Original Article

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Dentistry Section

Cuttlebone-derived Hydroxyapatite in Bone Tissue Engineering: A Systematic Review

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ABSTRACT

Introduction: Bone tissue engineering seeks to overcome limitations of conventional grafts in treating craniofacial and orthopaedic defects. Hydroxyapatite (HA) is a widely used substitute, but synthetic forms lack bioactive ions and remodeling capacity. Marine-derived biomaterials, especially Cuttlebone (CB), offer a natural architecture and trace elements that enhance osteogenesis.

Aim: To evaluate the regenerative potential of Cuttlebone-derived Hydroxyapatite (CB-HA) in preclinical in-vitro and in-vivo models.

Materials and Methods: Following Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines, a systematic search of PubMed, Scopus, Web of Science, and EBSCO was performed for studies published between 2005 and 2025. Eligible articles investigated CB-HA or its composites in bone regeneration. Data were extracted using the PICO framework, and quality was assessed with the modified SYRCLE tool.

Results: The present systematic review included 18 studies. In-vitro experiments demonstrated that CB-HA promoted osteoblast proliferation, alkaline phosphatase activity, mineral deposition, and osteogenic gene expression compared with controls. In-vivo studies in rat and rabbit models confirmed greater new bone volume, mineralisation, and vascularisation. Composites incorporating polymers or ion substitutions (Si, Sr, Mg, Mn) further improved bioactivity and mechanical performance. No adverse local or systemic effects were reported.

Conclusion: CB-HA shows superior osteogenic potential over conventional substitutes, attributed to its biomimetic architecture, porosity, and favourable surface chemistry. With standardised fabrication and long-term animal studies, CB-HA holds promise for future clinical translation in orthopaedic, craniofacial, and dental applications.

Keywords: Bone graft substitutes, Bone regeneration, Marine biomaterials

INTRODUCTION

Bone tissue constitutes about 15% of the total body weight and serves as the body's largest organ system [1]. It is composed of a dense outer cortical layer, which provides mechanical strength and support; and an inner cancellous layer with a honeycomb-like trabecular architecture contributing to its 80-90% porosity, facilitating metabolic activities such as mineral exchange and bone remodeling [2]. Bone not only maintains structural integrity, enables movement, protects vital organs but also regulates mineral homeostasis. However, its multifunctional nature also makes it vulnerable to injury and disease [3].

In clinical contexts, bone defects are commonly encountered in orthopaedic, reconstructive and dental specialties, arising from trauma, neoplastic resections, infections, degenerative disorders or congenital anomalies [4]. Specifically, craniomaxillofacial bone defects span a wide spectrum from minor alveolar bone loss and periodontal defects to severe skeletal discontinuities due to trauma or surgical resection. The restoration of such defects is particularly challenging due to the intricate three-dimensional architecture required for both functional and aesthetic rehabilitation. Failure to adequately reconstruct these defects may result in distortion of the adjacent tissues, compromised function and poor patient outcomes [5].

The repair of these bone defects necessitates the use of biomaterial-assisted regenerative strategies. While autogenous bone grafts remain the gold standard due to their osteoconductive, osteoinductive and osteogenic properties, their use is restricted by donor site morbidity, limited availability and risk of postoperative complications. Hence, alternatives like allografts, xenografts and alloplasts are considered [6].

Among alloplastic materials, HA with the composition of $Ca_{10}(PO_4)_6(OH)_2$- stands out for its close resemblance to the mineral phase of human bone. It promotes bone growth via osteoconduction and is biocompatible with no adverse local or systemic effects [7,8]. While synthetic HA offers high crystallinity and controlled Ca/P ratios, its drawbacks include poor resorption, lack of remodeling, and absence of bioactive trace elements such as Fe^{2+}, Mg^{2+}, Si^{2+}, Na^{+}, and F^{-} essential for bone metabolism [9,10].

To address these shortcomings, researchers have increasingly turned to biogenic sources of HA, mainly from marine-derived materials- including coral, fish bones, mollusk shells, sea urchins and CB [11,12]. These closely mimic natural bone in composition, retain native tissue architecture, and include beneficial trace elements, making it more bioactive, resorbable, and cost-effective. Its lower crystallinity enhances solubility and promotes better bone integration [13].

Among various marine-derived materials, CB from *Sepia officinalis* has emerged as a promising biogenic source for HA synthesis. Its $CaCO_3$-based aragonite composition, along with a well-organised lamellar architecture- featuring septae separated by distorted, S-shaped walls- makes CB highly suitable for scaffold fabrication and HA conversion [14]. Unlike other marine biomaterials, CB can be converted into CB-HA via hydrothermal conversion, wet chemical precipitation, or calcination, while retaining its native structure [15]. CB exhibits high porosity, mechanical strength, and biocompatibility, rendering it superior to other marine bioceramics. These properties facilitate cell growth, osteoconductivity, and bone integration, reinforcing its potential in bone defect repair and regeneration [16,17].

