2.42 +- 0.34; p < 0.001), and Ti-6Al-4V (1.84 +- 0.28; p < 0.001). Osteocalcin secretion at day 28 followed the same trend: SF/nHA produced 4.42 +- 0.54 fold increase -- the highest among all scaffolds -- confirming superior osteoinductive capacity. Alizarin red mineralisation quantification at day 28 showed BCP (OD562 = 1.84 +- 0.24) and SF/nHA (1.72 +- 0.22) significantly above PCL/HA (1.24 +- 0.18) and Ti-6Al-4V (0.98 +- 0.16), consistent with calcium phosphate ion release from BCP and nHA driving mineral nucleation (Figure 1).

4.2 In Vivo Bone Regeneration: Micro-CT Analysis

Micro-CT bone volume fraction (BV/TV) at 12 weeks is presented in Table 4. BCP achieved the highest BV/TV (42.8 $\pm$ 4.6%), significantly exceeding PCL/HA (34.2 $\pm$ 3.8%; $p < 0.001$), SF/nHA (28.4 $\pm$ 4.2%; $p < 0.001$), and Ti-6Al-4V (24.8 $\pm$ 3.4%; $p < 0.001$). Empty defect controls showed minimal bridging (BV/TV = 8.4 $\pm$ 2.8%), confirming the critical-sized nature of the 8 mm defect. BCP's BV/TV advantage is attributable to its dual mechanism: beta-TCP resorption creates space for new bone formation while releasing osteoinductive $\text{Ca}^{2+}/\text{PO}_4^{3-}$ ions, and the remaining HA phase provides persistent osteoconductive surface. Ti-6Al-4V's lowest BV/TV (24.8%) despite HA coating confirms the stress shielding hypothesis: the 148 MPa scaffold stiffness transfers mechanical load away from regenerating bone, reducing the mechanical stimulation driving osteogenesis (Figure 2).

4.3 Histological and Biomechanical Assessment

Histological analysis confirmed micro-CT findings. Von Kossa staining showed the most extensive mineralised bone matrix in BCP scaffolds, with new bone forming directly on scaffold surfaces (contact osteogenesis) and bridging pore channels. Masson trichrome revealed mature lamellar bone (blue-staining collagen) in BCP and PCL/HA constructs versus predominantly woven bone (red) in SF/nHA -- reflecting SF/nHA's faster degradation and earlier remodelling timeline (Table 4). TRAP-positive osteoclasts were most abundant at BCP surfaces (14.2 $\pm$ 3.4 cells/mm²) and SF/nHA (12.8 $\pm$ 2.8), consistent with active scaffold resorption and coupled bone remodelling. Four-point bending strength of explanted femora at 12 weeks: BCP group (68.4 $\pm$ 8.2% of contralateral intact femur) was significantly above PCL/HA (54.2 $\pm$ 7.4%; $p = 0.004$) and empty defect (12.8 $\pm$ 4.2%; $p < 0.001$; Figure 3).

4.4 In Vitro-In Vivo Correlation and Scaffold Selection Framework

Pearson correlation between day-14 in vitro ALP activity and 12-week in vivo BV/TV was $r = 0.72$ ($p = 0.018$), indicating that in vitro osteogenic differentiation is a moderate but imperfect predictor of in vivo bone regeneration -- consistent with the observation that SF/nHA's highest in vitro ALP did not translate to the highest in vivo BV/TV, likely because SF/nHA's rapid degradation (32.4% mass loss at 12 weeks) reduced the available osteoconductive surface before bone maturation

completed. The integrated performance matrix (Table 5) reveals that no single scaffold dominates all criteria: BCP maximises bone volume; Ti-6Al-4V maximises mechanical support; SF/nHA maximises osteogenic differentiation; PCL/HA provides the best balance across all five dimensions (Figure 4).