CB-HA scaffolds have shown promising results in in-vitro studies promoting osteoblast adhesion, ALP activity, and mineral deposition, as well as in animal models, demonstrating new bone formation and vascularisation. Despite growing interest, there remains a lack of comprehensive evaluation of CB-HA's biological performance, synthesis methods, and potential as a clinically translatable scaffold. Therefore, this systematic review was guided by the research question: "Does CB-HA exhibit regenerative potential?"

MATERIALS AND METHODS

The present systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and has been registered with the International Prospective Register of Systematic Reviews (PROSPERO) under the ID CRD420251085563.

Literature Search

A comprehensive literature search was conducted across leading scientific databases, including Scopus, PubMed, EBSCO and Web of Science, using predefined keywords, with the results summarised in [Table/Fig-1]. To ensure thoroughness and sensitivity, the search strategy incorporated a combination of controlled vocabulary (MeSH terms), synonymous expressions, alternate spellings, and relevant keywords. Boolean operators ("AND" and "OR") were employed to optimise retrieval of pertinent studies.

Database	Keywords used	Articles hit per database
Scopus	TITLE-ABS-KEY(cuttlebone) AND bone regeneration OR osteogenesis AND PUBYEAR > 2004 AND PUBYEAR<2026	61
PubMed	(((cuttlefish) OR (cuttlefish bone)) OR (cuttlebone)) AND (Hydroxyapatite)) AND ((((((((bone regeneration) OR (bone graft)) OR (osteogenic potential)) OR (osteogenesis)) OR (bone formation)) OR (bone substitute)) OR (Regeneration, Bone)) OR (Regenerations, bone)) OR (Osteoconduction))	34
EBSCO	cuttlefish OR cuttlebone AND bone regeneration OR osteogenic potential	72
Web of Science	Cuttlebone (CB), Hydroxyapatite (HA), Bone regeneration	102

[Table/Fig-1]: Summary of database search strategy and retrieved articles.

Inclusion and Exclusion criteria: Studies were included if they were original research articles assessing the osteogenic or regenerative potential of CB-HA or its composites, using either in-vitro cell models or in-vivo animal models. Only full-text articles published in English were considered. Studies were excluded if they did not focus on osteogenesis and bone regeneration, if CB-HA or its composites were not the main intervention, or if they were non-original works (e.g., reviews, pilot studies, abstracts, editorials, or opinion pieces). Articles without full text or not published in English were also excluded.

Study Procedure

Data were extracted using a structured form guided by the PICO framework.

In-vitro studies:

- **Population:** Relevant cell lines
- **Intervention:** CB-HA used alone or in combination with other materials
- **Comparator:** Commercial synthetic HA, xenograft-derived HA (e.g., bovine/porcine), or standard control groups
- **Outcome:** Osteogenic indicators including ALP activity, mineral deposition, and gene expression related to osteogenesis

In-vivo Studies:

- **Population:** Animal models with critical-sized bone defects, fractures, or skeletal deficiencies
- **Intervention:** CB-HA or its composite formulations
- **Comparator:** Commercial HA, xenograft-derived materials, or standard control scaffolds
- **Outcome:** Bone regeneration outcomes such as new bone volume, bone density, histological findings and histomorphometric analysis

To ensure the inclusion of contemporary research and evolving trends, the search was confined to studies published between 2005 and 2025.

Study selection followed three phases: Title screening, title/abstract screening, and full-text review, performed independently by two reviewers (A and B), with any conflicts resolved by a third reviewer (C). References were managed in Mendeley with duplicates removed, and the process is summarised in the PRISMA flowchart [Table/Fig-2]. Study quality was evaluated using the modified SYRCLE tool (in-vitro- [Table/Fig-3]) and the SYRCLE Risk of Bias tool (in-vivo- [Table/Fig-4]).

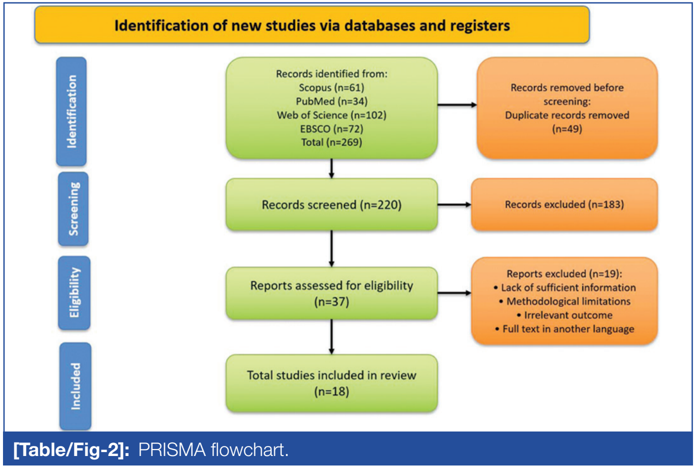
Quantitative Analysis

The included in-vitro and in-vivo studies showed wide variation in cell types, animal models, defect sizes, scaffold fabrication, material composition, and assessment methods. Owing to this heterogeneity, a meta-analysis was not feasible. Instead, results are presented separately for in-vitro and in-vivo studies in a descriptive, narrative form, enabling qualitative rather than pooled statistical comparisons.

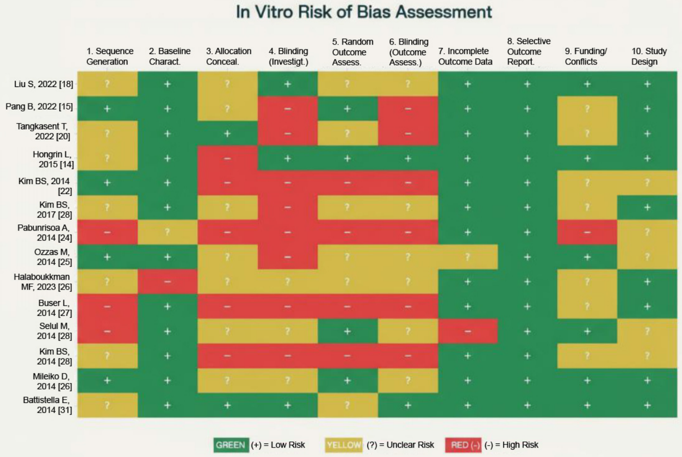
Qualitative Analysis

The qualitative synthesis was performed to assess CB-HA's regenerative potential across the following domains:

- Material characteristics- Examining fabrication techniques, porosity, crystallinity and bioactive element content of CB-HA and its composites.



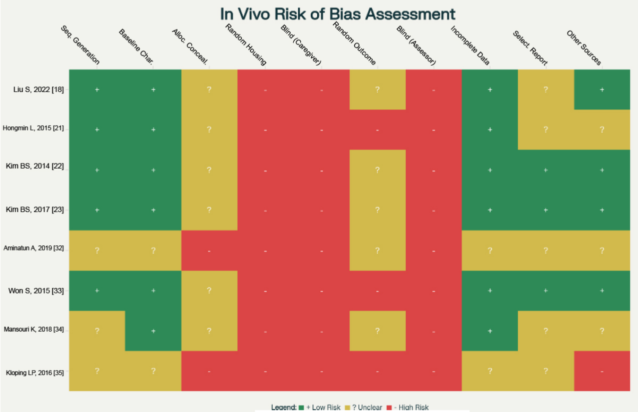
[Table/Fig-2]: PRISMA flowchart.


In Vitro Risk of Bias Assessment

	1. Sequence Generation	2. Baseline Charact.	3. Allocation Conceal.	4. Blinding (Investigt.)	5. Random Outcome Assess.	6. Blinding (Outcome Assess.)	7. Incomplete Outcome Data	8. Selective Outcome Report.	9. Funding/ Conflicts	10. Study Design
Liu S, 2022 [18]	?	+	?	+	?	?	+	+	+	+
Pang B, 2022 [15]	+	+	?	−	+	−	+	+	?	+
Tangkasent T, 2022 [20]	?	+	+	−	?	−	+	+	?	+
Hongrin L, 2015 [14]	?	+	−	+	+	+	+	+	+	+
Kim BS, 2014 [22]	+	+	−	−	−	−	+	+	?	?
Kim BS, 2017 [28]	?	+	?	−	?	?	+	+	?	+
Pabunrisoa A, 2014 [24]	−	?	−	−	−	−	+	+	−	?
Ozzas M, 2014 [25]	+	+	?	−	?	?	?	+	+	?
Halaboukkman MF, 2023 [26]	?	−	?	?	?	?	+	+	?	+
Buser L, 2014 [27]	−	+	−	−	−	−	+	+	?	+
Selul M, 2014 [28]	−	+	?	?	+	?	−	+	+	?
Kim BS, 2014 [28]	?	+	−	−	−	−	+	+	?	?
Mileiko D, 2014 [26]	+	+	?	?	+	?	+	+	+	+
Battistella E, 2014 [31]	?	+	+	+	?	+	+	+	+	+

🟩 GREEN (+) = Low Risk 🟨 YELLOW (?) = Unclear Risk 🟥 RED (-) (-) = High Risk

[Table/Fig-3]: Risk of Bias chart for in-vitro studies.