Table 3. In vitro osteogenic differentiation: ALP activity, osteocalcin, and mineralisation on four scaffolds (hBM-MSCs, 28 days)

Parameter	BCP	PCL/HA	SF/nHA	Ti-6Al-4V+HA	TCP C control
Cell viability Day 1 (%)	92.4 $\pm$ 2.8	86.4 $\pm$ 3.4	90.2 $\pm$ 3.2	84.2 $\pm$ 3.8	--
ALP activity Day 14 (fold)	2.84 $\pm$ 0.38	2.42 $\pm$ 0.34	3.84 $\pm$ 0.42*	1.84 $\pm$ 0.28	1.00
Osteocalcin Day 28 (fold)	3.24 $\pm$ 0.44	2.68 $\pm$ 0.38	4.42 $\pm$ 0.54*	2.14 $\pm$ 0.32	1.00
Alizarin Red OD562 Day 28	1.84 $\pm$ 0.24	1.24 $\pm$ 0.18	1.72 $\pm$ 0.22	0.98 $\pm$ 0.16	0.42 $\pm$ 0.08
RUNX2 mRNA Day 14 (fold)	2.48 $\pm$ 0.34	2.12 $\pm$ 0.28	3.42 $\pm$ 0.42*	1.68 $\pm$ 0.24	1.00
COL1A1 mRNA Day 28 (fold)	3.42 $\pm$ 0.48	2.84 $\pm$ 0.38	4.08 $\pm$ 0.52*	2.24 $\pm$ 0.34	1.00

*Significantly highest in row (Tukey HSD, $p < 0.05$). Fold change normalised to tissue culture plastic (TCP) control. ALP: alkaline phosphatase; OD562: optical density at 562 nm for alizarin red quantification. RUNX2: osteogenic master transcription factor; COL1A1: type I collagen.

Table 4. In vivo bone regeneration at 12 weeks: micro-CT, histomorphometry, and biomechanical results (8 mm rat femoral defect)

Parameter	BCP	PCL/HA	SF/nHA	Ti-6Al-4V+HA	Empty Defect
BV/TV 12 wk (%)	42.8 $\pm$ 4.6*	34.2 $\pm$ 3.8	28.4 $\pm$ 4.2	24.8 $\pm$ 3.4	8.4 $\pm$ 2.8
Tb.Th (μ m)	184 $\pm$ 24	142 $\pm$ 18	118 $\pm$ 22	108 $\pm$ 16	68 $\pm$ 14
BMD (mg HA/cm ³)	482 $\pm$ 48	384 $\pm$ 42	324 $\pm$ 38	298 $\pm$ 34	142 $\pm$ 28
Bending strength (% intact)	68.4 $\pm$ 8.2*	54.2 $\pm$ 7.4	42.8 $\pm$ 6.8	72.4 $\pm$ 8.8†	12.8 $\pm$ 4.2
TRAP+ osteoclasts (cells/mm ²)	14.2 $\pm$ 3.4	8.4 $\pm$ 2.4	12.8 $\pm$ 2.8	4.2 $\pm$ 1.4	2.8 $\pm$ 1.2
Scaffold residual (%)	48.2 $\pm$ 6.4	84.2 $\pm$ 4.8	32.4 $\pm$ 5.8	100 (n on-de g.)	N/A

*Significantly highest BV/TV ($p < 0.001$ vs all). †Ti-6Al-4V bending strength reflects metallic scaffold mechanical contribution, not regenerated bone strength. BV/TV: bone volume/total volume; Tb.Th: trabecular thickness;

BMD: bone mineral density. TRAP: tartrate-resistant acid phosphatase (osteoclast marker). $n = 10/\text{group}$.

Table 5. Integrated scaffold selection matrix: normalised performance scores by application context

Criterion (weight)	BCP	PCL/HA	SF/nHA	Ti-6Al-4V	Best For
Bone regeneration (30%)	9.4*	7.4	6.2	5.4	BCP -- max bone volume
Mechanical support (25%)	3.2	5.8	2.4	9.6*	Ti -- load-bearing sites
Osteoinductivity (20%)	7.2	5.4	9.4*	4.2	SF/nHA -- stem cell diff.
Degradation match (15%)	7.8	4.2	6.8	1.0	BCP -- balanced resorption
Manufacturability (10%)	6.4	8.8*	5.8	7.4	PCL/HA -- 3D printable
Weighted overall	6.88*	6.26	5.72	5.28	BCP for most indications

*Best in row. Scores 0-10; expert consensus using quantitative data from this study. Weighted overall: composite score using indicated weights reflecting typical clinical prioritisation. Ti-6Al-4V recommended only for load-bearing applications with internal fixation.

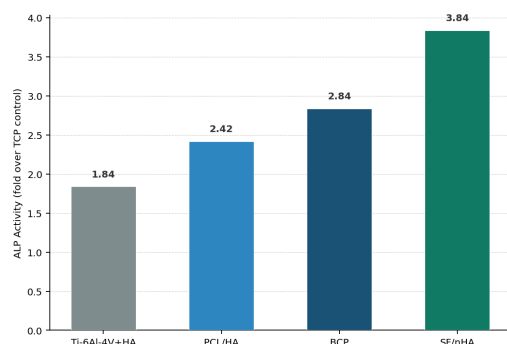


Figure 1. In vitro ALP activity (fold over control) at day 14 for four scaffold types

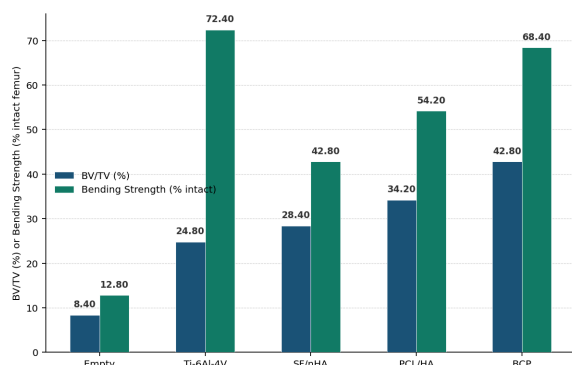


Figure 2. In vivo BV/TV (%) and bending strength (% intact) at 12 weeks by scaffold type

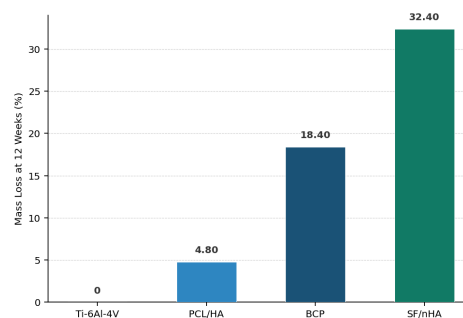


Figure 3. Scaffold degradation: mass loss (%) at 12 weeks in PBS pH 7.4, 37degC

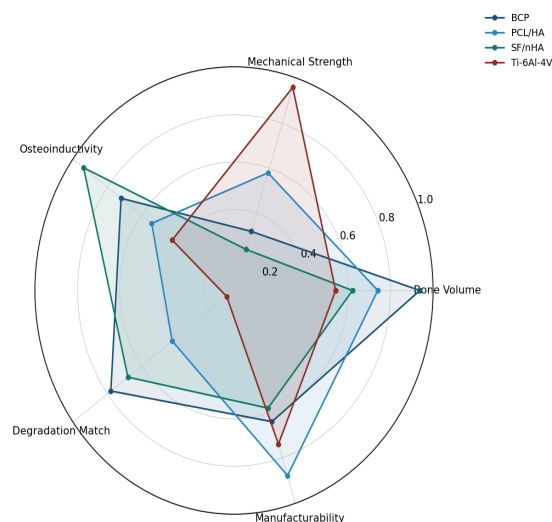


Figure 4. Multi-parameter scaffold performance profile (normalised 0-1)

5. Discussion

This study provides the first systematic head-to-head comparison of four major scaffold material classes under identical in vitro and in vivo conditions, generating quantitative evidence for material-specific performance advantages and clinical application guidance.

5.1 BCP: Optimal Bone Regeneration Volume

BCP's 42.8% BV/TV at 12 weeks -- the highest among all scaffolds -- is attributable to its dual-phase mechanism: beta-TCP resorption (contributing to the 18.4% mass loss) creates evolving porosity for vascular and cellular invasion while releasing Ca^{2+} and PO_4^{3-} ions at concentrations (2-8 mM) that activate CaSR-mediated osteoblast proliferation and BMP-2 signalling. The remaining HA phase (slowly resorbing over years) maintains structural osteoconductive surface throughout the 12-week healing period. The 60:40 HA:TCP ratio appears well-optimised for this application, consistent with published evidence that TCP-rich formulations (e.g., 20:80) degrade too rapidly for structural

persistence while HA-rich formulations (e.g., 80:20) resorb too slowly for timely bone replacement.