In Vivo Risk of Bias Assessment

	Seq. Generation	Baseline Char.	Alloc. Conceal.	Random Housing	Blind (Caregiver)	Random Outcome	Blind (Assessor)	Incomplete Data	Select. Report	Other Sources
Liu S, 2022 [18]	+	+	?	-	-	?	-	+	?	+
Hongmin L, 2015 [21]	+	+	?	-	-	-	-	+	?	?
Kim BS, 2014 [22]	+	+	?	-	-	?	-	+	+	+
Kim BS, 2017 [23]	+	+	?	-	-	?	-	+	+	+
Aminatun A, 2019 [32]	?	?	-	-	-	?	-	?	?	?
Won S, 2015 [33]	+	+	?	-	-	-	-	+	+	+
Mansouri K, 2018 [34]	?	+	?	-	-	?	-	+	?	?
Kloping LP, 2016 [35]	?	?	-	-	-	-	-	?	?	-

Legend: 🟩 + Low Risk 🟨 ? Unclear 🟥 - High Risk

[Table/Fig-4]: Risk of Bias chart for in-vivo studies.

- Biological performance- Reviewing cytocompatibility, osteogenic differentiation, mineralisation and histological evidence of bone formation in both in-vitro and in-vivo settings.
- Translational relevance - Contextualising experimental models, defect types, and follow-up durations to evaluate clinical applicability and identify future research needs.

RESULTS

From 269 retrieved articles, 49 duplicates were removed, leaving 220 for screening. After reviewing 37 full texts, 18 studies fulfilled the inclusion criteria [Table/Fig-1]. These were published between 2005 and 2025, with in-vitro and in-vivo experiments analysed separately.

The details of cell types, scaffolds, fabrication methods, models, defect sizes, and follow-up periods are summarised in [Table/Fig-5] [18-30] (in-vitro) and [Table/Fig-6] [18,21-23,31-34] (in-vivo). In-vitro models predominantly included MC3T3-E1, MG-63, human MSCs, DPSCs, and rat BMSCs. In-vivo studies involved rat and rabbit calvarial, tibial, and ectopic defects (1-10 mm), with follow-ups of 2-12 weeks. CB-HA was fabricated using hydrothermal conversion, ball milling, or calcination, often combined into composites with polymers or trace elements.