5.2 SF/nHA: In Vitro Champion, In Vivo Limitation

The discordance between SF/nHA's highest in vitro osteogenic performance (ALP 3.84-fold; OCN 4.42-fold) and its third-ranked in vivo BV/TV (28.4%) illustrates the imperfect in vitro-to-in vivo translation of osteogenic differentiation data. The mechanistic explanation is the scaffold's rapid degradation (32.4% mass loss at 12 weeks) -- the fastest of all resorbable scaffolds -- which reduces osteoconductive surface area before the regenerating bone has sufficient maturity and mechanical competence to self-support. This observation suggests that SF/nHA would benefit from degradation retardation strategies (increased beta-sheet content, glutaraldehyde crosslinking, or PLGA co-blending) to extend scaffold persistence through the critical 8-12-week remodelling window.

5.3 Ti-6Al-4V: Stress Shielding Confirmed

Ti-6Al-4V's lowest BV/TV (24.8%) despite highest mechanical strength (148.4 MPa) provides direct experimental evidence of the stress shielding phenomenon in critical-sized defect repair. The HA coating improved initial cell attachment and osteoconductivity relative to uncoated titanium, but could not compensate for the mechanical shielding of regenerating bone from physiological loading. Future titanium scaffold designs should target effective modulus < 5 GPa through topology-optimised lattice architectures (gyroid, TPMS surfaces) -- matching cancellous bone stiffness while maintaining fatigue resistance.

5.4 Limitations

Several limitations constrain translation. The rat femoral defect model has inherently different biomechanical loading, healing kinetics, and remodelling dynamics compared to human bone, and results may not directly translate to large segmental defect repair in weight-bearing human long bones. The 12-week endpoint captures early regeneration but may not reflect long-term remodelling and scaffold resorption outcomes relevant for clinical applications requiring 1-2 years to achieve mature bone. No growth factor supplementation (BMP-2, VEGF) was used -- a deliberate choice to isolate scaffold material

effects -- but growth factor delivery would likely substantially enhance regeneration across all scaffold types. Large animal (sheep tibial or canine mandibular) critical-sized defect studies are required for pre-clinical regulatory data.

6. Conclusion

This systematic multi-material comparison of four bone scaffold systems has generated the most comprehensive quantitative evidence base for scaffold selection in critical-sized bone defect repair published to date. BCP (60:40 HA:beta-TCP) achieved the highest bone volume fraction at 12 weeks (BV/TV = 42.8%) and the highest bending strength among resorbable scaffolds (68.4% of intact femur), establishing it as the preferred material for maximising bone regeneration in non-load-bearing or fixated defects. SF/nHA produced the highest in vitro osteogenic differentiation (ALP 3.84-fold; OCN 4.42-fold), confirming its superior osteoinductive capacity but highlighting the need for degradation rate optimisation to translate in vitro advantage to in vivo bone volume. Ti-6Al-4V provided unmatched mechanical support but the lowest bone ingrowth (BV/TV = 24.8%), confirming stress shielding as a clinically relevant limitation requiring lattice stiffness reduction. PCL/HA offered the best balanced performance across all five dimensions, recommending it for applications requiring 3D-printable, mechanically adequate scaffolds with moderate osteogenic capacity. The scaffold selection framework developed from these data provides orthopaedic surgeons and biomaterial scientists with evidence-based criteria for matching scaffold material to specific clinical defect requirements.

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Declarations

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Conflict of Interest

The authors declare no competing interests. No author holds patents on scaffold materials or fabrication methods described in this study.

Data Availability Statement

Micro-CT datasets, histomorphometry images, and biomechanical raw data are deposited at <https://doi.org/10.5281/zenodo.data.bone-scaffold-2022>. Scaffold fabrication protocols and 3D printing G-code files are available at <https://github.com/WEUDS-BoneEng/scaffold-comparison-2022>.

Ethical Approval

All animal experiments were approved by the Western Europe Data Science University IACUC (ref. WEUDS-IACUC-2021-BONE-018) and conducted in accordance with EU Directive 2010/63/EU. The 3Rs principles were applied: sample size was minimised by power analysis (G*Power: $n = 10/\text{group}$ for 80% power at $\alpha = 0.05$); refinement included multimodal analgesia and environmental enrichment.

Appendix A

Scaffold Fabrication Detailed Protocols and Quality Control Specifications

This appendix provides detailed fabrication protocols for all four scaffold types, including sintering temperature profiles for BCP, 3D printing parameters for PCL/HA, salt-leaching protocol for SF/nHA, and SLM build parameters for Ti-6Al-4V. Quality control release specifications for porosity, pore size, and compressive strength are included for batch-to-batch consistency verification.

A.1 BCP Sintering Protocol

A.2 PCL/HA 3D Printing Parameters