Author, year	Material preparation	Study model	Groups	Tests conducted	Key findings
Liu S et al., 2022 [18]	PVA/MCNTs/HA scaffold (freeze-dried + SBF)	MC3T3-E1	Control, PVA, PVA+MCNTs, PVA+MCNTs+HA	SEM, XRD, TGA, WCA, CCK-8, ALP, RT-qPCR	↑ Cell adhesion, ALP, osteogenic gene expression, mineralisation in PVA/MCNTs/HA aerogel scaffold
Pang B et al., 2024 [19]	CB by hydrothermal conversion calcination	L929, rBMSCs	HA, C-HA, C-BCP, C-βTCP	SEM, XRD, FTIR, EDS, degradation,Ca^{2+}/Sr^{2+} release, SBF, CCK-8, ALP, ARS, qPCR	C-βTCP → best Ca^{2+}/Sr^{2+} release, ALP & OCN expression; highest degradation & mineral deposition
Tangsuksant T et al., 2023 [20]	CB calcined + wet-chem/ hydrothermal conversion	hDPSCs	CB-HA, CB-βTCP, CB-BCP, commercial BCP, control	SEM, TEM, XRD, FTIR, ICP-OES, cytotoxicity, Live/Dead, ALP, ARS, OCN ELISA	CB-BCP → best mineralisation, Ca^{2+} release, OCN expression, highest ALP
Hongmin L et al., 2015 [21]	CB deproteinised + hydrothermal conversion	Mouse MSCs	Native CB, CB-HA	SEM, XRD, FTIR, protein adsorption, MTS, ALP, OCN	CB-HA showed superior protein adsorption, cell adhesion, proliferation, and osteogenic marker expression compared to native CB.
Kim BS et al., 2014 [22]	CB by hydrothermal conversion blended with PCL	MG-63	PCL, PCL/CB-HA	XRD, SEM, porosity, MTS, DNA, Live/Dead, ALP, SEM	CB-HA improved cell proliferation, ALP activity and surface attachment.
Kim BS et al., 2017 [23]	CB-HA by hydrothermal conversion with Si doping	hMSCs	CB-HA, Si-CB-HA	SEM, EDS, XRD, ICP, MTS, DNA, Live/Dead, ALP, qPCR	Si-CB-HAp enhanced MSC proliferation, viability, ALP activity and expression of Runx2, Col1A1, and OC.
Palaveniene A et al., 2019 [24]	CB-HA by hydrothermal conversion + regenerated cellulose	MG-63	RC, RC/CB-HA	SEM, micro-CT, compression, WST-1, proliferation, ALP, OCN, ARS, fluorescence microscopy	RC/CB-HA scaffold showed good cytocompatibility, improved mechanical strength, enhanced cell proliferation, ALP and OCN secretion, and notable mineralisation.
Cozza N et al., 2018 [25]	CB-HA ball milled + bioglass	MG-63	HA, HA+bioglass, CB-HA, CB-HA+bioglass	XRD, FTIR, SEM, compression, DNA, Alamar Blue, ALP	CB-based samples showed higher proliferation and mineralisation than synthetic HA.CB-HA+ bioglass had the highest ALP activity
Habiburrohman MR et al., 2025 [26]	CB (lamellar) calcined + hydrothermal conversion + nCHA + PEO/CS scaffold	MC3T3-E1	nCHA/PEO/CS ratios	XRD, FTIR, SEM, TEM, EDS, MTT, swelling, adhesion, ALP	nCHA/PEO/CS-11 → balanced pore architecture, mechanical stability, swelling; high cytocompatibility, ↑ cell attachment & ALP
Bauer L et al., 2024 [27]	CB-HA by hydrothermal conversion with Ion-substitution (Mn, Mg, Sr)	hMSCs	HA, Mn-HA, Mg/ Sr-HA, multi-ion	XRD, FTIR, SEM, qPCR, immunohistochemistry, H&E	PCL-coated 1-Mn-1-Mg-1-Sr-HAp → preserved porosity, supported hMSC attachment; ↑ ALP & DMP1 vs. HAp — early osteogenic differentiation; positive collagen I staining
Sukul M et al., 2016 [28]	CB (lamellar) → SBF immersion + collagen	Rat MSCs	CB, CB-HA, CB-HA-COL	SEM, porosity, compression, F-actin, MTT, ALP	CB-HA-COL → highest rBMSC proliferation & ALP; SEM showed improved porosity/topography.
Milovac D et al., 2014 [30]	CB-HA by hydrothermal conversion coated with PCL	MC3T3-E1	HA, PCL-CB-HA	MTT, SEM, fluorescence, ALP, collagen assays	PCL-coated scaffolds → better cell adhesion, cytoskeletal organisation, ↑ ALP, collagen production & type I collagen expression
Battistella E et al., 2010 [29]	CB-HA by hydrothermal conversion	MC3T3-E1	ET–/ET+ ,ST–/ST+ , CB HA–/HA+ ± osteogenic medium	MTT, ALP, OCN ELISA, SEM	CB-HA scaffolds → ↑ osteoblast proliferation/ differentiation, ALP, and OCN under osteogenic conditions.

[Table/Fig-5]: Summary of in-vitro studies included in the study evaluating cuttle bone derived HA & composites for bone regeneration [18-30].

Author, Year	Material Preparation	Study Model	Defect Type and Size	Groups	Tests Conducted	Key Findings	Follow- up
Liu S et al., 2022 [18]	PVA/MCNTs/HA scaffold (freeze-dried + SBF)	Sprague-Dawley rats	Calvarial, 5 mm	Control, PVA, PVA/ MCNTs, PVA/ MCNTs/HA	Micro-CT, MT	PVA/MCNTs/HA ↑ bone volume, density, collagen	8 week
Hongmin L et al., 2015 [21]	CB deproteinised + hydrothermal conversion to CB-HA	BALB/c mice	Ectopic model	Native CB, CB-HA	H&E	CB-HA induced bone formation	4 & 8 week
Kim BS et al., 2014 [22]	CB by hydrothermal conversion blended with PCL	New Zealand rabbits	Calvarial, 8 mm	PCL, PCL/CB-HA	Micro-CT, H&E, MT	CB-HA scaffold ↑ bone fill and integration, histology showed mature bone and integration.	2 & 8 week

Kim BS et al., 2017 [23]	CB-HA by hydrothermal conversion with Si doping	New Zealand white rabbits	Calvarial,8 mm	CB-HA, Si-CB-HA	Micro-CT, H&E, MT	Si-CB-HA ↑ bone volume & integration	8 week
Aminatun A et al., 2019 [31]	CB-HA by hydrothermal conversion	*Rattus norvegicus*	Femoral,1 mm	Control, bovine HA, CB-HA	XRD, histology	CB-HA promoted bone cell activity & woven/lamellar bone formation; regeneration more mature.	28 & 56 day
Won S et al., 2015 [32]	CB1: defatted, freeze-dried; CB2: +4% NaOCl; CB-HA: CB2 + hydrothermal conversion.	Rabbit	Calvarial, 5mm	CB1, CB1+BMP-2, CB2, CB-HA, CHA, blank	Radiographs & gray-level histogram , H&E, histomorphometry	CB-HA → visible bone regeneration, minimal inflammation; CB1 & CB2 fibrous encapsulation; CB1+rhBMP-2 → early bone formation; CHA highest regeneration, CB-HA comparable	4,8,12 week
Kim BS et al., 2014 [22]	CB-HA by hydrothermal conversion	New Zealand white rabbits	Calvarial, 10 mm	Empty, HA, CB-HA	BV% analysis, Micro-CT, H&E, MT	CB-HA ↑ new bone & integration	2 & 8 week
Mansouri K 2018 [33]	CB and CB-HAp prepared by hydrothermal conversion±PRP	New Zealand White rabbits	Tibial, 6 mm	Control, CB, CB-HA, CB+PRP, CB-HA+PRP	Fluoroscopy, histology, SEM	CB-HA+PRP → best bone trabeculae, porous integration	56 day
Kloping LP 2016 [34]	CB-HA powder + NaCl	Male *Rattus norvegicus*	Tibia fractured, repositioned, and fixated.	Control (NaCl), CB extract	Radiology, H&E	CB extract ↑ callus & osteoblasts	14 day

[Table/Fig-6]: Summary of in-vivo studies included in the study evaluating cuttle bone derived HA & composites for bone regeneration [18,21-23,31-34].

CB-HA and its composites showed high cytocompatibility. Liu S et al., (2022) reported peak viability at day 7 on PVA/MCNTs/HA versus other scaffolds [18]; Pang B et al., confirmed >90% activity in C-HA, C-BCP, and C-βTCP across 200-800 μg/mL [19]. Tangsuksant T et al., found >100% vitality in the DM group, followed by CB-HA [20]; Sukul M et al., noted the highest proliferation on CB-HA-COL [28]. Kim BS showed >2× higher proliferation on PCL/CB-HA than PCL at day 3 [22]; Kim BS found consistently higher OD on Si-CB-HA versus CB-HA [23]; Kim BS reported higher MG-63 density on CB-HA granules than pure HA [23]. Palaveniene P et al., recorded 55-67% viability on RC/CB-HA [24]; Habiburrohman MR et al., found >80% in all, with CS-11 outperforming CS-21 [26]. Cozza N et al., and Milovac D et al., showed improved activity and spreading with bioactive additives [25,30]; Battistella E et al., found HA less supportive than titanium [29].

The ALP is a key early marker of osteogenic differentiation, with activity rising during matrix maturation- just prior to mineralisation- and reflecting active cell-matrix interactions and osteoblast function. Across studies, CB-HAp and its composites consistently enhanced ALP activity, indicating their potential to promote early osteogenesis [30].

Liu S et al., found higher ALP on PVA/MCNTs/HA than controls at days 7-10, peaking on day 10 [18]. Pang B et al., reported significantly greater ALP in C-HA, C-BCP, and C-βTCP than commercial HA [19]; Tangsuksant T et al., observed elevated ALP in DPSCs with CB-HA and CB-BCP [20]. Hongmin L et al., recorded a 35.4% higher ALP on CB-HA versus native CB [21]. Kim BS showed >2× higher ALP on PCL/CB-HA than PCL [22]; Kim BS reported ~1.9-fold higher on Si-CB-HA versus CB-HA and also found ~2× higher ALP on CB-HA than pure HA [23]. Sukul M et al., noted stronger ALP expression on CB-HA-COL [28]. Palaveniene P et al., saw time-dependent increases on RC/CB-HA surpassing controls, while Cozza N et al., showed early upregulation by day 3 in CF30B composites [24,25]. Habiburrohman MR et al., found CS-11 scaffolds had greater ALP than CS-21 ($p<0.001$), indicating chitosan-driven differentiation [26]. Milovac D et al., reported ALP peaking at day 14 on HA/PCL, consistent with preosteoblast maturation, while Battistella E et al., confirmed HA's osteoinductive capacity, comparable to titanium [29,30]. Bauer L et al., observed ALP exceeding DMP1 in hMSCs on PCL-coated 1-Mn-1-Mg-1-Sr HA, suggesting retention in the early differentiation phase [27].

Alizarin Red Staining (ARS), a standard indicator of mineralisation and late-stage osteogenic differentiation, showed that CB-Hap-based materials enhance calcium deposition [32]. Pang B et al., observed the greatest mineralisation in C-βTCP, followed by C-HA and C-BCP, likely due to higher Ca^{2+} content promoting rBMSC differentiation [19]. Tangsuksant T et al., found CB-HA and CB-BCP induced greater mineralisation than standard osteogenic medium or CB-βTCP and DM particles [20]. Palaveniene P et al., reported marked calcium accumulation on RC/CB-HA scaffolds, absent in RC controls [24].

The CB-HA-based scaffolds consistently promoted both early and late osteogenic gene expression. Liu S et al., found that PVA/MCNTs/HA scaffolds yielded the highest Runx2, OCN, and Col(I) expression between days 7-21 [18]. Pang B et al., reported that C-βTCP produced the greatest upregulation of ALP (early marker) and OCN (late marker) among all tested groups [19]. Tangsuksant T et al., noted a non-significant trend toward higher OCN in CB-BCP [20]. Hongmin L et al., recorded a 21.2% increase in OCN expression on CB-HA compared with CB [21]. Kim BS et al., observed ~3.1-fold, ~7.6-fold, and ~1.9-fold increases in Runx2, Col1A1, and OCN, respectively, on Si-CB-HA [23]. Palaveniene P et al., reported significantly higher OCN secretion on RC/CB-HA than controls [24]. Bauer LA et al., demonstrated that ALP expression exceeded DMP1 in hMSCs on PCL-coated 1-Mn-1-Mg-1-Sr HA, suggesting retention in an early differentiation phase, consistent with previous PCL-coated CaP systems [27]. Battistella E et al., found OCN levels on HA derived from cuttlefish bone comparable to etched and sandblasted titanium scaffolds, indicating similar late-stage osteogenic performance [29].

Histological evaluation methods included Masson's trichrome, H&E staining, histopathology, histomorphometry, immunohistochemistry, and fluorescence imaging. Liu S et al., (2022) noted more collagen fibers with PVA/MCNTs/HA aerogels [18]; Hongmin L et al., (2015) observed 7.58% pore fill with new bone at 8 weeks in CB-HA, absent in native CB [21]. Kim BS et al., and Kim BS et al., reported more mature bone, osteoblast-like cells, and vascularisation in CB-HA composites than unmodified HA [22,23]. Bauer LA et al., confirmed type I collagen synthesis and bone-like matrix formation in PCL-coated 1-Mn-1-Sr-1-Mg-HA scaffolds [27]. Milovac D et al., found higher collagen in HA at day 7, shifting to HA/PCL at days 14-21 [30]. Aminatun A et al., reported increased osteoblasts and osteoclasts in the cuttlefish bone treatment group compared to controls [31]. Won S et al., (2015) showed faster cortical bone continuity and marrow formation in CHA and CB-HA [32]. Mansouri K et al., noted Haversian system formation in CB-HA+PRP and Kloping LP et al., reported higher osteoblast counts in treated groups [33,34].

Radiological assessments, mainly micro-CT, quantified bone regeneration alongside radiographs and bone volume analysis. Liu

S et al., showed accelerated new bone formation at 8 weeks in PVA/MCNTs/HA aerogel-filled defects [18]. Kim BS et al., reported significantly greater %BV and bone ingrowth in PCL/CB-HA scaffolds versus empty defects, with HA-PCL outperforming pure PCL [22]. Kim BS et al., found Si-CB-HA produced higher bone volume than CB-HA, supported by coronal micro-CT images [23]. Kim BS et al., noted BV% increases in pure HA (14.19±2.53%) and CB-HA (17.33±2.55%) over untreated controls (3.26±1.34%) [23]. Won S et al., observed markedly greater regeneration in CB-HA versus CB1, CB2, and CB1 + BMP at 12 weeks [32]. Kloping LP et al., reported ~1.5× thicker callus in the CB extract group, attributed to higher osteoblast production [34].

Comparisons were made between CB-HA and conventional materials such as HA, β-TCP, CB-BCP, native CB, commercial BCP, other grafts, or untreated controls. Overall, CB-HA demonstrated superior osteogenesis, mineralisation, and cellular integration in most cases, although some studies favored CB-BCP. Several investigations assessed CB-HA composites incorporating polymers or dopants such as PVA, PCL, silicon, regenerated cellulose scaffold, PEO/chitosan, and Mn-Sr [18,22,23,25,26,31], which often exhibited synergistic improvements in performance. Collectively, these findings highlight CB-HA's promise not only as a standalone biomaterial but also as a functional additive within composite scaffolds.

The risk of bias assessment for both in-vivo and in-vitro studies revealed consistent methodological challenges primarily related to incomplete reporting or implementation of allocation concealment and blinding procedures. In-vitro studies [Table/Fig-3] exhibited variable risk across parameters but also showed unclear or high risk in areas such as investigator blinding and funding disclosures. In-vivo studies [Table/Fig-4] generally maintained low risk in parameters related to sequence generation and baseline characteristics; however, high risk was identified in animal housing and blinding domains, which could introduce performance and detection biases. These findings highlight the need for improved standardisation and transparency in preclinical study design and reporting to enhance the reliability and reproducibility of research findings.

DISCUSSION

This systematic review evaluated the osteogenic potential of CB-HA across in-vitro and in-vivo models, and the collective results clearly indicate its superiority over conventional bone graft substitutes. CB-HA consistently supported new bone formation, mineralisation, and histological integration, which can be attributed to its unique biomimetic structure, trace element content, and favorable biodegradation profile.

Importantly, not only pure CB-HA but also its composites demonstrated strong osteogenic potential. In accordance with observations by Liu S et al., Tangsuksant T et al., and CB-HA and CB-BCP scaffolds promoted superior osteoblastic differentiation and viability in hDPSCs compared to commercial BCP, while the ordered porosity and surface roughness of CB-HA aerogels further enhanced nutrient transport, cell adhesion, and osteogenesis [18,20]. These effects were amplified by the incorporation of multi-walled carbon nanotubes, which significantly improved scaffold hydrophilicity and mechanical strength.

In contrast, further enhancement of CB-HA's performance was observed when modified with specific dopants or combined with polymeric matrices. Kim BS et al., demonstrated that PCL/CB-HA scaffolds significantly improved osteoblast proliferation and differentiation due to increased surface roughness and the release of bioactive magnesium ions [22]. Likewise, silicon doping (Kim BS et al., 2017 [23]) enhanced bone ingrowth and repair, underlining the osteogenic role of Si ions. Similarly, Palaveniene A et al., reported that regenerated cellulose/CB-HA scaffolds with multi-scale porosity mimicked native trabecular bone architecture, supporting cell adhesion, proliferation, and biomineralisation [24]. Habiburrohman MR et al., further confirmed that nCHA/PEO/CS scaffolds with fibrous ECM-like features and low crystallinity promoted vascularisation alongside osteogenic differentiation [26]. Additionally, Bauer L et al., showed that Mn^{2+}, Mg^{2+}, and Sr^{2+} substitutions in CB-derived calcium phosphates synergistically stimulated osteoblast activity, suppressed osteoclast function, and enhanced bone regeneration outcomes [27].

All the included studies were in strong accordance regarding the excellent biocompatibility of CB-HA and its composites. Liu S et al., Pang B et al., Tangsuksant T et al., Kim BS, Sukul M et al., and Kim BS et al., consistently demonstrated high cell viability, proliferation, and cytocompatibility across various CB-HA scaffolds [18-20,22,23,28]. Similarly, Palaveniene A et al., Cozza N et al., Habiburrohman MR et al., and Milovac D et al., confirmed the safe interaction of CB-HA with osteogenic cells, with no adverse reactions reported [24-26,30]. Together, these findings affirm CB-HA's excellent biological safety and suitability as a biocompatible bone graft material.

From a materials science perspective, CB-HAp possesses unique physicochemical characteristics that offer clear advantages over conventional calcium phosphate biomaterials. Its naturally occurring lamellar and porous architecture closely mimics the hierarchical structure of trabecular bone, making it a highly biomimetic material for bone tissue engineering. This inherent similarity not only facilitates cell adhesion and vascular ingrowth but also enhances osteoconductivity and bone regeneration potential [35].

Future studies should therefore focus on standardising scaffold fabrication protocols, extending evaluation to large-animal load-bearing models, and performing long-term analyses on resorption kinetics, mechanical remodeling, and immune responses. Exploration of GMP-compliant production and sterilisation methods is also essential to ensure scalability and regulatory acceptance. In summary, CB-HA demonstrates superior osteogenic potential compared with synthetic and xenogeneic substitutes, largely due to its biomimetic architecture, bioactive trace elements, and capacity for polymeric and ionic modifications. Collectively, these findings position CB-HA as a highly promising next-generation bone graft substitute for clinical applications in orthopaedics, craniofacial reconstruction, and dental regenerative medicine.

Limitation(s)

Despite these encouraging outcomes, some limitations need to be recognised. Considerable heterogeneity was observed across the studies, including differences in material preparation, cell lines, animal models, defect types, and follow-up durations, which precluded meta-analysis. Most studies were limited to small animal models and short observation periods (≤12 weeks), restricting insights into long-term remodeling and load-bearing performance. Additionally, gaps in methodological rigour such as incomplete reporting of randomisation, blinding, and allocation concealment limit the reliability of outcomes.

CONCLUSION(S)

The CB-HA consistently outperforms conventional calcium phosphate grafts in promoting osteogenesis, mineralisation, and tissue integration in preclinical models. Its success is attributed to biomimetic structure, trace bioactive ions, and enhanced composite or doped formulations. With no reported adverse reactions, CB-HA shows strong promise as a safe, bioactive scaffold. Future clinical translation requires standardised fabrication, long-term large-animal studies, and regulatory-aligned manufacturing. Optimised CB-HA composites are poised for early clinical trials in bone regeneration.

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